

PROCESS OPTIMIZATION FOR MICROGREEN BASED READY TO SERVE BEVERAGE

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Food Science and Technology

By

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LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2025

DECLARATION

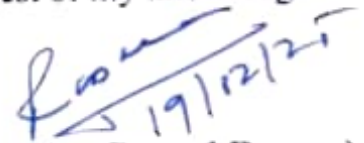
I, hereby declare that the presented work in the Project entitled “**Process Optimization for Microgreen Based Ready to Serve Beverage**” in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of **Dr. Prasad Rasane**, Professor and Head of Department (Food Technology and Nutrition) of School of Agriculture, Lovely Professional University, for the award of degree Ph.D. Food Science and Technology.



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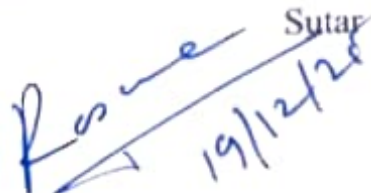
Food Technology and Nutrition

The Ph.D. Viva-Voce examination of research scholar Gunjal Mahendra Vishram has been held on 08/12/2025 and accepted for the award of Ph.D. Degree



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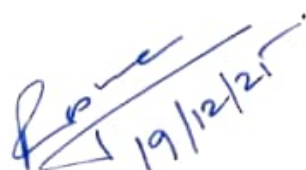


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Head of Department: Dr. Prasad Rasane

CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "**Process Optimization for Microgreen Based Ready to Serve Beverage**" submitted in fulfilment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the Department of Food Technology and Nutrition, School of Agriculture, is a research work carried out by Gunjal Mahendra Vishram, 12013899, is bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



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Place: Phagwara, Punjab (India)

Gunjal Mahendra Vishram

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ABSTRACT

Microgreens from different botanical species (*Amaranthaceae*, *Brassicaceae*, and *Linaceae*) were cultivated using three selected growing mediums (soil, cocopeat, and hydroponics), different LED light spectra (red, blue, yellow, and green), and foliar spray treatments (calcium chloride (CaCl_2) and potassium nitrate (KNO_3)). The various morphological properties (shoot length, fresh weight, and leaf area), proximate, chemical composition, and antioxidant activities of different botanical species of microgreens were examined. This study aims to address the effect of these treatments on growth parameters, bioactive compounds, and antioxidant activity, providing a comparative assessment of cultivation methods for optimizing nutritional values of microgreens. The findings revealed that the growing medium significantly influenced the quality parameters of the microgreens. Among the selected species, flax crops and radish sango microgreens exhibited superior morphological parameters such as shoot height, length, and yield compared to broccoli, cabbage, pak choi, beetroot, and red amaranthus microgreens. The moisture content in microgreens ranged from 91.92% to 95.33%, while relatively low levels of fat (2.23-7.58 g/100g) and protein (2.04-6.11 g BSA/100g) were observed. Comparative analysis indicated that flax crops and cabbage microgreens had the highest total chlorophyll content (26.28-29.16 $\mu\text{g/g}$), whereas beetroot microgreens exhibited the highest levels of carotenoids (7.42-8.09 $\mu\text{g/g}$). Cabbage microgreens contained the highest ascorbic acid content (329.20-438.71 mg/100g), while radish sango microgreens had elevated levels of anthocyanins (28.06-45.87 $\mu\text{mol}/100\text{g}$), total phenols (241.98-667.48 mg GAE/100g), total flavonoids (27.64-39.44 mg QE/100g), and antioxidant activity (DPPH 61.71-89.24%, FRAP 14.39-17.46 $\mu\text{mol TE/g}$). Further, all results were compared and verified by principle component analysis. Throughout the study, it was noticed and concluded that growing media is crucial for the growth and development of microgreens, and any modifications to the substrate will have an important effect on both the nutritional value and morphological traits of microgreens. Overall, the study concluded that based on the above mentioned results, among the growing medium (soil, cocopeat and hydroponics) the cocopeat-based medium was the most effective for enhancing plant growth (shoot height and leaf area), yield, and nutritional quality.

Further research focused on optimizing LED light treatments for microgreens from the *Brassicaceae* family, including broccoli, cabbage, pak choi, and radish sango. Growth under different LED light spectra (red, blue, yellow, and green) demonstrated that the highest growth for radish sango microgreens under red and blue LEDs light treatment for morphological

parameters such as fresh weight (3962.39 to 4614.32 g/m²), shoot height (6.27 to 9.72 cm), and leaf area (0.72 to 1.04 cm²). Blue LED treatments yielded the highest overall growth performance. The *Brassicaceae* microgreens exhibited varying levels of bioactive compounds, including total chlorophyll (9.05-53.48 µg/g), carotenoids (2.24-8.86 µg/g), anthocyanins (3.07-66.36 µmol/100g), total phenols (246.16-365.07 mg GAE/100g), flavonoids (13.67-42.33 mg QE/100g), and glucosinolates (17.60-39.25 µmol/g). Among all treatments, radish sango microgreens demonstrated the highest antioxidant activities, including radical scavenging activity (69.11-72.98%) and ferric-reducing antioxidant power (21.01-24.46 µmol TE/g). These findings highlight the potential of LED lighting for optimizing microgreen production by tailoring light exposure to enhance specific nutritional qualities.

In another study optimized foliar spray treatments of calcium chloride (CaCl₂) and potassium nitrate (KNO₃) to enhance the growth and biochemical properties of radish sango microgreens. Using response surface methodology (RSM), concentrations of CaCl₂ (4–18 mM) and KNO₃ (2–8 mM) were tested on parameters such as shoot height, fresh and dry weight, total phenolic and flavonoid content, chlorophyll, carotenoid, anthocyanin levels, and antioxidant activity (DPPH and FRAP). The optimal treatment (18 mM CaCl₂, 8 mM KNO₃) significantly improved growth and bioactive compound accumulation, achieving a desirability score of 0.809. The principal component analysis identified morphological parameters and antioxidant properties as key factors, explaining 83.1% of the variance. This research highlights the potential of tailored foliar applications to enhance the nutritional quality and antioxidant profile of microgreens.

Postharvest techniques were also explored, including washing treatments, packaging materials, and storage conditions, to extend the shelf life of radish sango microgreens. Various washing treatments, such as citric acid (0.50%, pH 2.6), sodium hypochlorite (150 mg/L, pH 8.3), ethanol (50%, pH 7.1), calcium chloride (10 mM, pH 6.4), acetic acid (0.5%, pH 2.9), and distilled water (pH 6.9), were assessed. Microgreens stored at 5°C in metalized polyester (MP) packaging with calcium chloride, citric acid, and sodium hypochlorite exhibited a significant reduction in microbial parameter, with total plate count maintained below 5.90 log CFU/g by the end of storage. Additionally, antioxidant retention was enhanced, with DPPH activity maintained above 70% and ascorbic acid levels exceeding 170 mg/100g, compared to rapid deterioration observed at 25°C in polystyrene tray packaging. Principal Component Analysis confirmed strong associations between antioxidant activity, bioactive compounds, and low-temperature storage, identifying optimal postharvest techniques to enhance commercial viability.

Drying techniques for radish sango microgreens were examined, specifically through tray drying under different temperature conditions (40°C, 50°C, and 60°C) and varying tray loading densities. The Midilli et al. model was identified as the most suitable for predicting drying patterns, with effective moisture diffusivity ranging from 5.35×10^{-7} to 1.273×10^{-6} m²/s. Tray drying at 60°C with optimized loading density proved most effective for energy efficiency and drying time reduction. Additional research investigated the impact of drying on bioactive compounds and antioxidant properties. Drying at 50°C with 0.057 g/cm² preserved the highest bioactive compounds, including anthocyanins (43.23 µmol/100 g), phenolics (189.45 mg GAE/100 g), and ascorbic acid (239.18 mg/100 g), with notable antioxidant activities (DPPH 43.13%, and FRAP 10.80 µmol TE/g). Degradation kinetics followed a first-order model, with thermal sensitivity varying among compounds.

Finally, the study developed and optimized microgreen-based dairy and Ready-to-Serve (RTS) beverages, integrating microgreens into functional food products. The optimized dairy beverage (lassi) formulation contained 70% dahi and 2.5% microgreen powder, yielding high levels of phenolics (114.00 mg GAE/100g), flavonoids (67.77 mg QE/100g), anthocyanins (48.23 µmol/100g), and ascorbic acid (152.77 mg/100g). Similarly, the 9.189% microgreen juice, 60% dahi, and 15.311% water achieved a desirability score of 0.781, significantly enhancing the beverage bioactive compounds. The optimized lassi beverage contained phenolic compounds (44.14 mg GAE/100g), flavonoids (22.62 mg QE/100g), anthocyanins (37.91 µmol/100g), and ascorbic acid (59.32 mg/100g), with an overall acceptability rating of 8.29. After 15 days at 5°C, the lassi exhibited the highest retention of bioactive compounds, alongside a lower total plate count (5.75 log cfu/mL) and yeast and mold count (4.69 log cfu/mL), ensuring microbial safety. The RTS beverage, incorporating microgreens (juice and powder) and pomegranate juice, displayed enhanced bioactive content of prepared beverage formulations. The kinetic degradation of key quality indices such as ascorbic acid, total phenolic content, antioxidant activity, and microbial stability was evaluated under different storage conditions (5°C, 25°C, and 37°C) using zero-, first-, and second-order reaction models. The results revealed that ascorbic acid degradation followed first-order kinetics. The Arrhenius, Eyring, and Ball models confirmed that higher temperatures significantly accelerated degradation, with ascorbic acid and phenolics displaying the highest temperature sensitivity. Refrigerated storage (5°C) effectively preserved bioactive compounds and sensory attributes, extending the beverage's shelf-life to 30 days. Overall, these findings provide a comprehensive framework for microgreen cultivation, processing, and functional food applications, supporting their role in sustainable agriculture and health-promoting diets.

CHAPTER-1

INTRODUCTION

With the rapid increase in urbanization and industrialization, mankind is facing several negative impacts on health and lifestyle worldwide (Voumik & Sultana, 2022). Lifestyle disorders such as diabetes, obesity, cardiovascular diseases (CVDs), hypertension, stroke, and cancer are becoming increasingly prevalent, posing significant challenges to global health (Powell-Wiley et al., 2021). However, scientific evidence suggests that proper nutrition and dietary modifications play a crucial role in preventing and managing these chronic conditions (Neuhouser, 2018). The growing awareness of health and wellness has fueled the demand for functional foods, which are rich sources of essential vitamins, minerals, and bioactive compounds. These protective foods offer numerous health benefits, including enhanced immunity, improved metabolic functions, and reduced risk of lifestyle-related diseases. While commonly consumed foods such as fruits and vegetables have long been recognized for their nutritional value, emerging trends in functional nutrition have spotlighted microgreens as a novel and potent source of bioactive compounds (Vignesh et al., 2024).

Over the last few decades, microgreens have gradually become popular as new forms of specialized vegetables and functional foods. They are very sensitive and immature leafy greens harvested from the first two pairs of leaves and marketed as a whole, including the leaves, stems, and cotyledons. Their cultivation started in the late 1980s, and their interest has widely increased due to their nutritional value (Treadwell et al., 2010). They are a relatively new form of product that has gained increasing popularity and are frequently referred to as the ‘vegetable confetti’. In addition to their potential health benefits, microgreen cultivation has different advantages, including ease of cultivation, a short cultivation duration, flexibility for indoor farming, and a high level of income for cultivars with an approximate price of \$30–50 per pound. It is more sustainable than mature crops (herbs and vegetables) because it requires a lower footprint of fertilizers, water, and space for cultivation (Kyriacou et al., 2020). Microgreens are young immature vegetables harvested 7 to 21 days after germination and are typically 1 to 3 inches tall. They consist of a stem, cotyledonary leaves, and true leaves. Unlike sprouts, which include seeds and roots, microgreens are consumed without roots. They present delicate textures and distinct flavors and are richer in minerals and micronutrients than fully-grown plants (Singh et al., 2024).

For the cultivation of microgreens, different species of seeds are used, such as vegetables (broccoli, radish, cabbage, kale, and mustard), herbs (basil, cilantro, parsley, dill,

and fenugreek), grains (wheatgrass, barley, and millet), cereals (sorghum and corn), legumes, and beans (lentils, chickpeas, mung beans, alfalfa) (Ebert, 2022). In this current study work different families of microgreens were used including, Amaranthaceae (Beetroot, Red amaranthus), Brassicaceae (Cabbage, Broccoli, Pak choi, Radish sango), and Linaceae (Flaxseed) microgreens all present notable health-promoting properties, rich bioactive compound profiles, and require specific cultivation practices to maximize their nutritional value. These microgreens contain high levels of polyphenols, flavonoids, dietary fibers, carotenoids, anthocyanins, glucosinolates, and antioxidants (Singh et al., 2024). The Amaranthaceae family microgreens such as (red amaranthus and beetroot) are good source of nutrient and bioactive compounds that possesses medicinal health benefits such as antioxidant, anti-inflammatory, cardiovascular protection, and immune support (Bhaswant et al., 2023). Next, from Brassicaceae families (Cabbage, Broccoli, Pak choi, Radish sango) microgreens are recognized as nutrient-dense foods with antioxidant, anti-inflammatory, anticancer, antidiabetic, and antimicrobial properties (Vučetić et al., 2025). The flaxseed microgreens provide health-promoting good properties such as antioxidant, anticancer, anti-inflammatory, antidiabetic, cardiovascular, and neuroprotective benefits due to enriched bioactive compounds (Mueed et al., 2022).

A wide range of growing methods are employed, including indoor, outdoor, and regulated environments such as greenhouses and creating systems (growing systems) that include soil and soilless (Di Gioia & Santamaria, 2015). Currently, illumination systems are used for the cultivation of microgreens, but these systems have not been systematically studied to enhance nutrients and bioactive compounds in detail (Xiao et al., 2014; Meas et al., 2020). The selection of appropriate species, growth methods, types of substrates, seed quality and purity, sowing methods and germination processes, irrigation practices, fertilizers used, harvesting methods, phytosanitary qualities, and postharvest storage conditions are the most important factors for the commercialization and home-growth cultivation of microgreens (Ebert, 2022). The bioactive composition of microgreens is significantly influenced by cultivation conditions, including temperature, humidity, photoperiods, and growing mediums. Hydroponic and soil-based systems, along with foliar nutrient applications and LED-light treatments, have been explored to enhance yield and nutritional profiles. However, comprehensive studies evaluating the impact of these factors on different microgreen varieties remain limited. The Principal Component Analysis (PCA) is a multivariate statistical technique widely used to reduce dimensionality and uncover hidden patterns in complex datasets. It helps identify correlations among variables and summarizes the data into principal components that

explain the maximum variance (Nayik & Nanda, 2016). In this study, PCA is important for evaluating the combined effect of different parameters including morphological, biochemical, and antioxidant properties. This approach facilitates the identification of optimal treatments and growing conditions by grouping similar responses and highlighting key contributing traits. This research aims to assess the effect of cultivation conditions on bioactive compounds in selected microgreens, optimizing growth parameters for superior nutritional quality.

Microgreens are highly perishable due to their high moisture content and delicate cellular structure, leading to rapid degradation in quality and bioactive compounds (Singh et al., 2024). Post-harvest treatments, including washing, packaging, and temperature control, play a crucial role in extending their shelf life (Sharma et al., 2023). This study seeks to determine optimal post-harvest storage strategies for prolonging the shelf life of microgreens while retaining their functional properties. Additionally, packaging methods are being explored to enhance microbial stability and reduce post-harvest losses. Apart from these, drying is a widely adopted preservation method used to extend the shelf life of microgreens while concentrating their bioactive components for diverse applications, including powders, extracts, and nutraceuticals (Sanyukta et al., 2023; Mansouri et al., 2024). Among the various drying techniques, convective drying is widely used due to its simplicity, cost-effectiveness, and scalability. This method involves the use of heated air to remove moisture, thereby reducing microbial activity and enzymatic degradation. However, the application of high temperatures during convective drying can lead to the degradation of thermolabile bioactive compounds, structural alterations, and changes in functional quality characteristics (Akbar et al., 2023; Khalangre et al., 2024). These changes are influenced by critical factors such as drying temperature, air velocity, and tray loading density, which must be optimized to retain the nutritional and functional quality of the final product (Chakraborty et al., 2023; Akbar et al., 2023).

Microgreens contain higher levels of antioxidants and phenolic compounds than edible seeds and mature leafy greens (Xiao et al., 2012; Mir et al., 2017; Marchioni et al., 2021). In addition, it contains high levels of phytonutrients and is considered a good functional food from a nutrient viewpoint (Xiao et al., 2015). It is mostly preferred in the form of salads and soups and uses stuffing ingredients for sandwiches; it is mostly used in restaurants to improve cuisine aesthetics and value (Turner et al., 2020). In the current era, the trend of functional food consumption and nutraceutical-based products has increased because they are potential sources of bioactive compounds, vitamins, trace elements, amino acids, and antioxidants and thus provide various health benefits (Zhang et al., 2020). These bioactive compounds are known to

alter the effects of oxidative stress, protect against cardiovascular diseases, control blood sugar levels, and improve vision, anti-inflammatory properties, and antimicrobial properties. Microgreens significantly improve the overall health of an individual, especially when consumed in fresh form (Lobiuc et al., 2017). Fermentation plays a key role in improving the functional properties of dairy products (Zhi et al., 2017). The metabolic activity of lactic acid bacteria, including *Lactobacillus*, *Streptococcus*, *Enterococcus*, and *Lactococcus*, along with probiotic strains such as *Bifidobacterium* and yeast species like *Saccharomyces* spp., enhances nutrient bioavailability and stabilizes bioactive compounds (Shah et al., 2016). Additionally, fermentation contributes to desirable sensory characteristics, such as texture and flavor, which are crucial for consumer acceptance (Ścibisz et al., 2019). The incorporation of radish sango microgreens into the fermentation process presents challenges, including maintaining bioactive compound stability and minimizing potential off-flavors (Singh et al., 2024).

Developing RTS beverages enriched with microgreens presents a novel approach to integrating their bioactive components into consumer-friendly products. The formulation process requires optimization of ingredient composition, processing techniques, and stability parameters. Response surface methodology (RSM) has been widely applied in food processing to determine optimal formulations and processing conditions (Kidane, 2021). In this study, RSM will be utilized to standardize and optimize the production process of microgreen-based Ready-to-Serve (RTS) beverages, ensuring desirable sensory, nutritional, and functional attributes. The incorporation of microgreens into RTS beverages necessitates a careful selection of complementary ingredients to balance flavor, texture, and nutrient bioavailability (Sharma et al., 2020). Pomegranate juice, for instance, has been identified as a potential base due to its high polyphenol and anthocyanin content, which synergistically interacts with the bioactive compounds of microgreens to enhance overall antioxidant capacity (Gardeli et al., 2019). Furthermore, the stabilization of RTS beverages through the addition of natural hydrocolloids, such as carboxymethyl cellulose, plays a crucial role in maintaining product consistency and preventing phase separation over storage periods (Staubmann et al., 2022).

The storage stability of RTS beverages is influenced by temperature, packaging materials, and biochemical interactions (Kumar et al., 2024). Degradation kinetics of bioactive compounds, microbial stability, and sensory acceptability are critical factors in determining shelf life (Hussain et al., 2014; Fikry et al., 2020). Advanced kinetic modeling, including Arrhenius, Ball, and Eyring equations, provides insights into degradation patterns under various storage conditions (Singh et al., 2024; Kumar et al., 2024). This study will evaluate the optimal storage conditions for microgreen-based RTS beverages, ensuring long-term stability

and consumer acceptability. The application of predictive shelf-life modeling allows for the accurate estimation of nutrient retention and quality deterioration over time, facilitating informed decision-making in commercial product development (Zhi et al., 2017). Understanding the synergistic interactions between microgreens and other beverage components is essential in achieving a well-balanced, palatable, and functionally enriched final product.

The primary objectives of this study are to assess the effect of cultivation conditions (growing medium, illumination, and foliar spray) on morphological parameters, compositional constituents and bioactive compounds in selected varieties of microgreens, determine post-harvest shelf life of microgreens at different storage conditions, standardize and optimize the process conditions for the preparation of microgreen-based ready-to-serve beverages, and determine the optimal storage conditions for the developed RTS microgreen-based beverages. This research bridges critical knowledge gaps in microgreen cultivation, post-harvest management, and value-added product development.

CHAPTER-2

REVIEW OF LITERATURE

2.1. Microgreens

Over the last few decades, microgreens have gradually become popular as new forms of specialized vegetables and functional foods. They are very sensitive and immature leafy greens harvested from the first two pairs of leaves and marketed as a whole, including the leaves, stems, and cotyledons. Their cultivation started in the late 1980s, and their interest has widely increased due to their nutritional value (Treadwell et al., 2010). They are a relatively new form of product that has gained increasing popularity and are frequently referred to as the ‘vegetable confetti’. In addition to their potential health benefits, microgreen cultivation has different advantages, including ease of cultivation, a short cultivation duration, flexibility for indoor farming, and a high level of income for cultivars with an approximate price of \$30–50 per pound. It is more sustainable than mature crops (herbs and vegetables) because it requires a lower footprint of fertilizers, water, and space for cultivation (Kyriacou et al., 2020). Microgreens are young immature vegetables harvested 7 to 21 days after germination and are typically 1 to 3 inches tall. They consist of a stem, cotyledonary leaves, and true leaves. Unlike sprouts, which include seeds and roots, microgreens are consumed without roots. They present delicate textures and distinct flavors and are richer in minerals and micronutrients than fully-grown plants. The primary distinctions between microgreens, baby greens, sprouts, and vegetables are shown in **(Figure 2.1)**.

For the cultivation of microgreens, different species of seeds are used, such as vegetables, herbs, grains, cereals, legumes, and beans (Ebert, 2022). A wide range of growing methods are employed, including indoor, outdoor, and regulated environments such as greenhouses and creating systems (growing systems) that include soil and soilless (Di Gioia F & Santamaria, 2015). Currently, illumination systems are used for the cultivation of microgreens, but these systems have not been systematically studied to enhance nutrients and bioactive compounds in detail (Xiao et al., 2014; Meas et al., 2020). The selection of appropriate species, growth methods, types of substrates, seed quality and purity, sowing methods and germination processes, irrigation practices, fertilizers used, harvesting methods, phytosanitary qualities, and postharvest storage conditions are the most important factors for the commercialization and home-growth cultivation of microgreens (Ebert, 2022).

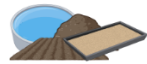
Parameters/Features	Sprouts	Microgreens	Baby greens	Mature counterparts
 Identification				
 Growing duration	2 to 7 Days	7 to 21 Days	14 to 28 Days	28 to 84 Days
 Harvesting stage	Germination stage (harvested as seedlings)	Cotyledon leaves (harvested above growing medium)	Young leaves (harvested as young leaves)	Fully developed (harvested as a mature stage)
 Growing medium	Soilless (water)	Soil, cocopeat, hydroponics, and growing pads/mats	Soil and hydroponics	Soil
 Pesticides and fertilizer	No need (pesticides and fertilizer residues absent)	No need (pesticides and fertilizer residues absent)	Need for during plant development stage (pesticides and fertilizer residues present)	Need for during plant development stage (pesticides and fertilizer residues present)
 Plants part used	Seed and roots	Leaves and stems	Leaves and stems	Full mature parts
 Flavor and texture	Mild flavor and tender texture	Intense flavor and tender texture	Mild to moderate flavor and tender texture	Moderate to strong flavor and firm texture
 Storage life	Short duration shelf life	Short duration shelf life	Longer shelf life	Longer shelf life
 Culinary use	Sandwiches, salads, toppings and stir-fries	Sandwiches, salads, smoothies, and garnishes	Sandwiches, wraps and salads	Salads, cooking
 Nutritional profile	Nutrient-dense, rich in vitamins and enzymes	High amount of vitamins, minerals, and antioxidants compounds	High amount of nutrients, vitamins, and minerals	Lower amount of vitamins and minerals compared to microgreens

Figure 2.1. The comparison between sprouts, microgreens, baby greens, and mature counterparts

Compared with seeds and mature leafy greens, micronutrients present relatively high levels of functional components, including vitamins (A, C, E, and K), minerals (Ca, Fe, Mg, Mn, Mo, Se, and Zn), antioxidants, and phenolic compounds (Xiao et al., 2012; Mir et al., 2017; Marchioni et al., 2021). In addition, it contains high levels of phytonutrients and is considered a good functional food from a nutrient viewpoint (Xiao et al., 2015). It is mostly preferred in the form of salads and soups and uses stuffing ingredients for sandwiches; it is mostly used in restaurants to improve cuisine aesthetics and value (Turner et al., 2020). In the current era, the trend of functional food consumption and nutraceutical-based products has increased because they are potential sources of bioactive compounds, vitamins, trace elements, amino acids, and antioxidants and thus provide various health benefits (Zhang et al., 2020). These bioactive compounds are known to alter the effects of oxidative stress, protect against cardiovascular diseases, control blood sugar levels, and improve vision, anti-inflammatory properties, and antimicrobial properties. Thus, microgreens significantly improve the overall health of an individual, especially when consumed in fresh form (Lobiuc et al., 2017).

2.2. Microgreen cultivation practices

Microgreens are cultivated at different levels, such as in households (small-scale production), industrial-based, and commercial marketing purposes. The various factors affecting the nutritional value of microgreens include the growing method (seed treatments, seed density, growing medium, biofortification, and illumination systems), harvesting duration, and postharvest handling practices, which are discussed in further sections (Stoleru et al., 2016).

2.2.1. Selection and treatments of microgreen seeds

The most common crops for cultivating microgreens belonging to several botanical families are legumes (Fabaceae: chickpea, lentil, alfalfa, clover, adzuki bean, and mungbean), cereals (Poaceae: barley, maize, oat, rice, rye, and wheat), Pseudocereals (Amaranthaceae: amaranth and Polygonaceae: buckwheat), oilseed (Asteraceae: sunflower, Linaceae: linseed/ flax crops and Pedaliaceae: sesame), vegetables and herbs (Amaranthaceae: beet, spinach, and onion), Amaryllidaceae: spring onion, leek, and chives, Apiaceae: celery, coriander, carrot, fennel, and parsley, Asteraceae: lettuce, *Brassicaceae*: purple mustard, Chinese kale, cabbage, purple kohlrabi, broccoli, pak choi, mizuna, turnip, flat cabbage/tatsoi, arugula, pepper cress, watercress, and daikon, Fabaceae: fenugreek, garden pea, and snow peas, Linaceae: lemon balm, sweet basil, and purple perilla) (Ebert, 2022). The germination rates of mustard, kale, cabbage, amaranth, swiss chard, and radish seeds are high, and these plants are suitable for microgreen cultivation (Nayak et al., 2021). To obtain good-quality microgreens, the selection

of seeds is the most important factor, as they must have good germination power and germination rates (above 95%). The seeds must be free from impurities as well as microorganisms such as bacteria and molds (Stoleru et al., 2016). The seeds should be handled with sanitary treatments to eliminate the presence of pathogenic bacteria, including those suggested for sprout cultivation by the US Food and Drug Administration (USFDA). Organic microgreen production without the use of chemical processes for sterilization of the seed surface and antimicrobial effects must be identified if the focus is organic cultivation. For seed disinfection treatments, the US FDA has suggested the use of a 2% calcium hypochlorite solution; some other commonly used treatments include ethanol, hydrogen peroxide, lactic acid, fatty acids, and peroxyacetic acid (Ding et al., 2013). The seeds were thoroughly rinsed with clean water after chemical treatment to remove chemical residues and impurities from the microgreen seeds. Seed density, seed type, seed weight, and population significantly affect the overall yield of microgreens (Singh, 2020).

The effects of different seed treatments and sowing densities on the cultivation of microgreen plants are presented in **(Table 2.1)**. Various researchers have studied the effects of seed treatments, such as soaking and priming, on seedling emergence before sowing (Li et al., 2021). Lee et al. (2004) observed the effects of seed treatment with matric priming (6 days in vermiculite, -1 MPa, and 12°C) and different soaking methods (water, 0.3% H₂O₂ for 48 h, 0.3 M HCl, 0.3 M NaCl for 2 h, and 4% NaOCl for 3 h) at 20°C on chard (*Beta vulgaris* L.) and table beet microgreen seeds. These experiments revealed that beet and chard had relatively high final germination percentages of 99% and 91%, respectively, after matric priming treatment. The seed treatment (H₂O₂) resulted in the earliest germination in both types of beet (*Beta vulgaris* L.). Compared with the vertical vermiculite orientation, the horizontal vermiculite orientation resulted in a greater percentage of germination with longer radicles, and a greater hypocotyl fresh weight was obtained in the horizontal vermiculite orientation (in chard) than in the vertical orientation. Cold water soaking of seeds is recommended only for large seeds (pea and radish) but not for small seeds, including cruciferous families and mucilaginous seeds (basil and lentils/ flax crops), which create jelly like coatings on the seeds when wet. The crop-specific sowing rates range from 1 seed/cm² in large-seeded species such as pea, sunflower, and chickpea to 4 seeds/cm² in small-seeded species such as watercress, arugula, and mustard, depending on the average seed weight, ability to germinate and desired shoot population density (Di Gioia & Santamaria, 2015). The experiments conducted on arugula microgreens in this study revealed that increasing the electrical conductivity of the nutrient solution and increasing the seed density significantly increased the shoot dry matter yield and total soluble

solids index. The optimal results for winter were achieved with 150 g/m² seeds at an electrical conductivity of 1 mS/cm, whereas in spring, the best outcomes required 175 g/m² seeds with at least 1 mS/cm electrical conductivity (Lerner et al., 2024). In another study, the four different microgreens, Rapini (landraces *Cima grande* and *Fasanese*), kale (landraces *Barese* and *Altamura*), and commercial cress, were grown in indoor environments with different seed densities, such as 3.0 to 5.0 seeds/cm². The study revealed that increasing the sowing density by up to 5 seeds/cm² resulted in a 19% increase in the microgreen yield compared with the lowest density. Additionally, sowing density influences dry matter content, particularly with longer growing cycles, and this effect varies among landraces, with the *Fasanese* rapini landrace showing a 7% higher dry matter content than the *Cima Grande* landrace does (Signore et al., 2024). The Microgreens seed density calculation software (version 20.1) has been reported to be a suitable tool for calculating the seed density of microgreens (Microgreens Seed Density Calculator, 2020). The increase in fresh yield per unit area of arugula and table beet microgreen has been studied, but its effect on fresh shoot cotyledon weight has not been fully elucidated. An increased sowing rate to increase yield increases production costs, whereas excessive stand density may result in variation in the elongation of shoots and restrict air circulation, which might favor fungal growth development (Murphy et al., 2010; Murphy & Pill, 2010). Nevertheless, many scientific gaps exist in the understanding of the effects of soaking pretreatment on microgreens to increase their production rates and nutrient profiles.

2.2.2. Microgreens growing media for cultivation

Microgreens are typically cultivated at the commercial level by using channels, benches, containers, or floating systems in controlled conditions, including greenhouses or high tunnels with soilless growing cultivation media (Di Gioia et al., 2017). During microgreen cultivation, growth medium parameters such as pH (5.5--6.5), water holding capacity (55--70%, v/v), aeration (20--30%, v/v), and low electrical conductivity must be considered. Cultivars prefer to grow microgreens in soil, whereas on a large scale, hydroponic growth methods are currently used. Soil substitutes, such as synthetic mats and peat-based mixes, are also used (Stoleru et al., 2016). Soil-based cultivation has numerous advantages, as it retains moisture content and serves as a rich source of micronutrients, which are helpful for plant growth (Wong et al., 2020; Gunjal et al., 2024).

As a sustainable option for microgreen production, natural fiber-based media, including cotton, jute, and cellulose, are currently attracting increasing interest (Muchjajib et al., 2015). In a previous study, a growing medium, *viz.* Hydroponic and vermicompost were used for the cultivation of cabbage and lettuce microgreens. The microgreens grown on vermicompost had

a greater nutrient profile than did the hydroponically grown microgreens. Furthermore, to increase the yield of microgreens, fertilizers such as calcium nitrate (150 mg/L) are also used. Typically, microgreens are harvested when they reach an appropriate height, and the first true leaf and cotyledon leaf appear at this stage. They are normally harvested at approximately 5-10 cm in height and depend on the microgreens family. The harvesting period is highly variable, ranging between 1 and 3 weeks; it depends on the period of sowing and the harvesting stage of the microgreens (Xiao et al., 2014). The harvesting of microgreens can be performed manually or automatically by cutting microgreens above the 1 cm growing medium. It is important to remove any unwanted materials from the growth medium and any seed integuments that may still be adhered to the cotyledons in some species (Di Gioia et al., 2017; Gunjal et al., 2024). Microgreens are highly perishable commodities because they contain a moisture content of approximately 92.1-95.1% and must be cleaned properly and chilled as soon as possible by following good handling practices (GHPs) to ensure food safety (Xiao et al., 2014; Xiao et al., 2015). After being harvested, microgreens are carefully packed into different types of packaging materials, such as polyethylene, modified atmosphere packaging, PET clamshell, and low-density polyethylene self-seal pouches, as they help reduce the microbial load and other environmental pollutants affecting quality during the storage of microgreens (Chandra et al., 2012; Paradiso et al., 2018).

2.2.3. Role of illumination systems in microgreens cultivation

Owing to its numerous advantages, such as its ability to improve yield, enhance various qualitative traits, and protect from environmental conditions, microgreen producers often use indoor growing systems instead of open sunlight (Lefsrud et al., 2006; Agarwal & Gupta, 2016). Thus, light plays a critical role in the formation and synthesis of phytoconstituents in microgreens. The commonly grown microgreens include dill, arugula cilantro, chervil, and amaranth, but illumination systems are also used to obtain better results. Different types of illumination systems, such as metal halide, incandescent, fluorescent, and high-pressure sodium (HPS) lamps, are frequently used for the cultivation of vegetables. Light-emitting diodes (LEDs) have good characteristics, such as high photon flux and spectral quality, which help to enhance the biochemical components in microgreens (Kyriacou et al., 2017). LEDs are light-emitting diodes that emit visible light, which is a vital factor that is responsible for promoting plant growth (Bula et al., 1991). These specialized diodes work similarly to P-N junction diodes in that they can transfer current in one direction but not in the other. It emits a specific wavelength depending on the semiconductor materials used, such as AlGaInP, which has red, orange, and yellow colors (Nair & Dhoble, 2015).

Table 2.1. Effects of cultivation practices on the quality of microgreens

Cultivation Practices	Microgreens Crops	Details of Treatments	Growing conditions parameters	Effects on microgreens quality	References
Seed Treatments	Table beet and chard (<i>Beta vulgaris</i> L.)	Seed soaking: 1. H ₂ O, 20 °C for 48 h; 2. H ₂ O ₂ , 0.3 % at 20 °C for 48 h; 3. HCl, 0.3 M at 20 °C for 2 h; 4. NaOCl, 4 % at 20 °C for 3 h.	Matrix priming: For 6 days in vermiculite (1:5 seed-to-vermiculite) imbued at 50 % dry weight with deionized H ₂ O. The growth chamber was kept in darkness at 12 °C.	Germination % increased and reduced harvesting days	Lee et al. (2004)
	Radish (<i>Raphanus sativus</i>), kale (<i>Brassica napus</i> var. <i>pabularia</i>), and amaranth (<i>Amaranthus tricolor</i>)	-	Matrix priming: For 6 days in vermiculite (1:5 seed-to-vermiculite) imbued at 50 % DW with deionized H ₂ O. Growth chamber in darkness at 12 °C.	Shoot dry weights increased	Lee & Pill (2005)
	Buckwheat (<i>Fagopyrum esculentum</i> Moench cv. 'Darja')	Seed soaking: Se at 10 mg L ⁻¹ I at 1000 mg L ⁻¹ ; selenate (SeO ₄ ²⁻ alone); selenite (SeO ₃ ²⁻ alone); iodate (IO ₃ ⁻ alone); iodide (I ⁻ alone); SeO ₄ ²⁻ + IO ₃ ⁻ ; SeO ₄ ²⁻ + I ⁻ ; SeO ₃ ²⁻ + IO ₃ ⁻ ; and SeO ₃ ²⁻ + I ⁻ and MilliQ water (control) soaked for 4 h in 200 mL	Growing medium: filter paper Growing parameters: day/night temperatures of 21 °C/ 16 °C, RH 60 – 80 %, and photoperiod 16 h daylight.	Se and I treatments had no significant effects on germination rates, microgreens yields increased, Se and I content increased in microgreens	Germ et al. (2019)

	Chia (<i>Salvia hispanica</i> L.)	Seed soaking: 6 and 12 h; the water at 70 – 80 °C and then allowed to cool down at room temperature for 6 and 12 h.	Growing medium: coconut coir mixed with sand and rice husk ash (1:1:1)	Germination %, microgreen height, and fresh weight increased	Junpatiw & Sangpituk (2019)
	Arugula (<i>Eruca vesicaria subsp. sativa</i>) and table beet (<i>B. vulgaris</i> L.)	Seed Soaking: 48 h in aerated deionized water	Growth chamber in darkness at 20 °C	Reduced the G50	Murphy et al. (2010)
Seed Sowing Density	Red radish (<i>Raphanus sativus</i>)	Seed Density: 6 seeds cell ⁻¹ (82.36 g m ⁻²), 8 seeds cell ⁻¹ (109.81 g m ⁻²), 10 seeds cell ⁻¹ (137.27 g m ⁻²) and 12 seeds cell ⁻¹ (164.72 g m ⁻²) Harvesting: 8 days	Growing medium: White sphagnum peat and coco coir dust	Seed density increased fresh weight microgreens increased	Thuong & Minh (2020)
	Chia (<i>Salvia hispanica</i> L.)	Seed density: 56, 93, 130, 167, and 204 g m ⁻² Harvesting: 7 days	Seed soaking: Soaked in water initially at 70 – 80 °C and then allowed to cool down at room temperature for 12 hours. Growing medium: coconut peat mixed with sand and rice husk ash (1:1:1)	Seed density increased fresh weight microgreens increased	Junpatiw & Sangpituk (2019)
	Arugula (<i>E. vesicaria subsp. sativa</i>) and table beet (<i>B. vulgaris</i> L.)	Seed Soaking: 48 h in aerated deionized water	Growth chamber in darkness at 20 °C	Increasing the sowing rate increased fresh yield per unit area and decreased mean shoot weight	Murphy et al. (2010); Murphy & Pill (2010)

	Arugula	Sowing densities: 50, 100, 150, and 200 g/m ² Electrical conductivity: 0.15, 1, 2, and 3 mS/cm	Growth chamber temperature at 20 °C	Increasing the electrical conductivity of the nutrient solution and seed density significantly enhanced shoot dry matter yield and the total soluble solids index	(Lerner et al., 2024)
	Rapini (<i>landraces Cima grande</i> and <i>Fasanese</i>), kale (<i>landraces Barese</i> and <i>Altamura</i>), and commercial cress	Sowing densities: 3.5, 4.0, and 4.5 seeds/cm ²	Growth chamber at the temperature of 20/20 °C (day/night), relative humidity (RH) ≥60%, photoperiod 18/6 (day/night), PPFD 30 μmol/m ² /s.	Increasing sowing density by up to 5 seeds/cm ² resulted in a 19% increase in microgreen yield.	(Signore et al., 2024)
Growing media	Chia (<i>Salvia hispanica</i> L.)	Growing media: coconut coir, rice husk ash, sand, coconut peat mixed with rice husk ash (1:1), and coconut peat mixed with sand and rice husk ash (1:1:1) Harvesting: 7 days	Seed soaking: Soaked in water initially at 70 – 80 °C and then allowed to cool down at room temperature for 12 hours. Growing medium: coconut peat mixed with sand and rice husk ash (1:1:1)	Seeds are sown in coconut coir and coconut coir mixed with sand and rice husk ash (1:1:1) providing the highest germination percentage range from 96-98%. Seeds are sown in coconut coir with sand and rice husk ash (1:1:1) providing the highest microgreen height (7.02 cm) and fresh weight (1.12 g/100 microgreens)	Junpatiw & Sangpituk (2019)
	Green basil (<i>Ocimum basilicum</i> L.), Red basil (<i>Ocimum basilicum</i>)	Growing medium: coconut fiber, vermiculite, and jute	Indoor growing method and controlled conditions	Growing medium effect on dry matter percentage and the nitrate and bioactive	Thuong & Minh (2020)

	‘Purpurascens’), and Rocket (<i>Eruca sativa</i> Mill.)			compounds concentration of microgreens all species.	
	Rapini (<i>Brassica rapa</i> L)	Growing medium: Peat Sure to Grow® Textile fibers Jute-kenaf fibers Harvesting: 11 days	Planting water: Nutrient solution Indoor growing method and controlled conditions	Growing media did not affect microgreen population density and fresh weight and growing media represent a potential source of microbial contamination for microgreens cultivation.	Di Gioia et al. (2017)
	Red amaranth (<i>Amaranthus Cruentus</i> L.)	Growing medium: Black soil, yellow soil, and orange soil All soils contain coconut compost (0 - 50 %)	-	Black soil showed the best results in growth, yields, and ascorbic acid and carotenoid contains	Lau et al. (2019)
	Broccoli (<i>Brassica oleracea</i>)	Growing medium: Hydroponics and soil	-	High bioactive compounds present in farm grown Soil-grown high Vitamin C present	Tan et al. (2020)
Nutrient Supplementation	Red Garnet amaranth (<i>Amaranthus tricolor</i>), Slow bolt arugula (<i>Eruca sativa</i>), Genovese basil (<i>Ocimum basilicum</i>), Waltham broccoli (<i>Brassica oleracea</i> var. <i>italic</i>), Red	Nutrients supplementation: 20 N - 8.7, P - 16.6, K, 0.05 % Mg, 0.05 % Fe, 0.025 % Mn, 0.013 % B, 0.013% Cu, 0.005 % Mo, and 0.025 % Zn, ICL Fertilizers, 100 mg L ⁻¹ N 4 days after planting. The pH of	Indoor growing method	Fertilization increased fresh shoot yield and macronutrient and micronutrient	Li et al. (2021)

	Acre cabbage (<i>Brassica oleracea</i> var. <i>capitata</i>), Daikon radish (<i>Raphanus sativus</i> var. <i>longipinnatus</i>, Red Russian kale (<i>Brassica napus</i> var. <i>pabularia</i>), Early White Vienna kohlrabi (<i>Brassica oleracea</i> var. <i>gongylodes</i>), Red Garnet mustard (<i>Brassica juncea</i>), and Green pea (<i>Pisum sativum</i>)	the fertilizer solution was 6.56 and EC 0.41 mS·cm⁻¹ Water (pH 7.54, EC 0.15 mS cm⁻¹) Soaking time: 6 h, T: 25 °C			
	Wild Rocket Napoli (<i>Diplotaxis tenuifolia</i>), Green Cabbage Copenhagen (<i>Brassica L. oleracea</i> var. <i>capitata</i>), and Green Brussels sprouts Mezzo Nano (<i>Brassica oleracea</i> var. <i>gemmifera</i>)	Nutrient supplementation: Hoagland nutrient solution Harvesting: 16 days	Growing method: Peat moss mix	Increase in total ascorbic acid and a 12 % increase in total anthocyanins.	El-Nakhel et al. (2021)
	Broccoli raab (<i>Brassica rapa</i> L. subsp. <i>sylvestris</i> L.	Nutrient supplementation: NH₄:NO₃ ratios (%):	Growing medium: hydroponic controlled conditions	Increased yield, mineral concentrations, and	Palmitessa et al. (2020)

	Janch. var. <i>esculenta</i> Hort), Broccoli (<i>Brassica oleracea</i> L. var. <i>italica</i>), and Cauliflower (<i>Brassica oleracea</i> L. var. <i>botrytis</i>)	5:95, 15:85, and 25:75. The element concentrations (mg L ⁻¹): N 105, P 16, K 117, Ca 8	Seed density: 4 seeds cm ⁻²	bioactive compounds contents	
	Broccoli (<i>Brassica oleracea</i> L. var. <i>italica</i> Cultivar: De Cicco)	Nutrient supplementation: 1 mM or 10 mM CaCl ₂ Harvesting: 10 days	Growing medium: hydroponic controlled conditions	Increased glucosinolates level, improved overall visual quality and storage life	Lu et al. (2018)
Illumination systems	Mustard (<i>Brassica juncea</i> ‘Red Lace’) and Kale (<i>Brassica napus</i> ‘Red Russian’)	Illumination systems: LED B: R light different ratios TFPD: 250 µmol m ⁻² s ⁻¹	Growing medium: hydroponic with deionized water and nutrient solution Water- deionized water NC [mg L ⁻¹]: N, 120; P, 20; K, 128; Ca, 72; Mg, 40; S, 53; Fe, 4; Mn, 0.08; Cu, 0.08; B, 0.16; Zn, 0.8. pH: 5.5 - 6.5 and EC: 1.3 - 1.7 mS cm ⁻¹	The increased percentage of blue light elongation of mustard and kale microgreens. Blue: Red light concentration changes in growth parameters and bioactive compounds	Brazaitytė et al. (2021)
	Red amaranth (<i>Amaranthus cruentus</i> L.) and Leafy vegetable amaranth (<i>Amaranthus gangeticus</i> L.)	Illumination systems: LED- Red (660 nm), Blue (447 nm), 70Red:30Blue and White (430 – 780 nm) light Harvesting: 8 days	Growing medium: Sphagnum peat moss (pH 6.0) controlled conditions	The combination of LED- 70Red:30Blue light enhanced fresh yield and bioactive compounds	Meas et al. (2020)
	Basil (<i>Ocimum basilicum</i> L., Sweet Genovese), Beet (<i>Beta vulgaris</i> L.,	Illumination systems LED- deep red (665 nm), red (638 nm), blue LEDs (447 nm),	Growing medium: Peat substrate Nutrient solutions (mg L ⁻¹): N-110, P ₂ O ₅ -50, K ₂ O-	UV-A irradiation treatments resulted in increased leaf area, fresh	Brazaitytė et al. (2015)

	bull's Blood), and Red pak choi (<i>Brassica rapa</i> var. <i>chinensis</i> , Rubi)	and far-red (731 nm) also high-power UV LEDs (366, 390, and 402 nm)	160, microelements: Fe, Mn, Cu, B, Mo, Zn. EC : 0.5-0.7 mS cm ⁻¹ . Controlled conditions	weight, and bioactive compounds	
	Mustard (<i>Brassica juncea</i> L., 'Red Lion'), Beet (<i>Beta vulgaris</i> L., 'Bulls Blood') and Parsley (<i>Petroselinum crispum</i> Mill., 'Plain Leaved or French')	Illuminations systems: LED Blue (447 nm), Red (638 nm and 660 nm), and Far-red (731 nm) PPFD - 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Growing medium: Peat substrate Nutrient solutions (mg L ⁻¹): N 110, P ₂ O ₅ 50, K ₂ O 160, and microelements Fe, Mn, Cu, B, Mo, and Zn Controlled conditions	Higher blue light intensities increase the accumulation of photosynthetic and carotenoid pigments	Samuolienė et al. (2017)
	Red amaranth (<i>Amaranthus Cruentus</i> L.)	Illuminations systems: Sunlight, red, blue, and red+blue light at 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Growing medium: Black soil	Sunlight showed the highest fresh weight, dry weight The red light highest amount of ascorbic acid	Lau et al. (2019)
	Basil (Sweet Genovese and Red Rubin)	Illumination system: LED White, Red, and Blue (120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 12 h each day)	Growing medium: Soil and Peat moss (2:1) Controlled conditions	Red and blue LED ratios improve growth and phenolic contents in both microgreens	Lobiuc et al. (2017)

The plant development stage and various physiological factors act as energy sources and signals. The major components of illumination systems are the wavelength, light intensity, light direction, and photoperiod (Jones, 2018). Liu et al. (2022) assessed the effects of different photoperiods and light intensities on the growth, nutrient, and antioxidant activity of Chinese kale and cabbage microgreens. The study involved the implementation of two different experiments, first, photosynthetic photon flux densities (PPFDs) of 30, 50, 70, and 90 $\mu\text{mol m}^{-2}\text{s}^{-1}$ with red:blue:green = 1:1:1 LEDs, followed by another experiment including five photoperiods of 12, 14, 16, 18 or 20 h/d. The hypocotyl length of both microgreens decreased when the light intensity increased. PPFDs of 70 and 90 $\mu\text{mol m}^{-2}\text{s}^{-1}$ yielded good results concerning the chemical composition of the microgreens. A photoperiod of 14 to 16 h/d may increase the biomass, nutritive quality, and antioxidant capacity of microgreens. Compared with white light, red light, blue light, and a combination of red and blue light have been found to increase photosynthesis and regulate plant metabolism more effectively (Alrifai et al., 2021). Different wavelengths of supplemental LED light affect antioxidant compounds, with green light improving the antioxidant properties of microgreens (Samuolienė et al., 2017). Blue light enhances growth, chlorophyll content, and anthocyanin pigments in microgreens, whereas red light predominantly increases phenolic content and free radical scavenging activity (Turner et al., 2021). Short-term red LED lighting increases total anthocyanins, total ascorbic acid, and specific mineral contents in various microgreens. Specific LED treatments, such as blue light applied shortly before harvest, significantly increase carotenoid, glucosinolate, and mineral contents in broccoli microgreens, highlighting the critical role of light quality in optimizing the nutritional quality of microgreens (Zhuang et al., 2022).

2.2.4. Role of foliar spray in the cultivation of microgreens

The foliar spray treatments are mostly contact sprays on various plant portions that aid in managing fertility, controlling insects and diseases, controlling plant development, and modifying the microenvironment (Suslow et al., 2003). Foliar spray is a term in which fertilizer spray is directly applied to a plant's leaves instead of being put into the soil (Antonoglou et al., 2018). Both the plant stomata and epidermis play a role in the way in which fertilizers are absorbed. Therefore, this method is appropriate for managing relatively small quantities of fertilizers, particularly micronutrients. Seeds provide adequate nutrients while in the developmental stage for young crops; therefore, most crops require very little or no fertilizer at all (Xiao et al., 2015). However, the use of organic fertilizers significantly increases the production rate (Murphy & Pill, 2010). Murphy & Pill (2010) examined the effects of various ranges of fertilizers on arugula microgreens. As an outcome, the use of nitrogen sources such

as $\text{Ca}(\text{NO}_3)_2$, NH_4NO_3 , and H_2NCONH_2 resulted in greater shoot fresh weight/ m^2 than $(\text{NH}_4)_2\text{SO}_4$.

Germ et al. (2019) studied the effects of the biofortification of buckwheat microgreens and seeds on yield and the physicochemical and biochemical properties of microgreens by adding selenium (Se), iodine (I), and selenium + iodine. The combination treatment of selenium + iodine resulted in a greater microgreens yield ($600\text{--}800\text{ gm}^{-2}$) than did treatment with selenium or iodine alone. Furthermore, the respiratory potential increased by 60–120% Fv/Fm , which was approximately 0.8% in all treated samples. The iodine content was high with IO_3^- treatment ($216\text{ }\mu\text{g g}^{-1}$), and the selenium content was reduced by 50% with the combination treatment. The impacts of selenium (Se) enrichment in kale microgreens via nutrient solution supplementation and foliar spraying were studied at various concentrations in a soilless cultivation system. The findings revealed that kale microgreens could accumulate significant amounts of Se, up to $893.3\text{ }\mu\text{g/kg}$ dry matter, with nutrient solution treatment without affecting yield. The optimal Se enrichment for fresh consumption was found to be $20\text{ }\mu\text{M}$ Se in nutrient solution (Tavan et al., 2024).

Eicher-Sodo et al. (2019) used an oxidizing agent (H_2O_2) on black oil sunflower, arugula, and radish microgreens. The foliar spray was used in the presence of water once daily at different concentrations ranging from 0 to 200 mg/L. The use of H_2O_2 did not significantly affect the yield or leaf area of any microgreen species. The maximum recommended concentrations for H_2O_2 foliar spray were 150 mg L^{-1} for radish, 100 mg L^{-1} for arugula, 125 mg L^{-1} for Othilie, 75 mg L^{-1} for Rouxai and 125 mg L^{-1} for Xandra lettuce. Goble (2018) studied the effects of Ca on radish microgreens grown hydroponically and evaluated their growth, yield, and nutrient content by using foliar spray Ca at concentrations ranging from 0–160 mM. The concentration of 20 mM Ca was found to have toxic effects on germination and growth, whereas 5 and 10 mM Ca resulted in high growth, hypocotyl length, and leaf surface area/plant. Lu et al. (2018) sprayed broccoli microgreens with CaCl_2 during the preharvest and postharvest storage periods. With the application of 10 mM CaCl_2 , they reported an increase in the level of total aliphatic glucosinolates. The overall visual quality, nutritional quality, stability of phytoconstituents, and storage life improved with the preharvest Ca spray of broccoli microgreens.

2.3. Nutritional profile of microgreens

The sprouts can be replaced with microgreens because of their vivid color, texture and flavor, nutrients, bioactive components, phytonutrient profile, and numerous health benefits (Puccinelli et al., 2019). Various studies have shown that microgreens contain higher levels of

phytonutrients than their seeds and mature parts (Yadav et al., 2018). Compared with developed vegetable crops, the *Brassicaceae* family microgreen contains more phytochemical compounds, such as vitamin A, ascorbic acid, phyloquinone, β -carotene, α -tocopherol, and glucoraphanin (Xiao et al., 2012; Choe et al., 2018). Compared with those in lettuce vegetables, the mineral (Ca, Mg, Fe, Mn, Zn, Se, and Mo) contents are greater, and a lower NO_3^- content is observed in lettuce microgreens (Pinto et al., 2015). Compared with mature leaves, celery microgreens present higher levels of minerals (N, P, Ca, Na, and S), except for K, Zn, Cu, and Mn (Singh et al., 2022). The concentrations of minerals (F, Mg, K, Ca, and total phosphorous), vitamins (C and B₉), and omega-3 fatty acids are 1 to 3 times greater in radish microgreens than in radish vegetables (Rani et al., 2018). Yadav et al. (2018) compared the antioxidant activities and mineral contents of microgreens and mature leafy greens in nine summer season crops. This study revealed that ascorbic acid, total phenolics, and total flavonoids ranged from 6.00 - 46.50 GAE mg 100 g⁻¹, 25.00 - 152.10 mg GAE 100 g⁻¹ and 9.58 - 142.39 mg CE 100 g⁻¹ in microgreens, whereas they ranged from 10.00 - 199.99 mg 100 g⁻¹, 69.01 - 313.92 mg GAE 100 g⁻¹ and 43.00 - 292.53 mg CE 100 g⁻¹ in mature leafy greens. The (Table 2.2) compares the nutritional components of microgreens and mature vegetables.

2.3.1. Nutritional components

As shown in (Table 2.3), the nutritional composition of the different botanical families of microgreens includes total dietary fiber ranging from 1.41–4.28 g 100 g⁻¹, insoluble dietary fiber ranging from 1.38–3.98 g 100 g⁻¹, soluble dietary fiber ranging from 0.02–0.31 g 100 g⁻¹, total protein ranging from 1.81–3.93 g 100 g⁻¹, total ash ranging from 0.48–1.67 g 100 g⁻¹ and total sugar ranging from 0.17–0.103 mg 100 g⁻¹ on a fresh weight basis (Ghoora et al., 2020). Other parameters, such as water content (%), dry weight (%), titratable acidity ($[\text{H}^+ \text{ mol/L}]$), pH value, and major mineral compounds, such as Na, K, P, Mg, Ca, and other microelements, such as Cu, Zn, and Se, are depicted in different botanical families of microgreens in (Table 2.3).

2.3.2. Bioactive compounds

The manufacturing of medicines, nutraceuticals, and functional foods, nutrient bioaccessibility, and bioavailability are more important for communities. A bioactive chemical is a nutrient that provides a biological signal that is converted to therapeutic properties when it is delivered to a living organism (Dima et al., 2020).

Table 2.3. Nutritional profiles of different selected families of microgreens

Biochemical Compounds	<i>Brassica</i> <i>ceae</i>	<i>Apiacea</i> <i>e</i>	<i>Amaryllida</i> <i>ceae</i>	<i>Malvac</i> <i>eae</i>	<i>Amarantha</i> <i>ceae</i>	<i>Astera</i> <i>ceae</i>	<i>Lamiaceae</i>	References
Microgreens Families Crops	39	7	1	1	4	2	4	
Water Content (%)	92.1-93.8	-	-	-	93.5-95.1	-	94.3	Xiao et al. (2015)
Dry weight (%)	6.56-7.92	5.47-10.6	5.26	8.9	6.95	8.36	5.72	Ghoora et al. (2020)
Titrateable Acidity ([H ⁺] mol L ⁻¹)	0.013-0.029	-	-	-	0.013-0.02	-	0.024	Xiao et al. (2015)
pH	5.67-5.86	-	-	-	6.37-6.43	-	5.82	Xiao et al. (2015)
Total dietary fiber (g 100 g ⁻¹ FW)	1.78-2.08	2.46-4.28	1.93	2.4	2.36	3.6	1.41	Ghoora et al. (2020)
Total ash (g 100 g ⁻¹ FW)	0.77-1.03	0.48-1.67	0.67	1.03	1.44	0.81	1.3	Ghoora et al. (2020)
Total protein (g 100 g ⁻¹ FW)	1.81-2.78	2.42-4.44	2.58	2.55	2.32	3.93	2.22	Ghoora et al. (2020)
Insoluble dietary fiber (g 100 g ⁻¹ FW)	1.5-1.92	1.39-3.98	1.69	2.11	2.22	3.38	1.38	Ghoora et al. (2020)
Soluble dietary fiber (g 100 g ⁻¹ FW)	0.16-0.28	0.09-0.31	0.24	0.29	0.14	0.25	0.02	Ghoora et al. (2020)
Fructose (mg 100 g ⁻¹)	0.5-0.56	-	-	-	0.13-0.33	-	0.08	Xiao et al. (2015)
Glucose (mg 100 g ⁻¹)	0.26-0.47	-	-	-	0.02-0.08	-	0.08	Xiao et al. (2015)
Sucrose (mg 100 g ⁻¹)	0.06	-	-	-	0.02-0.03	-	0.04	Xiao et al. (2015)
Total sugar (mg 100 g ⁻¹)	0.88-0.103	-	-	-	0.17-0.44	-	0.02	Xiao et al. (2015)

Ascorbic acid (mg 100 g ⁻¹ FW)	24.2-120.8	24.5-94.7	41.6-29.9	139.8-123.2	69-105.1	86.3-94	59-120.6	Ghoora et al. (2020); Ghoora et al. (2020); Xiao et al. (2016)
Lutein (mg 100 g ⁻¹ FW)	4-18.3	2.7-23.9	13.8	17.2	8.4-8.7	20.2	5.3-15.2	Ghoora et al. (2020); Xiao et al. (2012)
Chlorophyll a (mg 100 g ⁻¹ FW)	33-100	27.5-40.4	16.9	45.1	24.1	18.8-90	45-84	Ghoora et al. (2020); Lenzi et al. (2019)
Chlorophyll b (mg 100 g ⁻¹ FW)	17.3-55	15.8-34.3	12.6	25.9	11.2	14.5-65	27-66	Ghoora et al. (2020); Lenzi et al. (2019)
Total chlorophyll (mg 100 g ⁻¹ FW)	50.9-155	43.3-90.3	29.5	71	35.3	33.3-155	80-141	Ghoora et al. (2020); Lenzi et al. (2019)
Total phenolics (mg 100 g ⁻¹ FW)	49.3-61.8	23.5-63.5	21.4	73.6	14.6	39.4	28.6	Ghoora et al. (2020)
Total flavonoids (mg 100 g ⁻¹ FW)	1.1-2.1	3.8-5.5	1.7	6.5	2.4	5.9	2.9	Ghoora et al. (2020); Lenzi et al. (2019)
α-tocopherol (mg 100 g ⁻¹ FW)	13.8-58.8	5.1-35	15.2	10.3	17.1	48.7	16.5-24	Ghoora et al. (2020); Xiao et al. (2012)
β-carotene (mg 100 g ⁻¹ FW)	5.4-11.5	0.6-11.7	3.8	6.4	6.1-8.6	4.5	6.1-6.8	Ghoora et al. (2020); Xiao et al. (2012)
Dehydroascorbic acid (mg 100 g ⁻¹ FW)	9-40.8	9.8-22.5	-	-	26.5	-	09 12	Xiao et al. (2012); Xiao et al. (2014)
Phylloquinone (μg g ⁻¹ FW)	1.6-3.7	0.7-3.1	-	-	4.1	-	2-3.2	Xiao et al. (2012); Xiao et al. (2014)
Carotenoids (mg 100 g ⁻¹ FW)	18	-	-	-	-	11	34-10	Lenzi et al. (2019)
Anthocyanins (mg 100 g ⁻¹ FW)	13	-	-	-	-	13	1.52	Lenzi et al. (2019)
Violaxanthin (mg 100 g ⁻¹ FW)	1-3.1	1-7.7	-	-	4.4	-	2-2.7	Xiao et al. (2012)
γ-tocopherol (mg 100 g ⁻¹ FW)	5.6-16.7	3-8.3	-	-	11.2	-	6-8.3	Xiao et al. (2012)

Ca (mg 100 g ⁻¹ FW)	51.2-55.8	55.1-94.7	32	59.5	20.1	59-90.8	81.5	Ghoora et al. (2020); Xiao et al. (2016); Lenzi et al. (2019)
Mg (mg 100 g ⁻¹ FW)	30.1-35.2	38.1-52	22.8	63.5	71.2	47.1-59.79	24.92	Ghoora et al. (2020); Xiao et al. (2016); Lenzi et al. (2019)
K (mg 100 g ⁻¹ FW)	322.8	-	-	-	-	-	-	Ghoora et al. (2020); Xiao et al. (2016)
P (mg 100 g ⁻¹ FW)	23.8-251	118.5-480.5	273.9	214.6	69.7	61.56-331.1	328.6	Ghoora et al. (2020); Xiao et al. (2016); Lenzi et al. (2019)
Na (mg 100 g ⁻¹ FW)	64.7-74.1	52-228.3	40.9	57.17	20.8	163.2	-	Ghoora et al. (2020); Xiao et al. (2016)
Fe (mg 100 g ⁻¹ FW)	0.95-2.46	0.44-1.79	0.99	1.62	1.88	1.44-6.48	2.17	Ghoora et al. (2020); Xiao et al. (2016); Lenzi et al. (2019)
Zn (mg 100 g ⁻¹ FW)	1.17-1.21	0.41-1.57	1.35	1.69	1.33	0.24-0.19	0.79	Ghoora et al. (2020); Xiao et al. (2016); Lenzi et al. (2019)
Cu (mg 100 g ⁻¹ FW)	0.04-0.13	-	-	-	-	0.18	-	Xiao et al. (2016); Lenzi et al. (2019)
Mn (mg 100 g ⁻¹ FW)	0.4-3.48	-	-	-	-	2.34	-	Xiao et al. (2016); Lenzi et al. (2019)
Se (µg 100 g ⁻¹ FW)	1-1.93	1.25-3.2	0.65	3.95	1.28	1.68	1.5	Ghoora et al. (2020); Lenzi et al. (2019)

Compared with mature greens crops, micronutrients are good sources of various types of biochemical compounds, including pigments, antioxidants, phytochemicals, carotenoids, phenolic compounds, vitamins, and minerals (copper, zinc, and selenium), and they are beneficial for human health (Mir et al., 2017).

2.3.2.1. Vitamin C

Vitamin C or ascorbic acid is an essential cofactor for carrying out enzymatic reactions essential for synthesizing collagen, carnitine, and hormones (norepinephrine and epinephrine). It also inhibits oxidative damage caused by free radicals, replenishes other antioxidants, favors iron absorption, and aids in the conversion of cholesterol to bile acids (Cahill & El-Sohemy, 2011). Microgreens contain a high concentration of vitamin C, and the concentration of vitamin C in some selected botanical families ranges between 17.9 and 139.8 mg 100 g⁻¹ on a fresh weight basis (Choe et al., 2018). For example, Ghooora et al. (2020) measured the ascorbic acid content of 10 culinary microgreen species and reported that roselle microgreen constitutes 139.8 mg 100 g⁻¹ vitamin C on a fresh weight basis. In another study, an ascorbic acid content of 20.4–147.0 mg 100 g⁻¹ FW was observed in 25 botanical families of microgreens (Xiao et al., 2012). Clinical, biochemical, and epidemiological studies have revealed that a dietary intake of 90–100 mg of ascorbic acid per day helps reduce the risk of chronic health problems (Barrita & Sánchez, 2013). The amounts of ascorbic acid present in the different varieties of microgreens are listed in (Table 2.3). Most microgreens are consumed fresh without the use of different heat treatments (Lee et al., 2017).

2.3.2.2. Carotenoids

Carotenoids are fat-soluble photosynthetic pigments that are present mostly in fruits, vegetables, flowers, fungi, and a few kinds of animals in red, orange, and yellow colors (Ötles & Çagindi, 2007). Carotenoids exist in two forms, viz. carotenes (α -, β -, ψ -, γ -carotene and lycopene) and xanthophylls (zeaxanthin, β -cryptoxanthin, astaxanthin, fucoxanthin, lutein, and peridinin) (Mezzomo & Ferreira, 2016; Maoka, 2019). Microgreens are a good source of carotenoids, with 10 - 34 mg 100 g⁻¹ FW. Green basil microgreens presented the highest recorded carotenoid content of 34 mg 100 g⁻¹ FW, whereas the lowest carotenoid content was reported in red basil microgreens, i.e., 10 mg/100 g on a fresh weight basis (Lobiuc et al., 2017).

β -Carotene is regarded as a powerful fat-soluble antioxidant that imparts the characteristic red, yellow, and orange colors present in most fruits and vegetables. It is a precursor of vitamin A and plays a significant role in physiological functions, maintenance of eyesight, normal growth, and development of immune function (Johnson & Russell, 2010).

Table 2.2. Nutritional comparison between microgreens and mature vegetables

Crops	Nutritional components	Microgreens	Mature Vegetables	References
Red Cabbage	Total ascorbic acid (mg 100 g ⁻¹ FW)	147.00	24.40	Choe et al. (2018); Rizvi et al. (2022)
	Phylloquinone (µg g ⁻¹ FW)	2.8	0.04	
	β-carotene (mg 100 g ⁻¹ FW)	11.5	0.044	
	Tocopherol (mg 100 g ⁻¹ FW)	24.1	0.1	
	Lutein/ zeaxanthin (mg 100 g ⁻¹ FW)	8.6	0.3	
	Anthocyanins (µmol g ⁻¹)	12.44	33.36	
	Glucoraphanin (µmol g ⁻¹ DW)	4.80	0.88	
	Glucobrassicin (µmol g ⁻¹ DW)	1.15	1.26	
Broccoli	Glucoraphanin (µmol g ⁻¹ DW)	0.67-0.85	7.1±2.5	Choe et al. (2018); K. Khoja et al. (2020)
	Glucobrassicin (µmol g ⁻¹ DW)	10.13-10.81	1.1±0.4	
	Minerals (Fe, Ca, Cu, Mg, Mn, Mo, and Zn µg g ⁻¹ DW)	55.90, 25601.7, 6.40, 3702.53, 115.0, 2.76 and 39.65	54.37, 3911.91, 8.26, 1752.92, 25.67, 1.67 and 52.22	
Arugula	Total ascorbic acid (mg 100 g ⁻¹ FW)	45.8	15.0	Choe et al. (2018); Rizvi et al. (2022)
	Phylloquinone (µg g ⁻¹ FW)	1.6	1.1	
	β-carotene (mg 100 g ⁻¹ FW)	7.5	1.4	
	Tocopherol (mg 100 g ⁻¹ FW)	19.1	0.4	
	Lutein/ zeaxanthin (mg 100 g ⁻¹ FW)	5.4	3.6	
Radish	Minerals (F ⁻ , Mg, Na, K, Se, Ca, and P mg 100 g ⁻¹ FW)	181, 75, 192, 990, 0.05, 81 and 193	88, 22.13, 222, 394, 0.03, 44 and 49	Rani et al. (2018); Yadav et al. (2018); Rizvi et al. (2022)
	Vitamin B ₉ (mg 100 g ⁻¹ FW)	34.50	18.20	
	Vitamin C (mg 100 g ⁻¹ FW)	4.78-95.8	14.8-18.74	
	Phylloquinone (mg 100 g ⁻¹ FW)	180	2	
	Tocopherol (mg 100 g ⁻¹ FW)	19.7	0.0	

	β -carotene (mg 100 g ⁻¹ FW)	5.4	4.0	
	Omega 3 fatty acid (mg 100 g ⁻¹ FW)	15.65	9.38	
	Total phenolics (mg GAE 100 g ⁻¹)	135.74	225	
	Total flavonoids (mg CE 100 g ⁻¹)	39.83	42.20	
	Antioxidant activity (FRAP, CUPRAC, DPPH, TEAC μ mol TE g ⁻¹)	17.77, 26.82, 2.07 and 4.97	19.36, 34.13, 6.09 and 6.25	
Celery	Minerals (Zn, Cu, Fe, Mn, N, P, K, Ca, Na, and S mg g ⁻¹ DW)	0.05-0.009, 0.002-0.008, 0.039-0.070, 0.010-0.020, 46.1-63.9, 2.4-3.7, 11.8-17.0, 21.3-33.8, 4.2-6.4, 4.28-9.17	0.11-0.19, 0.011-0.024, 0.032-0.071, 0.017-0.028, 12.1-23.9, 0.6-1.0, 16.3-24.7, 14.3-26.2, 2.0-3.1, 2.45-5.95	Singh et al. (2022)
Lettuce	Minerals (P, K, Ca, Mg, S, Na mg/100g DW)	12.25-12.33, 117.3-127.4, 52.48-59.71, 22.95-23.62, 6.51-6.85, and 32.56-33.42	18.94-19.28, 419.8-430.4, 21.85-29.17, 10.50-12.49, 3.03-4.56, 4.02-4.86	El-Nakhel et al. (2020)
	Total ascorbic acid (mg AA 100 g ⁻¹ FW)	43.58-44.36	11.46-55.12	
	Chlorophyll a, Chlorophyll b, and Total Chlorophyll (mg 100 g ⁻¹ FW)	5.15-6.02, 1.01-1.58, and 6.16-7.60	8.78-17.80, 1.01-1.58, and 2.06-4.64	
	Lutein and β -carotene (μ g 100 g ⁻¹ FW)	300-528 and 1058-1567	558-1338 and 1516-2656	
	Total polyphenols (μ g g ⁻¹ FW)	314-1086	230-526	
Cilantro	Phylloquinone (mg 100 g ⁻¹ FW)	250	310	Rizvi et al. (2022)
	Ascorbic acid (mg 100 g ⁻¹ FW)	40.6	27.0	
	Tocopherol (mg 100 g ⁻¹ FW)	53	2.5	
	β -carotene (mg 100 g ⁻¹ FW)	11.7	0.3	
	Lutein/ zeaxanthin (mg 100 g ⁻¹ FW)	10.1	0.9	
Basil	Phylloquinone (mg 100 g ⁻¹ FW)	320	410	Rizvi et al. (2022)

	Ascorbic acid (mg 100 g ⁻¹ FW)	71.0	18.0	
	Tocopherol (mg 100 g ⁻¹ FW)	19.9	0.8	
	β-carotene (mg 100 g ⁻¹ FW)	8.4	3.1	
	Lutein/ zeaxanthin (mg 100 g ⁻¹ FW)	8.6	5.7	
Mustard	Phylloquinone (mg 100 g ⁻¹ FW)	190	260	Rizvi et al. (2022)
	Ascorbic acid (mg 100 g ⁻¹ FW)	62.2	70.0	
	Tocopherol (mg 100 g ⁻¹ FW)	22.1	2.0	
	β-carotene (mg 100 g ⁻¹ FW)	6.5	1.8	
	Lutein/ zeaxanthin (mg 100 g ⁻¹ FW)	4.9	3.7	
Fenugreek	Minerals (Fe, Ca, Cu, Mg, Mn, Mo and Zn µg g ⁻¹ DW)	82.32, 7658.93, 9.15, 1645.0, 24.94, 2.88 and 34.92	108.5, 26834.2, 6.72, 2687.4, 6.72, 2687.4, 41.24, 6.18 and 23.27	K. Khoja et al. (2020)
Rocket	Minerals (Fe, Ca, Cu, Mg, Mn, Mo, and Zn µg g ⁻¹ DW)	74.41, 22475.13, 9.93, 3472.46, 92.30, 7.91 and 90.34	130.82, 36753.4, 11.65, 3886.5, 119.93, 3.23, 80.29	K. Khoja et al. (2020)
Bottle gourd	Ascorbic acid (mg 100 g ⁻¹)	6.00	31.30	Yadav et al. (2018)
	Total phenolics (mg GAE 100 g ⁻¹)	67.14	313.92	
	Total flavonoids (mg CE 100 g ⁻¹)	23.15	201.75	
	Antioxidant activity (FRAP, CUPRAC, DPPH, TEAC µmol TE g ⁻¹)	8.35, 16.24, 2.22 and 4.50	31.25, 59.30, 14.64 and 12.62	
	Mineral content (Cu, Mn, and Fe mg kg ⁻¹)	2.72, 3.60 and 5.01	2.09, 10.19 and 5.01	
Cucumber	Ascorbic acid (mg 100 g ⁻¹)	25	17.45	Yadav et al. (2018)
	Total phenolics (mg GAE 100 g ⁻¹)	38.66	141.39	
	Total flavonoids (mg CE 100 g ⁻¹)	17.15	102.00	
	Antioxidant activity (FRAP, CUPRAC, DPPH, TEAC µmol TE g ⁻¹)	4.65, 9.79, 0.91 and 10.76	10.03, 12.74, 4.31 and 13.63	

	Mineral content (Cu, Mn, and Fe mg kg ⁻¹)	0.51, 2.28 and 3.65	1.22, 7.70 and 59.70	
Pumpkin	Ascorbic acid (mg 100 g ⁻¹)	24.01	29.00	Yadav et al. (2018)
	Total phenolics (mg GAE 100 g ⁻¹)	89.55	110.02	
	Total flavonoids (mg CE 100 g ⁻¹)	32.00	44.40	
	Antioxidant activity (FRAP, CUPRAC, DPPH, TEAC µmol TE g ⁻¹)	9.00, 12.12, 4.31 and 13.14	10.00, 12.98, 5.55 and 16.00	
	Mineral content (Cu, Mn, and Fe mg kg ⁻¹)	0.60, 5.20 and 3.00	1.00, 10.12 and 8.00	
Amaranthus (Amaranthus cv. Red, Amaranthus cv. Green, and Amaranthus cv. Katwa)	Ascorbic acid (mg 100 g ⁻¹)	32.12-37.50	84.00-199.99	Yadav et al. (2018)
	Total phenolics (mg GAE 100 g ⁻¹)	25.00-48.55	69.01-276.89	
	Total flavonoids (mg CE 100 g ⁻¹)	9.58-41.01	43.00-240.30	
	Antioxidant activity (FRAP, CUPRAC, DPPH, TEAC µmol TE g ⁻¹)	2.15-5.00, 3.62-12.58, 0.85-2.80, and 4.25-7.78	8.00-24.34, 3.62-12.58, 2.92-10.77, and 9.00-36.56	
	Mineral content (Cu, Mn, and Fe mg kg ⁻¹)	0.15-0.76, 3.34-9.54, and 2.64-18.00	0.75-7.70, 5.90-84.83, and 22.04-28.11	
Poi	Ascorbic acid (mg 100 g ⁻¹)	46.50	162.52	Yadav et al. (2018)
	Total phenolics (mg GAE 100 g ⁻¹)	80.35	95.73	
	Total flavonoids (mg CE 100 g ⁻¹)	17.73	53.52	
	Antioxidant activity (FRAP, CUPRAC, DPPH, TEAC µmol TE g ⁻¹)	3.90, 5.88, 0.60 and 6.28	6.59, 8.60, 1.72 and 8.10	
	Mineral content (Cu, Mn, and Fe mg kg ⁻¹)	0.08, 2.17 and 2.56	nd, 6.52 and 5.27	
Jute	Ascorbic acid (mg 100 g ⁻¹)	34.90	10.00	Yadav et al. (2018)
	Total phenolics (mg GAE 100 g ⁻¹)	152.10	309.44	
	Total flavonoids (mg CE 100 g ⁻¹)	142.39	292.53	
	Antioxidant activity (FRAP, CUPRAC, DPPH, TEAC µmol TE g ⁻¹)	15.55, 24.53, 3.15 and 7.18	38.11, 65.91, 29.72 and 16.00	

	Mineral content (Cu, Mn, and Fe mg kg ⁻¹)	1.14, 2.66 and 3.34	1.90, 28.78 and 12.27	
Water spinach	Ascorbic acid (mg 100 g ⁻¹)	17.40	17.40	Yadav et al. (2018)
	Total phenolics (mg GAE 100 g ⁻¹)	121.44	192.17	
	Total flavonoids (mg CE 100 g ⁻¹)	114.36	172.94	
	Antioxidant activity (FRAP, CUPRAC, DPPH, TEAC µmol TE g ⁻¹)	10.23, 24.16, 3.40 and 5.71	26.49, 43.48, 9.25 and 6.91	
	Mineral content (Cu, Mn, and Fe mg kg ⁻¹)	2.69, 6.84 and 5.66	1.73, 5.63 and 18.45	
Palak	Ascorbic acid (mg 100 g ⁻¹)	6.00	18.00	Yadav et al. (2018)
	Total phenolics (mg GAE 100 g ⁻¹)	60.00	123.50	
	Total flavonoids (mg CE 100 g ⁻¹)	22.66	100.40	
	Antioxidant activity (FRAP, CUPRAC, DPPH, TEAC µmol TE g ⁻¹)	6.71, 7.27, 1.20 and 4.53	11.60, 25.99, 7.33 and 9.12	
	Mineral content (Cu, Mn, and Fe mg kg ⁻¹)	0.45, 5.45 and 4.57	0.93, 7.14 and 10.88	

In microgreens, the β -carotene content ranged from 0.6 to 12.1 mg 100 g⁻¹ on a FW basis. The golden pea tendrils microgreens had the lowest β -carotene content, i.e., 0.6 mg 100 g⁻¹ FW, and the highest β -carotene content in red sorrel microgreens was 12.1 mg 100 g⁻¹ FW (Xiao et al., 2012). Most of the different botanical families of microgreens contain high amounts of β -carotene, with the exception of microgreen golden pea tendrils and popcorn shoots. The different botanical families associated with the β -carotene content of the microgreens are listed in **(Table 2.3)**.

Xanthophylls, another class of carotenoids, are yellow-colored pigments that are bioactive in nature. The major difference between xanthophyll and carotene pigments is that xanthophyll contains O₂ atoms in the form of hydroxyl groups or epoxides, whereas carotenes are hydrocarbon molecules with no O₂ (Vats, 2017). Most xanthophylls are found in fatty acid esters, glycosides, sulfates, and protein complexes, and 800 different types of xanthophylls have been discovered in nature (Maoka, 2019). Microgreens are a moderately good source of xanthophylls. The concentration of lutein in different families of microgreens ranged from 1.3–23.9 mg 100 g⁻¹ FW, and these data are presented in **(Table 2.3)**. Carrot microgreens belonging

to the Apiaceae botanical family present the highest lutein concentration of 23.9 mg 100 g⁻¹ FW (Ghoora et al., 2020), and the popcorn shoots of microgreens present the lowest lutein content of 1.3 mg 100 g⁻¹ FW (Xiao et al., 2012). Lutein is a lipid-soluble pigment that helps protect against photochemical reactions (Barrita and Sánchez, 2013). Violaxanthin belongs to the xanthophyll pigment orange-colored class, which is synthesized from zeaxanthin and comprises various fruits, green vegetables, and microalgae. It exhibits different properties, such as anti-inflammatory, antiproliferative, and proapoptotic activities, against human cancer cell lines (Wang et al., 2018). The concentration of violaxanthin in different botanical families of microgreens ranges from 0.9–3.9 mg 100 g⁻¹ FW, and these data are presented in **(Table 2.3)**.

2.3.2.3. Chlorophyll

Chlorophyll is a natural green pigment and bioactive compound present in the plant kingdom that plays a critical role in the photosynthesis process. This pigment is an important part of the human diet and is found in fruits and vegetables (Singh et al., 2020). Chlorophyll is used in clinical treatments such as thalassemia and hemolytic anemia because of its resemblance to hemoglobin. It is thought to create either heme or globin or both upon digestion (Niroula et al., 2019). The concentration of total chlorophyll in different botanical families of microgreens ranges from 29.5–155 mg 100 g⁻¹ FW, and these data are presented in **(Table 2.3)**. The highest concentration of total chlorophyll is present in the *Brassicaceae* family of wild mustard microgreen and common dandelion from the *Asteraceae* family of microgreen, at 155 mg 100 g⁻¹ FW, and the lowest concentration has been reported in onion microgreens belonging to the *Amaryllidaceae* family, at 29.5 mg 100 g⁻¹ FW.

2.3.2.4. Phylloquinone

Phylloquinone or vitamin K is a bioactive compound present in the green leaves of vegetables as well as in microgreens. It is a fat-soluble vitamin essential for blood coagulation and remodeling in humans (Kang et al., 2022). The concentration of phylloquinone among the different families ranged from 0.6–4.1 µg g⁻¹ FW, and the phylloquinone concentrations of the different families of microgreens are listed in **(Table 2.3)**. Among all varieties, the garnet amaranth microgreen variety presented the highest concentration of phylloquinone, i.e., 4.1 µg g⁻¹ FW, whereas the magenta spinach variety presented the lowest phylloquinone concentration of 0.6 µg g⁻¹ FW (Xiao et al., 2012). Choe et al. (2018) compared microgreens with mature vegetables and reported that microgreens contain relatively high concentrations of phylloquinone compared with mature vegetables.

2.3.2.5. Glucosinolates

Glucosinolates are plant secondary metabolites containing hydrophilic and sulfur compounds with more than 130 variants that are present in leafy Brassica vegetables and seeds. It has fungicidal, nematocidal and bactericidal properties (Alrifai et al., 2021). Cruciferae and its related microgene families, such as broccoli, cauliflower, cabbage, mustard, rapeseed, and horseradish, are good sources of glucosinolates. Research has revealed that glucosinolates have good antibacterial characteristics as well as regulatory functions in inflammation, producing stress responses, phase-I metabolism, and antioxidant activities (Bischoff, 2021). Huang et al. (2016) evaluated glucosinolate levels in microgreen and mature red cabbage plants. The content of micronutrients was 2-fold greater (17.1 mol g^{-1}) than that of their mature counterparts (8.3 mol g^{-1}), according to the findings. The glucosinolates show health-promoting activities, such as reducing cholesterol levels in humans, aiding the process of detoxification, lessening the ill effects of allergens, and preventing cancer formation. The hydrolytic products of glucosinolates, isothiocyanates, and indoles impart cancer-fighting properties via the apoptosis of cancer cells, the suppression of inflammation, the modulation of the cell cycle, and increased activity of detoxifying enzymes (Lu et al., 2018).

2.3.3. Minerals

Minerals play an essential role in the function of various human organs. They help in the prevention of various types of related disorders, such as diabetes, obesity, high blood pressure, and other cardiovascular diseases (Mohamed et al., 2021). Studies have suggested that microgreens are good sources of macro- and microminerals. For example, micronutrients are excellent sources of Ca (20.1 to 94.7 mg 100 g⁻¹ FW) and Mg (22.8 to 145.58 mg 100 g⁻¹ FW). The various types of minerals present in different microgreens along with their concentrations are presented in (Table 2.3).

2.4. Microgreens bioavailability and bioaccessibility of biocomponents

Bioefficiency is a vital parameter of foods used to fulfill the dietary needs of individuals and in functional food meals. If food provides the biochemical, mechanical, and energetic support required for the body's fundamental processes as well as aids in preserving human health, then it is bioefficient (Dima et al., 2020). Various factors affect the bioavailability of bioactive components, such as the structure of the cell wall, chemical compound structure, environmental conditions, processing conditions, and the individual gastrointestinal system (Le et al., 2020).

Microgreens contain large amounts of vitamins, minerals, and bioactive compounds (ascorbic acid, carotenoids, phenolic compounds, vitamin E, and glucosinolates) with relatively high antioxidant properties. Iron solubility and bioavailability in vegetables were determined

via simulated gastrointestinal in vitro digestion and subsequent measurement of ferritin in Caco-2 cells as a surrogate marker of iron uptake. The vegetables fenugreek and rocket presented higher levels of mineral iron than did their microgreens. Compared with mature fenugreek and broccoli, fenugreek and broccoli microgreens have low bioaccessibility and high-molecular-weight iron. Additionally, compared with mature vegetables, only fenugreek microgreens significantly increased the amount of iron that Caco-2 cells absorbed. Fenugreek and rocket vegetables, with the exception of broccoli, increased the absorption of FeSO₄ and the response to ferric ammonium citrate (FAC). Ascorbic acid dramatically increased the ability of mature fenugreek and rocket to absorb iron (K. Khoja et al., 2020).

The bioaccessibility of *Brassicaceae* family (broccoli, green curly kale, red mustard, and radish) microgreens was studied. After digestion, antioxidant compounds such as total soluble polyphenols and total isothiocyanates presented 43–70% and 31–63% bioaccessibility, respectively, while minerals presented 34–90% bioaccessibility. In this evaluation, radish and mustard microgreens show greater bioaccessibility of bioactive components and mineral contents (de la Fuente et al., 2019). The in vivo bioaccessibility and bioactive properties of polyphenols and glucosinolates from selected *Brassicaceae* family microgreens (kale, red cabbage, kohlrabi, and radish) were studied. In this study, lignans (14%), tyrosol derivatives (9%), and flavonoids (8%) presented the highest bioaccessibility. The bioaccessibility of glucosinolate compounds varies across four *Brassicaceae* microgreens, ranging from kale and kohlrabi microgreens to 2 and 43%, respectively. The anti-inflammatory, anticancer, antimicrobial, and antidiabetic activities are improved after digestion because of the relatively high bioaccessibility of bioactive compounds present in microgreens (Tomas et al., 2021).

2.5. Functional components of microgreens and their health-promoting properties

Microgreens are a new form of functional food crop that has the potential to diversify global food chain systems sustainably, make editions to urbanization and world climate change easier, and simultaneously contribute to health (Kou et al., 2013; Brazaitytė et al., 2015; Michell et al., 2020). Owing to the wide variety of nutrients and chemical compounds, microgreens have become a popular and novel source of biologically active substances. This smart food of the new generation has gained importance among health-conscious individuals because of its availability and high nutritive value, particularly for minerals, vitamins, and bioactive compounds (phenolic acid, flavonoids, glucosinolates, ascorbic acid, tocopherol, chlorophyll, anthocyanin, carotenoids, phylloquinone, and others) (Galieni et al., 2020). These bioactive compounds exhibit good health-promoting activities, including antioxidant, antidiabetic, antimicrobial, anti-inflammatory, and anticarcinogenic activities, as reported in

several studies, as shown in **(Table 2.4)** (Bahadoran et al., 2013; Le et al., 2019). The antioxidant properties of microgreens are among their most important health-promoting benefits. Antioxidants help protect the body from oxidative stress, which occurs when there is an imbalance between free radicals and antioxidants. Free radicals are highly reactive molecules that can damage cells, proteins, and DNA, contributing to the development of chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders (Paolillo et al., 2023). Microgreens are particularly rich in phenolic acids (chlorogenic acid and ferulic acid), which are potent antioxidants (Xiao et al., 2012; Le et al., 2020). These compounds work by donating hydrogen atoms or electrons to neutralize free radicals, thereby preventing cellular damage. Chlorogenic acid, for example, has been shown to reduce oxidative stress by inhibiting the oxidation of low-density lipoprotein (LDL) cholesterol, a key factor in the development of atherosclerosis (Macvanin et al., 2022). Flavonoids, another group of bioactive compounds found abundantly in selected families microgreens including Asparagaceae, Amaranthaceae, Asteraceae, *Brassicaceae*, and Poaceae that play a significant role in their antioxidant activity (Singh et al., 2024). The effect of different bioactive compounds presents in microgreens with respect to health benefits as shown in **(Figure 2.2)**.

Table 2.4. The selected family's common microgreens present functional components and their associated health-promoting properties.

Family	Microgreens crops	Functional components	Health-promoting properties	References
Asparagaceae	Asparagus, garlic, onion, leek	Allicin, sulfur, quercetin, kaempferol, flavonoids, ascorbic acid, vitamin A, and minerals (potassium, magnesium, and iron)	Anti-bacterial, anti-viral, cardioprotective, antioxidant, anti-inflammatory, anti-cancer	Ghoora et al. (2020); Guo et al. (2020); Negri et al. (2021)
<i>Brassicaceae</i>	Broccoli, cabbage, radish, arugula, kale, kohlrabi, mustard, mizuna	Sulforaphane, anthocyanin, glucosinolates, indole, carotenoids, tocopherols, vitamins (A, C, E)	Antioxidant, anti-cancer, anti-inflammatory, anti-microbial, support to detoxification and immune system	Weber (2017); Turner et al. (2020); Gao et al. (2021); Xiao et al. (2012)
Apiaceae	Cilantro, fennel, carrot, parsley	Linalool, coriandrin, quercetin, anethole, estragole, falcarinol, lutein, apigenin, luteolin	Anti-inflammatory, antioxidant, anti-microbial, anti-cancer, neuroprotection, digestive support.	Xiao et al. (2012); Ghoora et al. (2020)
Poaceae	Wheatgrass, barley grass, oat grass	Chlorophyll, flavonoids, saponins, superoxide dismutase, GABA, Avenanthramides, β -glucan, vitamins (A, C, E)	Antioxidant, detoxification, immune support, cholesterol-lowering, nervous system support, anti-inflammatory, anti-bacterial, anti-cancer	Gunjal et al. (2024); Singh et al. (2024); Niroula et al. (2019)
Amaranthaceae	Amaranth, red amaranth, quinoa, beet	Betalains, phenolic acids, flavonoids, betacyanins, betaxanthins, quercetin, kaempferol.	Anti-inflammatory, antioxidant, anti-cancer, cardiovascular and liver protection	Ebert et al. (2017); Meas et al. (2020)
Fabaceae	Fenugreek, alfalfa, pea, lentil, chickpea, mung bean	Phenolic compounds, diosgenin, saponins, isoflavones, vitamins (A, E, C, K, B ₆)	Anti-inflammatory, hypoglycemic, cholesterol-lowering, immune-boosting, antioxidant,	Huang et al. (2016); Priti et al. (2021); Kaur et al. (2022)

			estrogenic effects, anti-cancer	
Asteraceae	Sunflower, lettuce, chicory, arugula, basi	Chlorogenic acid, flavonoids, tocopherols, lactucaxanthin, β - carotene, phenolic acids, inulin, sesquiterpene lactones, polyphenols, glucosinolates, essential oil, apigenin, minerals (calcium, potassium), vitamins (A, C, K)	Antioxidant, anti- inflammatory, anti- aging, improve digestive health, anti-diabetic, anti- cancer	Puccinelli et al. (2019); Kathi et al. (2024); Mansouri et al. (2024)

Ascorbic acid is known to have potential anticancer activity (Visser and Dass, 2018), protective effect in sepsis (Wilson, 2008), improving bone health (Aghajanian et al., 2015), protective effect against photoaging, ultraviolet-induced immunosuppression, and photocarcinogenesis (Al-Niaimi and Zhen Chiang, 2017). In fact, ascorbate has an ability to chelate and reduce transition metal ions, especially Fe^{3+} and Cu^{2+} which contributes to its ability to enhance iron uptake from the diet (Visser and Das, 2018). Forms of vitamin E contain a phenolic group on the chromanol ring in their structure, which allows them to donate electrons to scavenge lipid peroxyl radicals, hence exhibiting antioxidant effect (Jiang et al., 2022). Yang et al. (2020) have reviewed various laboratory results and concluded that vitamin E can exhibit anticancer activity on certain conditions. Vitamin A has also been studied for its functions like promoting growth and reproduction and maintaining optimum mucosal epithelium secretion bone, epithelial tissue, eye vision. Additionally, vitamin A is believed to be responsible for immune cell differentiation and proliferation. Low levels of vitamin A are also associated with neonatal pneumonia (Li et al., 2020). Carazo et al. (2021) has reviewed various functions of vitamin A such as its role in cancer, vision, immunity, mucin synthesis.

Flavonoids such as quercetin and kaempferol not only scavenge free radicals but also enhance the body's antioxidant defenses by upregulating the expression of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase. This dual action makes flavonoids particularly effective in reducing oxidative stress and its associated risks (Gunjal et al., 2024). Xu et al. (2018) has reviewed the antioxidant properties of quercetin, for example its role in regulating GSH levels. The side phenyl ring of quercetin contains $-\text{OH}$ groups which are bound to major amino acid residues at the active site of two enzymes. This structure gives

a much-required inhibitory effect against enzymes such as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). In fact, quercetin is known to modulate signal transduction pathways, especially p38MAPK/iNOS signaling pathway, while downregulating MDA levels, and upregulating of SOD activity to enhance its antioxidant activity. Quercetin also exhibits anticancer activity by regulating cell apoptosis, inhibition of angiogenesis, inhibiting P-gp channel, alleviating oncogene expression and maintaining specific signaling pathways (Azeem et al., 2023). In various plants, luteolin (3,4,5,7-tetrahydroxy flavone) exists as a natural flavonoid. Luteolin as an antioxidant has a significant potential to inhibit proliferation and to suppress the expression of p-STAT3, p-EGFR, p-Akt, and p-Erk1/2 in MCF-7 breast cancer cells induced by EGF. It also suppresses the EGF-induced activities of EGFR signaling pathway in human breast cancer cell lines (Imran et al., 2019). Kang et al. (2017) studied the antioxidant potential of luteolin in apoptotic cell death in human colon cancer cells, and observed that mechanism of action of luteolin was associated with the elevated levels of glutathione (GSH). Studies have highlighted the strong bonding activity of luteolin with androgen receptor (AR) which inhibited its protein expression in lung cancer cell line A549 (Li et al., 2023) and higher affinity with α -amylase (Martinez-Gonzalez et al., 2019). Kaempferol is a common aglycone flavonoids in the form of glycosides. The four hydroxy groups are situated at the 3, 5, 7, and 4' positions, making it a tetrahydroxyflavone (Alkandahri et al., 2022). Kaempferol is also known to show anticancer, antioxidant, ameliorative (Shahbaz et al., 2023) hepatoprotective effect (Alkandahri et al., 2022). Via modulation of cell signaling pathways and inducing apoptosis and cell cycle arrest in cancer cells, kaempferol activates tumor suppressor genes, and inhibits angiogenesis, PI3K/AKT pathways, STAT3, transcription factor AP-1, Nrf2 and other cell signaling molecules and exhibits anticancer activity (Almatroudi et al., 2022).

The fat-soluble pigments are carotenoids (β -carotene and lutein), which have good antioxidant properties. β -Carotene is mostly found in *Brassicaceae* and *Asteraceae* family microgreens (Weber, 2017; Puccinelli et al., 2019). It is a precursor of vitamin A and is known for its ability to quench singlet oxygen, a highly reactive form of oxygen that can cause significant cellular damage (Xiao et al., 2012). β -carotene is also known to have an inhibitory effect on the production of NO (nitric oxide), prostaglandin E2, and superoxide dismutase. Additionally, it downregulates iNOS/cox-/NADPH oxidase proteins expression and mRNA, while inhibiting TNF- α to exhibit anti-inflammatory activity (Wu et al., 2022). Lutein, on the other hand, is particularly beneficial for eye health, as it accumulates in the retina and protects against oxidative damage caused by blue light and UV radiation, thus reducing the risk of age-

related macular degeneration (Kim & Park, 2016). Betalains are another type of pigment found in certain microgreens, such as beet microgreens, and contribute to their antioxidant activity (Hernández-Adasme et al., 2023). Betalains (betacyanins and betaxanthins) have been shown to protect cells from oxidative damage by increasing the activity of antioxidant enzymes and inhibiting lipid peroxidation. This makes them particularly effective in protecting against oxidative stress-related diseases (Meas et al., 2020; Sadowska-Bartosz & Bartosz, 2021)

Microgreens possess strong anti-inflammatory properties, largely due to their high contents of flavonoids, glucosinolates, and betalains (Weber, 2017; Le et al., 2020). Flavonoids, such as quercetin and kaempferol, exert their anti-inflammatory effects by modulating key signaling pathways involved in inflammation (Cicio et al., 2022). One of the primary mechanisms through which flavonoids reduce inflammation is by inhibiting the activation of the nuclear factor kappa B (NF- κ B) pathway. NF- κ B is a transcription factor that plays a major role in the production of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF- α) and interleukins. By inhibiting NF- κ B, flavonoids reduce the production of these inflammatory mediators, thereby mitigating inflammation (Moore et al., 2017; Marotti et al., 2021). Glucosinolates are another major bioactive compound; they contain sulfur-containing compounds found abundantly in microgreens from the *Brassicaceae* family and are hydrolyzed into bioactive compounds such as isothiocyanates (sulforaphane) upon consumption (Singh et al., 2024). All glucosinolates have a basic chemical structure containing β -D-thioglucose group, a sulfonate oxime group, and a side chain derived from a branched chain amino acid, methionine, alanine, phenylalanine, tyrosine, or tryptophan. They are generally biologically inactive in the organism, hydrolyzed by the enzyme myrosinase during chewing, leading to the formation of active derivatives such as isothiocyanates and indoles which have detoxifying effect as they inhibit neoplastic processes and adhesion, and intensifies tumor cell apoptosis (Esteve, 2020; Janda et al., 2019). The mechanism of action through which glucosinolates and their derivatives isothiocyanates stimulate apoptosis in cancer cell lines is summarized by Arumugam and Razis (2018). Lucarini et al. (2022) discussed evidence of the cytoprotective effects of hydrolysis-derived isothiocyanates by modulating the inflammatory response (NF- κ B pathway) and the oxidative stress (Nrf2/ARE pathway), two processes majorly involved in the pathogenesis of chronic pain syndromes. Sulforaphane has been extensively studied for its anti-inflammatory effects, which are mediated through its ability to inhibit the activation of NF- κ B and reduce the production of proinflammatory cytokines (Bouranis et al., 2023). Additionally, sulforaphane activates the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which upregulates the expression of antioxidant and detoxifying enzymes, further

contributing to its anti-inflammatory properties (Subedi et al., 2019). Another functional component is betalains, which are found in microgreens and exhibit strong anti-inflammatory effects (Rocchetti et al., 2020). Betalains inhibit the activity of proinflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), which are involved in the production of inflammatory mediators such as prostaglandins and leukotrienes. By reducing the production of these mediators, betalains help alleviate inflammation and protect against chronic inflammatory diseases (Doiphode et al., 2022). The anti-inflammatory properties of liquorice microgreens were investigated in lipopolysaccharide-induced Caco-2 cells (Marotti et al., 2021). Compared with no treatment, treatment with 2.50 g mL⁻¹ liquorice microgreen root extracts preserved cell viability and proliferation and inhibited the proinflammatory cascade from effectively damaging the cells. The effects of different micronutrients (asafoetida, fenugreek, fennel seeds, and curry leaves) on the enzymatic release of glucose were studied by Wadhawan et al. (2017). It was reported that 23.3 mg mL⁻¹ fenugreek microgreen extract reduced porcine-amylase activity, which is the enzyme responsible for the production of glucose from starch via the hydrolysis of α -1,4-glycosidic bonds. Furthermore, 2 mg mL⁻¹ fenugreek microgreen extract markedly reduced nonenzymatic glycation by 70%, indicating a positive effect on blood glucose regulation.

The anticancer properties of microgreens are largely due to their high levels of glucosinolates, flavonoids, and phenolic acid functional compounds (Zhang et al., 2021). These compounds exert their anticancer effects through a variety of mechanisms, including the induction of apoptosis, the inhibition of cell proliferation, and the suppression of angiogenesis. The anticancer properties of glucosinolates are particularly notable (Rizvi et al., 2022). Sulforaphane has been shown to induce apoptosis, or programmed cell death, in cancer cells by activating caspases, a family of protease enzymes involved in the apoptotic process. Additionally, sulforaphane inhibits the growth and proliferation of cancer cells by blocking the cell cycle at the G2/M phase, thereby preventing cells from dividing and spreading (Kaiser et al., 2021). Tomas et al. (2021) studied the in vitro bioaccessibility of the phytochemical and glucosinolate contents of four microgreen species of the *Brassicaceae* family, viz. kale, kohlrabi, red cabbage, and purple radish. He further reported that microgreens (kohlrabi and kale) presented high total phenolic and glucosinolate contents, respectively. The viability of tumoral colon cells treated with kale, radish, mustard, and broccoli microgreens was considerably lower than that of control cells ($P < 0.05$), indicating that microgreens have antiproliferative effects on colon cancer cell development (Fuente et al., 2020). Flavonoids, such as quercetin and kaempferol, also contribute to the anticancer properties of microalgae.

These compounds inhibit the activity of enzymes involved in the production of reactive oxygen species (ROS), which can cause DNA damage and promote cancer development (Chen & Chen, 2013). Moreover, flavonoids suppress angiogenesis, the process by which new blood vessels form to supply nutrients to tumors, by inhibiting the expression of vascular endothelial growth factor (VEGF). This action helps to starve tumors from the nutrients they need to grow and spread (Subbaraj et al., 2021). Phenolic acids, including chlorogenic acid and ferulic acid, also play a role in cancer prevention by protecting DNA from oxidative damage and modulating signaling pathways involved in cell proliferation and apoptosis (Yue et al., 2019). These compounds have been shown to inhibit the activity of matrix metalloproteinases (MMPs), enzymes that breakdown the extracellular matrix and facilitate cancer cell invasion and metastasis (Cabral-Pacheco et al., 2020).

The cardioprotective properties of microgreens are largely due to their ability to improve lipid profiles, reduce oxidative stress, and enhance endothelial function (Kaur et al., 2022). These benefits are attributed primarily to the presence of functional components such as tocopherols (vitamin E), phenolic acids, and carotenoids present in microgreens (Priti et al., 2021; Ghoola et al., 2020; Singh et al., 2024). α -Tocopherol is a powerful lipid-soluble antioxidant that protects cell membranes from oxidative damage by scavenging free radicals (Ghoola et al., 2020). This action is especially important in preventing the oxidation of low-density lipoprotein (LDL) cholesterol, a key factor in the development of atherosclerosis (Wojdyło et al., 2020). By reducing LDL oxidation, tocopherols help prevent the formation of arterial plaques and reduce the risk of cardiovascular diseases such as heart attack and stroke (Singh et al., 2024). Chlorogenic acid and ferulic acid also contribute to cardiovascular protection by promoting vasodilation and reducing arterial stiffness. These compounds inhibit the activity of angiotensin-converting enzyme (ACE), which is involved in the regulation of blood pressure (Lukitasari et al., 2020). By inhibiting ACE, phenolic acids help to lower blood pressure and improve blood flow, reducing the risk of hypertension and other cardiovascular conditions (Yu et al., 2022). Carotenoids, including lutein and β -carotene, further enhance cardiovascular health by reducing oxidative stress in blood vessels and improving endothelial function (Rani et al., 2024). Lutein, for example, has been shown to improve the elasticity of blood vessels and reduce the progression of atherosclerosis by protecting against oxidative damage (Xiao et al., 2012; Ghoola et al., 2020). Additionally, β -carotene, as a precursor of vitamin A, supports immune function and contributes to overall cardiovascular health (Singh et al., 2024). The major advantages of microgreens for maintaining blood glucose levels, managing weight, and controlling adipose tissue have not yet been completely investigated.

These findings lay the groundwork for further research into the potential value of microgreens in combating chronic health problems such as obesity, diabetes mellitus, and CVD (Huang et al., 2016). Huang et al. (2016) examined the effect of ingesting red cabbage microgreens on cardiovascular problems, i.e., hypercholesterolemia, in obese mice fed a high-fat diet. Compared with rats fed the same diet supplemented with mature red cabbage powder, those fed the same diet had considerably lower plasma low-density lipoprotein and hepatic triglyceride levels. In addition, the diacylglycerol O-acyltransferase 1 and sterol O-acyltransferase 1 genes were downregulated in animals fed with micro greens, which prevented the synthesis of cholesterol esters and triglycerides. Red cabbage microgreens have a favorable effect on lipid metabolism in the plasma and liver, and their consumption significantly reduces C-reactive protein and tumor necrosis factor mRNA expression in the liver, suggesting that red cabbage microgreens have an anti-inflammatory effect on inflammation caused by a high-fat diet (2016).

Microbes play a vital role in supporting metabolic health by improving glucose metabolism, enhancing insulin sensitivity, and reducing inflammation. These effects are largely due to the presence of flavonoids, phenolic acids, and fiber in microgreens (Bhaswant et al., 2023). Quercetin and kaempferol have been shown to modulate key pathways involved in glucose uptake and insulin signaling. These compounds enhance the activity of insulin receptors and glucose transporters, improving the uptake of glucose by cells and reducing blood sugar levels (Yang et al., 2022). Additionally, flavonoids inhibit the activity of enzymes involved in the production of advanced glycation end products (AGEs), which are harmful compounds that contribute to insulin resistance and the development of type 2 diabetes (Zhou et al., 2023). Additionally, phenolic acids, such as chlorogenic acid, further support metabolic health by inhibiting the activity of enzymes involved in carbohydrate digestion, such as α -amylase and α -glucosidase (Nyambe-Silavwe & Williamson, 2018). By slowing the breakdown of carbohydrates into glucose, these compounds help reduce postprandial blood sugar spikes and improve glycemic control (Singh et al., 2024). Fiber, particularly soluble fiber, also plays a crucial role in supporting metabolic health. Soluble fiber forms a gel-like substance in the gut that slows the absorption of glucose, helping to regulate blood sugar levels and reduce the risk of diabetes (Wu et al., 2024). Additionally, fiber promotes satiety, helping to manage appetite and reduce caloric intake, which is beneficial for weight management and preventing obesity (Ebert, 2022).

Microgreens contribute to digestive health and detoxification processes through their high fiber content and the presence of bioactive compounds such as inulin, glucosinolates, and

sulfur compounds (Gunjal et al., 2024; Singh et al., 2024). Inulin, a type of prebiotic fiber found in some microgreens, promotes the growth of beneficial gut bacteria, such as *Bifidobacteria* and *Lactobacilli* (Vera-Santander et al., 2023). Inulin is a linear fructan consisting of fructosyl units linked by β (2 $\rightarrow$ 1) bonds, commonly with a glucose moiety attached at the end through an α (1 $\rightarrow$ 2) linkage. Structurally, it is a polysaccharide made up of D-fructofuranose units, rendering it resistant to hydrolysis by human gastrointestinal enzymes due to its β -configuration. Studies have pointed out the positive effect of inulin on body weight levels when it is combined with either propionate ester, oligofructose, or green tea catechins. It is also believed to slow down the absorption of glucose in the intestine which helps regulating the elevation in blood glucose levels after meals, favoring glycemic homeostasis (Milagro and Aranaz, 2023). Further preclinical and clinical research is needed to examine the various biochemical activities and health benefits of microgreens in the context of their beneficial effects on chronic diseases. **(Figure 2.3)** shows the impacts of different biochemical activities and the health benefits of the microgreens.

2.6. Microgreens storage and shelf-life

The key factors are monitoring the perishability and overall quality of commercial microgreens during storage (Kou et al., 2014) because these greens have a low shelf life and are highly sensitive to harvest and postharvest handling practices, as their young tissues respire at a much higher rate than their mature counterparts do (Cantwell & Suslow, 2002). The effects of different storage practices on microgreens are shown in **(Figure 2.4)**. It requires careful harvesting, quick cooling, and a reduced respiration rate, which further assists in reducing spoilage and senescence (Treadwell et al., 2010).

The microgreen bioactive compounds and storage stability, harvesting time, and number of days significantly affect these quality parameters (Hasperué et al., 2016; Clarkson et al., 2005). The intensity of light and photoperiod is considered to be species-specific and more prominent in spring–summer. Clarkson et al. (2005) worked on end-of-day harvesting to extend the storage period of lollo rosso lettuce, baby red chard, and leaf roulette. The results revealed that the shelf life increased to 2–6 days following the end-of-day harvest. In ready-to-eat fresh-cut products, heat treatments such as pasteurization and sterilization are not needed, but washing is necessary for the removal of unwanted foreign materials and to reduce the microbial load. Different chemical treatments are used to reduce the microbial load from vegetables and fruits (Gonzalez et al., 2004).

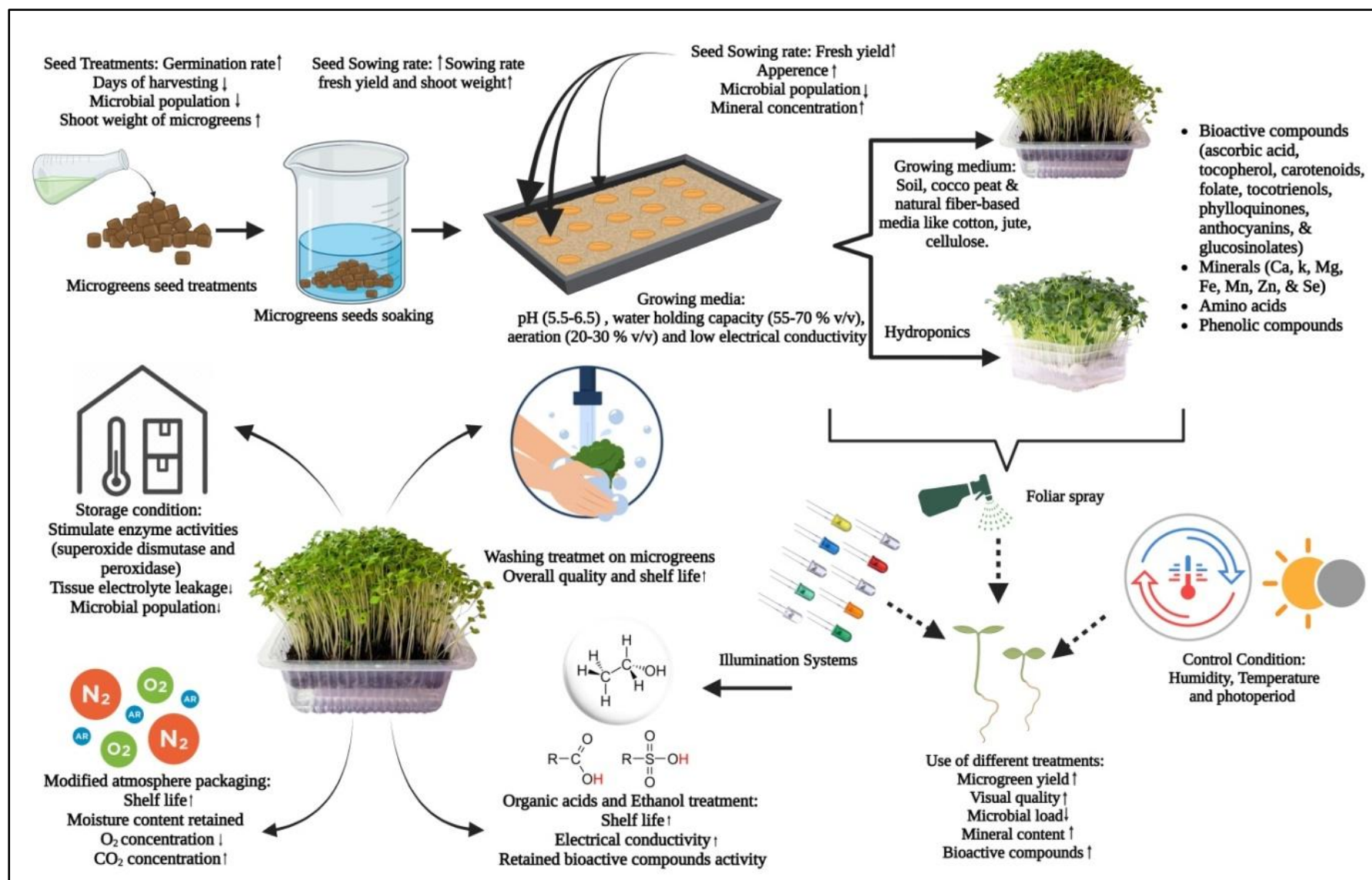


Figure 2.4. The effect of different cultivation and storage practices on microgreens

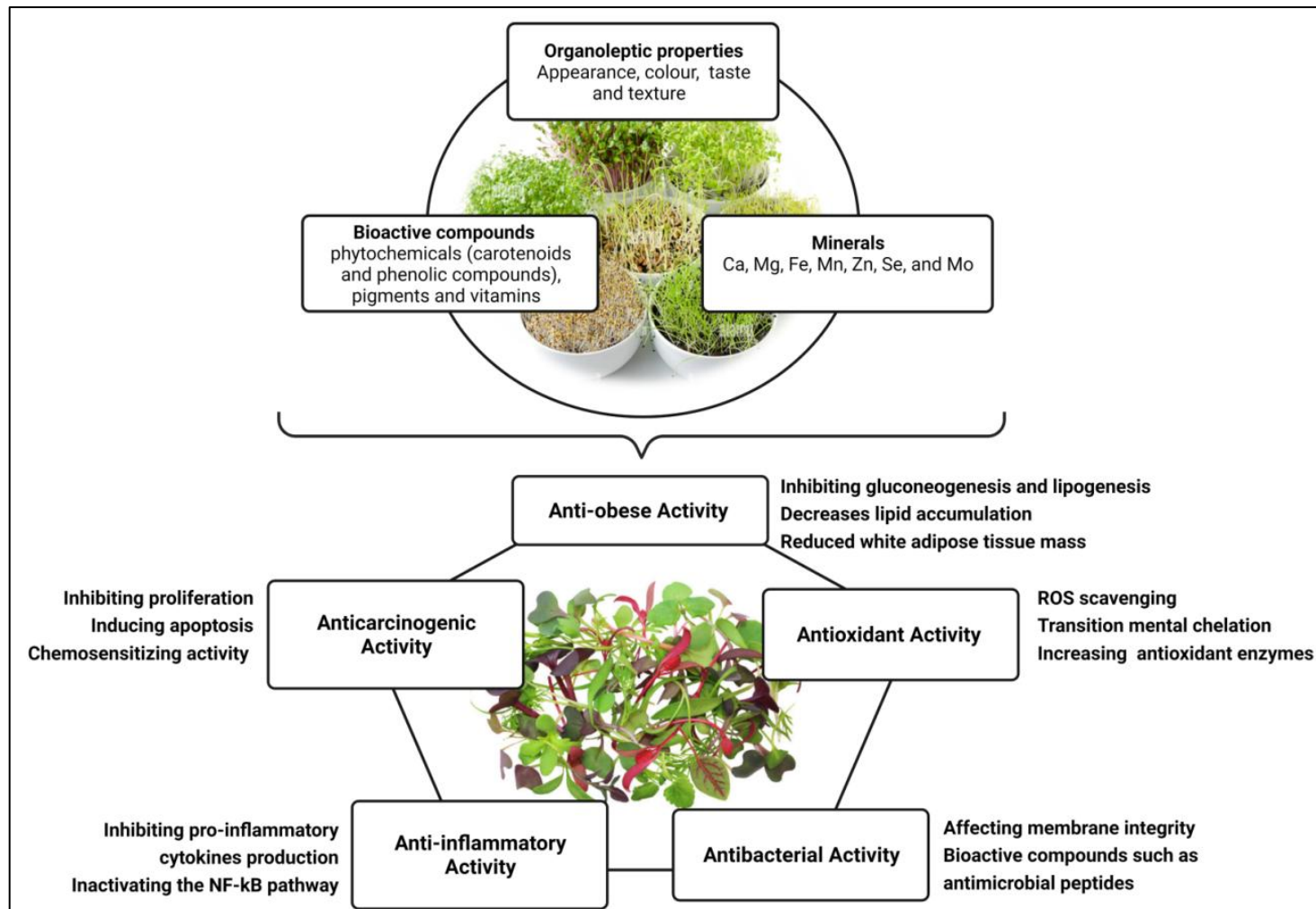


Figure 2.3. Biochemical activities and health benefits of microgreens (Tan et al., 2020; Xiao et al., 2016; Marotti et al., 2021; Ares et al., 2013; Yang et al., 2016).

The utilization of sprays including Ca amino acid chelate (1–20 mM), $C_6H_{10}CaO_6$ (1–20 mM), and especially $CaCl_2$ (pH 6.5, 10 mM concentration) resulted in the maintenance of the postharvest quality and shelf life of broccoli microgreens. Kou et al. (2014) used Ca treatment during preharvest conditions on broccoli microgreens. Preharvest Ca treatment with 10 mM $CaCl_2$ has much greater benefits, as it extends shelf-life, maintains overall quality, reduces electrolyte leakage from tissues, reduces the microbial load, and stimulates the activity of enzymes (peroxidase and superoxide dismutase). The yield of microgreens increased to 50%, in addition to increasing stem elongation; increasing bioactive compound, calcium, and glucosinolate contents; and increasing the activities of important ROS detoxification enzymes, which help to protect cell membranes from damage caused by lipid peroxidation (Kou et al., 2014; Sun et al., 2015).

In another study, selected microgreens such as radish ruby, tatsoi, radish green, cabbage red, and Brussels sprouts achieved shelf lifetimes ranging from 13–14 days at 1.6–4.44°C, whereas parsley Italian, cress upland, parsley curled, and celery sprouts lasted 20–21 days under the same conditions in clamshell containers or bags (Sharma et al., 2023). In a study conducted by Kou et al. (2013) to determine the quality and shelf life of buckwheat microgreens, 50 and 100 mg/L chlorine wash treatments were used to effectively reduce bacterial populations in the first 7 days of storage. Chandra et al. (2012) studied the effects of sanitizers on the microbial load and overall quality of Chinese cabbage microgreens. The different types of sanitizers used were 100 mL L⁻¹ chlorinated water (Cl), 0.25% (w/v) each of citric and ascorbic acid mixed solution, and 0.5% (w/v) citric acid (CA) solution followed by 50% (v/v) ethanol spray. The results revealed that the lowest microbial count was observed for the citric and ethanol sprays, which could be a good alternative to $CaCl_2$.

Temperature plays a key role during storage, as it is a major factor affecting the storage stability of microgreens by regulating the rate of respiration and metabolic activity (Xiao et al., 2014). Generally, microgreens have a shelf life of 2–4 days at ambient room temperature (15–25°C), but the shelf life may extend to 10–14 days when microgreens are stored at low temperatures, i.e., $5 \pm 1^\circ\text{C}$ (Chandra et al., 2012; Kou et al., 2013). The storage of microgreens below 1°C might result in chilling injury and physical damage to shoots and leaves (Berba & Uchanski, 2012). Modified atmospheric packaging is a packaging technology suitable for the storage of perishable commodities that helps minimize respiration rates, moisture loss, and environmental contamination and inhibits the growth of pathogenic microbes (Berba & Uchanski, 2012). Xiao et al. (2014) studied the effects of modified atmosphere packaging and light exposure treatment for 14 days at 1°C on the antioxidant activity, bioactive component

content, and sensory qualities of radish microgreens. The results of this study revealed that favorable O₂/CO₂ equilibrium and aerobic conditions led to physiological tissue damage. Moreover, a critical effect of temperature on shelf-life was observed in comparison with the effect produced by package film gas permeability. When various storage conditions are integrated, packaging treatment techniques provide the best results in extending the shelf life and maintaining the quality of microgreens, ensuring that they remain fresh and nutrient-rich for longer periods. Further research into novel packaging materials, advanced nanotechnology applications, and molecular breeding techniques will further increase the shelf life and quality of microgreens, meeting the growing consumer demand for fresh and nutritious produce.

2.7. Organoleptic properties of microgreens

Nutritional value and organoleptic properties (particularly appearance, texture, and flavor) influence consumer acceptance of food commodities in terms of organoleptic properties (Caracciolo et al., 2020). Consumer preference is dominated by appearance, although other factors encourage the repeat purchase of food products. Microgreens deliver a wide range of colors, including red, yellow, green, crimson, or multiple colors. In flavors, it tastes spicy, bitter, slightly sour, and neutral. In texture, it is juicy, crunchy, or regular (Di Gioia et al., 2017). Organoleptic properties are closely correlated with food material nutritional and biochemical properties; for example, phenolic content and pH are closely linked to the astringency, sourness, bitterness, and sweetness of microgreens (Xiao et al., 2015; Caracciolo et al., 2020). As a result, “overall liking” or “acceptability” without rating the products for organoleptic evaluations of individual attributes are rarely comparable across species. Xiao et al. (2015) coordinated a sensory assessment of six selected varieties of microgreens. Overall, the overall acceptability score of the pepper cress microgreens was the lowest, and that of the bull’s blood beet microgreens was the highest; moreover, no significant variation was found among the other four microgreens. The red amaranth microgreens and bull's blood beets had the second highest and highest overall eating quality scores, respectively. Another researcher, Caracciolo et al. (2020), conducted a sensory study of 12 selected microgreens and concluded that the total acceptability is based on organoleptic characteristics, which include sourness, aroma, grassy, heat, bitterness, and astringency. Microgreens that have a lower level of bitterness, grassy, and sourness, such as coriander and Swiss chard, are recommended over those with bitter and grassy tastes, such as mibuna, cress, and amaranth. Tan et al. (2020) carried out a study on sensory evaluations from local and commercial farms to evaluate broccoli microgreens and determined that their chlorophyll contents are relatively high, which results in a relatively high score of sweet taste for sugar production. Michell et al. (2020) studied the acceptability and

consumer sensitivity of six microgreen crops (tendrill pea, red cabbage, arugula, bull's blood beet, broccoli, and red garnet amaranth) in addition to hindering consumer acceptability and potential drivers. Many researchers have investigated consumer acceptance and reported certain factors affecting consumer acceptability, such as sensory perception, food neophobia, knowledge, accessibility and availability, cost, freshness, and shelf life. As a result, to promote this novel functional food, significant public awareness and acceptance of microgreens could be key factors.

2.8. Utilization of microgreens in value-added products and market analysis

In recent years, microgreens have become more famous in the culinary sector because of their nutritive value and organoleptic properties. They are used as ingredients in appetizer starters such as salads, soups, sandwiches, and for seasoning a great variety of main dishes (Ciuta et al., 2020). Microgreens are ideal candidates for the astronaut's functional salad in space missions (Kyriacou et al., 2017). Microgreens use was not unusual in the restaurant as a garnish on the dishes and beverages (Božić & Milošević, 2020).

2.8.1. Effect of processing on microgreens

The processing of microgreens can significantly influence their bioactive compounds through various mechanisms that alter their structural, functional, and nutritional properties. These changes can affect the availability, efficacy, and health benefits of these compounds. Processing methods such as washing, drying, blanching, freezing, packaging materials can disrupt the cellular structure of microgreens, leading to the release or degradation of bioactive compounds (Galieni et al., 2020).

Processing and storage conditions, such as temperature and packaging, can significantly affect the functional groups in microgreens, leading to changes in their nutritional and sensory qualities. For instance, optimal storage at 1°C without chilling injury helps maintain the stability of functional groups, such as chlorophyll and phenolic compounds. However, improper handling or higher temperatures may lead to degradation, reflected by changes in headspace composition and increased tissue electrolyte leakage. Additionally, while chlorine wash treatments can temporarily reduce microbial populations, they might also alter the microgreens functional group integrity over time (Xiao et al., 2014). Radish microgreens, when exposed to light, showed increased ascorbic acid content levels but deteriorated faster compared to dark storage conditions, which maintained carotenoid retention and quality (Xiao et al., 2014). Similarly, buckwheat microgreens stored at low temperatures with modified atmosphere packaging (MAP) retained freshness and exhibited lower tissue electrolyte leakage (Kou et al., 2013). The use of MAP combined with organic acid treatments in broccoli

microgreens significantly preserved bioactive compounds and reduced microbial load, suggesting an effective method to enhance shelf life and nutritional quality (Patil et al., 2024).

The Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) analyses of spinach, carrot, bathua, and Bengal gram microgreen powders reveal a correlation between their morphological structures and functional group compositions. SEM imaging shows distinct surface characteristics, such as nonuniform folds in spinach and elongated stalklike structures in Bengal gram, which likely influence the specific functional groups detected by FTIR spectroscopy. For instance, the rough surface of carrot microgreens correlates with the presence of alkanes, alcohols, and amines identified by FTIR. Similarly, the unique structural features observed in bathua and Bengal gram microgreens are reflected in their specific functional group peaks, such as alkenes and halo compounds. This combined analysis suggests that the physical morphology of microgreens may influence or reflect their chemical composition, providing insight into their potential functional properties (Sanyukta et al., 2023). The study focuses on the impact of processing on the structural integrity and functional properties of bioactive compounds, particularly pectic polysaccharides, in quinoa microgreens. Processing techniques, such as varying degrees of esterification, induce structural modifications in these compounds, affecting their molecular structure and functional groups. The degree of esterification significantly influences the structural integrity and bioactivity of these compounds, affecting their solubility, gelling properties, and interaction with other cellular components. These changes directly relate to the potential health benefits offered by quinoa microgreens, particularly in terms of their antioxidant, anti-inflammatory, and prebiotic activities (Li et al., 2024).

Thermal processing can cause the breakdown of cell walls and membranes, facilitating the release of phenolic acids, flavonoids, and other antioxidant compounds. The selected microgreen varieties carrot, spinach, bathua, and Bengal gram microgreens are dried in a tray dryer at 55°C for 8 to 9 h. Notably, the water holding capacity (3.40%), emulsion activity (56.37%), and emulsion stability (53.72%) were measured. On the other hand, Bengal gram microgreens presented the highest total phenolic content (32.2 mg GAE/g), total flavonoid content (7.57 mg QE/100g), and DPPH activity (90.60%). These findings highlight the potential of specific micronutrients as valuable ingredients in the food processing industry, suggesting their potential as nutritive additives (Sanyukta et al., 2023).

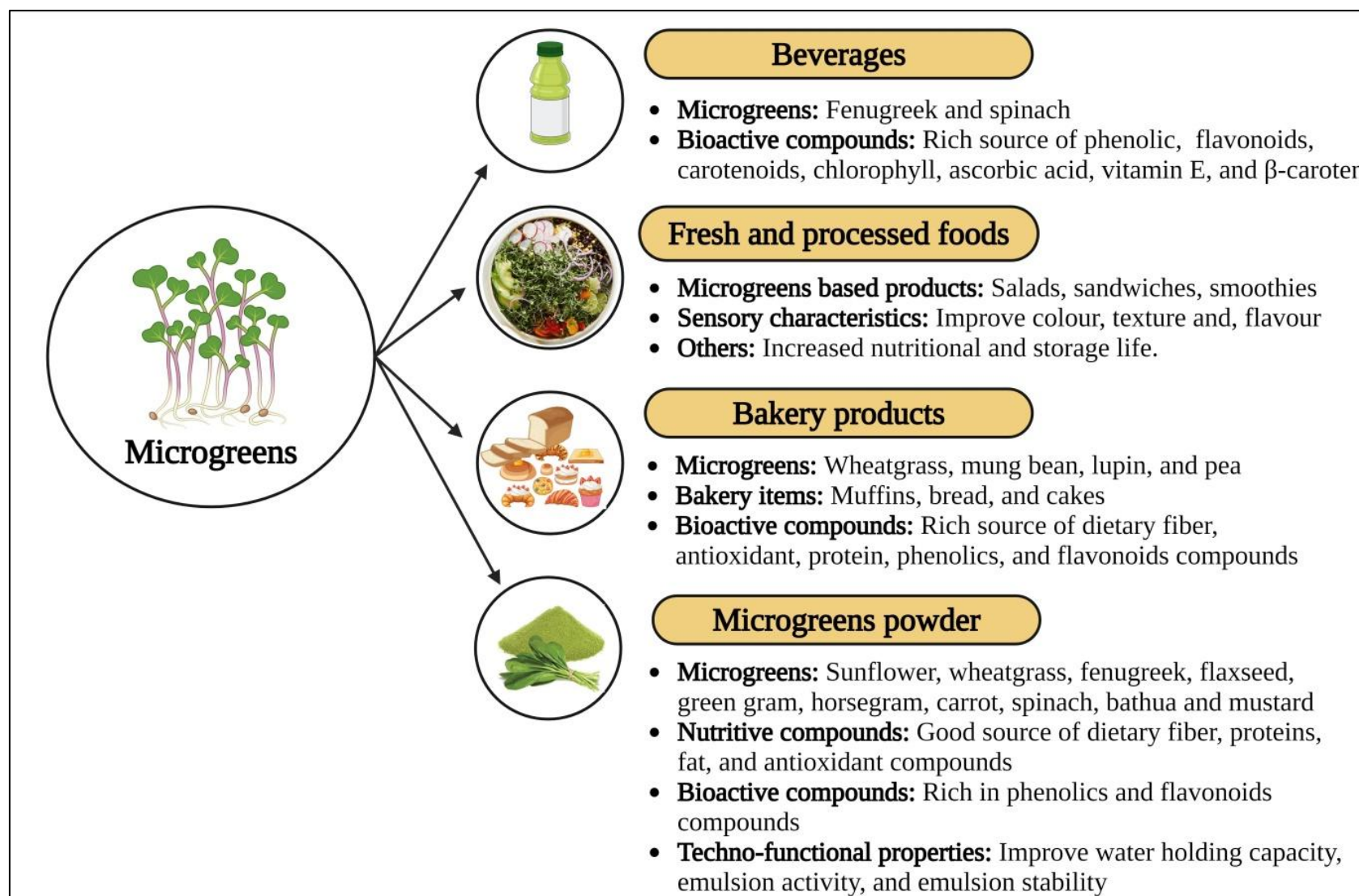


Figure 2.5. The utilization of microgreens into value added food products

The phenolic hydroxyl groups (-OH) are highly susceptible to oxidation, which can lead to the formation of quinones or the polymerization of phenolics. These structural changes can reduce the antioxidant activity of phenolic compounds and alter their interaction with other bioactive molecules, potentially diminishing the protective effects against oxidative stress and thereby affecting the health benefits of processed microgreens (Chen et al., 2020). Thermal processing and UV light exposure can break down the glycosidic bonds in flavonoids, leading to the formation of free aglycones. Additionally, the C=C bonds in flavonoid structures may undergo oxidative cleavage, affecting their stability and bioavailability. Such changes can reduce the antioxidant and anti-inflammatory activities of flavonoids (Gao et al., 2022).

In another study, broccoli microgreens were dried on various drying methods such as shade drying (room temperature), tray drying (55°C for 10 h), and microwave drying (1020 watts for 10 min). This study showed that microwave drying preserved the highest levels of ascorbic acid and chlorophyll but caused more degradation of β -carotene compared to shade drying. Shade drying, however, retained the highest total phenolic content, suggesting that the choice of drying method significantly influences the retention of specific bioactive compounds (Bhatt et al., 2023). However, excessive heat can denature proteins, degrade enzymes, and lead to the loss of structural integrity of delicate compounds like ascorbic acid. The thermal degradation can cause the oxidation of ascorbic acid into dehydroascorbic acid, which has a lower bioactivity (Galieni et al., 2020). Carotenoids are particularly vulnerable to isomerization and oxidation during processing, especially when exposed to heat and light (Khoo et al., 2011). The polyene chains in carotenoids can undergo cleavage, leading to the formation of epoxides or other oxidative products. This degradation reduces the provitamin A activity of carotenoids and diminishes their ability to protect cells from oxidative damage, thereby impacting the nutritional value of processed microgreens (Swapnil et al., 2021). Ascorbic acid (Vitamin C) also experiences functional group changes during processing. It can oxidize to dehydroascorbic acid (DHA), particularly under high temperatures or prolonged storage, leading to the loss of the enediol functional group that is essential for its antioxidant activity. This oxidation decreases the antioxidant potential of ascorbic acid (Yin et al., 2022).

The thermosonicated treated microgreen juice sample had no significant effect on the physicochemical parameters (total soluble solids, pH, and titratable acidity), but significant effects were observed on the total phenolic content, flavonoid content, and antioxidant activity of the microgreen juice. The use of thermosonication reduces the microbial population from the microgreen juice sample (Senthilnathan & Muthusamy, 2021). Heat during processing can lead to the oxidation of polyphenols and flavonoids, transforming them into quinones or other

oxidation products. While these oxidized forms may have different bioactivities, they often exhibit reduced antioxidant capacity. For instance, the catechol group in flavonoids can be oxidized, diminishing their ability to scavenge free radicals (Chaaban et al., 2017). Enzymatic or acid hydrolysis during processing can break down complex bioactive compounds into simpler forms. For example, glycosylated flavonoids may be hydrolyzed to aglycones, which may be more bioavailable and exhibit different health effects. This transformation can either enhance or reduce the overall bioactivity of the compound (Yang et al., 2020; König et al., 2023). For the extraction of juice from amaranth, red beet, and broccoli microgreens the advance cold-pressed processing method was used. The findings revealed a rich presence of phenolic compounds across all samples, with betalains in amaranth and red beet, and glucosinolates in broccoli microgreens. This processing method significantly altered the bioactive profiles, with various isothiocyanates appearing in broccoli juice and more phenolic acid aglycones and derivatives identified in all juices. The obtained microgreen juices demonstrated strong antioxidant activities and good sensory qualities, making them valuable as novel functional products (Belošević et al., 2024). Cold-pressed broccoli microgreen and sprout juices showed anti-cancer, anti-obesity, and preventive qualities against disorders linked to oxidative stress (Chartoumpekis et al., 2019; Li et al., 2021).

Processing can have varied effects on the nutritional content of microgreens, including both beneficial and detrimental changes. The ascorbic acid and tocopherols are sensitive to heat and light treatment, and their levels can decrease significantly during thermal processing (Gunjal et al., 2024). On the other hand, certain processing methods like steaming or blanching can inactivate oxidative enzymes (ascorbate oxidase), thereby preserving vitamin content (Xiao et al., 2017). The antioxidant activity of microgreens, primarily due to their polyphenolic content, can be influenced by processing. While mild processing might enhance the extractability and concentration of these antioxidants, severe processing can lead to their degradation (Bhaswant et al., 2023). For example, blanching can enhance the release of bound phenolics, while excessive drying or heating can cause significant losses (Xiao et al., 2017). Processing can alter the bioavailability of minerals by affecting anti-nutritional factors like phytic acid. For instance, fermentation and germination can reduce phytic acid levels, thereby enhancing the bioavailability of minerals such as iron and zinc. Conversely, certain processing methods might induce the formation of insoluble complexes that reduce mineral absorption (Nkhata et al., 2018). Future research should focus on optimizing processing methods to retain or enhance the bioactive compounds in microgreens, particularly in relation to their antioxidant and health-promoting properties. Investigating the synergistic effects of different processing

techniques on the bioavailability of nutrients and bioactives could provide valuable insights. Additionally, studies exploring the application of advanced processing technologies, such as pulsed electric fields and high-pressure processing, could open new avenues for developing functional food products with superior nutritional qualities.

2.8.2. Microgreens based value-added products

The versatility of microgreens makes them valuable assets in creating nutritious value-added food products. By including fresh microgreens in blends, the levels of health-promoting secondary metabolites can be increased. Microgreens can also be used to develop innovative food items such as beverages, seasonings, weaning foods, probiotic-infused products, yogurt, flour enhanced with powdered microgreens, bakery items, and teas, along with being eaten fresh. The utilization of microgreens in value-added food products and their advantages are shown in **(Figure 2.5)**. Over the last few years, researchers have focused on the preparation of microgreen-based functional beverages. For example, fenugreek and spinach microgreen juices are incorporated into different fruit juices, such as pomegranate, pineapple, and kinnow mandarin, which are used for the preparation of microgreen-based functional beverages (Sharma et al., 2021; Sharma et al., 2020).

Kaur et al. (2022) prepared gluten-free eggless rice muffins incorporated with a different range of microgreen powders (wheatgrass and mung bean). The incorporation of powder into the products enhanced the dietary fiber, antioxidant, protein, phenolic, and flavonoid properties of microgreen-based gluten-free eggless rice muffins. The addition of microgreen juice powder to muffins enhances the bioactive phenolic compounds, and the reduced bake loss is of industrial interest. Wheatgrass, fenugreek, and basil microgreen juice powders can be incorporated by up to 3% into baked products as a source of functional ingredients for health benefits (Kumar & Singh, 2024). The sunflower microgreen powder was used as a flour replacement in the cake preparation at concentrations ranging from 4 to 12%. The addition of sunflower microgreen powder had a significant effect on the moisture content, crude fiber, protein, fat, total phenolic content, antioxidant activity, mineral composition, and firmness but decreased the lightness, peroxide value, and overall acceptance. During a 14-day storage period, cakes with sunflower powder maintained greater firmness, chewiness, and moisture content with a more even hole distribution than did the control, suggesting the potential for functional and cost-effective gluten-free cakes (Mansouri et al., 2024). Klopsch et al. (2018) enhanced the nutritional value of bread by adding lupin and pea microgreens along with mature leaves to bread dough. The contents of biochemical compounds such as photosynthetic pigments (chlorophyll and carotenoids) and flavonoids significantly decreased

after baking, and significant pheophytin formation occurred in the end product. In bread prepared with lupin microgreens, the level of genistein is maintained, which shows anticarcinogenic properties and reduces the sugar level. The thermosensation method was used for the *Alternanthera sessilis* microgreen juice (frequency level, 44 kHz; temperature, 30–50°C; and duration, 20–35 min). In another study, ready-to-eat chutney powder was incorporated with microgreens and mature greens. The incorporation of microgreens into chutney powder increases the protein, phosphorous, and polyphenol contents of the prepared product (Nivedha and Lakshmy Priya, 2018).

2.8.3. Microgreens market trends

As a result of advancements in new crop production technologies over the last decade, the agricultural commodity market trend has shifted dramatically. Current technologies are adapted to meet consumer demand for healthy foods, increase the food supply, increase population income, increase genetic and technological advancement, increase access to information, increase the effects of biotic and abiotic constraints, the machinery revolution, the social and economic context, climatic changes and urbanization, which affect the production of agricultural goods, and citizens move from one region to another (Paraschivu et al., 2021). The microgreen market is divided into different segments on the basis of factors such as dominant crop type, farming or cultivation methods employed, type of growing medium used, geographical area of distribution, microgreen utilization in food and beverages, cosmetics, and others (Paraschivu et al., 2021).

The global CAGR (compound annual growth rate) of microgreens is 7.67%, and it is expected to reach a market size of US\$17,039.744 million in 2025 from US\$10,936.914 million in 2019. The major contributing regions to microgreen production are the United States, Canada, and Mexico (Microgreens Market, 2021). The microgrid market is emerging, but its many obstacles need to be overcome. Microgreens are similar to sprouts in several ways, and the incidence of microbial contamination caused by *Salmonella* and *Listeria* microbial species has been reported in Canada and the United States (Turner et al., 2020). The COVID-19 pandemic has brought about a change in consumer attitudes in terms of food choices, as people prefer more immune-boosting foods (health foods). Thus, microgreens are considered suitable contenders for meeting consumer expectations (Le et al., 2020).

2.9. Conclusion

Microgreens appear to be excellent low-calorie nutrient sources, such as phytonutrients, antioxidants, vitamins, minerals, glucosinolates, and other components, which improve human health. Current research examines progress in understanding cultivation practices, preharvest

factors that affect production and quality, postharvest factors influencing shelf-life, and the difficulties that lie ahead. Microgreens offer a rich source of bioactive compounds with significant health benefits, but several challenges must be addressed to fully realize their potential. Key issues include optimizing production methods to maximize yield and minimize microbial contamination, which poses a risk of food-borne illnesses. Various types of studies have shown that microgreens contain high levels of bioactive compounds and that these compounds are responsible for different types of biological activities, including antifungal, antimicrobial, anticancer, antidiabetic, anti-inflammatory, and antioxidant activities. This review contributes to the existing knowledge about micronutrient management, nutritional value, health benefits, and pre-and postharvest handling practices of this new specialty crop.

CHAPTER-3

HYPOTHESIS OF THE STUDY

It is hypothesized that cultivation conditions (growing medium, illumination system and foliar spray) significantly influence the bioactive compound composition of microgreens, leading to variations in their antioxidant properties and nutritional value. Additionally, optimized post-harvest storage conditions (washing, packaging and storage) will contribute to the extended shelf life and retention of functional bioactive compounds of microgreens. The standardization of Ready-to-Serve (RTS) beverage formulations incorporating microgreens will enhance their acceptability, stability, and health benefits. Furthermore, it is hypothesized that the selection of appropriate storage conditions for microgreen-based RTS beverages will ensure long-term preservation of bioactive compounds and sensory attributes, making them a viable functional food option for commercial applications.

CHAPTER-4

OBJECTIVES OF THE STUDY

The major objectives of the proposal research work are following:

1. To assess the effect of cultivation conditions on bioactive compounds in selected varieties of microgreens
2. To determine post-harvest shelf life of microgreens at different storage condition
3. To standardize and optimize the process condition for preparation of microgreens based ready-to-serve beverage
4. To determine the optimal storage condition for the developed ready-to-serve microgreen based beverage

Expected outcome of research work

- This research is expected to establish optimal cultivation practices that enhance the bioactive composition of microgreens, ensuring higher nutritional value and improved functional properties.
- The study will provide insights into effective post-harvest storage strategies, extending the shelf life of fresh microgreens while preserving their bioactive components.
- Additionally, the optimization of RTS beverage formulations will result in a nutritionally enriched, stable, and consumer-acceptable functional beverage.
- The findings will offer valuable guidance for commercial food producers, contributing to the development of sustainable and health-promoting microgreen-based food products.
- Furthermore, the study aims to advance scientific knowledge on degradation kinetics and storage stability of microgreen-based beverages, fostering their widespread adoption in the functional food industry.

CHAPTER-5

MATERIALS AND METHODOLOGY

The present investigation “**Process Optimization for Microgreen Based Ready to Serve Beverage**” was carried out at the Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India, during the year 2020-2025. The details of the materials used and methodology adopted during the research work investigation are detailed and explained below in this section.

5.1. Microgreen seeds

The study was conducted on different families of microgreens, including broccoli, cabbage, pak choi, radish sango, beetroot, red amaranthus, and flax crops were used, and its specification is shown in (Table 5.1). The above-selected microgreen seeds were provided by Seed Delivery LLP, Chandigarh, India. The selected microgreens were grown in a different growing medium based on experimental study work with three replications. The period from seed production to sowing was approximately six months.

5.2. Growing medium and microgreens cultivation

For the microgreens sowing, three different alternative growing mediums (Sandy loam soil, cocopeat, and hydroponics (burlap)) were used, and their physiochemical properties are shown in (Table 5.2). The growing media was sterilized thrice at 121°C and 15 psi for 15 min. Sterilized growth media and distilled water were used for the cultivation of microgreens. Firstly, all microgreen seeds were disinfected with calcium hypochlorite solution (2.00%) for 10 min, then rinsed and washed with distilled water thrice. These seeds were soaked in distilled water for 8 to 12 hrs at room temperature (approximately 25 – 30°C). After soaking, seeds were gently mixed in a growing medium and uniformly distributed into the growing cups. The sowing density of seeds and their details are shown in (Table 5.1). The growing condition was temperature $20\pm 2^{\circ}\text{C}$ and relative humidity of $65\pm 2\%$ in the dark for 3 days after that, 450 mg/L carbon dioxide, light intensity $55\ \mu\text{mol m}^{-2}\text{s}^{-1}$ and photoperiod 12/12 hrs (light/dark) maintained in the growing chamber (Therm-finsher, Plant Growth Chamber, Goa, India) with the dimensions $1200 \times 1200 \times 1200$ mm. Until the day of harvest, all microgreens were watered manually, usually two times per day (morning and evening). All microgreens’ seeds were grown in separate cups ($63.62\ \text{cm}^2$) and carefully harvested on the 9th day above the growing surface (1 cm) after the emergence of the first true leaves. Further, harvested fresh microgreens were used for research analysis only after washing them in tap water once and twice with drinking water.

Table 5.1. Selected microgreens families used for study and their specifications.

Families	Microgreens	Variety Name	Germination percentage (%)	Physical purity (%)	Genetic purity (%)	Sowing rate (g/m ²)
Amaranthaceae	Beetroot	Madhur	60	98	98	388.26
Amaranthaceae	Red amaranthus	Arun	70	98	98	88.03
<i>Brassicaceae</i>	Cabbage	Golden Acre	70	99	98	149.33
<i>Brassicaceae</i>	Broccoli	Calabrese	70	98	98	190.20
<i>Brassicaceae</i>	Pak choi	Urja All Green Pak Choi	75	98	98	125.75
<i>Brassicaceae</i>	Radish sango	Radish Purple Sango	70	99	98	292.37
Linaceae	Flax crops	Mukta	80	99	98	314.38

Note: The above-mentioned microgreen seeds were provided by Seed Delivery LLP., Chandigarh, India. The germination percentage, physical purity, and genetic purity values were obtained from the information provided on the packaging materials by the producer.

Table 5.2. Physiochemical properties of growing media

Parameters	Soil	Cocopeat
pH	7.60	6.09
Electrical conductivity(dsm ⁻¹)	0.25	1.43
Calcium carbonate (%)	6.00	4.00
Organic carbon (%)	0.58	20.55
Sodium (%)	0.03	0.05
Nitrogen (%)	0.01	0.02
Phosphorus (%)	0.02	0.04
Potassium (%)	0.32	0.47

5.3. Illumination systems for microgreens cultivation

After growing for 3 days in the dark, all the microgreens were transferred to various light treatments via LED light sources with emission wavelengths of red light (630–650 nm), blue light (450–455 nm), yellow light (480–490 nm) and green light (510–520 nm) (8 Watts, Philips Lumileds, USA). All the LED light sources were situated 35 cm from all the samples, and the photosynthetic photon flux density (PPFD), which was set as $45 \pm 2 \mu\text{mol m}^{-2} \text{s}^{-1}$ in all the treatments, and the photoperiod was maintained at 12/12 hrs (light/dark) for the next 6 days. All the microgreens were cultivated for 9th days until they reached the stage of emergence of the first true leaves.

Table 5.3. Experimental design of foliar spray treatments and amount of foliar spray applied to microgreens in each treatment grown in the cocopeat-based growing medium in control environmental conditions for 9 days growing periods

Treatments	Foliar spray treatment combination				pH	EC of solution ($\mu\text{S}/\text{cm}$)	Total foliar spray used for 6 days (mL)	Total foliar spray used for 6 days based on area (mL/cm^2)	Total base applied foliar spray	
	CaCl ₂ (mM)	KNO ₃ (mM)	CaCl ₂ (g/L)	KNO ₃ (g/L)					CaCl ₂ (g)	KNO ₃ (g)
T-01	18.000	2.000	1.998	0.202	7.24	2714.00	360.00	5.66	0.719	0.073
T-02	04.000	2.000	0.444	0.202	7.53	1230.00	360.00	5.66	0.160	0.073
T-03	11.000	5.000	1.221	0.506	7.21	2844.00	360.00	5.66	0.439	0.182
T-04	11.000	5.000	1.221	0.506	7.14	3012.00	360.00	5.66	0.439	0.182
T-05	20.900	5.000	2.319	0.506	6.84	4164.00	360.00	5.66	0.835	0.182
T-06	01.101	5.000	0.122	0.506	7.73	1368.00	360.00	5.66	0.044	0.182
T-07	18.000	8.000	1.998	0.809	7.14	4162.00	360.00	5.66	0.719	0.291
T-08	11.000	9.243	1.221	0.935	7.10	3448.00	360.00	5.66	0.439	0.336
T-09	11.000	5.000	1.221	0.506	7.04	2698.00	360.00	5.66	0.439	0.182
T-10	11.000	0.757	1.221	0.077	7.28	1428.00	360.00	5.66	0.439	0.028
T-11	11.000	5.000	1.221	0.506	7.32	2592.00	360.00	5.66	0.439	0.182
T-12	11.000	5.000	1.221	0.506	7.39	2714.00	360.00	5.66	0.439	0.182
T-13	04.000	8.000	0.444	0.809	7.60	2026.00	360.00	5.66	0.160	0.291

The selection of emission wavelengths was based on their physiological relevance to plant photoreceptors and photosynthetic pigments. Red and blue light correspond to the absorption peaks of chlorophyll a and b, which are essential for photosynthesis and photomorphogenesis. Green and yellow wavelengths, although less efficiently absorbed, contribute to canopy penetration and overall photosynthetic efficiency. These spectral ranges are consistent with previous studies on microgreen cultivation under controlled environments (Zhang et al., 2020).

5.4. Foliar spray treatments on microgreens

The different foliar spray treatment formulations and experimental work were carried out using the Design Expert (version 13), a statistical software package. The independent variables, such as calcium chloride (A) and potassium nitrate (B), were used in foliar spray treatments based on the Central Composite Rotatable Design (CCRD) treatments run (13) obtained. The current study works different types of independent variables within specified ranges: A ranged from 4.00 (-1) to 18.00 (+1), and B ranged from 2.00 (-1) to 8.00 (+1) were used. The optimization of different experiment treatments is represented in **(Table 5.3)**. For the entire set of experimental runs shown in **(Table 5.3)**, note that three replications were assigned to axial and factorial points, and six replications were allocated to a central point (i.e., 11 mM calcium chloride and 5.5 mM potassium nitrate). On day fourth, after sowing, 60.00 mL of foliar spray combination of calcium chloride (CaCl_2) + potassium nitrate (KNO_3) was applied in each treatment based on different treatments obtained from the response surface methodology using Central Composite Rotatable Design (CCRD) as shown in **(Table 5.3)**. Foliar treatments were applied uniformly on the cotyledonary leaves of microgreens on days four to nine after sowing using a handheld sprayer (Shah Agro Agency, Pune, India). Each replicate tray was placed on an electronic balance (WENSAR, Chennai, India) and zeroed on each spraying daytime. The spray every day morning and evening (time difference between spray 12 hours) was then applied until the balance registered 60.00 g, which accounts for 60.00 mL of the treatment solution as the density of the solution was considered to be equal to water (1 g/mL). The microgreens were cultivated for 9th days until they reached the stage of emergence of the first true leaves (Kathi et al., 2022). On the 9th day of cultivation, the microgreens were harvested and washed with drinking water, followed by air drying to remove surface water from the surface of the microgreens. The harvested samples were then packed into polypropylene packaging materials (25.4×20.3 cm) for further analysis.

The data obtained from dependent variables were subjected to response surface regression analysis using the least square methodology to determine the equations of actual factors fitting the quadratic model. The adequacy of each model was examined by evaluating the lack of fitness, coefficient of regression (R^2), precision adequacy, and F-value through variance analysis (ANOVA).

5.5. Post-harvest treatments on microgreens

The microgreens were harvested after 9 days of growth and washed with drinking water to eliminate any residual particles of the growing medium. The microgreens were then exposed to seven different washing treatments: distilled water (pH 6.9), citric acid (0.50%, pH 2.6), sodium hypochlorite (150 mg/L, pH 8.3), ethanol (50%, pH 7.1), calcium chloride (10 mM, pH 6.4), acetic acid (0.5%, pH 2.9), and unwashed (control) all conducted at room temperature (Dalal et al., 2020). For the washing of microgreens 1 L washing solution were used for 100 g of microgreens samples. Following the treatments, the microgreens were air-cooled for an additional 30 minutes to remove any remaining surface moisture, as depicted in **(Figure 5.1)**. The initial moisture content of the microgreens was found to be $92.82 \pm 0.29\%$ on a fresh weight basis. For the experiments, approximately 30 g of microgreens were packed into each container in triplicate. The packaging techniques employed included low-density polyethylene (LDPE) bags (20×15 cm, 150 μ m gauge, 6 pinholes), high-density polyethylene (HDPE) bags (20×15 cm, 175 μ m gauge, 6 pinholes), metalized polyester (MP) bags (20×15 cm, 50 μ m gauge, 6 pinholes), and polystyrene (PT) trays (250 mL, 200 μ m gauge, 6 pinholes), all of which were sealed. The prepared bags and trays were subsequently stored under different temperature conditions (5°C and 25°C). Refrigerators (Sharp 342, SJ-EP35T-SL, Mumbai, India) were used for the 5°C storage, while incubators (AKIVIN, Ajanta Export Industries, Ambala, India) were employed for the 25°C condition. Sampling was conducted on the initial day and continued every third day over 12 days.

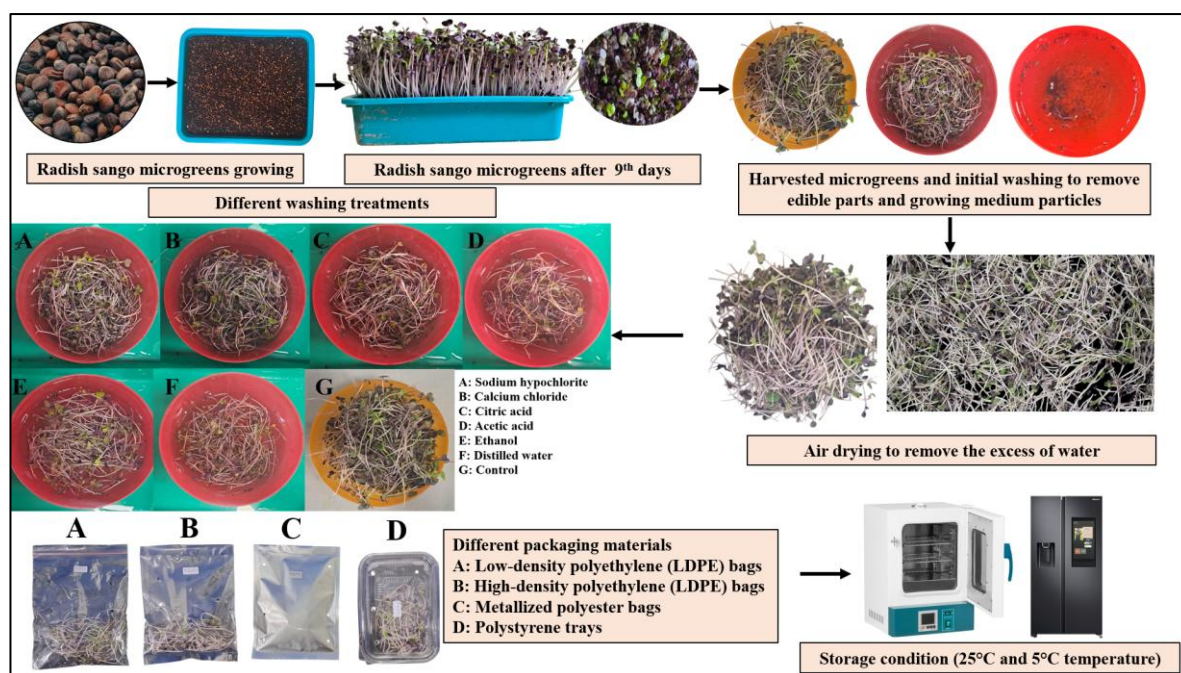


Figure 5.1. The experimental study plan for the effect of different washing treatments on microgreens stored in various packaging materials at 25°C and 5°C storage conditions.

5.6. Drying condition for microgreens

For drying, microgreen shoots were chopped to lengths of 1.5 to 2.0 cm and distributed in thin layers on stainless steel trays. Drying trials were conducted in triplicate in a tray dryer with forced air circulation, assembled locally at the School of Agriculture, LPU, Phagwara, Punjab, India, at temperatures of 40, 50, and 60°C and a constant air velocity of 1.5 m/s, until equilibrium moisture content was achieved. The sample loading density on the trays was 0.057 and 0.113 g/cm², spread uniformly to a thickness of 5 and 10 mm, respectively. Water content reduction was monitored through the gravimetric method, with sample weights recorded at 30 min intervals using an analytical balance (WENSAR, Chennai, India). Samples were collected at different time intervals to estimate the degradation of bioactive compounds such as total phenol content, anthocyanin contents, ascorbic acid content, and antioxidant activity DPPH in microgreens during the period of drying. Dried microgreens (1 kg) were ground into powder using a professional mixer grinder (Bajaj Electrical and Electronics Pvt. Ltd., Rex 500, Punjab, India) with a velocity of 20,000 r/min, running time of 5 min with an interval time of 10 min. The grinding was achieved at 25°C for 15 min. Collected samples were packed in LDPE polyethylene zipped bags (5 cm × 10 cm) and stored at -20°C for further analysis (**Figure 5.2**).

5.6.1. Drying data mathematical modeling

To analyze the drying kinetics of microgreens, various mathematical models were utilized. Moisture reduction data, collected under differing temperatures and tray load

conditions, were applied to ten distinct drying models (**Table 5.4**). Each model's performance was assessed using criteria such as the lowest root mean square error (RMSE), the smallest chi-square value (χ^2), and the highest coefficient of determination (R^2). The moisture ratio (MR) was determined using Equation (01):

$$MR = \frac{M_t}{M_0} \quad (01)$$

The moisture ratio (MR) was defined as the ratio of moisture at a given time (M_t) to the initial moisture content (M_0) (kg water/kg dry matter). Equilibrium moisture content was deemed negligible and therefore excluded from calculations to simplify the analysis while preserving accuracy.

5.6.2. Determination of effective moisture diffusivity

Effective moisture diffusivity (D_{eff}) indicates the rate at which internal moisture moves within the material. It is calculated based on Fick's second law of diffusion, which accurately describes the falling-rate period of drying (Aral & Beşe, 2016). For thin-layer drying, D_{eff} is determined using Equation (02).

$$MR = \frac{8}{\pi^2} \exp\left(-\frac{\pi^2 D_{eff} t}{4L^2}\right) \quad (02)$$

Where, the effective moisture diffusivity (D_{eff}) (m^2/s) and the thickness of the layer (L) (mm).

The effective diffusivity (D_{eff}) is determined by plotting the natural logarithm of the moisture ratio (MR) against drying time (t), resulting in a straight line with a negative slope, known as the drying constant (k). The value of k is then used to calculate (D_{eff}), as shown in Equation (03).

$$D_{eff} = \frac{4L^2 \cdot k}{\pi^2} \quad (03)$$

Table 5.4. The different mathematical models given by various authors for drying curves

Model name	Mathematical equation	References
Newton	$MR = \exp(-kt)$	(Pandiselvam et al., 2020)
Page	$MR = \exp(-kt^n)$	(Pandiselvam et al., 2020)
Hander and Pabis	$MR = a \cdot \exp(-kt)$	(Khalangre et al., 2024)
Logarithmic	$MR = a \cdot \exp(-kt) + b$	(Khalangre et al., 2024)
Verma et al	$MR = a \cdot \exp(-kt) + (1 - a) \exp(-gt)$	(Pandiselvam et al., 2020)

Approximation of diffusion mode	$MR = a. \exp(-kt) + (1 - a)\exp(-kbt)$	(Guemouni et al., 2022)
Midilli et al	$MR = a. \exp(-kt^n) + bt$	(Guemouni et al., 2022)
Two terms	$MR = a. \exp(-kt) + (b. \exp(-k_1t))$	(Guemouni et al., 2022)
Two-term expo.	$MR = a. \exp(-kt) + (1 - a). \exp(-kbt)$	(Guemouni et al., 2022)
Diffusion	$MR = a. \exp(-bt)$	(Khalangre et al., 2024)

5.6.3. Estimation of activation energy (E_a)

The activation energy (E_a) estimates the temperature sensitivity of drying, representing the energy required to remove a unit of moisture from a material at a specific moisture level (Fang et al., 2009). It is calculated using the Arrhenius equation, which describes the relationship between effective diffusivity (D_{eff}) and drying temperature. The Arrhenius Equation (04) is as follows:

$$D_{eff} = D_0 \exp\left(-\frac{E_a}{RT}\right) \quad (04)$$

Where D_0 is D_{eff} at infinite temperature (m^2/s), activation energy (E_a) is required for diffusion of one mole of moisture (kJ/mol), gas constant ($R=8.314 \times 10^{-3}$ kJ/mol), and T is the temperature in Kelvin (K).

The E_a is calculated by plotting $\ln(D_{eff})$ against $1/T$ in kelvin (K), which gives a slope ($k = -E_a/R$). The following Equation (05) was used.

$$E_a = -k \times R \quad (05)$$

5.6.4. Energy consumption

During our experimental study on microgreens drying at different temperature conditions and tray loading density, energy consumption can be quantified using two efficiency indices, such as specific electric energy consumption, as defined by Equation (06), and electrical energy (EE), as described by Equation (07) (Guemouni et al., 2022).

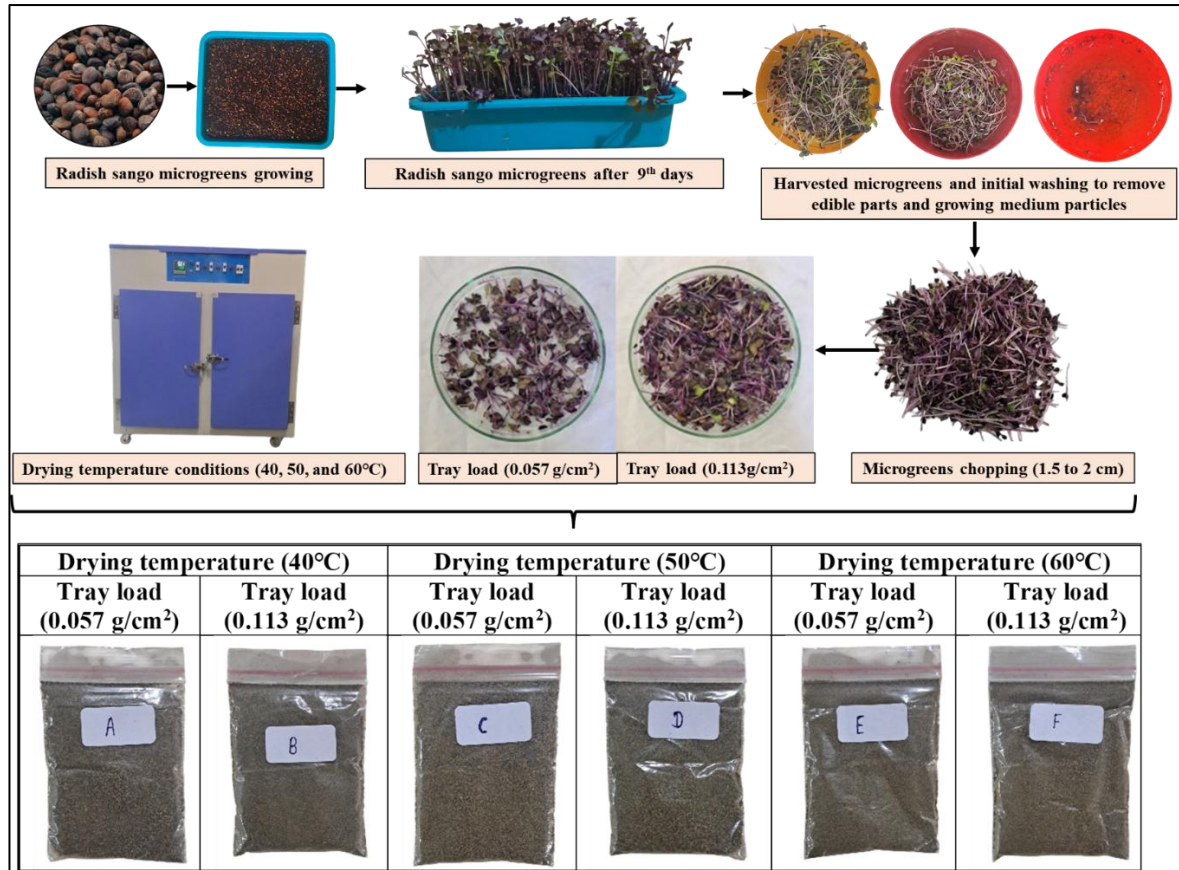


Figure 5.2. The harvested microgreen drying process at selected drying temperature and loading densities

$$SEC_e = \frac{3600E}{M_s(X_i - X_f)} \quad (06)$$

The specific electric energy consumption (SECe) is measured in (MJ/kg of water), while the total electric energy consumption (E) is expressed in (kWh). X_i and X_f represent the initial and final moisture contents (MCs) on a dry basis (d.b), respectively. M_s denotes the mass of dry solid matter in kilograms (kg).

$$EE = M_s(X_i - X_f) \frac{\Delta h_v}{3600E} \times 100 \quad (07)$$

Where EE is the electrical energy in (%), and Δh_v is the enthalpy of evaporation of water (2257 kJ/kg, at 100°C)

5.6.5. Bioactive compounds and antioxidant activity parameters degradation kinetics

The degradation of bioactive compounds and antioxidant activity parameters in microgreens was studied under drying conditions at temperatures of 40°C, 50°C, and 60°C, with varying loading densities of 0.057 and 0.113 g/cm². Samples were analyzed at hourly intervals to determine total phenolic content, anthocyanin content, ascorbic acid content, and

DPPH antioxidant activity. The observed changes in the concentration of these compounds were modeled using a first-order kinetic Equation (08), described as follows:

$$c = c_0 e^{\pm kt} \quad (08)$$

Here, c represents the values of total phenolic content, anthocyanin content, ascorbic acid content, and DPPH antioxidant activity at different time intervals during drying. The c_0 denotes the initial values of these parameters prior to the commencement of the drying process. The parameter k corresponds to the rate constant of the first-order kinetic model.

The half-life ($t_{1/2}$) for total phenolic content, anthocyanin content, ascorbic acid content, and DPPH antioxidant activity was calculated by following Equation (09).

$$t_{1/2} = \ln 2/k \quad (09)$$

The Arrhenius equation was employed to determine the temperature dependence of the degradation of total phenolic content, anthocyanin content, ascorbic acid content, and DPPH antioxidant activity. The Equation (10) is presented below.

$$k = -k_0 e^{E_a/RT} \quad (10)$$

In this equation, E_a is the activation energy (kJ/mol), R is the ideal gas constant (8.3145×10^{-3} kJ/mol. K), and T represents temperature (K).

5.7. Effect of particle size distribution on microgreens powder

Dried microgreens (1 kg) were ground into powder using a professional mixer grinder (Bajaj Electrical and Electronics Pvt. Ltd., Rex 500, Punjab, India) with a velocity of 20,000 r/min, running time of 5 min with an interval time of 10 min. The grinding was achieved at 25°C for 15 min. The powder was sifted with a vibratory sieve shaker (G Lab, India) at 1.50 ± 0.01 mm vibration amplitude for 5 min. Microgreen powders were sieved to obtain five British sieve sizes, 30, 36, 50, 100, and 200, which constituted the particle sizes 600, 425, 300, 150, and 75 μ m, respectively, as displayed in **(Figure 5.3)**. Then, the powders were packed in LDPE polyethylene zipped bags (10 cm $\times$ 15 cm) and stored at $4 \pm 2^\circ\text{C}$ before they were used for analysis.

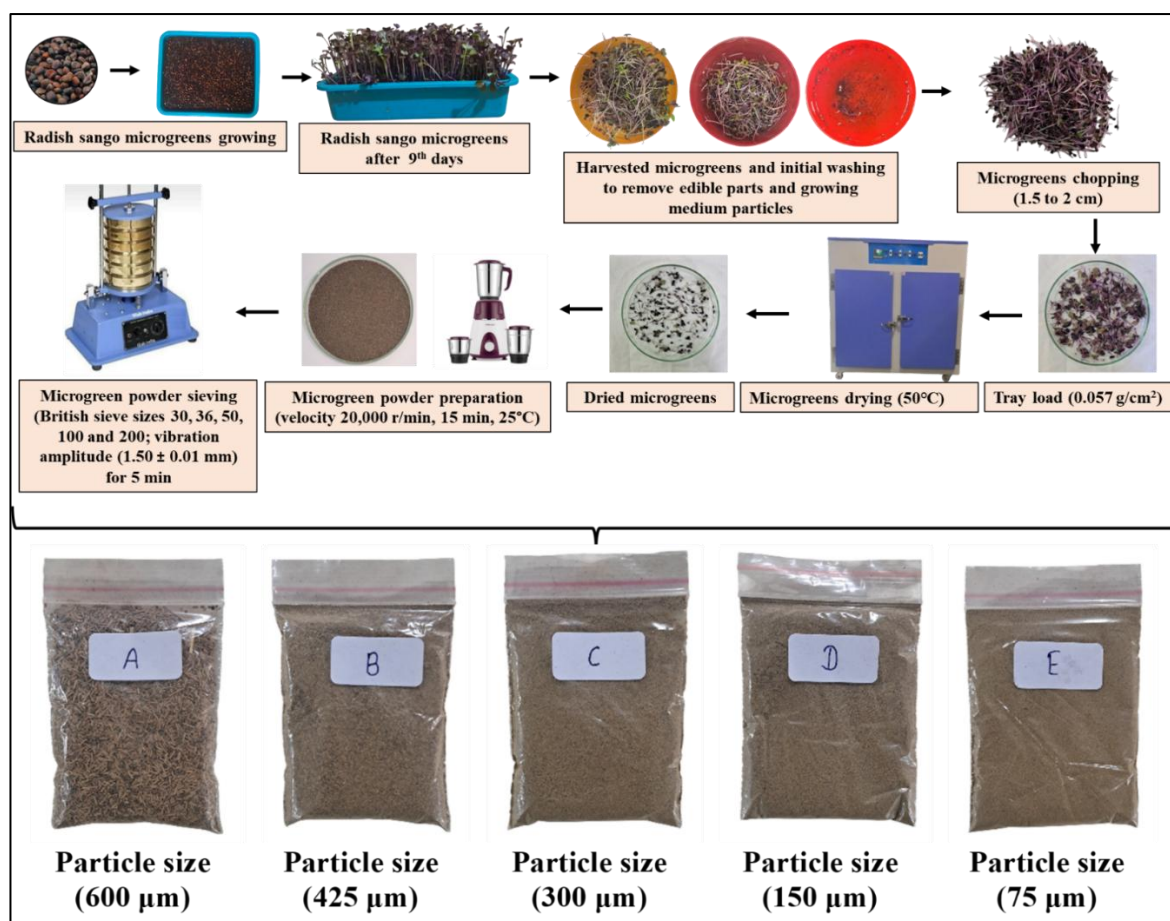


Figure 5.3. The process flow for microgreen powder preparation and size characterization of obtained powders by five British sieves.

5.8. Development of microgreen powder-based dairy (lassi) RTS beverage

The preliminary study was conducted aiming to determine the best formulation design from a sensory perspective in the form of trial and error. Dahi was prepared following the method described by Rasane et al. (2017). Fresh standardized milk was heat treated to 90°C for 15 min, followed by rapidly cooled to room temperature with chilled water and inoculated with 1.00 % (v/v) of a starter culture (*L. helveticus* MTCC 5463 and *S. thermophilus* MTCC 5460) were added. The prepared mixtures were incubated at 37°C for 4 to 5 hours until complete coagulation (pH level 4.5), then cooled overnight at 4±1°C. The development of microgreens-based lassi with various formulations was conducted using Design Expert software (version 13), a statistical analysis package. Independent variables, including Dahi (A) and microgreen powder (B), were selected for the formulation of lassi. A total of 13 experimental runs were generated using the Central Composite Rotatable Design (CCRD) model. The study explored the effects of these variables within specified percentage (%) ranges from Dahi (A) ranged from 70.00 (-1) to 75.00 (+1), and microgreen powder (B) ranging from 1 (-1) to 2.5 (+1) and the formulation of experimental treatments is summarized in (Table 5.5).

The sugar (12%) and CMC stabilizer (0.25%) fixed concentrations were used and to make the 100% formulation drinking water was used. The experimental study experimental flow diagram of the preparation of microgreen-based dairy (lassi) beverage using response surface methodology is shown in (Figure 5.4).

Table 5.5. Experimental design optimization of levels of various constituents in microgreen-based dairy (lassi) using response surface methodology

Treatments	Dahi (%)	Microgreen powder (%)	Sugar (%)	CMC (%)	Water (%)
1	72.50	1.75	12.00	0.25	13.50
2	72.50	1.75	12.00	0.25	13.50
3	68.96	1.75	12.00	0.25	17.04
4	76.04	1.75	12.00	0.25	9.96
5	70.00	1.00	12.00	0.25	16.75
6	72.50	2.81	12.00	0.25	12.44
7	70.00	2.50	12.00	0.25	15.25
8	75.00	2.50	12.00	0.25	10.25
9	72.50	1.75	12.00	0.25	13.50
10	72.50	1.75	12.00	0.25	13.50
11	75.00	1.00	12.00	0.25	11.75
12	72.50	0.69	12.00	0.25	14.56
13	72.50	1.75	12.00	0.25	13.50

Lassi preparation was performed based on the experimental runs. The ingredients were weighed according to the treatment specifications, and lassi was prepared following the method described by Kumar et al. (2023), with slight modifications (Figure 5.5). The prepared lassi samples were stored in 200 mL glass bottles under refrigeration conditions ($4\pm1^{\circ}\text{C}$) for subsequent analyses. The data obtained from dependent variables were analyzed using response surface regression analysis, employing the least squares methodology to fit the quadratic model. Model adequacy was evaluated by assessing the lack of fit, the coefficient of determination (R^2), adequate precision, and F-values through analysis of variance (ANOVA).

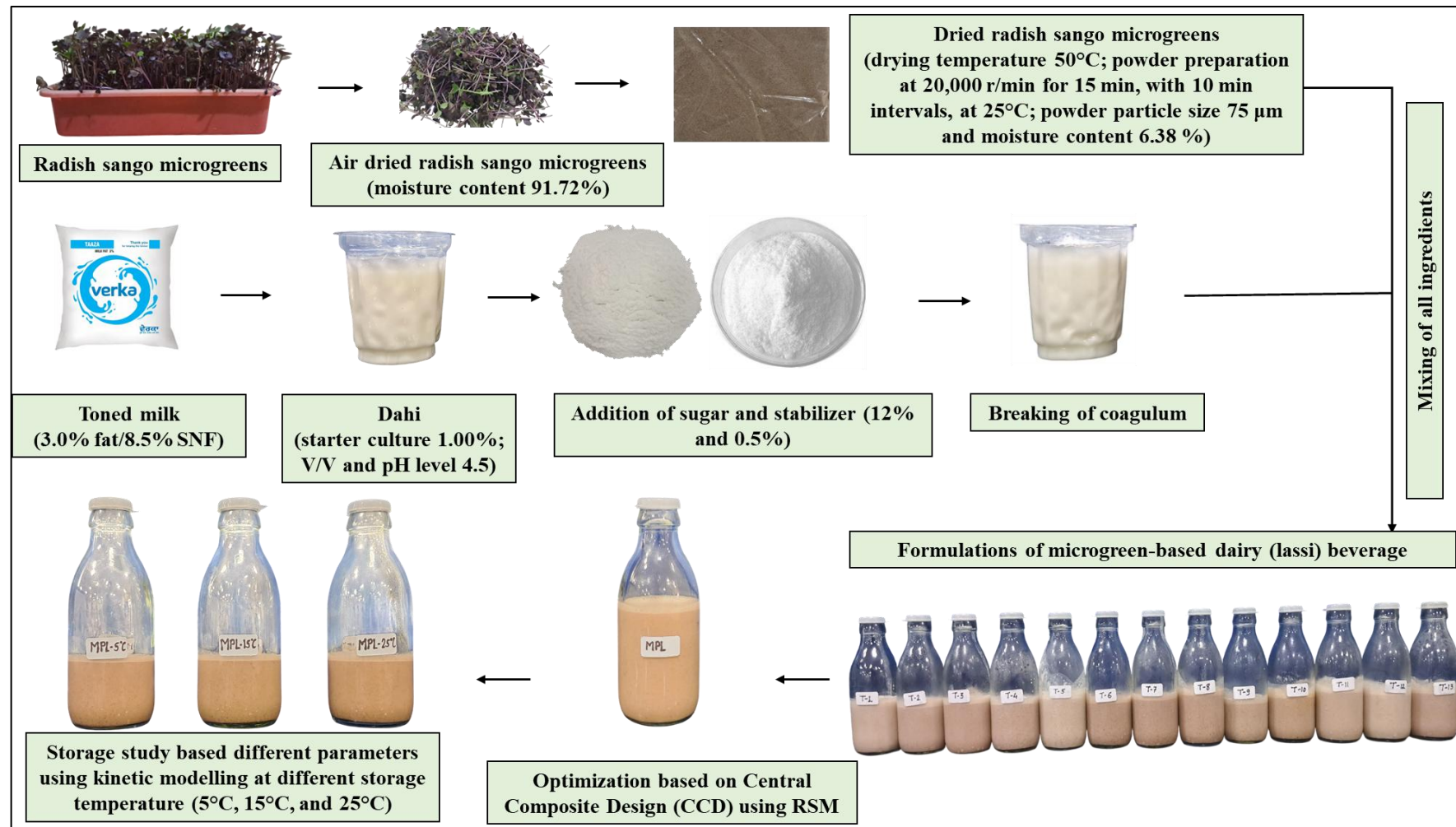


Figure 5.4. The experimental study experimental flow diagram of the preparation of microgreen-based dairy (lassi) beverage using response surface methodology

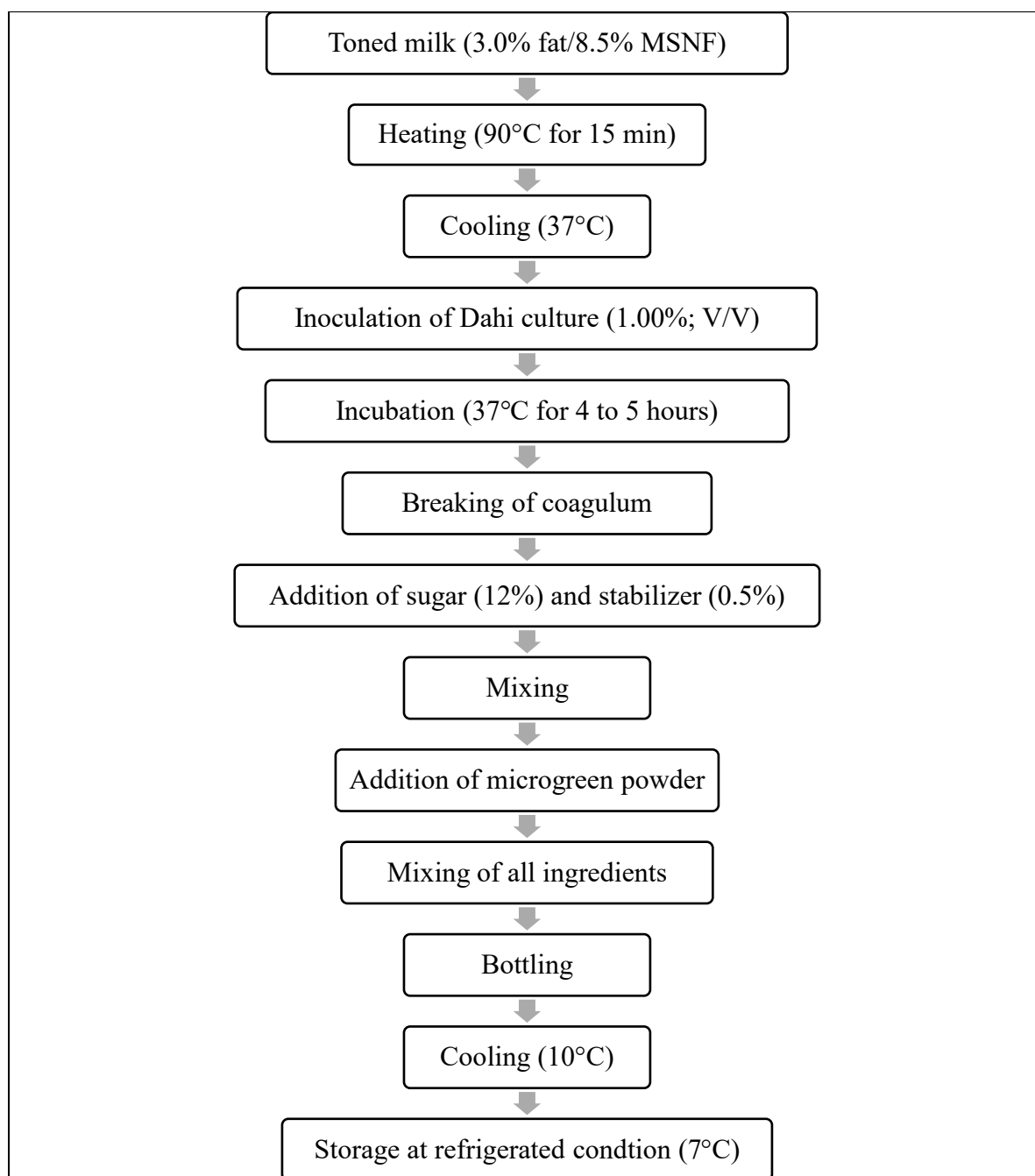


Figure 5.5. Flow chart for preparation of microgreen-based dairy (lassi) beverage using microgreen powder

5.9. Development of microgreen juice-based dairy (lassi) RTS beverage

Microgreens were harvested on the 9th day, washed with 0.50% (w/v) citric acid, and air-dried. The cleaned microgreens were pressed using a masticating juicer (Refurbished-Philips Masticating Slow Juicer 150 Watt - HR1887/81, Jalandhar, Punjab), and the obtained juices from microgreens were collected in plastic flasks and stored in glass bottles for further use at 4±1°C. Dahi was prepared as per the methodology explained in (Section 5.8).

The preliminary study was conducted aiming to determine the best formulation design from a sensory perspective in the form of trial and error. The development of microgreens juice-based lassi with various formulations was conducted using Design Expert software (version 13), a statistical analysis package. Independent variables, including microgreen juice (A), dahi (B), and drinking water (C), were selected for the formulation of lassi. A total of 17 experimental runs were generated using the D-optimal model. The study explored the effects of these variables within specified percentage (%) ranges from microgreen juice (A) ranged from 5 (-1) to 9.5 (+1) dahi (B) ranging from 60 (-1) to 64.5 (+1) and water (C) ranging from 15 (-1) to 19.5 (+1) and the formulation of experimental treatments is summarized in (Table 5.6).

Table 5.6. Experimental design optimization of levels of various constituents in microgreen juice-based dairy (lassi) using response surface methodology

Treatments	Dahi (%)	Microgreen extract (%)	Water (%)	Sugar (%)	CMC (%)
1	60.00	9.50	15.00	15.00	0.50
2	60.75	5.75	18.00	15.00	0.50
3	60.75	8.00	15.75	15.00	0.50
4	60.00	5.00	19.50	15.00	0.50
5	62.25	5.00	17.25	15.00	0.50
6	62.25	7.25	15.00	15.00	0.50
7	60.00	9.50	15.00	15.00	0.50
8	60.00	7.25	17.25	15.00	0.50
9	63.00	5.75	15.75	15.00	0.50
10	60.00	5.00	19.50	15.00	0.50
11	64.50	5.00	15.00	15.00	0.50
12	61.88	5.75	16.88	15.00	0.50
13	64.50	5.00	15.00	15.00	0.50
14	62.25	5.00	17.25	15.00	0.50
15	60.75	6.88	16.88	15.00	0.50
16	62.25	7.25	15.00	15.00	0.50
17	61.50	6.50	16.50	15.00	0.50

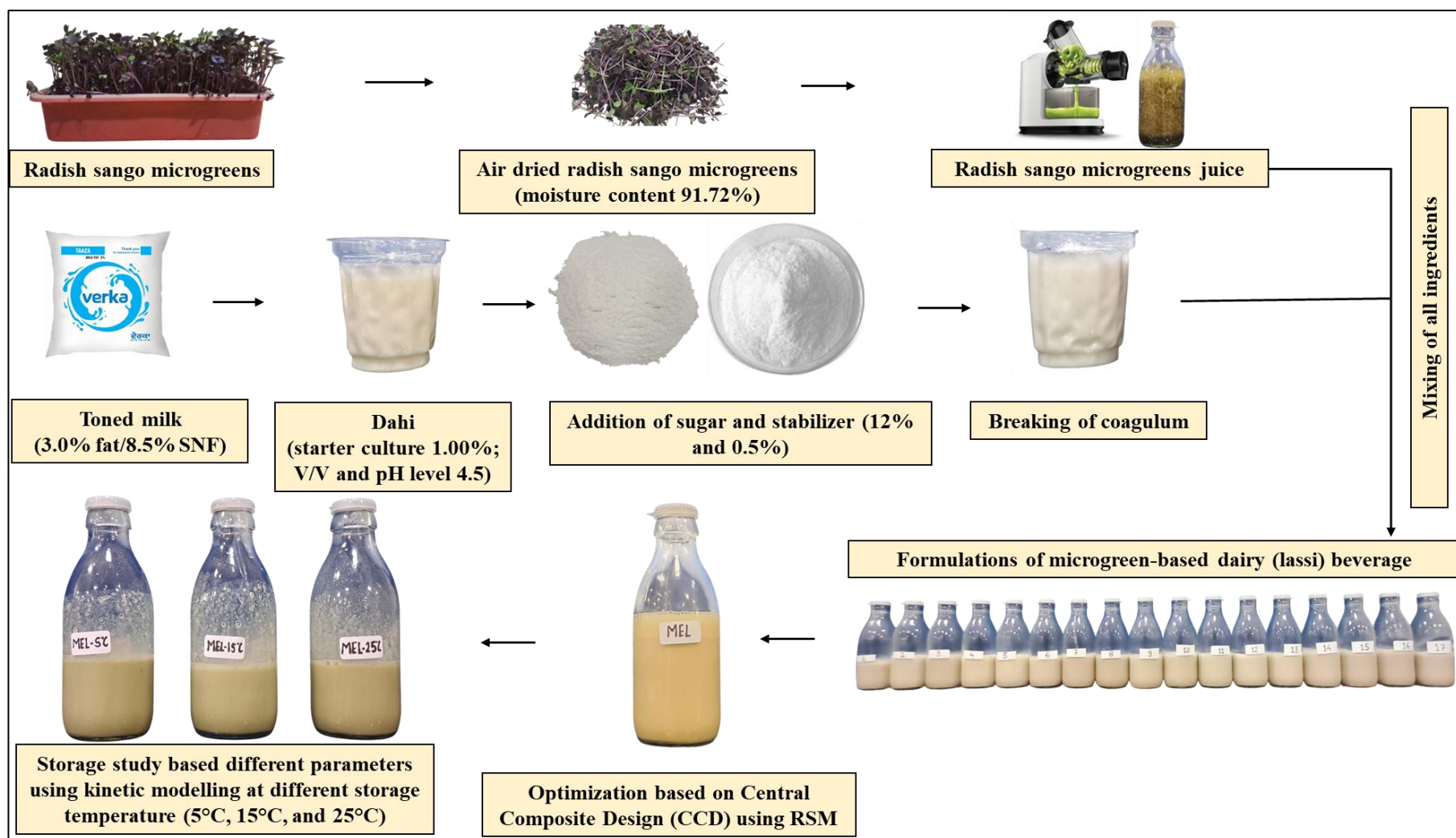


Figure 5.6. The different formulations of microgreen juice-based dairy (lassi) beverage using response surface methodology

The sugar (15%) and CMC stabilizer (0.5%) fixed concentrations were used. The experimental study experimental flow diagram of the preparation of microgreen juice-based dairy (lassi) beverage using response surface methodology is shown in **(Figure 5.6)**.

Lassi preparation was performed based on the experimental runs. The ingredients were weighed according to the treatment specifications, and lassi was prepared following the method described by Kumar et al. (2023), with slight modifications **(Figure 5.5)**. The prepared lassi samples were stored in 200 mL glass bottles under refrigeration conditions ($4\pm1^{\circ}\text{C}$) for subsequent analyses. The data obtained from dependent variables were analyzed using response surface regression analysis, employing the least squares methodology to fit the special cubic model. Model adequacy was evaluated by assessing the lack of fit, the coefficient of determination (R^2), adequate precision, and F-values through analysis of variance (ANOVA).

5.10. Development of microgreen powder-based RTS beverage

The microgreen powder was prepared as per the methodology explained **(Section 5.8)**. The extraction of pomegranate juice was conducted following the methodology outlined by Kashudhan et al. (2016). Fresh pomegranate fruits were thoroughly washed with clean water. The fruits were then cut open using a knife, discarding the peel adhered to the placenta, and the arils were carefully separated. These separated arils were extracted using a hand juice extractor (Krintonwel, India) and filtered through a muslin cloth, as illustrated in **(Figure 5.7)**. The obtained pomegranate juice was stored in airtight pre-sterilized glass bottles for further use at a temperature of $4\pm1^{\circ}\text{C}$.

The preliminary study aimed to identify the best formulation design from a sensory perspective through a trial-and-error approach. The development of microgreen powder-based RTS beverage with various formulations was carried out using Design Expert software (version 13), a statistical analysis package. Independent variables, including pomegranate juice (A), microgreen powder (B), and drinking water (C), were selected for the formulation. A total of 17 experimental runs were generated using the D-optimal model. The study examined the effects of these variables within specified percentage ranges: pomegranate juice (A) from 42.5% (-1) to 50% (+1), microgreen powder (B) from 0.5% (-1) to 2% (+1), and water (C) from 42.5% (-1) to 50% (+1). Fixed concentrations of sugar (5%) and CMC stabilizer (0.5%) were used for the formulation of experimental treatments as summarized in **(Table 5.7)**.

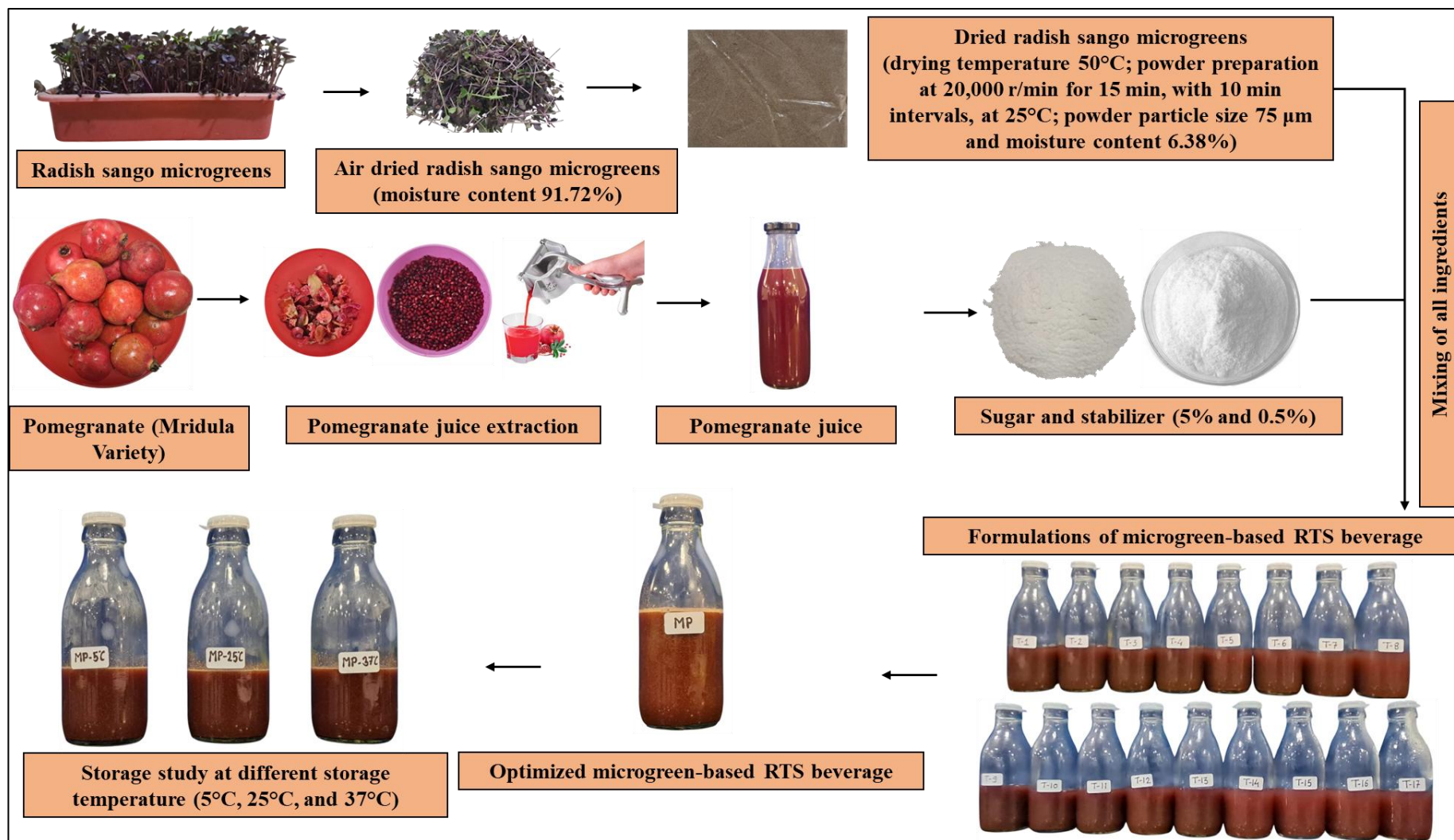


Figure 5.7. The experimental study experimental flow diagram of the preparation of microgreen-based RTS beverage.

Table 5.7. Formulations and ingredients used for the development of microgreen-based RTS beverage

Ingredients	RTS beverage formulation (%)	Minimum (%)	Maximum (%)
A: Pomegranate juice	A+B+C=94.5	42.5	50
B: Microgreen powder		0.5	2
C: Water		42.5	50
Sugar	5		
CMC	0.5		
Total	100		

The preparation of the microgreen-based RTS beverage was performed based on the experimental runs shown in (Table 5.8). The ingredients were weighed according to the treatment specifications, and the beverage was prepared following the method described by Sharma et al. (2021), with slight modifications (Figure 5.8). The prepared RTS beverage samples were stored in 200 mL glass bottles under refrigeration conditions ($4\pm1^{\circ}\text{C}$) for subsequent analyses. Data obtained from the dependent variables were analyzed using response surface regression analysis, employing the least squares methodology to fit the special cubic model. Model adequacy was evaluated by assessing the lack of fit, the coefficient of determination (R^2), adequate precision, and F-values through analysis of variance (ANOVA).

Table 5.8. Experimental design optimization of levels of various constituents in microgreen powder-based RTS beverage using response surface methodology

Treatments	Pomegranate juice (%)	Microgreen powder (%)	Water (%)
1	50.00	2.00	42.50
2	48.31	1.63	44.56
3	42.50	2.00	50.00
4	42.50	2.00	50.00
5	50.00	1.25	43.25
6	46.63	1.25	46.63
7	45.31	0.88	48.31
8	44.00	0.50	50.00
9	46.25	2.00	46.25
10	44.56	1.63	48.31
11	50.00	2.00	42.50
12	43.25	1.25	50.00
13	46.25	2.00	46.25
14	47.00	0.50	47.00
15	50.00	0.50	44.00
16	44.00	0.50	50.00
17	50.00	0.50	44.00

5.11. Development of microgreen juice-based RTS beverage

The preliminary study aimed to identify the best formulation design from a sensory perspective through a trial-and-error approach. Microgreen juice was prepared as per the methodology explained in (Section 5.8) and pomegranate juice was prepared as per the methodology explained in (Section 5.9). The development of microgreen juice-based RTS beverages with various formulations was carried out using Design Expert software (version 13), a statistical analysis package. Independent variables, including microgreen juice (A), pomegranate juice (B), and drinking water (C), were selected for the formulation. A total of 17 experimental runs were generated using the D-optimal model. The study examined the effects of these variables within specified percentage ranges: microgreen juice (A) from 10% (-1) to 20% (+1), pomegranate juice (B) from 40% (-1) to 54.5% (+1), and water (C) from 30% (-1) to 40% (+1). Fixed concentrations of sugar (5%) and CMC stabilizer (0.5%) were used for the formulation of experimental treatments as summarized in (Table 5.9).

Table 5.9. Formulations and ingredients used for the development of microgreen juice-based RTS beverage

Ingredients	RTS beverage formulation (%)	Minimum (%)	Maximum (%)
A: Microgreen juice	A+B+C=94.5	10	20
B: Pomegranate juice		40	54.5
C: Water		30	40
Sugar	5		
CMC	0.5		
Total	100		

The preparation of the microgreen juice-based RTS beverage was performed based on the experimental runs shown in (Table 5.10). The ingredients were weighed according to the treatment specifications, and the beverage was prepared following the method described by Sharma et al. (2021), with slight modifications (Figure 5.8). The prepared RTS beverage samples were stored in 200 mL glass bottles under refrigeration conditions ($4\pm1^{\circ}\text{C}$) for subsequent analyses. Data obtained from the dependent variables were analyzed using response surface regression analysis, employing the least squares methodology to fit the special cubic model. Model adequacy was evaluated by assessing the lack of fit, the coefficient of determination (R^2), adequate precision, and F-values through analysis of variance (ANOVA).

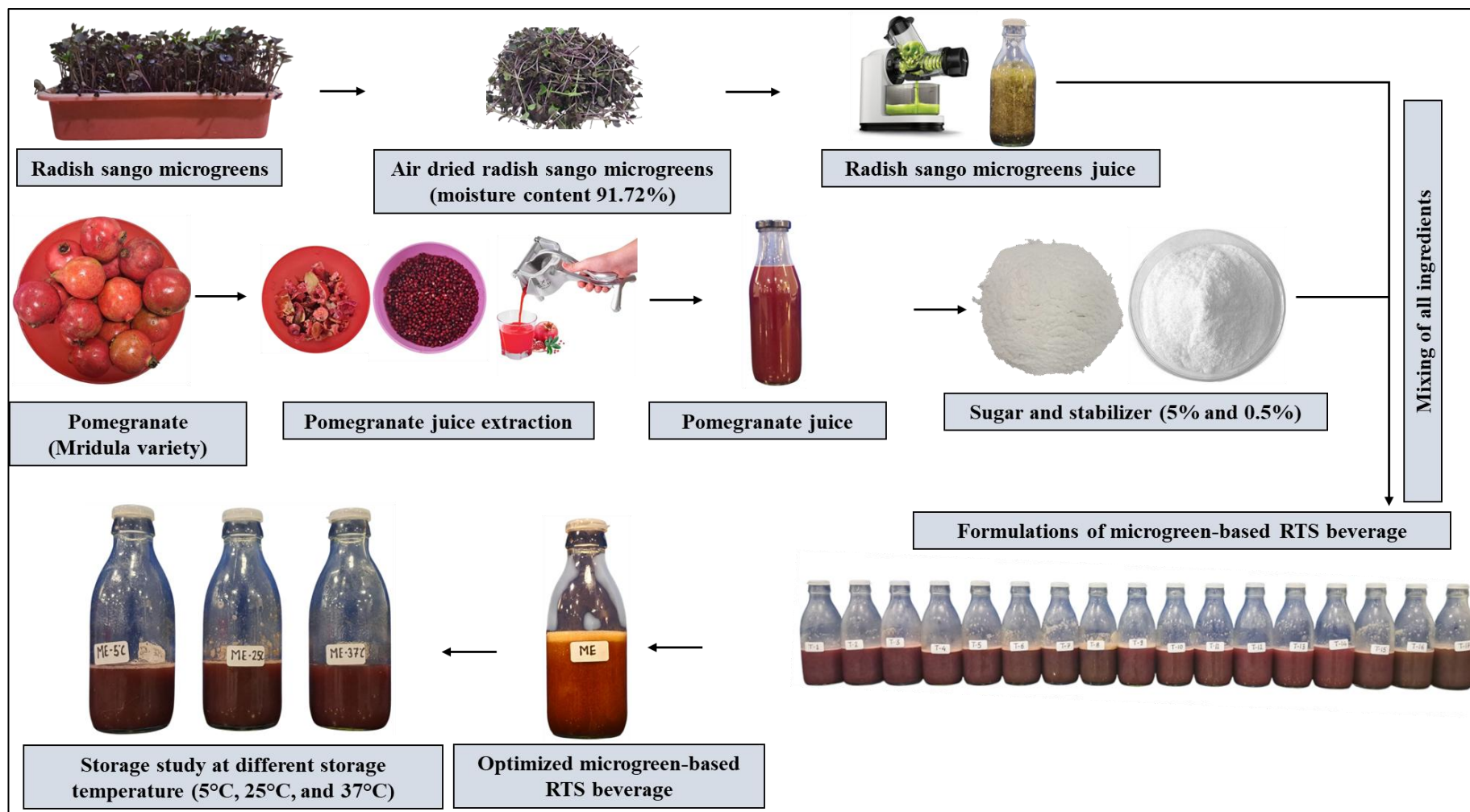


Figure 5.8. The experimental study experimental flow diagram of the preparation of microgreen juice-based RTS beverage.

Table 5.10. Experimental design optimization of levels of various constituents in microgreen juice-based RTS beverage using response surface methodology

Treatments	Microgreen juice (%)	Pomegranate juice (%)	Water (%)
1	10.00	44.50	40.00
2	10.00	49.50	35.00
3	15.00	49.50	30.00
4	10.00	44.50	40.00
5	13.33	47.83	33.33
6	20.00	40.00	34.50
7	17.25	40.00	37.25
8	20.00	44.50	30.00
9	10.00	54.50	30.00
10	15.00	49.50	30.00
11	16.67	46.33	31.50
12	10.00	49.50	35.00
13	11.50	46.33	36.67
14	10.00	54.50	30.00
15	14.90	44.70	34.90
16	14.50	40.00	40.00
17	20.00	44.50	30.00

5.12. Storage condition for the developed ready-to-serve microgreen-based beverage

5.12.1. Determination of shelf-life of optimized microgreen-based dairy RTS beverage

The storage conditions for the optimized microgreen-based dairy (lassi) beverage sample were storage temperature (5°C, 15°, and 25°C) based on various physicochemical, biochemical, and microbial parameters were tested for 15 days storage period days with 3 days intervals of time.

5.12.2. Determination of shelf-life of optimized microgreen-based RTS beverage

The storage conditions for the optimized microgreen-based RTS beverage sample were storage temperature (5°C, 25°C, and 37°C) based on various physicochemical, biochemical, and microbial parameters, which were tested for 30 day storage period days with 5 days intervals of time.

5.12.3. Establishment of the shelf-life model

The changes in overall acceptability, titratable acidity, free fatty acid content, thiobarbituric acid, ascorbic acid, and microbial parameters in optimized microgreen-based RTS beverage were studied under different storage temperature conditions for dairy-based RTS beverage (5°C, 15°C, and 25°C) and microgreen-based RTS beverage (5°C, 25°C, and 37°C). Samples were analyzed at 3- and 5-day intervals to determine changes in selected parameters. The observed changes in the concentration of these compounds were modeled using zero-order,

first-order, and second-order kinetics (Kumar et al., 2024). The general expression for the quality change has been shown in Equation (11), described as follows:

$$\frac{dC_t}{dt} = -kC_t^n \quad (11)$$

Where C_t represents the quality index varying with time, t represents storage time (d), k represents the rate of reaction (d^{-1}), and n represents the order of a reaction. After putting the value of $n = 0, 1$, and 2 and then integrating Equation (07), the zero-order, first-order, and second-order equation was obtained, as shown in Equations (12, 13, and 14).

$$C_t = C_0 - k \times t \quad (12)$$

$$C_t = C_0 \times e^{-k \times t} \quad (13)$$

$$\frac{1}{C_t} - \frac{1}{C_0} = k \times t \quad (14)$$

The temperature dependency of the k was modeled using the Arrhenius, Eyring, and Ball models, as shown in Equations (15, 17, and 19), respectively.

$$\ln k = \ln k_{\text{ref}} - \frac{E_a}{R_0} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}} \right) \quad (15)$$

$$T_{\text{ref}} = \frac{1}{n} \sum_{i=0}^n T_i \quad (16)$$

$$\ln \frac{k}{T} = -\frac{\Delta H}{R} \times \frac{1}{T} + \ln \frac{k_B}{h} + \frac{\Delta S}{R} \quad (17)$$

$$\log_{10} \left(\frac{D}{D_{\text{ref}}} \right) = - \left(\frac{T - T_{\text{ref}}}{Z} \right) \quad (18)$$

$$D = \frac{\ln 10}{k} \quad (19)$$

Where, ΔS represents the entropy of activation; ΔH represents the enthalpy of activation; h represents the Planck constant, 6.626×10^{-34} J s; k_B represents the Boltzmann constant, 1.381×10^{-23} J/K; R represents the ideal gas constant, 8.314 J/(mol·K); T represents the absolute storage temperature (K), D represent the storage time taken by the quality index to reduce by 90%, D_{ref} represents the D value at the reference temperature T_{ref} , Z represents the range of storage temperatures required the D value to reduce by 10 times ($^{\circ}\text{C}$), T_{ref} represents the reference temperature ($^{\circ}\text{C}$) (Equation 46).

5.13. Morphological parameters

5.13.1. Fresh yield

At harvest on the 9th day after germination, all the microgreens were harvested by cutting the seedlings just above the surface of the growing medium with clean scissors. After

cutting, all the selected microgreens for the study were weighed via a precision analytical balance (EPB8T, WENSAR, Chennai, India) to determine the fresh yield, and the obtained results were expressed in terms of grams per meter square (g/m²) (Di Gioia et al., 2023).

5.13.2. Fresh weight and dry weight

At harvest on the 9th day after germination, the fresh and dry weights of 25 microgreen shoots were measured according to the methods described by Dalal et al. (2019). A total of 25 microgreen shoots were randomly selected for analysis (n=3 observations). To determine the dry weights of the 25 microgreen shoot samples, they were dried at a constant temperature (55±2°C) for two days until they reached a constant weight, after which they were weighed on a precision scale. For the measurement of single shoot weight, 25 shoots of each microgreen were randomly selected, and the results are presented in mg/shoot weight.

5.13.3. Shoot height and width

A digital Vernier caliper (B0CHFVZN1M, SKADIOO, Perfect Sales India, India) was used to determine the microgreen shoot height and width. Twenty-five randomly selected microgreen shoots were considered, and the results are expressed as shoot height in centimeters (cm) and shoot width in millimeters (mm) (Flores et al., 2024).

5.13.4. Leaf length, width, and leaf area

A digital Vernier caliper was used to determine the microgreen length and width. Twenty-five microgreen shoots were randomly selected. The obtained results are expressed as the leaf length and width in centimeters (cm). The leaf length and width parameters were used to calculate the values of the leaf area via Equation (20), and the obtained results are expressed in centimeter squares (cm²) (Shi et al., 2019).

$$\text{Leaf area (cm}^2\text{)} = \pi \times (\text{Leaf length (cm)}/2) \times (\text{Leaf width (cm)}/2) \quad (20)$$

5.14. Physiological parameters

5.14.1. Weight loss

The weight loss of microgreens was assessed following the methodology of Patil et al. (2023). The weight loss of all the treatment samples was determined by accurately weighing the samples initially and periodically (every 3rd day) throughout storage duration in the packaging material using a weighing balance with a precision of 0.01 g (WENSAR, Chennai, India) used for all measurements. Weight loss was calculated using Equation (21) and expressed as a percentage (%) relative to the initial fresh weight of the microgreens. This process was performed for all treatment samples to evaluate the impact of washing treatments and packaging material on weight retention.

$$\text{Weight loss (\%)} \quad (21)$$

$$= \left(\frac{\text{Initial weight of sample (g)} - \text{Final weight of sample (g)}}{\text{Initial weight of sample (g)}} \right) \times 100$$

5.14.2. Electrolyte leakage

The physiological parameter of electrolyte leakage in microgreens was assessed using the method described by Patil et al. (2023), with modifications. The electrolyte leakage was measured with a digital conductivity meter (OH30058731-1EA, Ohaus, India). For each measurement, 1.00 g of microgreen sample was submerged in 30 mL of deionized distilled water at a temperature of $24 \pm 1^\circ\text{C}$. The samples were gently shaken for 30 minutes to allow for the release of electrolytes from the microgreens into the solution. The initial conductivity of the water was recorded using the conductivity meter, as described by Equation (22). Afterward, the same samples were frozen at $-20 \pm 1^\circ\text{C}$ to induce membrane rupture, followed by thawing, allowing for the complete release of cellular electrolytes. Conductivity was measured again after thawing to determine the total electrolyte leakage. The percentage (%) of electrolyte leakage was calculated by comparing the initial and final conductivity values, offering insights into the impact of treatments on the structural integrity of the cell membranes over the storage period.

$$\text{Electrolyte leakage (\%)} \quad (22)$$

$$= \left(\frac{\text{Conductivity after freezing} - \text{Conductivity before freezing}}{\text{Conductivity after freezing}} \right) \times 100$$

5.14.3. Overall quality and off-odor evaluation

The visual quality and off-odor of microgreens were evaluated using the methodology of Xiao et al. (2013) with modifications. Visual quality was assessed on a 9-point hedonic scale, where scores of 9, 8, 7, and 6 represented "like extremely," "like strongly," "like moderately," and "like slightly," respectively. A score of 5 indicated "neither like nor dislike," while 4, 3, 2, and 1 corresponded to "dislike slightly," "dislike moderately," "dislike strongly," and "dislike extremely," respectively. A score of 6 was considered the threshold for market acceptability. The off-odor evaluation was based on a 0 to 4 scale, where 0 indicated no off-odor, and scores of 1, 2, 3, and 4 indicated slight, moderate, strong, and extremely strong off-odor, respectively. Before the experiment, panelists received specialized training to familiarize them with the specific organoleptic properties of microgreens.

The sensory analysis was conducted in a dedicated sensory evaluation room designed to minimize external influences on panelists' assessments. The room was maintained at $22 \pm 2^\circ\text{C}$ with relative humidity between 50-60%, ensuring a comfortable environment. Even natural daylight fluorescent lighting (300-500 lux) was used to prevent color distortion in the samples, ensuring optimal visibility. Each panelist was seated in individual sensory booths separated by neutral-colored partitions to avoid distractions or interactions, ensuring unbiased evaluations. The room, measuring approximately 4×6 meters, provided sufficient space for the 30 semi-trained panelists ($n=30$; 15 males, 15 females) to work comfortably.

5.15. Physicochemical properties

The pH of the beverage samples was measured at 20°C using an electronic digital pH meter (Model: BRPH-10, BR Biochem Life Sciences Pvt Ltd, India) equipped with a glass electrode and a temperature probe. Before measurement, the pH meter was calibrated with standard buffer solutions of pH 4.0 and pH 9.0, both maintained at 20°C , as described by Hussain et al. (2014). The titratable acidity, expressed as percent lactic acid (%) for microgreen-based dairy RTS beverage and citric acid for fruits-based RTS beverage, was determined following the method outlined by Raje-Nimbalkar et al. (2023). Total soluble solids (TSS) content was analyzed using a digital refractometer (Model: HI-96811, Hanna Instruments, India) at 20°C , following the methodology explained by Pratap et al. (2017).

The water holding capacity (WHC) of the samples was assessed using the method of Ahmed et al. (2023) with slight modifications. A 5 g microgreen juice-based dairy beverage sample was centrifuged at $3282.7 \times g$ for 15 minutes at 20°C , after which the supernatant was discarded, and the remaining precipitate was weighed. WHC was calculated using Equation (23). Syneresis was evaluated by centrifuging the microgreen-based dairy beverage samples at $3000 \times g$ for 15 minutes at 10°C . The clear supernatant was weighed, and syneresis was expressed as the percentage (%) of the supernatant weight relative to the initial sample weight, calculated using Equation (24), as per the method of Ghaderi-Ghahfarokhi et al. (2024).

$$\text{WHC (\%)} = \frac{\text{Weight of sample (g)} - \text{Weight of sediments (g)}}{\text{Weight of sample (g)}} \times 100 \quad (23)$$

$$\text{Syneresis (\%)} = \frac{\text{Weight of Supernatant (g)}}{\text{Weight of sample (g)}} \times 100 \quad (24)$$

5.16. Proximate composition

The moisture, ash, and fat content of all selected microgreen samples were determined using AOAC methods (2010).

The moisture content was determined by weighing two grams of fresh sample in a petri dish and drying it at a temperature of 105°C by keeping it in a hot air oven (Model: PSI-030, Ahmedabad, Gujarat, India). The dry weight of all samples was recorded after 5 hrs until two consecutive constant readings. The moisture content was calculated by using the following Equation (25), and the results of the finding expressed in g/100g fresh weight basis.

$$\text{Moisture content (g/100g)} = \frac{\text{Initial weight of sample (g)} - \text{Final weight of sample (g)}}{\text{Initial weight of sample (g)}} \times 100 \quad (25)$$

Ash content in dried microgreen samples was measured according to the gravimetric method incinerated two grams of oven-dried microgreen samples in a muffle furnace (Model: NSW-101, Arsh Enterprise, Ahmedabad, India) at 550°C for 5-6 hrs until a white ash was obtained. The loss in weight represented the organic matter and residue being the ash content, which was calculated using Equation (26) and results of the finding expressed in g/100g dry weight basis.

$$\text{Ash content (g/100g)} = \frac{\text{Weight of ash (g)}}{\text{Weight of moisture free sample (g)}} \times 100 \quad (26)$$

The total fat content in the dried microgreens sample was determined according to the acid hydrolysis method using Soxhlet extractor at 60°C until constant weight. The fat content was calculated using Equation (27), and the results of the finding expressed in g/100g dry weight basis.

$$\text{Fat content (g/100g)} = \frac{W_2 - W_1}{W} \times 100 \quad (27)$$

Where,

W = Weight of sample (g)

W_1 = Weight of flask (g)

W_2 = Weight of flask + fat present in flask (g)

The crude fiber content of the dried microgreen samples was determined using the acid-alkali method outlined by Sadasivam and Manikam (1992). The five grams of moisture and fat-free samples were weighed accurately and boiled with 200 mL of sulphuric acid (1.25%, V/V) for 30 min. The acid-digested mixture was filtered through a muslin cloth, and the residue was washed with hot distilled water for the removal of acid. The residue was then transferred to the same beaker and boiled for 30 min with 200 mL of sodium hydroxide (1.25%, W/V) solution. Again, the alkali-digested mixture was filtered through a muslin cloth, and the residue was washed with hot distilled water for alkali removal and finally washed with alcohol and

ether. The acid and alkali digested residue was transferred to a pre-weighed crucible, dried, cooled, and weighed. It was then ignited for 2 to 3 hrs in a muffle furnace (Model: NSW-101, Arsh Enterprise, Ahmedabad, India) at 600°C, then cooled in desiccators and reweighed. The fiber content of the microgreens sample was calculated from the loss in weight on ignition using Equation (28) and expressed in g/100g dry weight basis.

$$\text{Crude fibre content (g/100g)} = \frac{\text{Weight of crucible with fiber (g)} - \text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100 \quad (28)$$

The soluble protein content of the dried microgreen samples was determined using the Lowry method Lowry et al. (1951). Standard curves of the Bovine Serum Albumin (BSA) solution ($y = 0.0015x + 0.1305$; $R^2 = 0.9942$) used for the calculation of the amount of protein and the estimated protein results were expressed in g BSA/100g dry weight basis.

5.17. Photosynthetic pigments

The photosynthetic pigments, such as chlorophyll and carotenoid content were determined by following the method described by Martnez-Ispizua et al. (2022). Briefly, microgreen samples of a known amount were homogenized in 10 mL of 80% acetone solution. The prepared extracts were centrifuged at 6000×g for 5 min, and the absorbance (Abs) values were taken at different wavelengths (470, 646.6, and 663.6 nm) using a UV-Vis spectrophotometer (LB-925, Labcare, Mumbai, India). The chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoid content were calculated by using Equations (29, 30, 31, and 32), and the obtained result was expressed as µg/g on a fresh weight basis.

$$\text{Chlorophyll a (}\mu\text{g/g)} = 12.25 \times \text{Abs } 663.6 - 2.79 \times \text{Abs } 646.6 \quad (29)$$

$$\text{Chlorophyll b (}\mu\text{g/g)} = 21.3 \times \text{Abs } 646.6 - 5.1 \times \text{Abs } 663.6 \quad (30)$$

$$\text{Total carotenoid content (}\mu\text{g/g)} \quad (31)$$

$$= \frac{[(1000 \times \text{Abs } 470) - (1.82 \times \text{Chlorophyll a}) - (85.02 \times \text{Chlorophyll b})]}{198}$$

$$\text{Total chlorophyll content (}\mu\text{g/g)} = 7.15 \times \text{Abs } 663.6 + 18.71 \times \text{Abs } 646.6 \quad (32)$$

5.18. Bioactive compounds and antioxidant activity

To detect various antioxidant compounds (total phenol content, total flavonoid content, and antioxidant activities (DPPH, FRAP, and ABTS) in a fresh microgreen sample, 1.00 g of the sample was weighed and subsequently blended with 100 mL of 80% ethanol. Followed by centrifuging the extract at 6000 g for 15 minutes. The ethanol-based supernatant was stored in glass vials at -20°C and quantified within 24 hours to ensure the stability of the antioxidant compounds.

The quantifications of total phenolic content (TPC) were determined by following the method described by Dhaka et al. (2023). Briefly, 1.0 mL of ethanolic extract was dissolved in 0.5 mL of Folin–Ciocalteu (FC) reagent followed by 1.5 mL of 20% Na₂CO₃, and then 7 mL of deionized water was added to the test sample. This prepared solution was then allowed to incubate at room temperature for 10 min, following which the absorbance was measured at 765 nm using a UV-Vis spectrophotometer. The obtained results were represented in mg GAE (Gallic Acid Equivalents)/100g fresh weight basis.

The total flavonoid concentration in samples was assessed following the protocol outlined by Alam & Sharma (2020). Briefly, 0.3 mL of 5% sodium nitrite and 0.3 mL of 10% aluminum chloride were added to 1 mL of the ethanolic extract. The resulting solution was then diluted to a final volume of 10 mL with distilled water. Subsequently, the absorbance of the prepared test sample was measured at a wavelength of 510 nm using a UV-Vis spectrophotometer. The total flavonoid content was determined in mg QE (Quercetin Equivalents)/100g by referencing a calibration curve constructed with standard quercetin.

The radical scavenging potential of the sample was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following the procedure outlined by Kowitcharoen et al. (2021). Briefly, ethanolic extracts of 0.1 mL were mixed with a DPPH solution (2.9 mL), and the absorbance of the resultant solution was measured at wavelength (517 nm) using a UV-Vis spectrophotometer after a 30 min incubation period. The inhibition percentage of DPPH radical scavenging activity was determined using Equation (33).

The ferric-reducing antioxidant power (FRAP) of the sample was determined using the methodology described by Xiao et al. (2020). The FRAP reagent was prepared using a mixture of 0.3 mol/L acetate buffer (pH 3.6), 0.01 mol/L 2,4,6-tripyridyl-s-triazine (TPTZ) prepared in 0.04 mL/L HCl, and 0.02 mol/L ferric chloride hexahydrate (FeCl₃·6H₂O) in a 10:1:1 volume ratio. To perform the assay, 1.8 mL of the FRAP working solution was combined with 100 µL of the supernatant, ensuring the mixture was mixed thoroughly before incubating at 37°C for 15 minutes in the dark. The absorbance of the test sample was subsequently measured at a wavelength of 593 nm using a spectrophotometer. The results of the FRAP assay were quantified based on a Trolox calibration curve and expressed as µmol TE (Trolox equivalents) per gram of fresh weight (µmol TE/g).

The 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical scavenging activity of the microgreens was determined following the procedure described by Sanyukta et al. (2023). Briefly, ethanol extract (0.1 mL) was mixed with ABTS solution (2.9 mL) and vortexed. Subsequently, the absorbance of the sample was measured at 734 nm using

a UV-Vis spectrophotometer after a 6 min incubation period. The percentage of ABTS scavenging activity was calculated using Equation (34).

$$\text{DPPH radical scavenging activity (\%)} \quad (33)$$

$$= (\text{Abs. of control} - \text{Abs. of sample}) / (\text{Abs. of control}) \times 100$$

$$\text{ABTS scavenging activity (\%)} \quad (34)$$

$$= (\text{Abs. of control} - \text{Abs. of sample}) / (\text{Abs. of control}) \times 100$$

The anthocyanin content was spectrophotometrically determined as described by Martinez-Ispizua et al. (2022). Briefly, microgreen fresh samples of 0.1 g were homogenized in 5 mL of solution containing Methanol:HCl:H₂O (90:1:9). The prepared samples mixture was vortexed and stored in the dark place for 60 min at room temperature. Then, they were centrifuged at 6000×g for 10 min, and the supernatant was used for the analysis. The obtained supernatant solution absorbance (Abs.) was measured at 534, 643, and 661 nm wavelength using a spectrophotometer, with the methanol:HCl:H₂O solution used as the blank. The anthocyanin content was calculated using Equation (35) and expressed in μmol/100 g.

$$\text{Anthocyanin content (}\mu\text{mol/100g)} \quad (35)$$

$$= ((0.0821 \times \text{Abs. at 534 nm}) - (0.00687 \times \text{Abs. at 643 nm}) - (0.002426 \times \text{Abs. at 661 nm})) \times 5$$

The determination of total glucosinolate content in microgreen samples following the procedure outlined by Mawlong et al. (2017). The 0.1 g of microgreen powder was blended with 10 mL of 80% methanol, then the resulting mixture was centrifuged at 3000 rpm for 4 minutes and left to settle for 12 h at room temperature. A methanol-based extract (0.1 mL) was mixed with double distilled water (0.3 mL) in test tubes, followed by the addition of 3 mL of 2 mM sodium tetrachloropalladate. After that, the prepared test sample was incubated for 60 min, and the absorbance was taken at 425 nm and the findings were represented in μmol/g of fresh weight.

The ascorbic acid content in the microgreens was quantified using the protocol outlined by Jagota & Dani (1982). The samples 0.1 g were homogenized in 2 mL of 10% trichloroacetic acid, and then the prepared mixture was centrifuged at 6000 rpm for 5 minutes. After that, 0.1 mL of extract was added to 2 mL of 10% trichloroacetic acid and 0.3 mL of Folin Ciocalteu's reagent. Furthermore, test samples were incubated at room temperature for 20 minutes, and absorbance was taken spectrophotometrically at 760 nm wavelength. The ascorbic content was calculated in mg/100g on a fresh weight basis by using the calibration curve (10–50 μg/mL) prepared using standard L-ascorbic acid.

5.19. Quantifications of antinutrients

The tannins in microgreen samples were determined using the Folin Ciocalteu's method, employing tannic acid as a reference standard concentration (100–500 µg/mL), and the results were quantified in mg/100g, as outlined by Sirisangsawang & Phetyim (2023).

Oxalic acid content analysis, microgreens underwent potassium permanganate titration following the protocol by Bok et al. (2022). The 1 mL of extract was added to 5 mL of 2.00 N sulfuric acid and 2.00 mL of 3 µmol KMnO₄ reagent. Furthermore, test samples were incubated at room temperature for 10 minutes, and absorbance was taken spectrophotometrically at 520 nm. The oxalic acid was calculated in mg/100g by using the calibration curve (100–500 µg/mL) prepared using standard oxalic acid.

The phytic acid content in samples was determined as per the method described by Adegbusi et al. (2023). The microgreens sample (75 mg) was homogenized with (10 mL) of 2.4% HCl solvent and incubated for 30 min at 25°C. After all the test samples were centrifuged at 3000 rpm for 30 min, and from the obtained supernatant, 3 mL aliquots and different concentrations of hydrated sodium phytates salt (0-40 µg/mL) were taken after that, 1 mL of Wade reagent were added. The absorbance was taken at 500 nm using a UV-Vis spectrophotometer after 10 min. The total phytic acid content was calculated by using the below Equation (36), and results were expressed in mg/100g on a fresh weight basis.

$$\begin{aligned} \text{Phytic acid content (}\mu\text{g/g)} & \quad (36) \\ &= [\text{Abs. of sample} - \text{Abs. of blank} \\ &\quad - \text{Intercept}] \times 10/\text{Slope} \times \text{Weight of sample} \times 3 \end{aligned}$$

5.20. Thiobarbituric acid and free fatty acid content

The thiobarbituric acid (TBA) and free fatty acid (FFA) content in the microgreen juice-based dairy beverage sample was analyzed following the method outlined by Singh et al. (2024). To determine the TBA content, 4 g of RTS beverage sample was mixed with 100 mL of 20% trichloroacetic acid and 100 mL of distilled water. The mixture was allowed to stand at room temperature for 10 min before being filtered through Whatman No. 1 filter paper. A 5 mL aliquot of the filtrate was then combined with 5 mL of 10 mM thiobarbituric acid solution and incubated at 95°C for 30 min. After incubation, the absorbance of the sample was measured at 532 nm using a spectrophotometer. The TBA content was subsequently calculated using Equation (37).

For the determination of FFA content, 2 g of the microgreen juice-based dairy beverage sample was dissolved in 50 mL of ethanol in a 250 mL conical flask. A few drops of

phenolphthalein indicator (1% phenolphthalein in 95% ethanol) were added, and the solution was titrated against a 0.10 N potassium hydroxide solution until a stable pink color persisted for 15 sec. The titration value was used to calculate the acid value and FFA content using Equation (38).

$$\text{TBA content (mg MDA/kg)} = 7.8 \times \text{Abs. 532 nm} \quad (37)$$

$$\text{FFA value (}\mu\text{g/g)} = \left(\frac{\text{Titrate value} \times \text{Normality of KOH} \times 282.47 \times 1000}{\text{Weight of sample (g)}} \right) \quad (38)$$

Where, 1 mL N/10 KOH = 0.028 g oleic acid.

5.21. Physical properties of microgreens powder

5.21.1. Water activity

The water activity (aw) of microgreen powder samples was measured using the method outlined by Budnimath et al. (2023). A small quantity of the powder was added to the sample holder up to the marked level and placed in the sensing groove of the water activity meter. The device's biosensor, located on the lid, performed the measurement, with three beeps signaling the endpoint, after which the water activity value was digitally displayed. Measurements were taken in triplicate using a Labswift water activity meter (Novasina, Lachen, Switzerland).

5.21.2. Bulk and tapped density

The bulk density and tapped density of the different microgreen powders were carried out using the methodology described by Singh et al. (2022). A known amount of microgreen powder was placed into a 100 mL measuring cylinder, and the volume occupied by the powder was measured. The bulk density of the microgreen powder sample was measured in triplicates, and the obtained results were expressed in terms of (g/cm³). The bulk density was calculated using the following Equation (39).

$$\text{Bulk density (}\frac{\text{g}}{\text{cm}^3}\text{)} = \frac{\text{Weight of powder sample (g)}}{\text{The initial volume of powder sample (cm}^3\text{)}} \quad (39)$$

The tapped density was calculated by tapping (50±5 taps/min) the measuring cylinder for 5 min until no visible decrease in volume was noticed. The final volume was then read and used to calculate, and tapped density was calculated using Equation (40). The tapped density of the microgreen powder sample was measured in triplicates, and the obtained results were expressed in terms of (g/cm³).

$$\text{Tapped density (}\frac{\text{g}}{\text{cm}^3}\text{)} = \frac{\text{Weight of powder sample (g)}}{\text{The final volume of powder sample (cm}^3\text{)}} \quad (40)$$

Based on bulk density and tapped density obtained results the Hausner ratio and Carr index were calculated following Equations (41 and 42).

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad (41)$$

$$\text{Carr index (\%)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \quad (42)$$

5.21.3. Angle of repose

The angle of repose ($^{\circ}$) for microgreen powder samples was measured following the method outlined by Budnimath et al. (2023). The powder was carefully poured through a funnel positioned at a fixed height of 2.0 cm above a hard surface. Pouring continued until the apex of the formed powder pile reached the funnel's tip. The angle of repose was then calculated using Equations (43) and (44) provided below.

$$\tan \theta = \frac{h}{r} \quad (43)$$

$$\theta = \tan^{-1} \left(\frac{h}{r} \right) \quad (44)$$

5.21.4. Foaming capacity

The foaming capacity of microgreen powder samples was analyzed following the method previously reported by Budnimath et al. (2023). Briefly, 2 g of microgreen powder sample was whipped with 100 mL of distilled water for 5 min and then transferred to a 10 mL measuring cylinder. The foaming capacity was calculated using Equation (45) mentioned below.

$$\text{Foaming capacity (\%)} = \frac{\text{Foam volume after mixing (mL)}}{\text{Starting volume of slution phase (mL)}} \times 100 \quad (45)$$

5.21.5. Swelling index

The swelling index of microgreen powder samples was determined according to the method reported by Ma & Mu (2015). The 1.0 g sample of microgreen powder was placed in a 10 mL measuring cylinder, and 5 mL of distilled water was gently added. The initial volume occupied by the sample was recorded. After allowing the sample to stand undisturbed for 1 h, the volume post-swelling was measured. The swelling index was calculated using the following Equation (46).

$$\text{Swelling index (\%)} = \frac{\text{Volume before swelling (mL)}}{\text{Volume after swelling (mL)}} \times 100 \quad (46)$$

5.21.6. Water and oil absorption capacity

Water and oil absorption capacity were assessed by the method given by Singh et al. (2022). The water and oil absorption capacity of microgreen powder samples were calculated using Equation (47 and 48).

$$\text{Water absorption capacity (g/g)} = \frac{(\text{Initial} - \text{Final solution volume}) \times 1}{\text{Sample weight (g)}} \times 100 \quad (47)$$

$$\text{Oil absorption capacity (g/g)} = \frac{\text{Weight of sample} + \text{oil}}{\text{Sample weight}} \times 100 \quad (48)$$

5.22. Characterization of dried microgreen powder

5.22.1. Functional groups determination

Fourier Transform Infrared (FTIR) spectroscopy was employed to analyze the functional groups present in dried microgreen powder according to the method followed by (Sanyukta et al., 2023). Briefly, 100 mg of the microgreens powder sample was mixed with spectroscopic grade potassium bromide (KBr) in a ratio of 1:100 and ground to ensure homogeneity. The mixture was then compressed into a thin, transparent pellet using a hydraulic press under high pressure. The prepared pellet was placed in the sample holder, and the FTIR spectra were recorded in the mid-infrared region (4000–400 cm^{-1}) using an FTIR spectrometer. The resolution was set to 4 cm^{-1} , and 32 scans were averaged for each spectrum to improve the signal-to-noise ratio. The obtained spectra were analyzed to identify the characteristic absorption bands corresponding to various functional groups in the sample, providing insights into the chemical composition of the microgreen powder.

5.22.2. Surface morphological study

The surface morphology of dried microgreen powder was analyzed using scanning electron microscopy (SEM) by following the method proposed by (Mansouri et al., 2024). A 100 mg of sample was mounted onto an aluminum stub using double-sided carbon adhesive tape to ensure stability. To enhance electrical conductivity and prevent charging during analysis, the sample was sputter-coated with a thin layer of gold under a vacuum using a sputter coater. The coated sample was then placed in the SEM chamber, and micrographs were obtained at different accelerating voltages. Images were captured at different magnifications to observe the surface morphological structure, including particle size, shape, and surface texture.

5.22.3. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was conducted to determine the thermal properties of dried microgreen powder by using a method (Reddy et al., 2024). A 10 mg sample was accurately weighed and sealed in an aluminum pan, while an empty aluminum pan was used as a reference. The analysis was performed using a DSC instrument under a nitrogen atmosphere to prevent oxidation, with a flow rate of 50 mL/min. The sample was subjected to a controlled heating program, typically ranging from 25°C to 450°C, at a constant heating rate of 10°C/min. The thermal transitions, such as glass transition temperature (T_g), melting

temperature (T_m), and enthalpy changes (ΔH), were recorded. The obtained thermograms were analyzed to understand the thermal behavior and stability of the dried microgreen powder.

5.22.4. Thermogravimetric analysis

Thermogravimetric analysis (TGA) was performed to evaluate the thermal stability and decomposition profile of dried microgreen powder according to the method proposed by (Sanyukta et al., 2023). Briefly, 10 mg of the sample was accurately weighed and placed in a platinum crucible. The analysis was carried out using a thermogravimetric analyzer under a nitrogen atmosphere with a flow rate of 50 mL/min to prevent oxidative degradation. The sample was heated from room temperature to 650 °C at a constant heating rate of 10°C/min. The weight loss of the sample was recorded as a function of temperature, and the thermogram was analyzed to identify key thermal events such as moisture loss, decomposition of organic components, and residual ash content.

5.22.5. Particle size and zeta potential

The particle size and zeta potential of dried microgreen powder were determined using dynamic light scattering (DLS) with a zeta potential analyzer by following the procedure of (Kumar & Singh 2024). For the analysis, 100 mg of the sample was dispersed in 10 ml of deionized water. The dispersion was sonicated for 5 min to prevent aggregation and ensure uniformity. The prepared sample was then introduced into the sample cell of the analyzer. For particle size measurement, the instrument measured the intensity of scattered light at an angle of 90°, providing data on the average hydrodynamic diameter (z-average) and size distribution. For zeta potential, the electrophoretic mobility of the particles was measured under an applied electric field, and the data were used to calculate the zeta potential, reflecting the surface charge and colloidal stability of the particles.

5.23. Microbial analysis

Microbial analysis, including total plate count and yeast and mold counts of the sample, was conducted following the method by Kumar et al. (2023). A 1 g sample was aseptically mixed with 9 mL of distilled water and thoroughly shaken to ensure homogenization. Serial dilutions were prepared, and samples were incubated at 37°C for 24 to 48 hours. The total plate count was determined on nutrient agar, while yeast and mold counts were assessed on potato dextrose agar. Results for total plate count, yeast and mold, and coliform counts were expressed as colony-forming units per gram (cfu/mL) based on colony counts from petri dishes.

5.24. Sensory properties

Sensory evaluation of the microgreen-based RTS beverage samples was performed using a 9-point hedonic scale and descriptive analysis, assessing attributes such as color and

appearance, flavor and sweetness, consistency, and overall acceptability. A panel of 30 semi-trained individuals (15 male, 15 female) participated, with documented consent and understanding. The lassi samples were served in identical clear glass cups, labeled with random three-digit codes, and presented at a temperature of 7-10°C to simulate typical consumption conditions (Singh et al., 2024).

5.25. Cost estimation of beverage

The cost analysis of the optimized microgreen-based beverage was calculated by considering the expenses for raw materials, processing, and labor, including both fixed and variable costs, as outlined in the methodology described by Pandhi and Poonia (2021).

5.26. Statistical analysis

The data collected from the experiment underwent a Two-way ANOVA analysis within different types of treatment utilizing SPSS version 23.0 (Chicago, USA). The factors considered in the two-way ANOVA were applied, and a Tukey's HSD test was used to determine whether the groups differed from one another ($P < 0.05$). The outcomes were displayed as mean $\pm$ standard deviations.

The principal component analysis (PCA) was carried out to analyze the relationship treatments and parameters using Minitab Statistical Software (Version 17.0, Minitab Inc., Enterprise Drive State College, PA). PCA was applied to different datasets, including morphological traits, biochemical and antioxidant attributes, as well as combined parameters and postharvest storage parameters. The different study, principal components were selected based on eigenvalues greater than 1, and the percentage (%) of variance explained by each component was used to evaluate their contribution. The obtained PCA results were visualized using score plots to display treatment clustering and loading plots to identify the strength and direction of variable contributions to each principal component. Traits with high positive loadings on PC-1 or PC-2 were interpreted as major contributors to the observed variability among treatments.

The drying data was statistically analyzed and modeled utilizing the Solver add-in in MS Excel 2021, which was also used to determine effective moisture diffusivity. The model's performance was evaluated based on metrics such as the coefficient of determination (R^2), root mean square error (RMSE), and chi-square (χ^2), conducted using MS Excel 2019. Degradation kinetic analysis, graphical representations, and data visualization were created using OriginPro 9.0. The optimal proportions of microgreen (juice and powder), dahi, pomegranate juice, and water for the development of microgreen-based dairy RTS beverage and microgreen-based fruit RTS beverage were determined using regression analysis and the Design Expert 13 software

from Stat Ease Inc. in MN, USA. Multiple regression models were used for the analysis of the data, and ANOVA was utilized to determine the statistical significance of each outcome. Responses were collected based on the physicochemical, biochemical, and antioxidant parameters of the microgreen-based RTS beverage.

CHAPTER-6

RESULTS AND DISCUSSION

6.1. The effect of cultivation conditions on morphological and bioactive compounds

There has been a growing interest in promoting a healthy lifestyle including the consumption of nutritious and quality foods, and microgreens offer a very good option. However, growing mediums and conditions are affecting their quality parameters such as morphological and nutritional quality parameters. Thus, this study research work aimed to study the effects of growing medium, illumination system and foliar spray treatments on the morphological and nutritional quality parameters of selected microgreens by sustainable development goals.

6.1.1. The effect of growing mediums on morphological, proximate, and biochemical parameters in selected varieties of microgreens

The selected microgreens grown in three different growing mediums (soil, cocopeat, and hydroponics) and changes in growth period during 9 days of cultivation were shown in (Figures 6.1 and 6.2) and the different selected microgreens and their parts such as shoots and true leaf were shown in (Figure 6.3).

6.1.1.1. Shoot height and width

The selected microgreens grown in three different growing mediums (soil, cocopeat, and hydroponics) showed significant effects on the height and width of microgreen shoots as shown in (Table 6.1). Among all the microgreens, flax crops (*Linaceae*) microgreens reflect the highest shoot height in soil (7.39 ± 0.86 cm) and cocopeat (6.80 ± 1.08 cm) growing medium. Moreover, the trend observed was flax crops > radish sango (*Brassicaceae*) > broccoli (*Brassicaceae*) > cabbage (*Brassicaceae*) in the soil-based medium, while pak choi (*Brassicaceae*), beetroot (*Amaranthaceae*), and red amaranthus (*Amaranthaceae*) performed better in cocopeat. It is important to note that comparing morphological traits across different botanical families, such as *Linaceae* (flax), *Brassicaceae* (radish sango), and *Amaranthaceae* (beetroot), should be done cautiously, as inherent genetic, physiological, and anatomical differences affect growth patterns (Kyriacou et al., 2016; Xiao et al., 2016). Hence, it is more appropriate to compare morphological trends within a single family, where similar genetic backgrounds can provide more meaningful insights.

Microgreen shoot width was affected by the interaction between selected microgreens and both growing mediums. Radish sango microgreens showed higher shoot width compared to other microgreens in cocopeat (1.19 ± 0.12 mm) growing medium (Table 6.1). The red

amaranthus microgreen shoots revealed very low shoot width in soil (0.52 ± 0.03 mm) and cocopeat (0.50 ± 0.03 mm) growing medium compared to other microgreens grown in both growing medium. All selected microgreens shoot width measured were in the range between (0.50 and 1.19 mm). However, significant differences were observed in the shoot height and width of selected microgreens based on their growing substrate. Within the *Brassicaceae* family, contradictory responses were observed for cabbage showed superior shoot height in soil, whereas broccoli, pak choi, and radish sango performed better in cocopeat. This suggests species-specific responses even within the same family, likely due to differences in water uptake efficiency, nutrient absorption, root morphology, and adaptation to the physical properties of the medium (Hoang and Vu, 2022; Saleh et al., 2022).

The comparison between different growing media is presented in (Table 6.1). The soil and cocopeat based growing medium showed better results for shoot height and shoot width of selected microgreens. Ten microgreens (amaranth, arugula, basil, broccoli, cabbage daikon, radish, kale, khorabi, mustard, and pea) were grown in the peat-based soilless growing medium. From this study shoot heights were varied among all selected microgreens grown in (Nov, 2018 and Jan, 2019) the values of shoot height ranging from (3.8 – 9.9 cm) were observed (Li et al. 2021). Dhaka et al. (2023) investigates the six crops (mungbean, lentil, red radish, pearl-millet, mustard, and red cabbage) as microgreens grown under the light with a 16 h light/8 h dark cycle. From this investigation height of different crops on the 9th day was reported as lentil (8–12 cm), red radish (6–9 cm), mungbean (7–14 cm), pearl millet (7–12 cm), and mustard (6–11 cm) respectively. Likewise, according to a study by Ntsoane et al. (2023), used the different microgreens of the *Brassicaceae* family (cabbage, rocket, and radish) were grown in different mediums (Hygromix, and Promix) with different seed densities (4, 8, and 12 seeds per cavity). This study showed a significant ($P<0.05$) effect on the growing medium and seed densities on microgreen shoot height (22.3 – 47.97 mm). The red cabbage, broccoli, mizuna, green mustard, and pak choi microgreens were grown in substrates including (sand, organic soil, coco coir, rice husk, white sphagnum peat, and vermiculite) with different formulations, the shoot height of 5.8 to 7.2 cm were observed (Hoang and Vu, 2022). Similar findings of shoot length of microgreens were reported for, different formulated soilless media such as PitMoss, vermicast, sawdust, mushroom compost, perlite, and Pro-mix BXTM were used for the kale, swiss chard, arugula, and pak choi microgreens (Saleh et al., 2022). Previous studies showed significant differences in microgreen shoot height, mostly due to the variation in harvest times and some at the cotyledon stage, others at first true leaves, or after a set number of days (Senevirathne et al., 2019). These differences, along with microgreens seed sowing density (Signore et al., 2024)

and light irradiance (Luo et al., 2019), significantly influenced this parameter results. Furthermore, the results also suggested that the variations in plant height of microgreens to the different media were dependent on genotypic differences (Saleh et al., 2022).

6.1.1.2. Shoot fresh and dry weight

The results on fresh and dry shoot weights of different selected microgreens grown under two growing mediums are presented in **(Table 6.2)**, respectively. The fresh single shoot weight of selected microgreens cultivated in soil, cocopeat and hydroponics is shown in **(Table 6.2)**, respectively. Progressive changes were observed in the fresh weight and dry weight of microgreens with growing medium. Among all the microgreens, radish sango (*Brassicaceae*) reported the highest fresh weight (single shoot and 25 shoots) and dry weight in both soil and cocopeat-based growing media, compared to the other six microgreens studied. This superior performance is likely due to radish sango robust hypocotyl development, thick tissue structure, and higher water-binding capacity, which promote biomass accumulation under optimal conditions (Ntsoane et al., 2023; Hoang and Vu, 2022).

Soil and cocopeat were found to significantly affect the fresh and dry weights of microgreens. Specifically, flax (*Linaceae*), cabbage (*Brassicaceae*), pak choi (*Brassicaceae*), and red amaranthus (*Amaranthaceae*) showed the highest fresh and dry weights of 25 shoots in the soil-based medium, while cabbage, radish sango, and pak choi also performed well in the cocopeat-based medium. This suggests that soil growing medium content good mineral content supports biomass production in some species, whereas cocopeat growing medium superior water retention and aeration favor others, depending on species specific root and nutrient uptake strategies (Kyriacou et al., 2018; Ferrarezi et al., 2022). Significant differences were observed among microgreen species and growing media for fresh and dry weight measurements. The highest single shoot weight was recorded in radish sango (133.89 ± 35.59 mg in cocopeat), while the lowest was seen in red amaranthus (4.24 ± 0.62 mg in cocopeat) (Table 6.2). These differences can be attributed to the inherent genotypic traits of each species, where *Brassicaceae* species like radish sango have larger cotyledons and thicker stems compared to the slender morphology of *Amaranthaceae* members like red amaranthus (Xiao et al., 2016; Kyriacou et al., 2016).

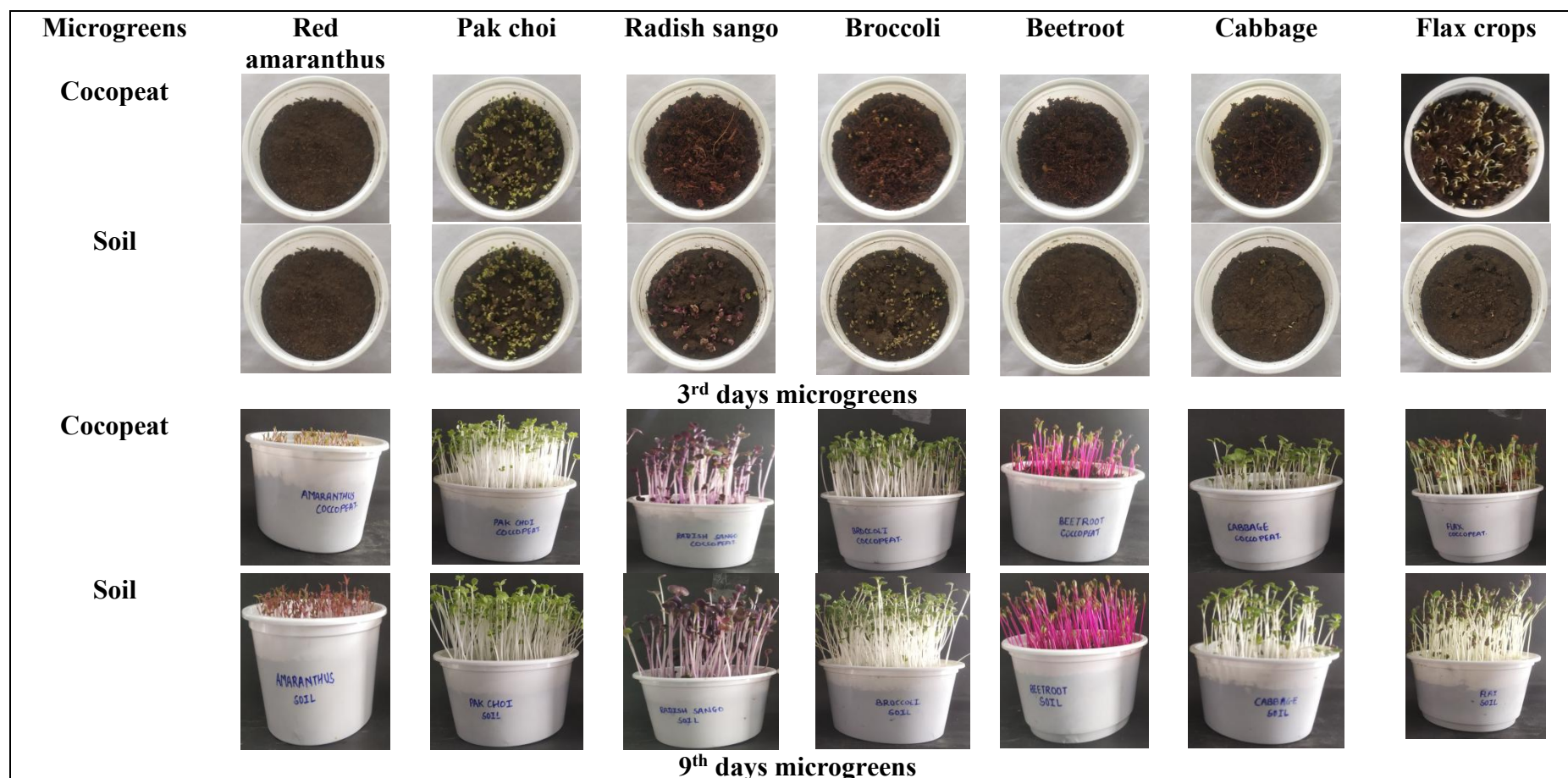


Figure 6.1. The selected microgreens grown in cocopeat and soli-based growing medium.



Figure 6.2. The different families selected microgreens grown in soil less growing medium

Table 6.1. Morphological characteristics of different selected microgreens grown in three growing mediums

Microgreens	Growing medium	Height of shoot (cm)	Width of shoot (mm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm ²)
Cabbage	Cocopeat	3.09±0.73 ^{ijC**}	0.77±0.09 ^{deC**}	0.41±0.08 ^{fgC*}	0.59±0.12 ^{bcB*}	0.20±0.08 ^{cdB*}
	Soil	4.16±0.72 ^{fghC***}	0.70±0.13 ^{efgC*}	0.40±0.08 ^{fc*}	0.59±0.12 ^{bb*}	0.22±0.08 ^{cb*}
	Hydroponics	3.71±0.74 ^{hiC*}	0.87±0.07 ^{bcC*}	0.47±0.11 ^{fgC*}	0.58±0.13 ^{bcB**}	0.19±0.07 ^{cdB**}
Broccoli	Cocopeat	4.49±0.69 ^{efghB**}	0.64±0.11 ^{gE**}	0.32±0.06 ^{hC*}	0.42±0.10 ^{efgC*}	0.11±0.04 ^{fghiC*}
	Soil	5.60±1.12 ^{cdB***}	0.64±0.12 ^{gE*}	0.38±0.08 ^{ghC*}	0.49±0.12 ^{dc*}	0.16±0.06 ^{defgC*}
	Hydroponics	4.69±1.01 ^{efgB*}	0.69±0.08 ^{efgE*}	0.38±0.07 ^{ghC*}	0.52±0.12 ^{cdC**}	0.15±0.06 ^{defC**}
Pack choi	Cocopeat	5.12±0.64 ^{deB**}	0.77±0.04 ^{deD**}	0.33±0.06 ^{hd*}	0.48±0.07 ^{deC*}	0.12±0.04 ^{efghC*}
	Soil	5.00±0.76 ^{defB***}	0.67±0.07 ^{fgD*}	0.32±0.05 ^{hd*}	0.46±0.09 ^{defC*}	0.12±0.04 ^{efghiC*}
	Hydroponics	4.41±0.42 ^{efghB*}	0.66±0.06 ^{gD*}	0.38±0.10 ^{ghD*}	0.52±0.08 ^{cdC**}	0.16±0.05 ^{defgC**}
Radish sango	Cocopeat	5.22±0.64 ^{cdA**}	1.19±0.12 ^{aA**}	0.65±0.13 ^{deB*}	0.91±0.17 ^{aA*}	0.48±0.17 ^{abA*}
	Soil	6.16±1.67 ^{bcA***}	1.18±0.13 ^{aA*}	0.72±0.13 ^{abB*}	0.91±0.18 ^{aA*}	0.53±0.20 ^{aA*}
	Hydroponics	5.66±1.36 ^{cdA*}	1.13±0.08 ^{aA*}	0.61±0.13 ^{ebB*}	0.90±0.18 ^{aA**}	0.44±0.16 ^{ba**}
Beetroot	Cocopeat	4.36±1.11 ^{efghC**}	0.91±0.10 ^{bbB**}	0.79±0.18 ^{aA*}	0.14±0.03 ^{ie*}	0.09±0.03 ^{hijD*}
	Soil	3.82±0.54 ^{hiC***}	0.81±0.16 ^{cdB*}	0.60±0.12 ^{ea*}	0.13±0.01 ^{ie*}	0.06±0.01 ^{ijD*}
	Hydroponics	3.77±0.48 ^{hiC*}	0.76±0.08 ^{defB*}	0.77±0.15 ^{abA*}	0.16±0.08 ^{ie**}	0.10±0.06 ^{ghijD**}
Red amaranthus	Cocopeat	2.54±0.47 ^{ikC**}	0.50±0.05 ^{hF**}	0.45±0.07 ^{fgC*}	0.15±0.03 ^{ie*}	0.05±0.01 ^{je*}
	Soil	2.02±0.38 ^{kC***}	0.52±0.03 ^{hF*}	0.47±0.09 ^{fc*}	0.14±0.02 ^{ie*}	0.05±0.01 ^{je*}
	Hydroponics	2.82±0.44 ^{ikC*}	0.50±0.07 ^{hF*}	0.44±0.05 ^{fgC*}	0.15±0.02 ^{ie**}	0.05±0.01 ^{je**}
Flax crops	Cocopeat	6.80±1.08 ^{abA**}	0.66±0.03 ^{gE**}	0.70±0.04 ^{cdA*}	0.15±0.02 ^{hd*}	0.17±0.02 ^{cdeB*}
	Soil	7.39±0.86 ^{aA***}	0.65±0.05 ^{gE*}	0.71±0.12 ^{bcdA*}	0.40±0.04 ^{ghD*}	0.19±0.04 ^{cdB*}
	Hydroponics	4.09±0.42 ^{ghA*}	0.65±0.06 ^{gE*}	0.71±0.05 ^{bcdA*}	0.40±0.04 ^{fgD**}	0.22±0.03 ^{cb**}

Two-way ANOVA was applied to estimate the effect of growing medium on selected microgreens and results were presented as mean ± standard deviations of different morphological parameters. Tukey's HSD test was performed to underline differences in the morphological parameters of selected microgreens. Different lowercase letters in the column indicate significant (P<0.05) differences among the microgreens grown in three growing mediums. Different uppercase letters in the column indicate significant (P<0.05) differences among the microgreens. The symbol (*, **, ***) in the column indicates the significant (P<0.05) differences among the growing medium.

Table 6.2. Morphological characteristics of different selected microgreens grown in three growing mediums

Microgreens	Growing medium	Weight of single shoot (mg)	Microgreens 25 shoot fresh weight (g)	Microgreens 25 shoot dry weight (mg)	Yield (g/m ²)
Cabbage	Cocopeat	30.95±7.55 ^{efghB*}	0.74±0.01 ^{efgBC*}	62.88±0.59 ^{fd**}	688.49±102.49 ^{fd**}
	Soil	42.12±11.02 ^{cdB*}	0.92±0.02 ^{cdBC*}	63.40±1.18 ^{fd**}	688.49±102.49 ^{fd**}
	Hydroponics	44.31±7.00 ^{cdeB*}	1.15±0.03 ^{defBC*}	64.25±0.95 ^{fd*}	642.38±28.66 ^{fd*}
Broccoli	Cocopeat	30.90±5.55 ^{efghC*}	0.96±0.01 ^{defCD*}	63.37±1.31 ^{fd**}	1548.32±111.74 ^{cdeC**}
	Soil	27.84±5.47 ^{ghC*}	0.70±0.03 ^{fgCD*}	64.67±0.59 ^{fd**}	1768.39±104.75 ^{abcC**}
	Hydroponics	36.84±8.60 ^{cdefgC*}	0.69±0.06 ^{fgCD*}	63.11±0.90 ^{fd*}	1341.36±63.04 ^{cC*}
Pak choi	Cocopeat	32.78±12.60 ^{defghC*}	0.85±0.04 ^{defgD*}	77.65±0.58 ^{cC**}	1804.02±97.88 ^{abBC**}
	Soil	32.62±5.71 ^{defghC*}	0.57±0.04 ^{efgD*}	68.53±0.42 ^{cC**}	1609.63±168.20 ^{abcdBC**}
	Hydroponics	28.12±6.38 ^{fghC*}	0.80±0.07 ^{gD*}	52.09±2.58 ^{gC*}	1490.69±69.76 ^{deBC*}
Radish sango	Cocopeat	133.89±35.59 ^{aA*}	3.19±0.15 ^{abA*}	188.05±0.54 ^{aA**}	1883.14±52.54 ^{aA**}
	Soil	111.56±28.60 ^{bA*}	3.53±0.26 ^{bA*}	187.45±0.25 ^{abA**}	1838.60±118.91 ^{abA**}
	Hydroponics	115.08±24.60 ^{bA*}	2.90±0.34 ^{aA*}	185.37±0.54 ^{cA*}	1600.72±44.16 ^{bcdA*}
Beetroot	Cocopeat	21.98±4.54 ^{hD*}	0.54±0.04 ^{gD*}	25.09±0.18 ^{ie**}	487.29±48.73 ^{fghE**}
	Soil	26.65±4.49 ^{ghD*}	0.63±0.03 ^{fgD*}	31.23±0.30 ^{he**}	625.62±69.54 ^{fe**}
	Hydroponics	24.05±4.00 ^{ghD*}	0.72±0.06 ^{fgD*}	30.30±0.26 ^{he*}	540.21±41.00 ^{fgE*}
Red amaranthus	Cocopeat	4.24±0.62 ^{ie*}	0.11±0.01 ^{he*}	10.78±0.12 ^{jF**}	288.71±15.90 ^{ghF**}
	Soil	6.21±1.96 ^{ie*}	0.11±0.01 ^{he*}	10.41±0.15 ^{jF**}	244.69±17.67 ^{hF**}
	Hydroponics	3.92±0.33 ^{ie*}	0.12±0.02 ^{he*}	10.08±0.18 ^{if*}	294.99±14.26 ^{ghF*}
Flax crops	Cocopeat	41.16±4.87 ^{cdefB*}	1.08±0.08 ^{cdeB*}	72.95±0.06 ^{dB**}	1815.55±91.17 ^{abAB**}
	Soil	46.92±8.47 ^{cB*}	0.87±0.04 ^{cB*}	73.37±0.33 ^{dB**}	1609.63±168.20 ^{bcdAB**}
	Hydroponics	34.76±5.99 ^{cdefghB*}	1.31±0.14 ^{defgB*}	71.92±0.59 ^{dB*}	1619.58±20.21 ^{bcdAB*}

Two way- ANOVA was applied to estimate the effect of growing medium on selected microgreens and results were presented as mean ± standard deviations of different morphological parameters. Tukey's HSD test was performed to underline differences in the morphological parameters of selected microgreens. Different lowercase letters in the column indicate significant (P<0.05) differences among the microgreens grown in three growing mediums. Different uppercase letters in the column indicate significant (P<0.05) differences among the microgreens. The symbol (*, **, ***) in the column indicates the significant (P<0.05) differences among the growing medium.

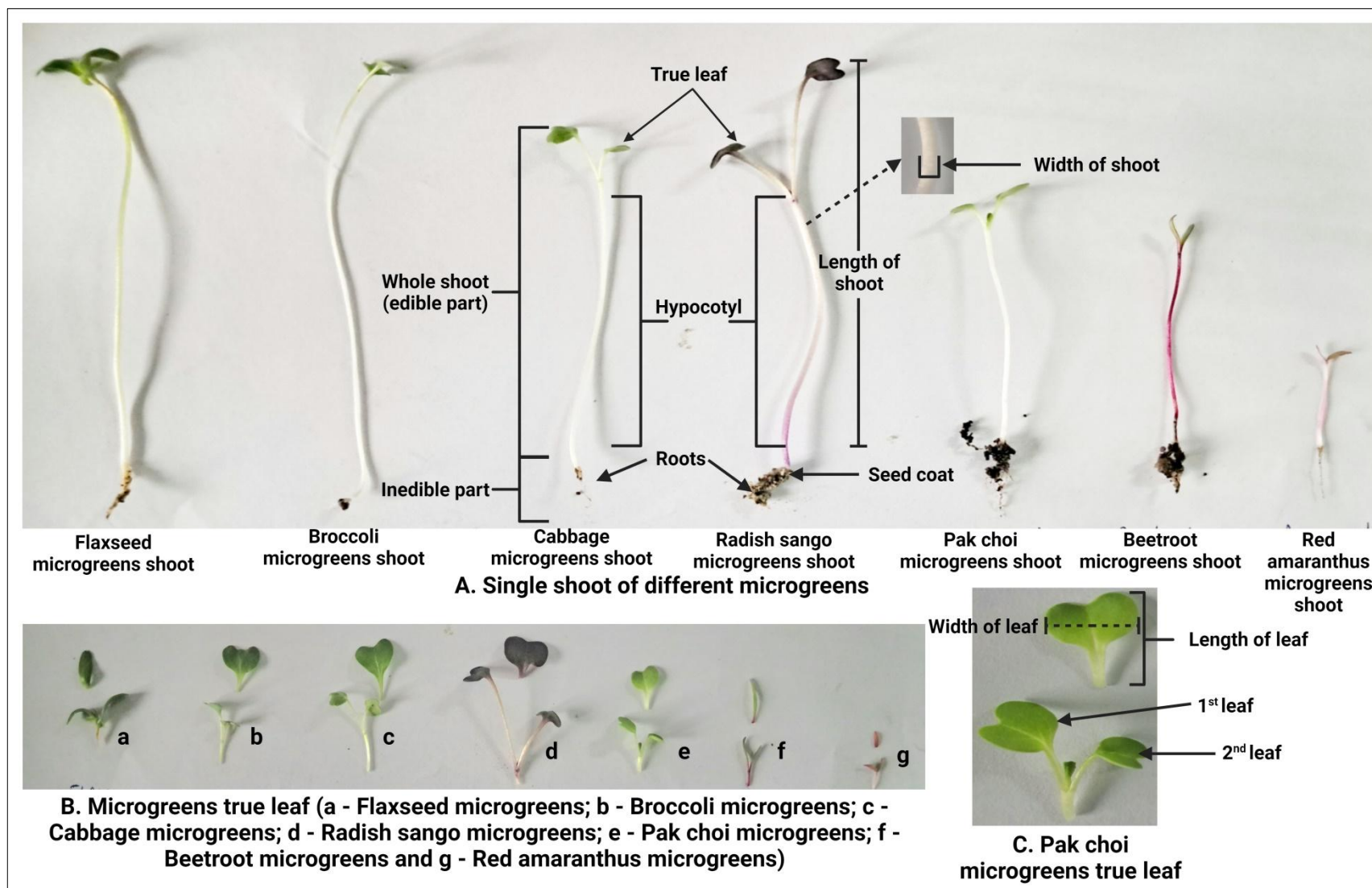


Figure 6.3. The different selected families microgreens and their part

Moreover, fresh weight variations reflect not only species genetics but also growing medium properties, such as aeration, porosity, nutrient availability, and water-holding capacity, which directly influence plant metabolic activity and tissue hydration levels (Signore et al., 2024; Luo et al., 2019). For instance, while hydroponic systems may offer uniform nutrient delivery, they often produce lower fresh weights due to reduced mechanical support for root anchorage, affecting water uptake dynamics. These findings are consistent with previous studies on *Brassicaceae* microgreens, where radish, cabbage, and rocket showed superior fresh and dry weight performances under optimized substrate and seed density conditions (Ntsoane et al., 2023; Saleh et al., 2022). Additionally, calculating moisture content from these fresh and dry weight values allows the assessment of microgreen succulence, which is crucial for determining post-harvest behavior, shelf life, textural quality, and storage or drying suitability (Kyriacou et al., 2018; Ferrarezi et al., 2022). In summary, the observed differences in fresh and dry weights across species and substrates highlight the strong interplay between genotype (species specific growth traits) and environment (growing medium properties), underlining the importance of carefully selecting both crop and substrate for optimizing microgreen biomass production (Kyriacou et al., 2016; Hoang and Vu, 2022).

6.1.1.3. Leaf length, leaf width, and total leaf area

The results on fresh and dry shoot weights of different selected microgreens grown under three growing mediums (soil, cocopeat, and hydroponics) are presented in **Table 6.2**. The fresh single shoot weight, fresh weight of 25 shoots, and dry weight of 25 shoots were measured for each microgreen species. Progressive differences were observed across the growing media, confirming that substrate type significantly influences biomass accumulation. The changes in leaf length, leaf width, and total leaf area of microgreens were recorded and are presented in **Table 6.1**. Leaf dimensions are visually illustrated in **Figure 6.3**. Among the selected microgreens, beetroot (*Amaranthaceae*) grown in cocopeat reported the highest leaf length, while pak choi (*Brassicaceae*) grown in soil showed the highest values in its group. Radish sango (*Brassicaceae*), grown in both soil and cocopeat, exhibited the greatest leaf width, while pak choi grown in soil recorded the lowest leaf width.

Leaf length and width were significantly affected by both the growing medium and their interaction with the specific microgreen species ($P < 0.05$). These significant differences within families, especially among *Brassicaceae* microgreens (such as cabbage, broccoli, pak choi, and radish sango), underline the diverse growth patterns observed when grown in soilless mediums. This diversity can be linked to each species' unique genetic background, leaf morphology, and physiological adaptations (Kyriacou et al., 2016; Hoang and Vu, 2022). The

total leaf area varied widely, with radish sango showing the largest area ($0.53 \pm 0.20 \text{ cm}^2$) and red amaranthus (Amaranthaceae) recording the smallest ($0.05 \pm 0.01 \text{ cm}^2$). These results suggest that, while some species (like radish sango) invest in both vertical and lateral leaf expansion, others (like red amaranthus) focus more on shoot elongation or slender structures, reflecting different ecological strategies and resource allocation patterns (Xiao et al., 2016; Saleh et al., 2022).

Our results showed that cocopeat and soil-based growing media promoted variations in shoot height, leaf dimensions, and biomass (Li et al., 2021), consistent with previous reports in other microgreens including groundnut, rapini, broccoli raab, cauliflower, broccoli, **fenugreek**, amaranth, spinach, mint and fennel (Sharma et al., 2013; Di Gioia et al., 2016; Palmitessa et al., 2020; Sinha & Thilakavathy, 2021). Cocopeat, derived from coconut husks, offers excellent moisture retention, aeration, and lightness, supporting healthy root and leaf development by maintaining sufficient N:P:K nutrient supply without waterlogging (Xiong et al., 2017; Widiwujani et al., 2020). Soil-based media, by contrast, supply richer mineral nutrients and microbial activity, influencing growth dynamics depending on the species' nutrient requirements (Al-Busaidi et al., 2021).

The properties of the growing medium, including pH, electrical conductivity, organic carbon, sodium, nitrogen, phosphorus, and potassium, significantly influence plant growth. A slightly acidic to neutral pH (6.0–7.0) is optimal for nutrient availability, while too high an alkaline pH can reduce iron, magnesium, and zinc uptake (Ferrarezi et al., 2022). High electrical conductivity may indicate excessive salts, leading to osmotic stress and nutrient imbalances, ultimately affecting leaf size and plant health (Stoleru et al., 2016). Maintaining these parameters within ideal ranges is essential for optimizing leaf expansion and area in microgreens. Importantly, our study highlights that morphological traits such as leaf length, width, and area are influenced not just by the growing medium but also by inherent genotype differences and family-specific characteristics. This means that even under identical environmental conditions, species-specific genetic factors determine how resources are allocated to shoot elongation versus leaf expansion, affecting overall morphological outcomes (Kyriacou et al., 2016; Xiao et al., 2016; Luo et al., 2019).

6.1.1.4. Fresh yields

The harvested microgreens were weighed to determine the fresh yield (FW) per cup (63.62 cm^2). Both the type of microgreen and the growing medium significantly affected fresh yields, as shown in **Table 6.2**. Among all species, radish sango (*Brassicaceae*) grown in cocopeat reported the highest yield, while red amaranthus (Amaranthaceae) grown in soil

showed the lowest. Radish sango (grown in both soil and cocopeat), flax (*Linaceae*, grown in soil and cocopeat), and broccoli (*Brassicaceae*, grown in soil) showed no statistically significant differences in yield between soil and cocopeat. Similarly, broccoli and cabbage (*Brassicaceae*) grown in both soil and cocopeat did not show major differences, although slight variations were noted depending on the medium.

Notably, the highest overall fresh yield was consistently observed in *Brassicaceae* species, especially radish sango, followed by pak choi, broccoli, and cabbage, highlighting the superior biomass-producing potential of this family under optimized substrate conditions. By contrast, the *Amaranthaceae* member red amaranthus consistently produced the lowest yield, which may reflect its inherently slender and delicate morphology, resulting in less harvested biomass per area (Xiao et al., 2016; Kyriacou et al., 2018). In assessing microgreen yield, the sustainability and efficiency of production depend heavily on the characteristics of the growing medium. Soilless substrates such as cocopeat enhance water retention, nutrient availability, and root aeration, which can significantly increase microgreen productivity, especially for high-performing species like *Brassicaceae* members (Galieni et al., 2020; Ferrarezi et al., 2022). Conversely, soil provides more mineral-rich conditions, benefiting species that prefer heavier substrates, although it can sometimes reduce aeration and slow growth in delicate crops.

The fresh yield data in this study align closely with previous reports: Di Gioia et al. (2023) reported yields ranging from 409.3 to 2258.8 g/m² across seventeen microgreen species, while *Brassicaceae* microgreens such as kale, cabbage, arugula, and mustard showed fresh yields between 535.6 to 871.2 g/m² under varied media like sand, organic soil, coco coir, rice husk, and humic acid blends (Jones-Baumgardt et al., 2019). The observed differences in fresh yields can be attributed to multiple factors such as, species specific growth habits (compact vs. leafy), nutrient uptake efficiency, water use patterns, and substrate root interactions (Kyriacou et al., 2018; Hoang and Vu, 2022).

For instance, *Brassicaceae* species like radish sango and pak choi typically develop thicker hypocotyls and larger cotyledon areas, enhancing fresh weight compared to the slender, fine-leaved red amaranthus. Furthermore, the fundamental properties of the growing medium including nutrient content, water-holding capacity, aeration, medium stability, and pH are critical for maximizing yield. An optimal pH range (6.0–7.0) ensures balanced nutrient availability, while excessive salt levels (high electrical conductivity) can hinder water uptake and suppress biomass production (Ferrarezi et al., 2022; Stoleru et al., 2016). Managing these factors is essential to overcoming one of the major limitations faced by the microgreens industry: achieving consistent, high yields in commercial production (Singh et al., 2024).

6.1.1.2. Proximate composition

Results of the proximate analysis of the seven microgreens grown in three different growing substrates are shown in (Table 6.3). Moisture content is the most important parameter that affects the shelf life and quality of fresh produce. The various kinds of factors influencing moisture content include environmental parameters, method of processing, and harvesting and storage conditions. The moisture content showed significant differences among all the microgreens and their growing medium. Radish sango microgreens cultivated in a soil-based growing medium showed the highest range moisture content i.e. 95.33 ± 0.10 g/100g, FW. In broccoli microgreens, the lowest 90.72 ± 0.21 g/100g, FW moisture content was observed in the hydroponic-based growing medium compared with other microgreens. Pak choi, beetroot, and red amaranthus microgreens had no significant changes in moisture content. Kowitcharoen et al. (2021) found the moisture content 87.29 – 94.67 g/100g in 13 different microgreens grown in a controlled environment. In another study, a moisture content of 80.83 – 92.63% was found in four varieties of microgreens including carrot, spinach, bathua, and Bengal gram. The effect of cultivation substrate on different sprouts of sorghum, wheat, horse gram, cowpea, mungbean, and fenugreek the moisture content similar range (90.28 – 90.74 g/100g, FW), was observed (Eswaranpillai et al., 2023). In radish sango microgreens the moisture content was 90.84 to 92.20%, FW was grown in four distinct growing substrates containing soil, vermicompost, and cocopeat growing medium (Eswaranpillai et al., 2023). Another investigation was done on mustard, leaf mustard, radish, and cabbage microgreens grown in various soilless media and discovered nearly the same moisture content of 88.0 to 92.0%, FW was observed (Polash et al., 2019). Our findings closely align with this study finding results.

Table 6.3. Proximate composition of microgreens grown in three growing mediums

Microgreens	Growing medium	Moisture content (g/100g, FW)	Ash content (g/100g, DW)	Fat content (g/100g, DW)	Carbohydrate content (g/100g, DW)	Fiber content (g/100g, DW)	Protein content (g BSA/100g, DW)
Cabbage	C	95.26±0.02 ^{aB**}	9.62±0.65 ^{defgB***}	6.87±0.12 ^{abcdB**}	2.41±0.07 ^{cd*}	11.97±0.11 ^{abcAB*}	4.86±0.02 ^{bB**}
	S	91.92±0.01 ^{cdeB**}	10.38±0.02 ^{cdB**}	6.21±0.14 ^{cdeB**}	2.50±0.08 ^{cd*}	10.79±0.24 ^{cdefAB**}	4.08±0.24 ^{bcdB**}
	H	91.20±0.28 ^{deB*}	8.73±0.74 ^{fghB*}	6.03±0.08 ^{cdeB*}	2.41±0.06 ^{cd**}	10.69±0.20 ^{cdefAB*}	3.86±0.04 ^{cdefB*}
Broccoli	C	95.14±0.24 ^{aAB**}	11.17±0.08 ^{cdC***}	7.07±0.08 ^{abcA**}	2.58±0.03 ^{cd*}	9.85±0.08 ^{defgC*}	3.52±0.17 ^{cdefCD**}
	S	94.84±0.04 ^{abAB**}	7.38±0.18 ^{hijC**}	7.58±0.06 ^{aA**}	2.61±0.08 ^{cd*}	11.42±0.46 ^{abcdeC**}	2.95±0.11 ^{efghCD**}
	H	90.72±0.21 ^{cAB*}	7.12±0.47 ^{ijC*}	6.39±0.53 ^{abcdeA*}	2.17±0.08 ^{cd**}	9.41±0.40 ^{fghC*}	2.87±0.26 ^{fghCD*}
Pak choi	C	94.65±0.47 ^{abA**}	10.23±0.19 ^{defB***}	4.17±0.59 ^{fc**}	2.31±0.08 ^{cd*}	11.64±0.90 ^{abcdA*}	2.19±0.06 ^{hd**}
	S	94.74±0.52 ^{abA**}	9.33±0.05 ^{efgB**}	3.66±0.12 ^{fc**}	2.33±0.31 ^{cd*}	13.22±0.49 ^{aA**}	3.54±0.76 ^{cdefD**}
	H	93.69±0.88 ^{abcdA*}	8.60±0.13 ^{ghiB*}	3.35±0.15 ^{fgC*}	2.23±0.18 ^{cd**}	10.81±0.39 ^{aA*}	2.34±0.04 ^{ghD*}
Radish sango	C	95.27±0.19 ^{aA**}	9.63±0.71 ^{defgD***}	3.13±0.18 ^{fgD**}	3.90±0.07 ^{cdB*}	10.56±0.28 ^{cdefBC*}	6.11±0.21 ^{aA**}
	S	95.33±0.10 ^{aA**}	6.20±0.08 ^{hijD**}	2.23±0.19 ^{gD**}	4.02±0.14 ^{bcdB*}	10.74±0.24 ^{cdefBC**}	4.34±0.37 ^{bcA**}
	H	91.82±0.26 ^{cA*}	6.20±0.08 ^{ijD*}	2.13±0.03 ^{gD*}	3.78±0.16 ^{dB**}	10.56±0.20 ^{cdefBC*}	4.28±0.04 ^{bcA*}
Beetroot	C	94.56±0.78 ^{abA**}	18.55±0.04 ^{aA***}	6.24±0.21 ^{cdeB**}	4.76±0.13 ^{aA*}	11.30±0.08 ^{bcdAB*}	3.39±0.40 ^{cdefgC**}
	S	94.99±0.18 ^{abA**}	15.40±0.51 ^{bA**}	6.29±0.16 ^{bcdB**}	4.81±0.21 ^{aA*}	11.35±0.41 ^{bcdAB**}	4.11±0.54 ^{bcdC**}
	H	94.29±0.71 ^{abcA*}	12.11±0.25 ^{cA*}	6.08±0.19 ^{cdeB*}	4.57±0.07 ^{abA**}	11.03±0.52 ^{bcdAB*}	2.89±0.11 ^{fghC*}
Red amaranthus	C	94.24±0.03 ^{abcAB**}	16.39±0.54 ^{bA***}	6.17±0.20 ^{cdeAB**}	2.68±0.10 ^{cd*}	7.84±0.86 ^{hd*}	2.04±0.18 ^{hE**}
	S	94.59±0.46 ^{abAB**}	15.64±0.30 ^{bA**}	7.51±0.51 ^{abAB**}	2.73±0.39 ^{cd*}	9.47±0.60 ^{fghD**}	2.27±0.13 ^{ghE**}
	H	92.54±2.13 ^{bcdAB*}	14.92±0.25 ^{bA*}	5.71±0.04 ^{deAB*}	2.62±0.10 ^{cd**}	8.28±0.11 ^{ghD*}	2.04±0.07 ^{hE*}
Flax crops	C	93.56±0.50 ^{abcdAB**}	7.72±0.53 ^{hijE***}	6.18±0.84 ^{cdeB**}	4.70±0.07 ^{aA*}	9.81±0.82 ^{efgBC*}	3.39±0.40 ^{defghCD**}
	S	94.34±0.52 ^{abcAB**}	7.12±0.54 ^{ijE**}	6.10±0.06 ^{cdeB**}	4.70±0.21 ^{aA*}	12.76±0.12 ^{abBC**}	4.14±0.54 ^{cdefCD**}
	H	93.02±0.45 ^{abcdeAB*}	4.91±0.18 ^{ke*}	5.50±0.24 ^{cdB**}	4.51±0.08 ^{abcA**}	9.79±0.28 ^{efgBC*}	2.89±0.11 ^{fghCD*}

Two way- ANOVA was applied to estimate the effect of growing medium on selected microgreens and results were presented as mean ± standard deviations of different parameters. Tukey's HSD test was performed to underline differences in parameters. Different lowercase letters in the column indicate significant (P<0.05) differences among the microgreens grown in three growing mediums. Different uppercase letters in the column indicate significant (P<0.05) differences among the microgreens. The symbol (*, **, ***) in the column indicates the significant (P<0.05) differences among the growing medium. Where, C: Cocopeat; S: Soil; H: Hydroponics

The growing mediums showed a very significant effect on the moisture content of selected families of microgreens. Cocopeat growing medium has the ability to bind water because it has the nature of crumbs so that air, water, and roots easily enter the growing media and can bind water as compared to soil-based growing medium. Cocopeat growing medium essential nutrient content such as sodium, nitrogen, phosphorus, and potassium content was high as compared to soil growing medium (Widiwujani et al., 2020).

Ash content or total ash measures the number of total minerals present in biomass it is an important parameter in food products. The ash content of the studied microgreens shows significant differences among all microgreens grown in two growing mediums. The ash content of microgreens ranges from 6.20 to 18.55 g/100g, DW. Among all microgreens, the beetroot variety cultivated in the cocopeat medium shows the highest ash content and the radish sango cultivated in the soil and hydroponic based medium shows the lowest ash content (**Table 6.3**). The ash content highest concentration found in Amaranthaceae and the lowest in Linaceae microgreens were observed. Moreover, growing medium shows significant effects on the ash content of microgreens. The ash content in two species of cabbage (red and white) microgreens 16.06-25.60 g/100g was found. This study showed that LED light treatments (white, white with red, and white with blue) significantly effect on ash content of cabbage microgreens (Podsędek et al., 2023). Paradiso et al. (2018) characterized the nutritional profile of six genotypes of microgreens and from this study, the ash content was found in between 0.9 to 1.1 g/100g, DW. Kowitcharoen et al. (2021) reported an ash content of 0.34 – 0.75 g/100g in 14 microgreens belonging to different families. The various factors influencing the ash content of microgreens due to variations in cultivation methods, type of growing medium, illumination intensity, and insect or pest attack these factors influence on ash content of microgreens (Kyriacou et al. 2019; Ferrarezi et al. 2022). The variations in the ash content of microgreens could be attributed to genotypic differences (Ghoora et al., 2020). Based on these research works, we found a higher amount of ash content in all selected microgreens. As a result, microgreens may be regarded as an excellent source of minerals in the human diet to fulfil dietary requirements.

The fat or lipid content of all selected microgreens varied in growing mediums i.e. 7.58 ± 0.06 g/100g, DW in broccoli microgreens in soil-based growing medium higher amount and 2.13 ± 0.03 g/100g, DW in radish sango microgreens grown in hydroponic, respectively in (**Table 6.3**). A significant difference was observed in the fat content of all microgreens grown in growing mediums. Moreover, in soil-based growth medium higher amount of fat content was observed except in pak choi, radish sango, and cabbage microgreens. The various factors showed a significant effect on the fat content of microgreens including species and variety,

growing medium, and growing conditions (Khatoon and Singh, 2022; Podsędek et al., 2023; Sanyukta et al. 2023). Microgreens contain a lower amount of fat content as compared with green leafy vegetables (Paradiso et al., 2018). The fat content is within a similar range to that reported in white cabbage and red cabbage microgreens (3.78 to 7.97 g/100g) as per Podsędek et al. (2023). In the *Brassicaceae* (broccoli, Chinese kale, purple radish, radish rat-tailed radish, and red cabbage) and *Fabaceae* (lentil) microgreens the fat content was observed in the range of 0.38 to 0.50 g/100g, FW and 0.43 g/100g, FW observed in soilless growing medium (Kowitcharoen et al., 2021). Our findings suggest that microgreens, particularly those from the *Amaranthaceae* and *Linaceae* families, may have higher moisture content and lower fat content, may help prevent type-2 diabetes and weight gain because of their higher moisture and lower fat content (Saleh et al., 2022). Foods with a higher moisture content have more volume and weight without adding more calories, which helps with weight management by encouraging satiety and lowering caloric consumption overall. A lower-fat diet, especially one low in saturated fat, is also linked to a lower chance of insulin resistance and type 2 diabetes. Lower fat diets help to enhance insulin sensitivity and lipid profiles, both of which are important for both controlling and preventing type-2 diabetes (Ordovas et al., 2018).

The selected growing medium significantly influences the carbohydrate content of microgreens. The carbohydrate content of microgreens ranges from 2.17 to 4.81 g/100g, DW. Among all microgreens, the beetroot variety cultivated in the cocopeat medium shows the highest carbohydrate content and the pak choi microgreens shows the lowest carbohydrate content (Table 6.3). Different substrates can lead to variations in the nutritional composition of microgreens, including their carbohydrate content. The carbohydrate content in microgreens vary depending on the growing medium. Hydroponic growing medium generally have the lowest carbohydrate content compared to soil and cocopeat-based growing medium. Specifically, broccoli, pak choi and radish sango microgreens grown hydroponically have significantly lower carbohydrate levels than those grown in soil or cocopeat. This is likely due to the nutrient composition and water availability in hydroponic systems, which can reduce carbohydrate accumulation (Khatoon & Singh, 2022). Conversely, soil-based growing medium physicochemical properties (pH, electrical conductivity, organic carbon, N, P, and K) these enhances photosynthesis and carbohydrate synthesis due to better nutrient cycling and aeration (Wei et al., 2024). Cocopeat, with its higher water retention capacity and moderate nutrient release, offers an intermediate effect, promoting carbohydrate accumulation (Tuckeldoe et al., 2023). Significant differences ($p < 0.05$) in carbohydrate content across the growing media highlight the influence of environmental and nutrient factors in carbohydrate synthesis and

storage in microgreens. These findings underscore the importance of the growing medium, with soil and cocopeat providing better nutritional profiles compared to hydroponics based growing medium.

The fiber content of selected microgreens ranged from 7.84 ± 0.86 to 13.22 ± 0.49 g/100g DW observed respectively in **(Table 6.3)**. Among all microgreens, pak choi microgreens grown in soil-based growing medium reflected the highest 13.22 ± 0.49 g/100g, DW fiber content and red amaranthus grown in cocopeat medium lowest 7.84 ± 0.86 g/100g DW fiber content was observed. Our results are notably lower than those reported by Devi et al. (2018) for dried wheatgrass powder, which had higher fiber content. Dietary fiber plays a crucial role in supporting digestive, cardiovascular, and skin health, as well as in lowering blood cholesterol and glucose levels (Polash et al., 2018). The protein content showed significant differences among all selected microgreens as well as both growing mediums. Radish sango microgreens cultivated in a cocopeat-based growing medium showed the highest range protein content i.e. 6.11 ± 0.21 g BSA/100g, DW. In red amaranthus microgreens, the lowest 2.04 ± 0.18 g BSA/100g, DW protein content was observed in a cocopeat-based growing medium compared with other microgreens presented in **(Table 6.3)**. Kowitcharoen et al. (2021) reported a protein content of 1.88-6.47 g/100g FW in 14 microgreens belonging to different families. *Brassicaceae* microgreens reported a higher amount of protein reported by Paradiso et al. (2018). Meanwhile, our study results also supported *Brassicaceae* (cabbage, broccoli, radish sango, and pak choi) containing high protein content, followed by *Linaceae* (flax crops) and *Amaranthaceae* (red amaranthus and beetroot) families of microgreens (Sanyukta et al., 2023). Ghoola et al. (2020) also found comparable protein content in ten culinary microgreens, ranging from 1.8 to 4.4 g/100g. The physicochemical properties of growing medium are crucial for plant growth during the development stage (Kyriacou et al., 2018). The variances observed in the proximate composition including moisture, ash, fat, fiber, and protein of microgreens can be attributed to disparities in their cultivation techniques (Eswaranpillai et al., 2023), growing environments, microgreen varieties, and nutrient solutions (Polash et al., 2019). Additionally, the nutritional profile of microgreens is affected by factors like light intensity, consistent provision of specific supplements in nutrient solutions, and issues related to insects or pests (Sanyukta et al., 2023).

6.1.1.3. Photosynthetic pigments

The photosynthetic pigment contents of different selected microgreens grown in different growing medium are shown in **(Table 6.4)**. The chlorophyll content of the selected microgreens ranged from 11.72 ± 1.31 – 29.16 ± 0.91 $\mu\text{g/g}$, FW, with the highest concentration in

cabbage microgreen grown in cocopeat medium chlorophyll a 15.95 ± 0.62 $\mu\text{g/g}$, FW and least in beetroot microgreens grown in cocopeat 3.75 ± 0.06 $\mu\text{g/g}$, FW was found. Flax crops microgreens grown in cocopeat growing medium have 6.49 ± 0.08 $\mu\text{g/g}$ FW reported the highest chlorophyll b and a low concentration was found in the red amaranthus microgreens grown in soil 2.52 ± 0.40 $\mu\text{g/g}$, FW. The order of chlorophyll content across selected families was *Linaceae* > *Brassicaceae* > *Amaranthaceae*. Significant variations ($P < 0.05$) were noted in photosynthetic pigments content among different families and microgreens. This similar range was found in selected legumes (sanifon, alfalfa, red cover, mung bean, chickpea, maize, lentils, and black pea) for chlorophyll a $6.25 - 24.7$ $\mu\text{g/g}$, FW, chlorophyll b $5.117 - 11.385$ $\mu\text{g/g}$, FW and total chlorophyll $8.423 - 36.632$ $\mu\text{g/g}$, FW (Altuner, 2021). Comparable results were also obtained in wheat landrace (kose and kirik), wheat (alparslan), barley (beysehir), and oats (seydisehir, sari, and Feith) microgreens in terms of chlorophyll a $12.410 - 27.989$ $\mu\text{g/g}$, FW, chlorophyll b $3.773 - 7.450$ $\mu\text{g/g}$, FW and total chlorophyll $16.184 - 35.438$ $\mu\text{g/g}$, FW (Altuner et al., 2021). In another study, cabbage, and rocket microgreens were grown in cocopeat-based media with nutrient supplementation, this study showed chlorophyll a $5.0 - 5.5$ $\mu\text{g/g}$, FW, chlorophyll b $2.1 - 2.2$ $\mu\text{g/g}$, FW and $7.2 - 7.5$ $\mu\text{g/g}$, FW (El-Nakhel et al., 2021). The current findings results are slightly higher than those of previous research works. These obtained data confirm that the photosynthetic pigment content is highly varied in microgreen varieties according to their growing methods. Both the growing mediums show significant differences in chlorophyll content of selected botanical families microgreens. The nutrient profile (N, P, and K) level is high in comparison to a soil-based growing medium. The chlorophyll compound contains the Mg, N and one Mg atom binds four atoms. The outcome demonstrated that the composition of the growing medium can meet the nutrient needs of microgreens and it can support the metabolic process and help plant growth as well as the photosynthesis process (Widiwujani et al., 2020). Apart from chlorophylls health-enriching properties, it also helps in enriching the appearance of microgreens. Microgreens with a high chlorophyll concentration also have greener-coloured leaves. Microgreens have a range of green hues that enhance their aesthetic appeal. These differences in chlorophyll content highlight the influence of genotype and microgreen species. Evidence suggests that the concentrations of chlorophylls in younger leaves of the same species are lower compared to mature leaves, likely due to physiological factors. Additionally, some studies have not observed a significant impact of the growing media on phytochemical compounds when the harvesting cycle is short (Thepsilvisut et al., 2023).

Table 6.4. Photosynthetic pigments content of microgreens grown in three growing mediums

Microgreens	Growing medium	Chlorophyll a (µg/g, FW)	Chlorophyll b (µg/g, FW)	Chlorophyll a+b (µg/g, FW)	Total carotenoid (µg/g, FW)
Cabbage	Cocopeat	15.95±0.62 ^{aB***}	6.21±0.19 ^{aA**}	29.16±0.91 ^{aA**}	7.31±0.07 ^{abcB**}
	Soil	14.23±0.19 ^{cB*}	6.49±0.08 ^{aA**}	26.28±0.01 ^{bA**}	6.05±0.10 ^{efgB*}
	Hydroponics	12.78±0.15 ^{bB**}	5.68±0.08 ^{abA*}	26.59±0.40 ^{bA*}	6.05±0.10 ^{defB*}
Broccoli	Cocopeat	12.03±0.11 ^{cC***}	4.94±0.26 ^{bcB**}	23.96±0.33 ^{cB**}	5.89±0.07 ^{defD**}
	Soil	9.77±0.29 ^{dC*}	4.99±0.14 ^{bcB**}	22.23±0.60 ^{cdB**}	4.88±0.13 ^{ghD*}
	Hydroponics	10.33±0.29 ^{dC**}	4.27±0.08 ^{cdeB*}	20.72±0.46 ^{dB*}	4.39±0.26 ^{hijD*}
Pak choi	Cocopeat	5.45±0.16 ^{fgE***}	3.34±0.20 ^{efghC**}	15.80±0.32 ^{efgD**}	3.47±0.04 ^{iE**}
	Soil	6.12±0.23 ^{efE*}	3.56±0.37 ^{dcfgC**}	16.41±0.61 ^{ed**}	3.72±0.00 ^{ijE*}
	Hydroponics	6.13±0.23 ^{efE**}	3.00±0.04 ^{fghiC*}	15.99±0.16 ^{efD*}	3.74±0.08 ^{ijE*}
Radish sango	Cocopeat	10.22±0.27 ^{dD***}	4.03±0.28 ^{cdefC**}	21.14±0.60 ^{dC**}	6.27±0.03 ^{defC**}
	Soil	3.39±0.32 ^{hD*}	2.57±0.20 ^{ghiD**}	20.58±0.16 ^{hiC**}	4.36±0.01 ^{hijC*}
	Hydroponics	10.06±0.12 ^{dD**}	3.61±0.13 ^{fghiD*}	12.81±0.49 ^{dC*}	6.39±0.36 ^{cdeC*}
Beetroot	Cocopeat	3.75±0.06 ^{hE***}	2.56±0.24 ^{iD**}	13.26±0.24 ^{hiE**}	7.42±0.02 ^{abA**}
	Soil	6.73±0.04 ^{efF*}	3.05±0.21 ^{ghiD**}	11.72±1.31 ^{efE**}	8.09±0.39 ^{aA*}
	Hydroponics	3.44±0.62 ^{hF**}	1.95±0.04 ^{ghiD*}	16.68±0.04 ^{iE*}	6.12±0.76 ^{defA*}
Red amaranthus	Cocopeat	4.49±0.13 ^{ghF***}	2.55±0.81 ^{ghiD**}	13.99±1.03 ^{fghE**}	4.66±0.11 ^{ghiD**}
	Soil	4.54±0.59 ^{ghF*}	2.52±0.40 ^{ghiD**}	14.04±0.17 ^{fghE**}	4.82±0.33 ^{ghD*}
	Hydroponics	4.51±0.10 ^{ghF**}	2.25±0.03 ^{hiD*}	13.69±0.01 ^{ghiE*}	4.68±0.02 ^{ghiD*}
Flax crops	Cocopeat	16.03±0.49 ^{aA***}	5.07±0.13 ^{bcB**}	28.13±0.49 ^{abA**}	6.53±0.29 ^{bcdC**}
	Soil	14.30±0.10 ^{bA*}	5.65±0.36 ^{abB**}	26.92±0.40 ^{bA**}	5.32±0.06 ^{fghC*}
	Hydroponics	15.82±0.20 ^{aA**}	4.47±0.22 ^{cdB*}	27.18±0.01 ^{abA*}	5.48±0.04 ^{efgC*}

Two way- ANOVA was applied to estimate the effect of growing medium on selected microgreens and results were presented as mean ± standard deviations of different pigments content parameters. Tukey's HSD test was performed to underline differences in the parameters of selected microgreens. Different lowercase letters in the column indicate significant (P<0.05) differences among the microgreens grown in three growing mediums. Different uppercase letters in the column indicate significant (P<0.05) differences among the microgreens. The symbol (*, **, ***) in the column indicates the significant (P<0.05) differences among the growing medium.

The carotenoids are another pigment with a similar pattern to total chlorophyll content, ranging from 3.47 ± 0.04 to 8.09 ± 0.39 $\mu\text{g/g}$, FW as reported in (**Table 6.4**). Higher carotenoid content was found in beetroot microgreens 8.09 ± 0.39 $\mu\text{g/g}$ FW grown in soil and least observed in pak choi microgreens 3.47 ± 0.04 $\mu\text{g/g}$, FW grown in cocopeat substrates. The lower content was recorded in microgreens pak choi, while radish sango microgreens displayed the highest. Significant variations ($p < 0.05$) were noted in carotenoid content among families and microgreens. This similar range was reported in selected legumes (sanifon, alfalfa, red cover, mung bean, chickpea, maize, lentils, and black pea) carotenoid content ranged from 4.327 - 5.631, $\mu\text{g/g}$ FW and in lentils microgreen carotenoid content reported 5.230 $\mu\text{g/g}$, FW, the mentioned range was very similar to flax crops/lentils microgreens carotenoid content results (Altuner, 2021). Additionally, carrot and dill microgreens were cultivated hydroponically with protein hydrolysate in the nutrient solution and carotenoid levels ranging from 0.291 to 0.340 $\mu\text{g/g}$, FW, were recorded (El-Nakhel et al., 2021). The difference in microgreen carotenoid pigments is associated with the light intensity, and spectral quality used during growing conditions/ day of harvest and based on differences arising with the microgreen species and variety used for cultivations (Kopsell and Sams, 2013; Samuolienė et al. 2017). From our examination, it is concluded chlorophyll content is higher than carotenoid pigments (Mroczek-Zdyrska et al., 2016; Klopsch et al. 2018).

6.1.1.4. Biochemical compounds

6.1.1.4.1. Anthocyanin content

Anthocyanin possesses a range of colors including red, orange, blue, or purple coloration to plant tissues (Kowitcharoen et al., 2021). The radish sango, beetroot, and red amaranthus exhibit purplish-red hypocotyls owing to the accumulation of anthocyanins. The anthocyanin content of all selected microgreens was varied in both growing mediums i.e. 2.04 ± 0.08 – 45.87 ± 0.16 $\mu\text{mol}/100\text{g}$, FW, respectively in (**Table 6.5**). All selected microgreens showed significant differences in anthocyanin content grown in growing mediums. Radish sango microgreens reported higher anthocyanin content compared to other microgreens grown in both mediums and flax crops microgreens reported lower content in both growing substrates. Notably, significant variations in anthocyanin content were observed among the selected crop families (*Amaranthaceae*, *Brassicaceae*, and *Linaceae*) ($P < 0.05$). Martínez-Ispizua et al. (2022) studied the 11 lettuce varieties that evaluated anthocyanin content in the three development stages (microgreen, baby, adult) in lettuce microgreens 36.12 – 72.34 $\mu\text{mol}/100\text{g}$, FW, where our results are similar to this research works. Moreover, in growing mediums significant differences were observed in anthocyanin content. The composition of growing

medium such as pH has shown a significant effect on the anthocyanin content of selected families of microgreens. The pH content of the cocopeat (6.09) growing medium is slightly acidic compared to the soil-based (7.60) growing medium. The slightly acidic pH of the growing substrate helps to enhance the availability of Zn in root development and this in turn increases the accumulation of plant anthocyanin content. In addition, micronutrients (iron, manganese, and zinc) are responsible for pH decreases in the plant-growing medium (Saleh et al., 2022; Khandaker et al., 2019). The pH, acidity, and composition of the growing medium these parameters influence pigment accumulation, particularly anthocyanins (Khandaker et al., 2019).

6.1.1.4.2. Ascorbic acid

The ascorbic acid content in selected microgreens grown in three different growing mediums was shown in (Table 6.5). Ascorbic acid ranged from 177.59 ± 0.91 to 438.20 ± 1.15 mg/100g, FW, with the highest concentration in cabbage microgreens grown in cocopeat medium and lowest in flax crops microgreens grown in hydroponic medium observed. Rohmanna and Mulyawan (2022) studied the effect of growing media (organic soil and rockwool) on *Cassia alata* L. (Gelinggang) microgreens. From this study, ascorbic acid concentration found in *Cassia alata* L. microgreens ranged from 563.16 – 628.857 mg/100g, FW. In another study, in five different microgreens (kale, radish, beetroot, green peas, and amaranth) the ascorbic acid content reported was 6.6 – 31.1 mg/100, FW (Wojdyło et al., 2020). In another study, cabbage and rocket microgreens were grown in peat-based media with nutrient supplementation, from this research work 246.3 – 390.6 mg/100g, FW ascorbic acid content was reported (El-Nakhel et al., 2021). Xiao et al. (2012) reported a range of total ascorbic acid contents ranging from 20.4–147.0 mg/100g, FW in 25 commercially available microgreens and the study claimed microgreens have higher total ascorbic acid concentrations than mature plants. Various factors are responsible for variation in the ascorbic content of microgreens such as species and varieties, oxygen, water, and temperature. The types of growing medium substrate can accumulate oxygen and affect ascorbic acid content in plants. The growing medium particle size and its fiber content help to accumulate oxygen and provide good aeration for plant growth (Rohmanna and Mulyawan, 2022). Various factors such as environmental conditions, growing medium, and light influence the accumulation of ascorbic acid in soilless grown leafy vegetables. Notably, a lower pH level in the growth medium was found to enhance ascorbic acid content in plants (Ntsoane et al., 2023). As microgreens are generally consumed fresh, ascorbic acid can be largely retained without cooking (Lee et al., 2017; Yadav et al., 2018).

Table 6.5. Biochemical parameters of microgreens grown in three growing mediums

Microgreens	Growing medium	Anthocyanin ($\mu\text{mol}/100\text{g}$, FW)	Ascorbic acid ($\text{mg}/100\text{g}$, FW)	Total glucosinolate ($\mu\text{mol}/\text{g}$, FW)	TPC (mg GAE/100g, FW)	TFC (mg QE/100g, FW)
Cabbage	Cocopeat	3.85 $\pm$ 0.23 ^{fghiD***}	438.71 $\pm$ 1.15 ^{aA***}	36.74 $\pm$ 0.08 ^{cdD**}	357.21 $\pm$ 0.51 ^{bB***}	33.26 $\pm$ 0.17 ^{bA***}
	Soil	3.54 $\pm$ 0.42 ^{fghiD*}	329.20 $\pm$ 0.91 ^{dA**}	35.72 $\pm$ 0.17 ^{deD**}	350.81 $\pm$ 0.64 ^{cB**}	39.74 $\pm$ 0.28 ^{aA**}
	Hydroponics	3.63 $\pm$ 0.51 ^{fghiD**}	243.15 $\pm$ 1.25 ^{iA*}	24.47 $\pm$ 0.76 ^{fD*}	319.64 $\pm$ 0.38 ^{fB*}	15.44 $\pm$ 0.14 ^{iA*}
Broccoli	Cocopeat	3.65 $\pm$ 0.05 ^{fghiD***}	305.17 $\pm$ 0.69 ^{cC***}	35.54 $\pm$ 0.25 ^{deC**}	328.38 $\pm$ 0.76 ^{cC***}	27.99 $\pm$ 0.07 ^{efD***}
	Soil	4.03 $\pm$ 0.21 ^{fghiD*}	369.36 $\pm$ 1.36 ^{cC**}	35.24 $\pm$ 0.17 ^{deC**}	320.23 $\pm$ 0.70 ^{fC**}	30.46 $\pm$ 0.11 ^{cD**}
	Hydroponics	3.94 $\pm$ 0.13 ^{fghiD**}	256.46 $\pm$ 1.14 ^{hC*}	34.33 $\pm$ 0.25 ^{eC*}	298.56 $\pm$ 0.64 ^{gC*}	10.94 $\pm$ 0.28 ^{lD*}
Pak choi	Cocopeat	4.54 $\pm$ 0.13 ^{fd***}	204.84 $\pm$ 1.15 ^{kG***}	38.00 $\pm$ 0.34 ^{cB**}	257.98 $\pm$ 0.83 ^{iG***}	20.18 $\pm$ 0.59 ^{eE***}
	Soil	2.86 $\pm$ 0.09 ^{ijD*}	199.60 $\pm$ 0.57 ^{lG**}	37.88 $\pm$ 0.51 ^{cB**}	167.30 $\pm$ 0.25 ^{oG**}	28.94 $\pm$ 0.28 ^{deE**}
	Hydroponics	4.11 $\pm$ 0.18 ^{fgD**}	189.52 $\pm$ 2.28 ^{noG*}	38.18 $\pm$ 1.10 ^{cB*}	131.67 $\pm$ 0.95 ^{pG*}	18.14 $\pm$ 0.28 ^{hE*}
Radish sango	Cocopeat	45.87 $\pm$ 0.16 ^{aA***}	374.28 $\pm$ 0.35 ^{bB***}	49.79 $\pm$ 0.17 ^{aE**}	667.12 $\pm$ 0.51 ^{aA***}	28.02 $\pm$ 0.06 ^{defB***}
	Soil	28.06 $\pm$ 0.15 ^{cA*}	328.55 $\pm$ 0.45 ^{dB**}	49.85 $\pm$ 0.25 ^{aE**}	241.98 $\pm$ 0.38 ^{lA**}	39.51 $\pm$ 0.16 ^{aB**}
	Hydroponics	28.25 $\pm$ 0.67 ^{cA**}	266.29 $\pm$ 1.37 ^{gB*}	47.38 $\pm$ 0.51 ^{bE*}	260.27 $\pm$ 1.27 ^{jA*}	16.14 $\pm$ 0.28 ^{jB*}
Beetroot	Cocopeat	34.97 $\pm$ 0.06 ^{bB***}	369.52 $\pm$ 0.91 ^{cD***}	NA	340.99 $\pm$ 0.51 ^{dD***}	28.59 $\pm$ 0.07 ^{defA***}
	Soil	27.94 $\pm$ 0.18 ^{dB*}	252.91 $\pm$ 0.69 ^{hiD**}	NA	339.10 $\pm$ 0.13 ^{dD**}	40.01 $\pm$ 0.10 ^{aA**}
	Hydroponics	23.91 $\pm$ 0.11 ^{cB**}	195.40 $\pm$ 1.48 ^{lmD*}	NA	248.79 $\pm$ 0.06 ^{kD*}	17.84 $\pm$ 0.14 ^{hiA*}
Red amaranthus	Cocopeat	9.38 $\pm$ 0.01 ^{cC***}	277.74 $\pm$ 0.45 ^{fE***}	6.13 $\pm$ 0.34 ^{gA**}	350.81 $\pm$ 0.64 ^{cE***}	20.74 $\pm$ 0.85 ^{gF***}
	Soil	10.29 $\pm$ 0.34 ^{cC*}	249.56 $\pm$ 0.52 ^{iE**}	5.23 $\pm$ 0.25 ^{ghA**}	286.27 $\pm$ 0.57 ^{hE**}	27.26 $\pm$ 0.17 ^{ffF**}
	Hydroponics	9.86 $\pm$ 0.34 ^{cC**}	192.74 $\pm$ 2.28 ^{mnE*}	4.09 $\pm$ 0.17 ^{hA*}	284.19 $\pm$ 0.70 ^{hE*}	13.44 $\pm$ 0.14 ^{kF*}
Flax crops	Cocopeat	2.04 $\pm$ 0.08 ^{jiE***}	275.73 $\pm$ 1.03 ^{fiE***}	NA	271.76 $\pm$ 0.45 ^{fiE***}	30.47 $\pm$ 0.10 ^{cC***}
	Soil	3.07 $\pm$ 0.05 ^{ghijE*}	187.10 $\pm$ 1.14 ^{oF**}	NA	235.77 $\pm$ 0.38 ^{mF**}	29.44 $\pm$ 0.99 ^{cdC**}
	Hydroponics	3.07 $\pm$ 0.05 ^{hijE**}	177.59 $\pm$ 0.91 ^{pF*}	NA	226.49 $\pm$ 0.64 ^{nF*}	16.64 $\pm$ 0.14 ^{ijC*}

Two way- ANOVA was applied to estimate the effect of growing medium on selected microgreens and results were presented as mean $\pm$ standard deviations of different biochemical parameters. Tukey's HSD test was performed to underline differences in the biochemical parameters of selected microgreens. Different lowercase letters in the column indicate significant ($P<0.05$) differences among the microgreens grown in three growing mediums. Different uppercase letters in the column indicate significant ($P<0.05$) differences among the microgreens. The symbol (*, **, ***) in the column indicates the significant ($P<0.05$) differences among the growing medium. Where, TPC: Total phenol content; TFC: Total flavonoid content; GAE: Gallic acid; QE: Quercetin.

6.1.1.4.3. Total glucosinolate

The total glucosinolate content in selected families of microgreens ranged from 4.09 to 49.85 ± 0.25 $\mu\text{mol/g}$, FW with the radish sango exhibiting the highest value and red amaranthus the lowest, as shown in (Table 6.5). These findings align with recent research findings on radish (35.31 to 45.61 $\mu\text{mol/g}$, FW), cabbage (22.04 to 30.24 $\mu\text{mol/g}$, FW), and rocket (24.02 to 39.83 $\mu\text{mol/g}$, FW) microgreens (Ntsoane et al., 2023). Another study observed total glucosinolate content ranging from 1.0 to 535.5 $\mu\text{mol}/100$ g fresh weights across 30 *Brassicaceae* microgreen varieties (Xiao et al., 2018). Our research revealed the highest total glucosinolate content in *Brassicaceae*, followed by *Amaranthaceae* families of microgreens. The concentration of total glucosinolate content in different crops is significantly affected by factors such as developmental stage, environmental conditions (temperature and light), nutrient availability in the growing medium, plant variety, and density (Ishida et al., 2014; Mawlong et al., 2017).

6.1.1.4.4. Total phenol content

In microgreens, phenolic compound is the third important class of phytochemicals. The total phenolic content of different selected microgreens grown in soil and cocopeat-based growing medium was reported to range from 131.67 ± 0.95 to 667.48 ± 1.02 mg GAE/100g, FW and the results are shown in (Table 6.5). The highest total phenol content was recorded in radish sango microgreens grown in cocopeat at 667.48 ± 1.02 mg GAE/100g, FW while the least content was found in pak choi microgreens grown in hydroponic medium at 131.67 ± 0.95 mg GAE/100g, FW. Significant differences in total phenolic content were observed across the three microgreen families: *Amaranthaceae*, *Brassicaceae*, and *Linaceae*.

These values were comparable with those obtained in mungbean microgreens 190.92 – 241.75 mg GAE/100g and lentil microgreens 212.60 – 321.35 mg GAE/100g, FW. This study shows that diverse genotypes of mungbean and lentil were grown as microgreens under plain-altitude (Delhi) and high-altitude (Leh) conditions, which showed significant genotypic variations for phytochemicals (Priti et al., 2021). Another study on 30 commercial varieties of *Brassicaceae* microgreens total phenolic content of 88.6 – 811.2 mg GAE/100g, FW was reported. In ruby radish microgreens 811.2 mg GAE/100g, FW has been reported (Xiao et al., 2018). Within the *Brassicaceae* family, consisting of 14 microgreen crops substantial variations in total phenolic content were observed ranging from 9.22 to 268.99 mg GAE/100g, FW (Kowitcharoen et al., 2021). In our experimental work radish sango microgreens grown in selected mediums showed higher results of the phenolic compounds compared to other microgreens. The phytochemicals profiles are significantly different in microgreen species, these species contained some flavan-3-ols (catechins and polymeric procyanidins), phenolic

acids (mainly caffeoylquinic, ferulic, sinapic, chlorogenic, and their derivatives), flavonols (mainly quercetin, kaempferol, isorhamnetin), and flavones (daidzein and genistein). Some samples (radish, beetroot, amaranth) comprised also anthocyanins, mainly cyanidin derivatives. Phenolic acids, such as caffeoylquinic, ferulic, sinapic, or chlorogenic acid, and their derivatives are present (Wojdyło et al., 2020). The different kinds of intrinsic and extrinsic factors are known to affect phenolic compounds, like growing conditions, species/varieties, harvesting stage, and preparation of samples (Ghoora et al., 2020). Overall, the present results show that using a cocopeat medium enhances the concentration of phytonutrients content in microgreens. The cocopeat-based growing medium provided optimum physicochemical conditions to support the growth and increased various microgreens biochemical parameters. The maximum level of bulk density, water-holding, and air-holding capacities, and porosity shows a significant effect on growth and biochemical parameters (Kyriacou et al., 2019; El-Nakhel et al., 2021). The soilless substrate exhibited superior moisture and water retention capabilities compared to other substrates. This likely induced physiological stress to the roots triggered enhanced metabolic activity, and activated defensive responses in microgreens, leading to an increased accumulation of total phenol content (Ntsoane et al., 2023).

6.1.1.4.5. Total flavonoid content

Total flavonoid content varied among all microgreens in both growing mediums are shown in **(Table 6.5)**. The concentration of total flavonoid content ranged from 10.94 ± 0.28 – 40.01 ± 0.10 mg QE/100g, FW in among the growing mediums. The highest flavonoid content was observed in beetroot microgreens 40.01 ± 0.10 mg QE/100g, FW in soil medium and least observed in broccoli microgreens 40.01 ± 0.10 mg QE/100g, FW grown in hydroponic medium, respectively. Similar trends were observed in the flavonoid content among selected summer season leafy greens compared at mature and microgreens stage 9.58 – 240.30 mg CE/100g, FW (Yadav et al., 2018). In another study twelve vegetable sprouts (broccoli, red radish, radish, mizuna, kale, taatsai, pak choi, Chinese cabbage, turnip, rapeseed, chicory, and alfalfa) were grown for seven days. The total flavonoid contents of broccoli, red radish, and radish sprout were found 25.36 , 25.26 , and 25.16 mg CE/100g, FW (Lee et al., 2021). The growing medium, species, and cultivation systems are all factors considerably reported to affect flavonoid synthesis (Koh et al., 2021). These findings underscore the significant influence of intrinsic and extrinsic factors, including species, growing conditions, harvesting days, and pre-harvest/postharvest conditions, on the variation in total phenolic and flavonoid content across microgreens (Ntsoane et al., 2023; Acharya et al., 2021).

6.1.1.5. Antioxidant activities

The antioxidant capacity of selected microgreens grown in different growing mediums was determined using the ferric reducing antioxidant potential (FRAP), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. The results obtained from this assay are summarized in (Table 6.6).

6.1.1.5.1. Antioxidant activity DPPH

The antioxidant activity DPPH ranges from 56.21 ± 0.08 – $89.24 \pm 0.91\%$, FW in all selected microgreens that are grown in both growing mediums. Higher antioxidant capacities were found in radish sango microgreens $89.24 \pm 0.91\%$, FW grown in cocopeat and in contrast, red amaranthus microgreens $56.21 \pm 0.08\%$, FW grown in soil substrates showed minimal inhibition activity. Similarly, the antioxidant capacity in spinach microgreens is 39.16 % and in fenugreek microgreens, 41.19% has been reported (Sharma et al. 2021; Sharma et al. 2020). A study comparing the antioxidant capacity of the lettuce crops in three development stages, this comparative study indicated that fully developed lettuce microgreens had 12.65 – 88.46 % scavenging effects whereby at the baby stage 32.61 – 87.02 %, and the adult stage 3.58 – 39.41 % scavenging effects were reported, respectively (Martínez-Ispizua et al., 2022). The soil and cocopeat-based growing medium showed a significant effect on the enhancement of antioxidant activity in the selected microgreens. In general, the occurrences of higher phenolic contents enhance the antioxidant property of the selected microgreens grown in a cocopeat growing medium because phenols possess ideal structural properties for scavenging free radicals and thus they prevent oxidative damages. The antioxidant ability assessment by DPPH radical scavenging revealed the greatest DPPH scavenging ability of the microgreens. Total flavonoid content has been high in microgreens grown in cocopeat-based growing medium and significant differences observed growing medium cocopeat and soil (Wei et al., 2018).

6.1.1.5.2. Antioxidant activity ABTS

The ABTS assay evaluates a substance's capacity to counteract ABTS radicals, with greater inhibition percentages indicating stronger antioxidant properties. Among the selected microgreens, notable variations in ABTS free radical scavenging activity were observed. Radish sango, belonging to the *Brassicaceae* family, demonstrated the highest ABTS scavenging activity at $89.71 \pm 0.10\%$, FW, followed by red amaranthus, pak choi, flax crops, beetroot, broccoli, and cabbage, which also exhibited ABTS scavenging activity. Additionally, comparable findings of ABTS scavenging activity ranging from 29.36 to 81.85%, FW were documented for spinach, carrot, bathua, and Bengal gram (Sanyukta et al., 2023). In other findings the various wheatgrass juices (black, purple, and blue) were the ABTS scavenging

activity ranging from 24.18 to 51.40%, FW was observed (Sharma et al., 2020). These outcomes were inferior to our findings, possibly attributable to variations in microgreen types.

Table 6.6. Antioxidant activities (DPPH, ABTS, and FRAP) of microgreens grown in three growing mediums

Microgreens	Growing medium	Antioxidant activity DPPH (% _{FW})	Antioxidant activity ABTS (% _{FW})	Antioxidant activity FRAP (μmol TE/g _{FW})
Cabbage	Cocopeat	74.03±0.22 ^{deC***}	89.28±0.71 ^{aF***}	9.20±1.00 ^{dB**}
	Soil	71.82±0.10 ^{efC*}	83.60±0.40 ^{cF**}	11.62±0.59 ^{cB**}
	Hydroponics	67.84±0.45 ^{hiC**}	72.16±0.30 ^{iF*}	7.14±0.91 ^{efB*}
Broccoli	Cocopeat	74.18±0.43 ^{dD***}	88.35±0.81 ^{abDE***}	7.96±0.41 ^{deC**}
	Soil	64.36±0.14 ^{ijklD*}	88.06±0.21 ^{abcDE**}	6.49±0.33 ^{efgC**}
	Hydroponics	60.38±0.18 ^{nD**}	79.50±0.51 ^{hDE*}	5.55±0.33 ^{fghC*}
Pack choi	Cocopeat	69.14±0.33 ^{ghD***}	89.64±0.41 ^{bB***}	3.24±0.08 ^{ijDE**}
	Soil	62.07±0.81 ^{lmnD*}	87.99±0.11 ^{abcB**}	4.31±0.08 ^{hiDE**}
	Hydroponics	66.31±0.28 ^{ijD**}	85.04±0.21 ^{efB*}	4.01±0.34 ^{hijDE*}
Radish sango	Cocopeat	89.14±0.33 ^{aA***}	87.13±0.30 ^{bcdA***}	17.46±0.50 ^{aA**}
	Soil	61.71±0.80 ^{mnA*}	89.71±0.10 ^{aA**}	14.39±0.50 ^{bA**}
	Hydroponics	76.82±0.54 ^{cA**}	88.49±0.41 ^{abA*}	12.62±0.33 ^{bcA*}
Beetroot	Cocopeat	72.40±0.21 ^{defB***}	87.41±0.31 ^{bcE***}	5.25±0.42 ^{fghD**}
	Soil	80.22±0.19 ^{bB*}	85.11±0.51 ^{efE**}	4.43±0.25 ^{hiD**}
	Hydroponics	71.09±0.90 ^{fgB**}	81.51±0.51 ^{gE*}	4.25±0.50 ^{hijD*}
Red amaranthus	Cocopeat	69.46±0.31 ^{ghE***}	87.12±0.10 ^{bcdC***}	2.30±0.25 ^{jE**}
	Soil	56.21±0.08 ^{klE*}	86.26±0.30 ^{cdeC**}	5.01±0.75 ^{ghiE**}
	Hydroponics	64.21±0.90 ^{oE**}	85.33±0.40 ^{defC*}	3.78±0.50 ^{hijE*}
Flax crops	Cocopeat	65.68±1.32 ^{ijkE***}	86.76±0.61 ^{bcdC***}	4.90±0.59 ^{ghiDE**}
	Soil	63.81±0.13 ^{klmE*}	86.26±0.71 ^{cdeCD**}	4.78±0.25 ^{ghiDE**}
	Hydroponics	62.74±0.63 ^{lmE**}	83.60±0.61 ^{fCD*}	4.13±0.17 ^{hijDE*}

Two way- ANOVA was applied to estimate the effect of growing medium on selected microgreens and results were presented as mean ± standard deviations of different antioxidant parameters. Tukey's HSD test was performed to underline differences in the antioxidant parameters of selected microgreens.

6.1.1.5.3. Antioxidant activity FRAP

Total ferric reducing antioxidant potential (FRAP) varied among all microgreens cultivated in three growing mediums are shown in **(Table 6.6)**. The maximum FRAP activity was observed in radish sango microgreens grown in cocopeat 17.46±0.50 μmol TE/g, FW and minimum FRAP was observed in red amaranthus microgreens grown in cocopeat growing medium 2.30±0.25 μmol TE/g, FW. These values were comparable with those obtained in mungbean microgreens 5.70 – 20.44 μmol TE/g, FW and lentil microgreens 14.70 – 55.00 μmol TE/g, FW. This study shows that diverse genotypes of mungbean and lentil were grown

as microgreens under plain-altitude (Delhi) and high-altitude (Leh) conditions, which showed significant genotypic variations in the antioxidant activity of microgreens (Priti et al., 2021). The seedling soilless media (Hygromix, Promix, and TS1) were used for the cultivation of radish, cabbage, and rocket microgreens, and the FRAP antioxidant activity in ranged 1.2 to 7.0 mM TEAC/g, FW was observed (Ntsoane et al., 2023). The FRAP antioxidant activity in leafy greens is influenced by multiple factors such as horticultural practices, including the type of substrate utilized, the growing conditions, the plant species and cultivar, the stage of maturity, and the extraction methods employed. Each of these factors can potentially change the FRAP antioxidant content (Kullaj, 2016). The growing medium shows a significant effect on the antioxidant activity of microgreens. Many studies reported that strong correlation between the bioactive compounds and antioxidant activities. Our results are consistent with findings that the increase in antioxidant activity is due to the bioactive compounds (ascorbic acid, phenolic, and flavonoid content) in microgreens and growing medium soil and cocopeat (Virdi et al., 2020; Saleh et al., 2022).

6.1.1.4. Antinutrients

Antinutrients are the chemical compounds found in diverse types of foods that can interfere with the absorption of nutrients and these compounds show adverse effects on health when consumed in large amounts (Petroski and Minich, 2020). The different types of antinutrient contents present in microgreens are shown in **(Table 6.7)**. Tannins are naturally occurring plant compounds that possess both positive and negative properties. On the positive side, tannins exhibit strong antibacterial properties, helping to inhibit the growth of harmful pathogens, and they possess potent antioxidant properties that can reduce oxidative stress and inflammation, contributing to overall health. However, tannins can also act as antinutrients by binding to proteins and minerals, potentially reducing their absorption and bioavailability (Huang et al., 2017). The level of tannin content in all the microgreens ranged from 48.26 ± 0.90 to 286.27 ± 1.23 mg/100g, FW. The cabbage and radish sango microgreens exhibit higher levels of tannin content and flax crops microgreens were shown the lowest concentration for tannin content. Similar trends were observed in the tannin content for the lotus seed sprouts in water at different temperatures (25 and 35°C) i.e., 74 to 93 mg/100g, FW (Sathithon, 2012). In another study, the tannin content in lentil sprouts grown under different germination times (3 to 9 days) ranged from 0.60 to 77% was observed. From this study was shown that the increase in the germination days significantly reduced the tannin content (Aktaş et al., 2022).

Table 6.7. Antinutritional compounds of microgreens grown in three growing mediums

Microgreens	Growing medium	Tannin (mg/100g, FW)	Phytic acid (mg/100g, FW)	Oxalic acid (mg/100g, FW)
Cabbage	Cocopeat	232.38±1.34 ^{bA***}	471.53±0.43 ^{efC**}	46.78±0.06 ^{cC**}
	Soil	197.62±0.68 ^{cA**}	468.79±1.29 ^{fC*}	46.75±0.04 ^{cC**}
	Hydroponics	136.98±1.12 ^{eA*}	480.82±0.21 ^{cdC*}	51.57±0.54 ^{cC*}
Broccoli	Cocopeat	78.65±0.57 ^{iD***}	441.25±1.07 ^{iE**}	46.11±0.02 ^{fB**}
	Soil	82.86±0.90 ^{iD**}	411.41±1.08 ^{KE*}	45.48±0.06 ^{gB**}
	Hydroponics	72.86±0.34 ^{kD*}	414.00±1.29 ^{jkE*}	55.04±0.06 ^{bB*}
Pak choi	Cocopeat	71.83±1.24 ^{kF***}	464.69±1.07 ^{gD**}	46.58±0.08 ^{efD**}
	Soil	64.60±0.23 ^{lF**}	462.71±0.86 ^{gD*}	46.58±0.04 ^{efD**}
	Hydroponics	53.18±0.67 ^{mF*}	452.97±0.86 ^{hD*}	49.55±0.04 ^{dD*}
Radish sango	Cocopeat	286.27±1.23 ^{aB***}	108.52±1.07 ^{nG**}	45.52±0.08 ^{gA**}
	Soil	162.30±1.01 ^{dB**}	196.02±0.39 ^{lG*}	45.56±0.02 ^{gA**}
	Hydroponics	97.54±0.79 ^{hB*}	145.32±0.26 ^{mG*}	58.94±0.06 ^{aA*}
Beetroot	Cocopeat	124.92±0.23 ^{fC***}	478.49±0.49 ^{dB**}	46.89±0.06 ^{fF**}
	Soil	134.29±1.35 ^{eC**}	482.96±0.64 ^{cB*}	46.83±0.04 ^{eF**}
	Hydroponics	109.84±0.45 ^{gC*}	473.66±1.29 ^{eB*}	28.33±0.09 ^{iF*}
Red amaranthus	Cocopeat	78.34±0.78 ^{iD***}	513.86±0.43 ^{aA**}	46.45±0.08 ^{efG**}
	Soil	83.26±1.46 ^{iD**}	511.26±0.64 ^{aA*}	46.84±0.02 ^{eG**}
	Hydroponics	76.91±0.56 ^{jD*}	507.46±0.86 ^{bA*}	16.16±0.04 ^{jG*}
Flax crops	Cocopeat	95.64±1.24 ^{hE***}	413.40±0.86 ^{jk**}	46.81±0.06 ^{eE**}
	Soil	61.43±0.67 ^{lD**}	411.41±1.08 ^{kF*}	46.54±0.10 ^{efE**}
	Hydroponics	48.26±0.90 ^{nE*}	416.34±1.01 ^{jF*}	42.78±0.06 ^{hE*}

Two way- ANOVA was applied to estimate the effect of growing medium on selected microgreens and results were presented as mean ± standard deviations of different morphological parameters. Tukey's HSD test was performed to underline differences in the morphological parameters of selected microgreens.

The (**Table 6.7**) presents the phytic acid content found in various types of soilless grown microgreens. Phytic acid content varied between 108.52±1.07 to 513.86±0.43 mg/100g, FW, with the highest concentration detected in red amaranthus microgreens and the lowest in radish sango microgreens. Notably, pea and sunflower microgreens exhibited lower phytic acid levels compared to unsoaked and soaked (1.06 to 0.91 and 1.91 g/100g) for peas, and unsoaked (1.67 to 1.91 g/100g) sunflower seeds. Additionally, research by Aktaş et al. (2022) on lentil sprouts cultivated over varying germination periods (3 to 9 days) revealed phytic acid content ranging from 0.59 to 80% was observed.

Oxalic acid presents a significant level in green leafy vegetables and it is recognized as a major antinutritional compound. Its primary action involves obstructing the absorption of divalent cations like calcium through the formation of insoluble salts (Gemede, 2014). The

microgreens had oxalate content ranging from 16.16 ± 0.04 to 58.94 ± 0.06 mg/100g, FW in radish sango microgreens exhibited the highest, and red amaranthus microgreens showed the lowest concentration of oxalic acid content. The level of oxalic acid in microgreens is lower than its mature counterpart (Longvah, 2017). Similar findings were reported in ten cultivated culinary microgreens ranging from 14.3 to 68.2 mg/100g, FW oxalic acid (Singh et al., 2022). The phytase enzyme activities were on the rise and the reduction in phytic acid content at the germination process stage was observed (Poudel et al., 2023). Similarly, the decrease in tannin content of seeds during the germination process may be due to the leaching of tannins into water and subsequent binding of polyphenols with different organic substances (Aktaş et al., 2022). The growing medium fertility exerts effects on the oxalate levels of some plants. High-growing medium fertility associated with high carbon, nitrogen-available phosphorus, and potassium is associated with high total oxalate in *Amaranthus* and *Basella* (Syed et al., 2020).

6.1.1.5. Principal component analysis (PCA) of selected parameters in response to growing medium for microgreens

The principal component analysis (PCA) allowed us to summarize the main behaviors outlined with the univariate analysis of variance, highlighting the effect of the selected microgreens and three growing mediums on morphological, proximate, biochemical, and antioxidant parameters. PCA was also applied specifically to the morphological parameters, including plant height, leaf length, leaf width, fresh weight, and dry weight across the seven microgreen species crops and three growing media. The first two principal components (PC-1 and PC-2) accounted for a combined 84% of the total morphological variation (**Table 6.8**), with PC-1 explaining 70.6% and PC-2 contributing 13.4%. The PCA score plot (**Figure 6.4 A**) shows that radish sango in cocopeat formed a distinct grouping, characterized by greater plant height and fresh weight, distinguishing it from other species-media combinations. Hydroponic treatments again showed tighter clustering, reflecting uniform morphological development, likely due to consistent water and nutrient delivery. Soil and cocopeat treatments displayed broader scatter, highlighting variability in morphological outcomes influenced by environmental and substrate factors.

The PCA loading plot (**Figure 6.4 B, Table 6.8**) revealed that PC-1 was strongly influenced by plant height (0.410) and fresh weight (0.388), indicating that samples scoring high on PC-1 had taller and heavier plants. PC-2 was largely shaped by leaf width (0.365) and dry weight (0.332), capturing differences in leaf size and biomass retention. Notably, negative loadings on PC axes (leaf length -0.145 on PC-1) do not signify poor morphological performance but rather reflect the opposite trend relative to positive-scoring traits. These results

emphasize that radish sango in cocopeat optimally expressed morphological traits like height and fresh biomass, making it stand out in the PCA space. Hydroponically grown samples tight grouping confirms consistent morphological performance, while the broader scatter seen in soil and cocopeat groups suggests more heterogeneous morphological expression due to variable physical and nutrient characteristics of those media.

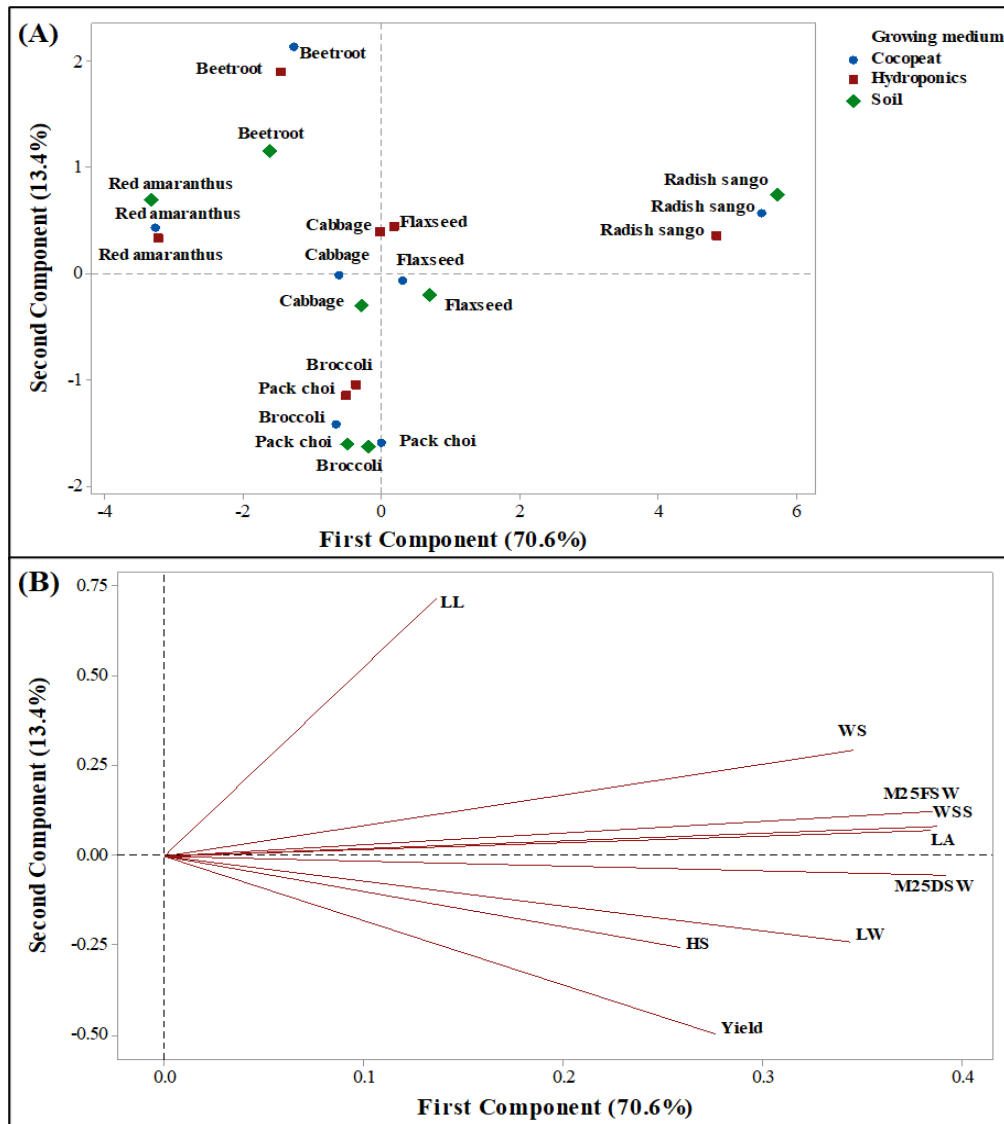


Figure 6.4. Principal component analysis score plot (A) and loading plot (B) describe the relationship among different morphological parameters of microgreens obtained from selected microgreens families varieties grown under different growing conditions.

Table 6.8. Loadings of the first three principal components based on factor analysis different morphological parameters of selected microgreens families varieties grown in different growing medium.

Factor loadings	Principal components (PC)		
	PC-1	PC-2	PC-3
Microgreens shoot height (SH)	0.259	-0.254	-0.645
Microgreens shoot width (WS)	0.346	0.294	0.161
Microgreens leaf length (LL)	0.136	0.718	-0.479
Microgreens leaf width (LW)	0.344	-0.238	0.363

Microgreen total leaf area (LA)	0.384	0.072	0.140
Microgreens weight of single shoot (WSS)	0.388	0.081	0.099
Microgreens 25 shoot fresh weight (M25FSW)	0.386	0.124	0.095
Microgreens 25 shoot dry weight (M25DSW)	0.392	-0.055	0.079
Yield	0.277	-0.496	0.232
Eigenvalue	6.3550	1.2035	1.0604
Total variance (%)	70.6	13.4	11.8
Cumulative variance (%)	70.6	84.0	95.8

Principal component analysis was carried out for a total of 15 dependent variables (proximate, biochemical, and antioxidant parameters) in selected microgreens grown in cocopeat and soil growing medium. Two components with eigenvalues >1 were retained, together explaining 52.8 % of the total variance (Table 7). PC1 accounted for 32.40 % of the variance and PC2 for 20.40 %. In the PCA score plot (**Figure 6.5 A**), radish sango microgreens grown in cocopeat separated distinctly from all other treatments, indicating a unique combination of high protein, phenolic content, and antioxidant capacity. Hydroponically grown samples clustered tightly regardless of species, reflecting consistent nutrient uptake under controlled conditions, whereas soil- and cocopeat-grown samples were more dispersed, pointing to greater heterogeneity in their compositional profiles.

The loadings plotted in (**Figure 6.5 B, Table 6.9**) reveal the key drivers of variation for the PC-1 is positively correlated with protein (0.370), ascorbic acid (0.325), and FRAP activity (0.346), and negatively with ash content (−0.117) and fat content (−0.113), suggesting that samples scoring high on PC-1 are richer in protein and antioxidants but lower in ash and lipid content. Next, PC-2 is dominated by carotenoids (0.340) and DPPH activity (0.298), indicating that this axis captures variation in pigment-related antioxidant potential. The distinct clustering of radish sango in cocopeat along both principal component (PC) underscores its superior nutritional and functional profile in that medium. The tight hydroponic clusters confirm that controlled nutrient delivery yields reproducible compositional outcomes. Broader spreads in soil and cocopeat treatments reflect variability in water retention and nutrient availability inherent to those substrates. Negative PCA scores simply denote inverse alignment with the axis direction and do not imply poor quality but rather opposite trait emphasis relative to positive loadings. Together, these findings demonstrate that medium choice markedly influences microgreen composition, and PCA effectively highlights both the dominant variables and the degree of consistency afforded by each cultivation system.

Table 6.9. Loadings of the first five principal components based on factor analysis different biochemical and antioxidant parameters of selected microgreens families varieties grown in different growing medium.

Factor loadings	Principal components (PC)				
	PC-1	PC-2	PC-3	PC-4	PC-5
Moisture content (MC)	0.085	-0.261	0.061	-0.475	0.023
Ash content (AC)	-0.117	-0.323	0.343	0.008	0.025
Fat content (FC)	-0.113	0.129	0.480	-0.094	0.164
Fiber content (FIC)	0.125	0.061	-0.098	-0.405	-0.580
Protein content (PC)	0.370	0.014	0.008	0.009	-0.225
Chlorophyll a (Chla)	0.160	0.413	0.133	0.020	-0.014
Chlorophyll b (Chlb)	0.182	0.406	0.141	-0.089	0.061
Total carotenoid (TC)	0.192	-0.039	0.374	0.102	-0.356
Anthocyanin (Ant)	0.186	-0.374	-0.041	0.207	-0.218
Chlorophyll a + b (Chl a+ b)	0.168	0.417	0.137	-0.014	0.013
Ascorbic acid (AA)	0.282	-0.064	0.192	-0.113	0.378
Total glucosinolate content (TG)	0.243	0.107	-0.370	-0.072	0.197
Total phenol content (TPC)	0.264	-0.129	0.225	0.227	0.248
Total flavonoid content (TFC)	0.200	-0.079	0.145	-0.446	-0.006
Tannin content (T)	0.330	-0.102	0.140	0.091	0.063
Phytic acid (PA)	-0.276	0.074	0.323	-0.162	-0.046
Oxalic acid (OA)	0.149	0.182	-0.142	-0.065	-0.050
Antioxidant activity DPPH (DPPH)	0.271	-0.158	0.131	0.160	-0.205
Antioxidant activity ABTS (ABTS)	0.087	-0.205	-0.114	-0.436	0.290
Antioxidant activity FRAP (FRAP)	0.346	-0.047	-0.143	0.127	0.169
Eigenvalue	6.4861	4.0754	3.0377	2.1180	1.0795
Total variance (%)	32.4	20.4	15.2	10.6	5.4
Cumulative variance (%)	32.4	52.8	68.0	78.6	84.4

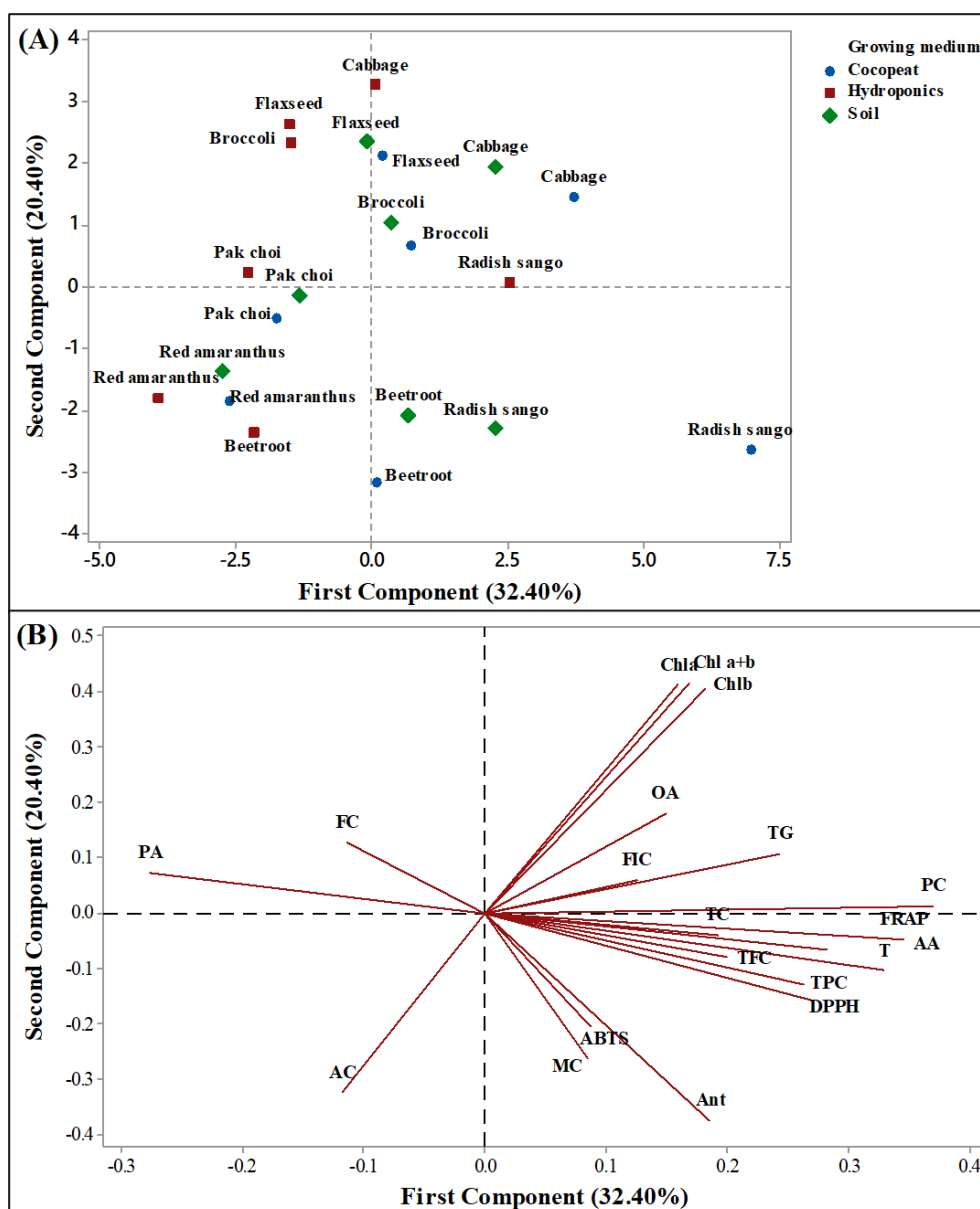


Figure 6.5. Principal component analysis score plot (A) and loading plot (B) describe the relationship among different morphological parameters of microgreens obtained from selected microgreens families varieties grown under different growing conditions.

6.1.2. The effect of illumination systems on morphological parameters and bioactive compounds in selected varieties of microgreens

6.1.2.1. Effect of LED light on morphological parameters

The selected microgreens subjected to various LED light treatments revealed significant differences ($P < 0.05$) during growth in select *Brassicaceae* family microgreens, including broccoli, radish sango, cabbage, and pak choi growth parameters. The LED light treatments employed in this study included blue (450-455 nm), green (510-520 nm), red (630-650 nm), and yellow (480-490 nm) lights. Each light treatment had a unique influence on growth parameters, which is critical for optimizing the indoor cultivation of microgreens. The

changes in the growth of *Brassicaceae* family (broccoli, cabbage, pak choi, and radish Sango) microgreens during the selected growth period are shown in (Figure 6.6). The *Brassicaceae* family microgreens harvested on the 9th day after sowing are shown in (Figure 6.7).

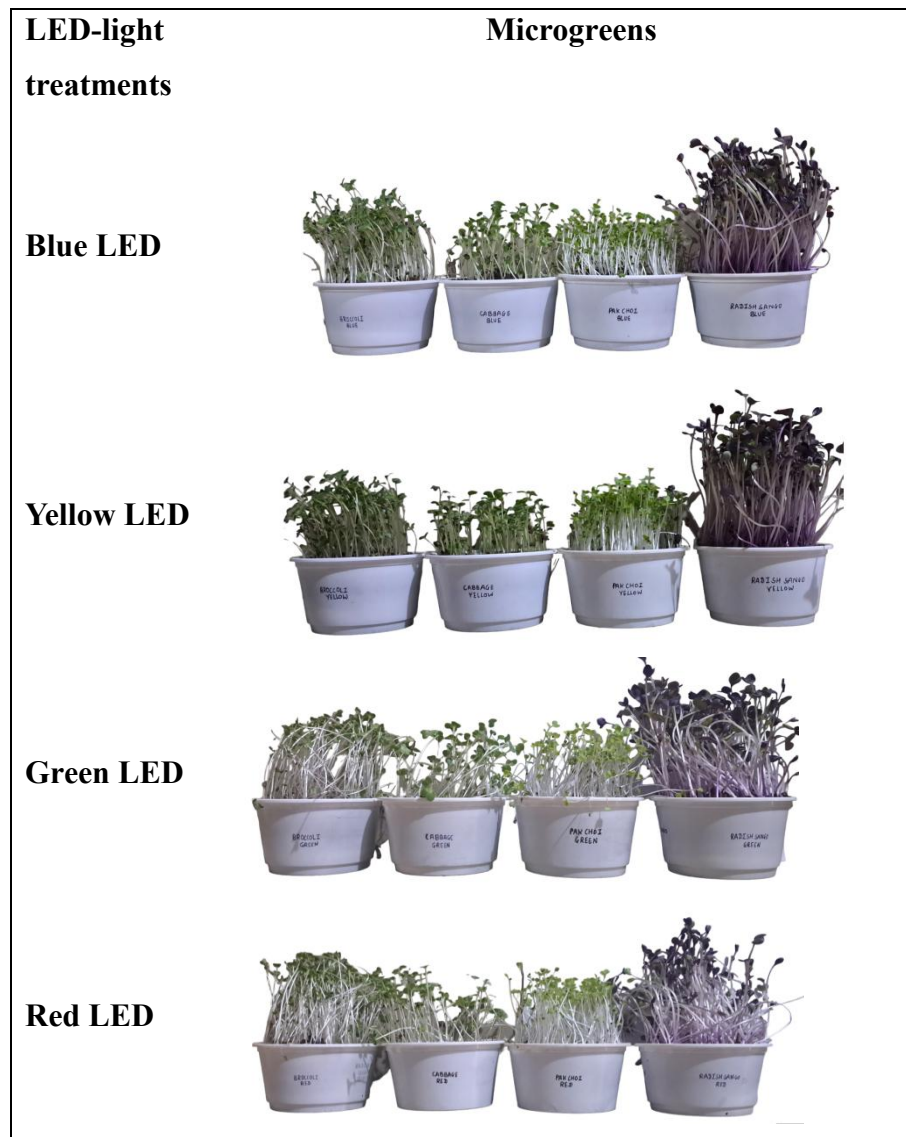


Figure 6.6. Growth changes in selected *Brassicaceae* families microgreens grown in different LED-light treatments.

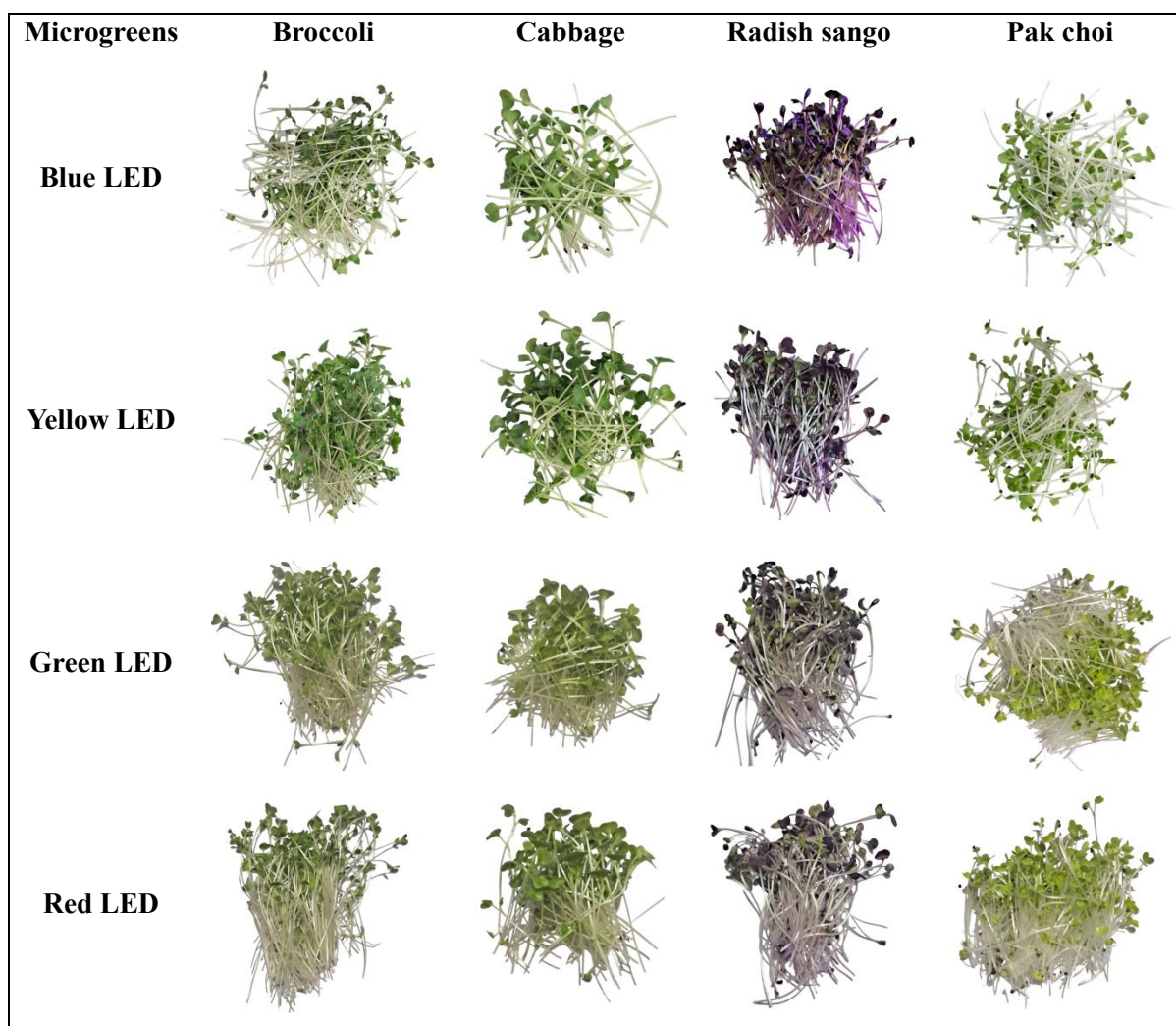


Figure 6.7. The selected *Brassicaceae* families microgreens were harvested on the 9th day

6.1.2.1.1. Shoot weight, height, and width

Pak choi microgreens cultivated under the selected LED light treatments presented the lowest weight of a single shoot, ranging from 31.68 to 32.30 mg/shoot, as shown in **(Table 6.10)**. On the other hand, compared with the other LED light treatments, the green and yellow LED light treatments resulted in greater single shoot weights, indicating a good growth rate, but these treatments were still less optimal. Among all the microgreens, the radish cultivar presented the highest single shoot weight, ranging from 176.08 to 226.92 mg/shoot. The weights of the *Brassicaceae* families cabbage and pak choi microgreens did not drastically change under the different LED light treatments. The yellow LED light treatment promoted a good growth rate and increased single-shoot weights in the microgreens. The growth-related parameters of plant weight are critical parameters that help indicate overall plant health and biomass production rate (Amitrano et al., 2023). Amaranthus and turnip greens were grown in LED treatments (white, blue, and red), and shoot weights ranging from 18.5 to 66.4 mg/shoot were observed. This study revealed that the on-shoot height of microgreen species, light, and

their interaction significantly ($P<0.05$) influenced shoot height (Toscano et al., 2021). The lowest single shoot weights of broccoli and pak choi microgreens under blue LED light conditions are consistent with previous research indicating that blue light can suppress stem elongation and leaf expansion while increasing the chlorophyll content and secondary metabolites (Kopsell et al., 2014). This suppression may lead to smaller, more compact plants. On the other hand, radish sango microgreens presented the highest single shoot weight under the green, red, and yellow LED light treatments, suggesting that this spectrum might penetrate deeper into plant tissues, enhancing the photosynthesis and growth of plants (Thuong & Minh, 2020).

Table 6.10. Effect of different LED lights on morphological parameters (shoot weight, height, and width) of *Brassicaceae* families microgreens

Microgreens	LED	Weight of single shoot (mg)	Height of Shoot (cm)	Width of Shoot (mm)
Broccoli	Blue	37.34±13.28 ^{cB}	6.00±1.44 ^{bcB}	0.86±0.10 ^{defgA}
	Green	54.28±15.58 ^{cA}	6.72±1.25 ^{aA}	0.82±0.09 ^{defgA}
	Red	44.28±15.25 ^{cAB}	5.04±1.17 ^{cdA}	0.81±0.08 ^{defgA}
	Yellow	52.22±11.93 ^{cA}	4.60±0.95 ^{deC}	0.78±0.07 ^{fgA}
Cabbage	Blue	60.57±14.81 ^{cB}	4.70±1.40 ^{deB}	0.92±0.12 ^{dA}
	Green	61.44±18.95 ^{cA}	4.72±0.96 ^{deA}	0.75±0.11 ^{gA}
	Red	58.38±17.66 ^{cAB}	3.65±1.01 ^{efA}	0.87±0.11 ^{defgA}
	Yellow	59.08±15.31 ^{cA}	3.30±0.87 ^{fC}	0.88±0.07 ^{defA}
Pak choi	Blue	31.68±8.65 ^{cB}	3.68±0.81 ^{efB}	0.89±0.15 ^{defA}
	Green	31.52±9.74 ^{cA}	4.08±0.83 ^{defA}	0.78±0.22 ^{efgA}
	Red	31.86±8.55 ^{cAB}	4.08±0.82 ^{defA}	0.89±0.10 ^{defA}
	Yellow	32.30±8.27 ^{cA}	4.34±0.65 ^{defC}	0.90±0.09 ^{deA}
Radish sango	Blue	176.08±42.38 ^{bB}	6.27±1.37 ^{bB}	1.28±0.15 ^{bcA}
	Green	226.92±77.50 ^{aA}	8.66±1.76 ^{aA}	1.50±0.06 ^{aA}
	Red	215.96±74.09 ^{aAB}	9.72±2.11 ^{aA}	1.38±0.17 ^{abA}
	Yellow	214.36±64.27 ^{aA}	6.30±1.55 ^{bC}	1.24±0.17 ^{cA}

The results were presented as mean±standard deviations; (n=3). The different lowercase letters within the column indicate significant ($P<0.05$) differences among all treatments (treatments=16) and different uppercase letters within the column indicate significant ($P<0.05$) differences among the LED light (blue, green, red, and yellow) among the parameters.

The cabbage and pak choi microgreens in the selected LED light treatments presented the shortest shoot heights, ranging from 3.65 to 4.72 cm, and the radish Sango microgreens presented the highest shoot heights, ranging from 6.27 to 9.72 cm, as shown in (Table 6.10). However, the green and red LED light treatments had a significant effect ($P<0.05$), generally resulting in the tallest shoots, reflecting their positive impact on the microgreen growth parameters. The length of shoot plants depends on the species. Similar findings in earlier studies on *Brassicaceae* (cabbage, kale, mizuna, and mustard) microgreens grown under

different intensities, such as low ($120 \pm 5.06 \mu\text{mol m}^{-2}\text{s}^{-1}$), medium ($160 \pm 3.60 \mu\text{mol m}^{-2}\text{s}^{-1}$), and high ($210 \pm 5.91 \mu\text{mol m}^{-2}\text{s}^{-1}$) shoot heights ranging from 2.9 ± 0.1 to 9.1 ± 0.1 cm, were reported (Flores et al., 2024). In another study, similar growth rates were observed for 28 microgreen species and cultivars belonging to Alliaceae, Amaranthaceae, Apiaceae, Asteraceae, Boraginaceae, *Brassicaceae*, Chenopodiaceae, Fabaceae, Lamiaceae and Tropaeolaceae, ranging from 2.0 to 26.2 cm under different light conditions (Frąszczak & Kula-Maximenko, 2022).

The changes in shoot width during the growth of *Brassicaceae* family (broccoli, cabbage, pak choi, and radish Sango) microgreens are shown in (Table 6.10). Among all the LED light treatments, the regenerated cultivar presented the greatest shoot width, ranging from 1.24 to 1.50 mm. The broccoli, cabbage, and pak choi microgreens presented similar shoot widths under the selected light treatments. In addition, the different LED light treatments had no significant effect ($P > 0.05$) on shoot width. However, no similar study has investigated the shoot width of microgreens. Previous studies have shown significant differences in microgreen morphological parameters, mostly due to the variation in harvest time periods and some at the cotyledon stage, others at first true leaves, or after a set number of days (Senevirathne et al., 2019). These differences, along with microgreen seed sowing density (Signore et al., 2024) and light irradiance (Luo et al., 2024), significantly influence these morphological parameters.

6.1.2.1.2. Leaf length, width, and leaf area

The different LED light treatments had significant effects ($P < 0.05$) on the leaf length, width, and leaf area of *Brassicaceae*-selected microgreens in our experiment (Table 6.11). The greatest leaf length and width, ranging from 0.81 to 1.00 cm and from 1.26 to 1.34 cm, respectively, were observed for the radish sango microgreens under the different light treatments. The lowest values for the pack choi microgreens under the different LED light treatments ranged from 0.46 to 0.48 cm in length, and 0.67 to 0.69 cm in width were observed. The morphological parameters of the microgreen leaf length and width are critical indicators of the photosynthetic surface area and overall plant health. The narrow leaves of broccoli, cabbage, and pak choi microgreens under blue LED light treatment may restrict leaf expansion, limiting the photosynthetic area. The ability of radish sango microgreens to grow the widest leaves under yellow LED light treatment highlights the potential to promote leaf expansion. Two different varieties of broccoli microgreens were grown for 19 days after sowing, and the leaf length and width of these microgreens ranged from 25.2 to 17.7 mm (Di Bella et al., 2020). The radish sango microgreens presented a total leaf area of 0.72 to 1.04 cm² under the selected light treatments, followed by broccoli, cabbage, and pak choi microgreens. The significant

differences ($P<0.05$) within the species highlight the diverse growth patterns of *Brassicaceae* microgreens under different light treatments.

Table 6.11. Effect of different LED lights on morphological parameters (leaf length, width, and leaf area) of *Brassicaceae* families microgreens

Microgreens	LED	Length of leaf (cm)	Width of leaf (cm)	Leaf area (cm ²)
Broccoli	Blue	0.56±0.11 ^{cdeB}	0.79±0.09 ^{cdB}	0.36±0.10 ^{cB}
	Green	0.59±0.07 ^{cdAB}	0.77±0.07 ^{cdA}	0.35±0.05 ^{cA}
	Red	0.63±0.12 ^{cAB}	0.86±0.16 ^{cAB}	0.44±0.17 ^{cAB}
	Yellow	0.63±0.13 ^{cA}	0.83±0.16 ^{cA}	0.43±0.16 ^{cA}
Cabbage	Blue	0.63±0.10 ^{cB}	0.83±0.10 ^{cB}	0.42±0.10 ^{cB}
	Green	0.60±0.09 ^{cdAB}	0.87±0.13 ^{cA}	0.42±0.12 ^{cA}
	Red	0.62±0.09 ^{cAB}	0.79±0.11 ^{cdAB}	0.39±0.10 ^{cAB}
	Yellow	0.64±0.13 ^{cA}	0.84±0.13 ^{cA}	0.43±0.15 ^{cA}
Pak choi	Blue	0.48±0.06 ^{deB}	0.67±0.07 ^{dB}	0.26±0.05 ^{cB}
	Green	0.48±0.05 ^{deAB}	0.69±0.08 ^{dA}	0.26±0.04 ^{cA}
	Red	0.48±0.06 ^{deAB}	0.69±0.07 ^{dAB}	0.26±0.05 ^{cAB}
	Yellow	0.46±0.07 ^{eA}	0.69±0.09 ^{dA}	0.25±0.07 ^{cA}
Radish sango	Blue	0.81±0.08 ^{bB}	1.14±0.13 ^{bB}	0.72±0.12 ^{bB}
	Green	0.95±0.21 ^{aAB}	1.34±0.18 ^{aA}	1.02±0.35 ^{aA}
	Red	0.91±0.22 ^{abAB}	1.26±0.21 ^{abAB}	0.92±0.37 ^{aAB}
	Yellow	1.00±0.22 ^{aA}	1.29±0.24 ^{aA}	1.04±0.41 ^{aA}

The results were presented as mean±standard deviations; (n=3). The different lowercase letters within the column indicate significant ($P<0.05$) differences among all treatments (treatments=16) and different uppercase letters within the column indicate significant ($P<0.05$) differences among the LED light (blue, green, red, and yellow) among the parameters.

6.1.2.1.3. Shoot fresh weight, dry weight and yield

The effects of different LED light treatments on the fresh weight, dry weight, and yield of *Brassicaceae* microgreens are shown in (Table 6.12). The selected microgravity and LED light treatments had a significant effect ($P<0.05$) on yield parameters. The highest microgreen shoot yield was reported for radish sango microgreens under yellow LED light (4697.31 g/m²), followed by red (4614.32 g/m²), green (4509.26 g/m²) and blue (3962.39 g/m²) LED light. Compared with the other microgreens, the cabbage microgreens presented consistent and lowest yields (766.04 to 1018.49 g/m²) across the selected LED light treatments, as shown in (Table 6.12). The radish sango microgreens (3.98 to 5.45 g/25 shoots and 0.211 to 0.237 g/25 shoots) presented the highest 25 shoot fresh and dry weights under the different LED light treatments, and the lowest fresh and dry weights were observed for the pak choi microgreens (0.78 to 0.92 g/25 shoots and 0.033 to 0.035 g/25 shoots) under the selected LED light treatments, as depicted in (Table 6.12). Our findings are consistent with those of previous studies on the fresh and dry weights of radish, cabbage, and rocket microgreens cultivated under different seed densities and growth media. a varied condition of selective media and seed

density ranges (L. L. Ntsoane et al., 2023). The lowest yield of broccoli under the green LED-light treatments aligns with the parameter's suppression by green wavelengths, leading to less biomass accumulation. Red and yellow light is known to drive the light-dependent reactions of photosynthesis, resulting in relatively high yields. The selected microgreens presented the highest yields under yellow and red LED light, again highlighting the benefits of a broader spectrum in promoting optimal growth conditions and biomass production (Zhang et al., 2020). The current study revealed the significant effects of different LED light treatments on the growth and morphological parameters of *Brassicaceae* microgreens. These findings suggest that LED light treatments can be optimized on the basis of the desired growth characteristics and yield outcomes for different microgreens. However, further studies are needed to explore the physiological mechanisms underlying the responses of other microgreen species and their growth conditions. The precise control offered by LED light systems can significantly increase micronutrient production in indoor and vertical farming systems, increasing the efficiency and sustainability of agricultural practices.

Table 6.12. Effect of different LED lights on morphological parameters (shoot fresh weight, dry weight and yield) of *Brassicaceae* families microgreens

Microgreens	LED	Yield (g/m ²)	Microgreens 25 shoot fresh weight (g)	Microgreens 25 shoot dry weight (mg)
Broccoli	Blue	1393.23±52.14 ^{deC}	1.02±0.03 ^{defB}	0.064±0.004 ^{cdA}
	Green	1509.55±39.53 ^{cdB}	1.18±0.04 ^{defB}	0.066±0.003 ^{cdB}
	Red	1643.68±47.85 ^{cdA}	1.32±0.03 ^{defA}	0.065±0.004 ^{cdAB}
	Yellow	1731.45±17.95 ^{ca}	1.21±0.17 ^{defB}	0.070±0.003 ^{cdAB}
Cabbage	Blue	807.96±144.30 ^{gC}	1.44±0.10 ^{dB}	0.132±0.018 ^{ba}
	Green	766.04±48.05 ^{gB}	1.15±0.04 ^{defB}	0.067±0.002 ^{cdB}
	Red	943.04±52.26 ^{fgA}	1.38±0.02 ^{deA}	0.074±0.008 ^{cdAB}
	Yellow	1018.49±50.22 ^{fgA}	1.37±0.08 ^{deB}	0.082±0.002 ^{cb}
Pak choi	Blue	1182.33±70.54 ^{efC}	0.78±0.02 ^{fb}	0.035±0.004 ^{dA}
	Green	1370.02±42.96 ^{deB}	0.82±0.09 ^{efB}	0.033±0.003 ^{dB}
	Red	1601.56±55.33 ^{cdA}	0.92±0.03 ^{defA}	0.035±0.002 ^{dAB}
	Yellow	1600.56±91.64 ^{cdA}	0.80±0.09 ^{efB}	0.033±0.003 ^{dAB}
Radish sango	Blue	3962.39±166.78 ^{bc}	3.98±0.17 ^{cb}	0.237±0.006 ^{aA}
	Green	4509.26±91.80 ^{aB}	4.78±0.54 ^{bB}	0.211±0.021 ^{aB}
	Red	4614.32±228.83 ^{aA}	5.45±0.46 ^{aA}	0.237±0.040 ^{aAB}
	Yellow	4697.31±52.64 ^{aA}	4.35±0.09 ^{bcB}	0.234±0.024 ^{aAB}

The results were presented as mean±standard deviations; (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments (treatments=16) and different uppercase letters within the column indicate significant (P<0.05) differences among the LED light (blue, green, red, and yellow) among the parameters.

6.1.2.2. Effects of different LED lights on pigments

As shown in **(Table 6.13)**, the chlorophyll (chlorophyll a, chlorophyll b, and total chlorophyll) contents of cabbage microgreens were measured under yellow LED light, and the lowest chlorophyll a, chlorophyll b, and total chlorophyll contents were detected in radish sango microgreens under green LED light. The chlorophyll content significantly differed ($P<0.05$) between the *Brassicaceae* species microgreens and the LED light treatments. The yellow LED-light treatment had the highest chlorophyll content, followed by the green, red, and blue LED-light treatments. Similar findings were reported for the total chlorophyll content in selected legume microgreens grown for 7-20 days, whose content varied between 8.423 and 36.632 $\mu\text{g/g}$ (Altuner et al., 2022). In another study, a combination of Se and LED light quality treatments was used for broccoli sprouts, and the chlorophyll content on the 7th day ranged from 1.68 to 2.00 mg/g (He et al., 2020). The LED light treatments were important for the accumulation of pigments in the microgreens. Yellow LED light treatment results in the highest chlorophyll content in selected microalgae because its optimal wavelength effectively stimulates chlorophyll synthesis and photosynthetic efficiency (Zhang et al., 2020; Rahman et al., 2021). Yellow light penetrates plant tissues uniformly, activating photoreceptors that regulate the chlorophyll biosynthesis process (Cavallaro et al., 2022).

The carotenoid content in all the selected *Brassicaceae* family microgreens ranged from 2.24 to 9.02 $\mu\text{g/g}$, FW was observed, as shown in **(Table 6.13)**. The selected *Brassicaceae* family microgreens and different LED light treatments had significant effects ($P<0.05$) on the carotenoid content. Broccoli microgreens presented the highest carotenoid content under yellow LED light treatment, whereas radish cultivar microgreens under green LED light treatment presented the lowest carotenoid content. In all the LED light treatments, yellow LED light increased the carotenoid content, followed by red, green, and blue LED light. Similar findings were reported for *Brassicaceae* microgreens, including cabbage, kale, mizuna, and mustard, which were grown under low ($120\pm5.06 \mu\text{mol m}^{-2}\text{s}^{-1}$), medium ($160\pm3.60 \mu\text{mol m}^{-2}\text{s}^{-1}$), and high ($210\pm5.91 \mu\text{mol m}^{-2}\text{s}^{-1}$) intensities in which the carotenoid content ranged from 6.3 to 36.2 $\mu\text{g/g}$, FW was observed (Flores et al., 2024). Carotenoids are crucial for photoprotection and light harvesting, and the increased carotenoid content under yellow light indicates an adaptive response to manage excess light energy and protect the photosynthetic device (Wu et al., 2024). LED lighting generally promotes the accumulation of carotenoids in microalgae. For example, supplementing with LED yellow light (595 nm) has been shown to increase the total carotenoid content in microgreens by 16% (Brazaitytė et al., 2015), and a similar effect has been observed in chickpea sprouts grown under yellow light (Khattak et al., 2008). Additionally, supplemental blue, red, and green LED lights have been

reported to increase α -carotene and β -carotene levels in microgreens (Brazaitytė et al., 2015; Kamal et al., 2020).

Table 6.13. Effect of different LED lights on photosynthetic pigment contents of *Brassicaceae* families microgreens

Microgreens	LED	Chlorophyll a ($\mu\text{g/g}$, FW)	Chlorophyll b ($\mu\text{g/g}$, FW)	Chlorophyll a+b ($\mu\text{g/g}$, FW)	Carotenoid ($\mu\text{g/g}$, FW)
Broccoli	Blue	25.48 $\pm$ 0.20 ^{cD}	11.01 $\pm$ 0.17 ^{eD}	36.71 $\pm$ 0.38 ^{eD}	6.63 $\pm$ 0.05 ^{dD}
	Green	24.35 $\pm$ 0.62 ^{fB}	11.34 $\pm$ 0.93 ^{deB}	35.90 $\pm$ 1.56 ^{eB}	6.53 $\pm$ 0.05 ^{deC}
	Red	21.89 $\pm$ 0.15 ^{gC}	9.32 $\pm$ 0.20 ^{fC}	31.39 $\pm$ 0.34 ^{fC}	5.73 $\pm$ 0.03 ^{fB}
	Yellow	27.96 $\pm$ 0.84 ^{dA}	11.91 $\pm$ 0.21 ^{dA}	40.10 $\pm$ 1.05 ^{dA}	7.52 $\pm$ 0.18 ^{cA}
Cabbage	Blue	22.39 $\pm$ 0.64 ^{gD}	9.98 $\pm$ 0.32 ^{fD}	32.56 $\pm$ 0.96 ^{fD}	5.62 $\pm$ 0.12 ^{fD}
	Green	34.49 $\pm$ 0.23 ^{bB}	15.20 $\pm$ 0.18 ^{bB}	49.99 $\pm$ 0.40 ^{bB}	8.86 $\pm$ 0.08 ^{aC}
	Red	29.96 $\pm$ 0.11 ^{cC}	13.20 $\pm$ 0.05 ^{cC}	43.42 $\pm$ 0.14 ^{cC}	8.50 $\pm$ 0.04 ^{bB}
	Yellow	36.80 $\pm$ 0.05 ^{aA}	16.37 $\pm$ 0.04 ^{aA}	53.48 $\pm$ 0.07 ^{aA}	9.02 $\pm$ 0.03 ^{aA}
Pak choi	Blue	10.59 $\pm$ 0.07 ^{kD}	3.93 $\pm$ 0.15 ^{iD}	14.60 $\pm$ 0.22 ^{ijD}	3.11 $\pm$ 0.01 ^{jD}
	Green	11.65 $\pm$ 0.04 ^{jB}	4.49 $\pm$ 0.11 ^{iB}	16.24 $\pm$ 0.16 ^{iB}	4.08 $\pm$ 0.01 ^{iC}
	Red	11.69 $\pm$ 0.20 ^{jC}	4.37 $\pm$ 0.14 ^{iC}	16.14 $\pm$ 0.34 ^{iC}	4.57 $\pm$ 0.07 ^{hB}
	Yellow	19.37 $\pm$ 0.15 ^{hA}	7.52 $\pm$ 0.15 ^{gA}	27.04 $\pm$ 0.30 ^{gA}	5.14 $\pm$ 0.02 ^{gA}
Radish sango	Blue	10.72 $\pm$ 0.05 ^{jiD}	4.63 $\pm$ 0.03 ^{iD}	15.44 $\pm$ 0.08 ^{iD}	3.13 $\pm$ 0.01 ^{jD}
	Green	6.07 $\pm$ 0.12 ^{mB}	2.93 $\pm$ 0.20 ^{jB}	9.05 $\pm$ 0.32 ^{kB}	2.24 $\pm$ 0.01 ^{kC}
	Red	9.45 $\pm$ 0.02 ^{iC}	4.01 $\pm$ 0.03 ^{iC}	13.54 $\pm$ 0.06 ^{jC}	4.62 $\pm$ 0.03 ^{hB}
	Yellow	15.70 $\pm$ 0.05 ^{iA}	6.58 $\pm$ 0.08 ^{hA}	22.42 $\pm$ 0.13 ^{hA}	6.31 $\pm$ 0.31 ^{eA}

The results were presented as mean $\pm$ standard deviations; (n=3). The different lowercase letters within the column indicate significant ($P<0.05$) differences among all treatments (treatments=16) and different uppercase letters within the column indicate significant ($P<0.05$) differences among the LED light (blue, green, red, and yellow) among the parameters.

6.1.2.3. Effects of different LED lights on bioactive compounds

6.1.2.3.1. Anthocyanin content

As shown in (Table 6.14), radish sango microgreens presented the highest anthocyanin content under blue LED light (66.36 $\pm$ 0.01 $\mu\text{mol}/100\text{g}$, FW) and the lowest anthocyanin content under green LED light (3.07 $\pm$ 0.23 $\mu\text{mol}/100\text{g}$, FW). In addition, all the LED light treatments had a significant effect ($P<0.05$) on the anthocyanin content. The highest anthocyanin content was detected in the blue LED-light treatment, followed by the red, yellow, and green LED-light treatments. Similar results were shown in another study of 11 different lettuce varieties of microgreens, in which the anthocyanin content ranged from 36.12 to 72.34 $\mu\text{mol}/100\text{g}$, FW (Martínez-Ispizua et al., 2022). In basil, Swiss chard, and rocket microgreens, a lower anthocyanin content ranging from 8.83 to 14.41 $\mu\text{g/g}$, FW was observed (Bulgari et al., 2016). In baby leaf lettuce, blue light increased the anthocyanin content (Demir et al., 2023). The results obtained in this study revealed that blue, red, and green LED light treatments effectively increased the anthocyanin content in selected microgreens. Similar findings were reported for

strawberry fruits with supplemental light-emitting diodes in the red (623 nm), far-red (727 nm), and blue (470 nm) regions of the spectrum at three different densities (Pérez-Romero et al., 2024). Light plays a crucial role in anthocyanin biosynthesis and is influenced by anthocyanin quality and intensity. Short-wavelength light (blue and UV) effectively increases the anthocyanin content (Podsędek et al., 2023). Anthocyanins are important for photoprotection and antioxidant activity, and the increased content under blue LED light treatment suggests a protective response to light stress, increasing the plant's resilience to environmental stressors (Trojak & Skowron, 2017). Apart from these factors, seedling density and fertilization treatment influence the anthocyanin content of microgreens (Cowden et al., 2024).

6.1.2.3.2. Total phenolic content and flavonoid content

The total phenol content (TPC) in *Brassicaceae* family microgreens also showed considerable variation under the different LED light treatments. Radish sango microgreens presented the highest TPC under blue LED light (325.35 mg GAE/100g, FW), whereas pak choi under green LED light presented the lowest phenol content (246.16 mg GAE/100g, FW), as shown in **(Table 6.14)**. In selected *Brassicaceae* family microgreens, trends for phenolic compounds, radish sango, cabbage, broccoli, and pak choi were observed. These results suggest that blue LED light is effective in promoting phenolic compound accumulation in microalgae, followed by red, yellow, and green LED light. A similar range was reported for TPC in *Brassicaceae* microgreens, including cabbage, kale, mizuna, and mustard, which varied between 51.5 and 248.5 mg GAE/100g, FW (Flores et al., 2024). Similarly, in the *Brassicaceae* family, the TPC of fourteen microgreen crops (Kowitcharoen et al., 2021) and thirteen microgreens (Xiao et al., 2019) substantially varied, ranging from 9.22 to 811.2 mg GAE/100g, FW, respectively. Microgreens contain various phenolic compounds that are vital antioxidants that play crucial roles in protecting cells from oxidative damage (Tallei et al., 2023). The greater total phenol content observed under blue light can be attributed to the activation of the phenylpropanoid pathway, which is responsible for the synthesis of phenolic compounds (Tan et al., 2020). LED light treatment helps enhance the nutritional quality of microgreens, as a higher phenolic content translates to better antioxidant properties (Podsędek et al., 2023).

The total flavonoid content (TFC) in the selected microgreens varied significantly with different LED light treatments, as shown in **(Table 6.14)**. The highest TFC was observed in broccoli microgreens (42.33 mg QE/100g, FW), whereas the lowest TFC was observed in pak choi, ranging from 13.67 to 38.67 mg QE/100g, FW. The experimental study data revealed that yellow LED light may be more effective at promoting flavonoid biosynthesis than blue, red, or green LED light. The TFC of in-summer leafy greens in microgreens ranged from

Table 6.14. Effect of different LED lights on biochemical parameters of *Brassicaceae* families microgreens.

Microgreens	LED	Biochemical compounds						
		Anthocyanin ($\mu\text{mol}/100\text{g}$, FW)	Total phenol content (mg GAE/100g, FW)	Total flavonoid content (mg QE/100g, FW)	Ascorbic acid (mg/100g, FW)	Total glucosinolate ($\mu\text{mol}/\text{g}$, FW)	Tannin (mg/100g, FW)	Protein content (g BSA/100g, FW)
Broccoli	Blue	7.27 $\pm$ 0.15 ^{fA}	305.32 $\pm$ 0.91 ^{bcA}	33.67 $\pm$ 1.15 ^{cdB}	362.79 $\pm$ 0.18 ^{gA}	29.62 $\pm$ 0.18 ^{dA}	61.85 $\pm$ 1.21 ^{iB}	1.93 $\pm$ 0.15 ^{abcB}
	Green	6.09 $\pm$ 0.14 ^{gC}	299.91 $\pm$ 0.90 ^{bcdB}	25.00 $\pm$ 2.00 ^{efC}	339.25 $\pm$ 1.86 ^{iB}	17.60 $\pm$ 0.42 ^{fC}	59.63 $\pm$ 1.08 ^{iD}	2.01 $\pm$ 0.50 ^{abcB}
	Red	9.01 $\pm$ 0.72 ^{eB}	303.21 $\pm$ 0.52 ^{bcB}	16.60 $\pm$ 1.44 ^{gC}	330.97 $\pm$ 0.32 ^{jC}	18.28 $\pm$ 0.62 ^{fC}	70.69 $\pm$ 1.02 ^{hC}	2.20 $\pm$ 0.22 ^{abcA}
	Yellow	9.20 $\pm$ 0.11 ^{eB}	298.11 $\pm$ 0.90 ^{bcdB}	42.33 $\pm$ 1.15 ^{aA}	347.52 $\pm$ 1.59 ^{hB}	24.25 $\pm$ 0.78 ^{eB}	78.49 $\pm$ 0.07 ^{gA}	2.17 $\pm$ 0.22 ^{abcAB}
Cabbage	Blue	6.20 $\pm$ 0.41 ^{gA}	325.35 $\pm$ 0.62 ^{bA}	41.00 $\pm$ 2.00 ^{abB}	439.46 $\pm$ 1.49 ^{aA}	39.14 $\pm$ 0.40 ^{abA}	240.53 $\pm$ 0.66 ^{cB}	1.50 $\pm$ 0.23 ^{bcdB}
	Green	7.35 $\pm$ 0.31 ^{fC}	304.59 $\pm$ 0.24 ^{bcB}	16.67 $\pm$ 2.14 ^{gC}	411.61 $\pm$ 1.16 ^{bB}	39.12 $\pm$ 0.05 ^{abcC}	244.60 $\pm$ 0.96 ^{bD}	1.41 $\pm$ 0.25 ^{bcdB}
	Red	6.11 $\pm$ 0.12 ^{gB}	307.57 $\pm$ 0.36 ^{bcB}	23.87 $\pm$ 0.95 ^{fC}	376.24 $\pm$ 0.37 ^{cC}	39.29 $\pm$ 0.43 ^{aC}	238.84 $\pm$ 0.80 ^{cC}	1.62 $\pm$ 0.04 ^{abcdA}
	Yellow	9.24 $\pm$ 0.18 ^{eB}	307.33 $\pm$ 0.44 ^{bcB}	37.00 $\pm$ 3.46 ^{bcA}	407.20 $\pm$ 1.49 ^{bB}	39.25 $\pm$ 0.37 ^{aB}	254.39 $\pm$ 0.36 ^{aA}	1.19 $\pm$ 0.04 ^{dAB}
Pak choi	Blue	6.06 $\pm$ 0.37 ^{gA}	272.28 $\pm$ 1.37 ^{cdeA}	18.40 $\pm$ 2.25 ^{gB}	148.82 $\pm$ 1.78 ^{mA}	38.08 $\pm$ 0.30 ^{abcA}	60.69 $\pm$ 0.60 ^{iB}	1.23 $\pm$ 0.01 ^{dB}
	Green	3.07 $\pm$ 0.23 ^{hC}	246.16 $\pm$ 1.88 ^{eB}	13.67 $\pm$ 1.15 ^{gC}	165.05 $\pm$ 1.86 ^{kB}	37.88 $\pm$ 0.36 ^{bcC}	50.26 $\pm$ 0.66 ^{jD}	1.36 $\pm$ 0.09 ^{cdB}
	Red	3.55 $\pm$ 0.23 ^{hB}	249.46 $\pm$ 1.80 ^{eB}	14.87 $\pm$ 1.72 ^{gC}	118.49 $\pm$ 1.30 ^{nC}	38.04 $\pm$ 0.30 ^{abcC}	52.27 $\pm$ 0.37 ^{jC}	1.69 $\pm$ 0.20 ^{abcdA}
	Yellow	6.39 $\pm$ 0.35 ^{fgB}	261.17 $\pm$ 0.90 ^{deB}	38.67 $\pm$ 0.58 ^{abcA}	159.79 $\pm$ 0.18 ^{lB}	37.92 $\pm$ 0.69 ^{bcB}	79.53 $\pm$ 0.89 ^{gA}	1.55 $\pm$ 0.09 ^{bcdAB}
Radish sango	Blue	66.36 $\pm$ 0.01 ^{aA}	365.07 $\pm$ 51.25 ^{aA}	29.73 $\pm$ 1.10 ^{deB}	393.06 $\pm$ 0.16 ^{cA}	38.80 $\pm$ 0.25 ^{abcA}	143.07 $\pm$ 0.92 ^{eB}	1.27 $\pm$ 0.11 ^{dB}
	Green	35.14 $\pm$ 0.37 ^{cC}	306.52 $\pm$ 0.52 ^{bcB}	26.47 $\pm$ 2.20 ^{efC}	367.51 $\pm$ 1.10 ^{fB}	37.84 $\pm$ 0.25 ^{cC}	110.16 $\pm$ 1.10 ^{fD}	1.43 $\pm$ 0.25 ^{bcdB}
	Red	39.65 $\pm$ 0.46 ^{bB}	320.63 $\pm$ 2.38 ^{bB}	29.93 $\pm$ 1.29 ^{deC}	382.60 $\pm$ 3.23 ^{dC}	38.24 $\pm$ 0.12 ^{abcC}	109.47 $\pm$ 1.21 ^{fC}	1.65 $\pm$ 0.11 ^{abcdA}
	Yellow	33.47 $\pm$ 0.25 ^{aB}	311.62 $\pm$ 2.38 ^{bB}	35.80 $\pm$ 1.06 ^{bcA}	373.94 $\pm$ 2.09 ^{eB}	38.36 $\pm$ 0.52 ^{abcB}	154.60 $\pm$ 0.96 ^{dA}	1.44 $\pm$ 0.04 ^{bcdAB}

The results were presented as mean $\pm$ standard deviations; (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments (treatments=16) and different uppercase letters within the column indicate significant (P<0.05) differences among the LED light (blue, green, red, and yellow) among the parameters.

9.58 to 142.39 mg CE/100g, FW (Yadav et al., 2018). Broccoli microgreens were grown at different temperatures, and the flavonoid content ranged from 19.84 to 23.05 mg QE/g, DW (Šola et al., 2024). Flavonoid compounds are a diverse group of phytonutrients, and these compounds possess diverse properties, such as antioxidant, anti-inflammatory, and anticancer properties (Singh et al., 2024). The increase in flavonoid content under yellow LED light treatment suggests that specific wavelengths within the yellow spectrum may activate enzymes phenylalanine ammonia-lyase (PAL), chalcone synthase, and flavonol synthase involved in the flavonoid biosynthesis pathway (Fayezizadeh et al., 2024). In addition, the lower flavonoid content under red LED light implies that red wavelengths may be less effective in stimulating the flavonoid pathway (Luo et al., 2024). The significant differences in total flavonoids under the various LED light treatments highlight the potential for tailoring light quality to optimize the bioactive composition of various microgreens.

6.1.2.3.3. Ascorbic acid content

The ascorbic acid content was highest in cabbage microgreens under blue LED light (439.46 mg/100g, FW) and lowest in pak choi microgreens under red light (118.49 mg/100g, FW), as shown in **(Table 6.14)**. Significant variation in ascorbic acid content was detected among *Brassicaceae* family microgreens subjected to different LED light treatments. Among the LED light treatments, blue light is particularly effective at increasing ascorbic acid levels in microgreens, followed by yellow, green, and red LED light. A similar trend was observed in basil microgreen cultivars (such as red rubin, ablagh, and Kapoor) under different LED light spectra ranging from 153.29 to 405.46 mg/100g, FW for ascorbic acid content (Fayezizadeh et al., 2024). In another study on the biofortification of arugula microgreens through ascorbic acid, the concentration of ascorbic acid in arugula microgreens ranged from 372 to 891 mg/100g, FW (Kathi et al., 2022). Ascorbic acid is a vital antioxidant that plays a crucial role in maintaining immune function and preventing oxidative stress (Singh et al., 2024). The higher ascorbic acid content under blue light can be attributed to the activation of genes involved in ascorbic acid biosynthesis. Blue light has been reported to increase the activity of enzymes such as GDP-L-galactose phosphorylase, which is a key enzyme in the ascorbic acid biosynthetic pathway. This response to blue light can significantly increase the nutritional composition of microgreens, increasing their ascorbic acid content (Bournonville et al., 2023).

6.1.2.3.4. Total glucosinolates

The glucosinolate content varied significantly among the selected *Brassicaceae* family microgreens and among the different LED light treatments, as shown in **(Table 6.14)**. Among *Brassicaceae* family microgreens, radish cultivated under blue LED light presented the highest

glucosinolate content (29.73 $\mu\text{mol/g}$, FW), whereas broccoli cultivated under green LED light presented the lowest glucosinolate content (17.60 $\mu\text{mol/g}$, FW). The results revealed that blue LED light treatment is particularly effective in promoting glucosinolate accumulation in microgreens. Our findings are in accordance with those of Demir et al. (2023), who reported that an increase in the blue light ratio positively influenced glucosinolate accumulation in broccoli, cabbage, and radish microgreens and that the effects of different light wavelengths were variable depending on the type of glucosinolate. In another study, the total glucosinolate contents in radish (35.31 to 40.26 $\mu\text{mol/g}$), cabbage (22.04 to 30.24 $\mu\text{mol/g}$), and rocket (22.87 to 39.83 $\mu\text{mol/g}$) microgreens were reported. This study revealed that the differences in glucosinolate content in plants are mostly due to environmental conditions, climate, soil nutrient availability, plant location and variety, plant density, and developmental stage (Ntsoane et al., 2023). Broccoli microgreens were grown at different temperatures, and the total intact glucosinolate content ranged from 34.97 to 51.06 mg SinE/g; DW was also measured (Šola et al., 2024). Glucosinolates are sulfur-containing compounds known for their cancer-preventive properties (Mawlong et al., 2017). The high glucosinolate content under blue LED light can be attributed to the activation of glucosinolate biosynthetic genes, which are known to respond to blue light exposure. Blue light may increase the expression of transcription factors such as MYB28 and MYB29, which regulate the glucosinolate biosynthesis pathway (Lee et al., 2023). On the other hand, the decreased glucosinolate content in response to green light implies that green wavelengths might not be as successful in triggering the glucosinolate pathway. The lower efficacy of green light in driving secondary metabolite production may result in reduced glucosinolate synthesis (Abe et al., 2015).

6.1.2.3.5. Tannin content

The selected *Brassicaceae* family microgreens had a significant effect on the tannin content under the different LED light treatments. Among the all-microgreens cabbage (238.84 to 254.39 mg/100g, FW) microgreens presented the highest tannin content, followed by radish sango (109.47 to 154.60 mg/100 g, FW), broccoli (59.63 to 78.49 mg/100g, FW) and pak choi (50.26 to 79.56 mg/100g, FW) microgreens (**Table 6.14**). Yellow LED light significantly affected the tannin content in *Brassicaceae* family microgreens, followed by blue, red, and green LED light. The tannin content in fenugreek and *Brassica carinata* microgreens (61.7 ± 1.1 and 50.63 ± 0.25 mg/g, respectively) was significantly greater than that reported in our previous study (Nakakaawa et al., 2023; Jayaraman & Ramasamy, 2024). The higher tannin content in microalgae under yellow LED light can be attributed to the activation of specific enzymes involved in the tannin biosynthesis process. Yellow light may influence the expression of genes

such as leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR), which are key enzymes in the tannin biosynthesis pathway (Mora et al., 2022). On the other hand, the lower tannin content under green LED light suggests that green light may be less effective at promoting tannin synthesis. The lower efficacy of green light in driving secondary metabolite production may result in reduced tannin accumulation (Marchant et al., 2022).

6.1.2.3.6. Protein content

The protein content in the microgreens varied depending on both the type of microgreen and the LED light treatment used, as shown in **(Table 6.14)**. The results revealed that broccoli microgreens generally presented greater protein contents when exposed to red and yellow LED light, with values of 2.20 ± 0.22 g BSA/100g, FW and 2.17 ± 0.22 g BSA/100g, FW, respectively. The red LED light treatment resulted in a high protein content, and the blue, green, and yellow LED light treatments had similar effects on the protein content in all the microgreens. The protein contents of ten selected culinary microgreens, carrot, fennel, fenugreek, French basil, mustard, onion, radish, roselle, spinach, and sunflower microgreens (1.81 to 4.44 g/100g, FW), were observed (Ghoora et al., 2020). In another study, *Brassica* microgreens (broccoli raab, broccoli, and cauliflower) were grown in nutrient solutions with three $\text{NH}_4\text{:NO}_3$ molar ratios, and the protein content ranged from 2.19 to 4.20 g/100g, FW (Renna et al., 2020). The variation in protein content among microgreens under different LED light treatments can be attributed to the differential impact of light wavelengths on plant metabolic processes, particularly protein synthesis (Gao et al., 2021). Red light is known to increase the expression of genes related to chlorophyll synthesis and photosynthesis-related enzyme activity, which may contribute to increased protein accumulation in microgreens. Compared with red light, blue LED light, which is effective at regulating stomatal opening and photomorphogenesis, may not sufficiently promote protein synthesis pathways (Zhou et al., 2024).

6.1.2.4. Effects of different LED lights on antioxidant activity

The DPPH assay measures the ability of microgreens to scavenge free radicals, an indicator of antioxidant capacity. According to the results presented in **(Table 6.15)**, different microalgae presented varying levels of DPPH radical scavenging activity under different LED light treatments. The DPPH activity was highest for broccoli (73.80%, FW) microgreens under (73.80%, FW) blue LED treatment and lowest for pak choi (56.73%, FW) microgreens under red LED light treatment. The LED light treatment significantly increased the antioxidant capacity, and the yellow LED light treatment resulted in the highest antioxidant activity, followed by the blue, red, and green LED light treatments. In different cultivars and genotypes of basil microgreens, DPPH radical scavenging activity ranging from 8.26-86.76% was

observed, and this study revealed that cultivar and genotype significantly affect antioxidant activity (Fayezizadeh et al., 2023). A similar trend was observed for the DPPH antioxidant activity of the basic microgreen cultivars (i.e., red rubin, ablagh, and Kapoor) under different LED light spectra, which ranged from 34.18 to 78.35%, FW. The biochemical composition of microgreens affects antioxidant activity parameters (Fayezizadeh et al., 2024). In another study by Dhaka et al. (2023), in six different microgreens, an antioxidant capacity of 72–87% was reported. Kale microgreens were grown under different LED light illumination systems, and the DPPH radical scavenging activity ranged from 87.19 to 90.50% (Lee et al., 2023). Compared with the other light treatments, the blue and yellow LED light treatments significantly increased the antioxidant capacity of *Brassicaceae* family microgreens. These findings suggest that blue light is more effective at stimulating the production of antioxidant compounds in microgreens, which may be attributed to the activation of specific photoreceptors or metabolic pathways responsive to blue light (Lee et al., 2023). The higher DPPH activity under blue light may be due to the increased synthesis of phenolic compounds and flavonoids, which are known for their radical scavenging properties (Fayezizadeh et al., 2023).

The FRAP assay assesses the reducing power of microgreens, another indicator of antioxidant potential. The results revealed varying responses to different LED light treatments across the studied *Brassicaceae* family microgreens, as shown in **(Table 6.15)**. The highest reducing power was observed for radish sango (21.01 to 27.15 $\mu\text{mol TE/g}$, FW) under the selected LED light treatment among all the microgreens, followed by cabbage, broccoli, and pak choi microgreens. Compared with the other LED light treatments, the blue LED light treatment increased the level of reducing power. Similarly, results were reported for 8 species of the *Brassica* genus of microgreens, where increasing blue light increased antioxidant activity (Alrifai et al., 2020). The changes in FRAP antioxidant activity in microgreen samples are impacted by various microgreen cultivation practices, including the growing medium, growing conditions, species and cultivar of microgreens, stage of harvesting, and methods used for the extraction of compounds (L. L. Ntsoane et al., 2023). The enhanced FRAP activity under blue light could be due to the increased production of anthocyanins and other phenolic compounds that contribute to the overall antioxidant capacity (Zhang et al., 2020). The FRAP assay results indicate that different wavelengths of LED light can differentially influence the antioxidant activity of microgreens, with blue light showing more pronounced effects than the other LED light treatments. Further research is needed to explore the underlying mechanisms of these responses and to develop guidelines for the commercial production of antioxidant-rich micronutrients.

Table 6.15. Effect of different LED lights on antioxidant activities of *Brassicaceae* families microgreens.

Microgreens	LED	Antioxidant activities	
		DPPH (% FW)	FRAP (μmol TE/g, FW)
Broccoli	Blue	73.80±0.37 ^{aB}	9.02±0.17 ^{efA}
	Green	69.95±0.50 ^{bD}	9.59±1.20 ^{efB}
	Red	73.01±0.40 ^{aC}	10.07±0.58 ^{eC}
	Yellow	69.30±0.12 ^{bcA}	10.07±0.29 ^{eB}
Cabbage	Blue	72.60±1.02 ^{aB}	19.57±0.29 ^{cA}
	Green	69.00±0.07 ^{bcD}	15.44±1.09 ^{dB}
	Red	68.54±0.40 ^{bcC}	14.87±0.15 ^{dC}
	Yellow	69.07±0.51 ^{bcA}	15.44±0.33 ^{dB}
Pak choi	Blue	57.52±0.41 ^{dB}	6.81±1.16 ^{gA}
	Green	54.28±0.07 ^{eD}	6.52±0.72 ^{gB}
	Red	56.73±1.16 ^{dC}	7.58±0.44 ^{fgC}
	Yellow	69.11±0.27 ^{bcA}	7.48±0.29 ^{fgB}
Radish sango	Blue	72.17±0.71 ^{aB}	24.46±1.00 ^{bA}
	Green	68.04±0.35 ^{cD}	21.39±0.83 ^{cB}
	Red	69.88±1.17 ^{bcC}	21.01±0.87 ^{cC}
	Yellow	72.98±0.41 ^{aA}	27.15±0.44 ^{aB}

The results were presented as mean±standard deviations; (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments (treatments=16) and different uppercase letters within the column indicate significant (P<0.05) differences among the LED light (blue, green, red, and yellow) among the parameters.

6.1.2.5. Principal component analysis (PCA)

PCA was conducted on *Brassicaceae* microgreens (broccoli, cabbage, pak choi, and radish sango) to assess the impacts of different LED light treatments (blue, green, red, and yellow) on various morphological, biochemical, and antioxidant parameters. PCA was employed to reduce the dimensionality of the data and to identify the key variables that contribute to the variation observed in the dataset. The results of the PCA provide valuable insights into the relationships among different parameters and how they are influenced by different LED light treatments.

PCA on nine morphological traits, including weight of single shoot (WSS), height of shoot (HS), shoot width (SW), yield, leaf length (LL), leaf width (LW), total leaf area (LA), 25-shoot fresh weight (25 MFW), and 25-shoot dry weight (25 MDW)—revealed that the first two components captured 96.0 % of the total variance. Specifically, PC1 explained 92.1 % and PC2 accounted for 3.9 % of the variation, with eigenvalues of 8.289 and 0.351, respectively, as shown in (Table 6.16). The PCA loading plot (Figure 6.8) illustrates that all nine traits loaded strongly and positively on PC-1 (loadings ranging from 0.292 to 0.345), indicating that PC-1 represents an overall growth-vigor axis, where higher scores correspond to larger, heavier

plants across all measured traits. In contrast, PC-2 exhibited divergent loadings, with shoot height loading negatively (-0.875) and other traits such as leaf length (0.297) and 25 MDW (0.223) loading positively (range -0.109 to 0.297).

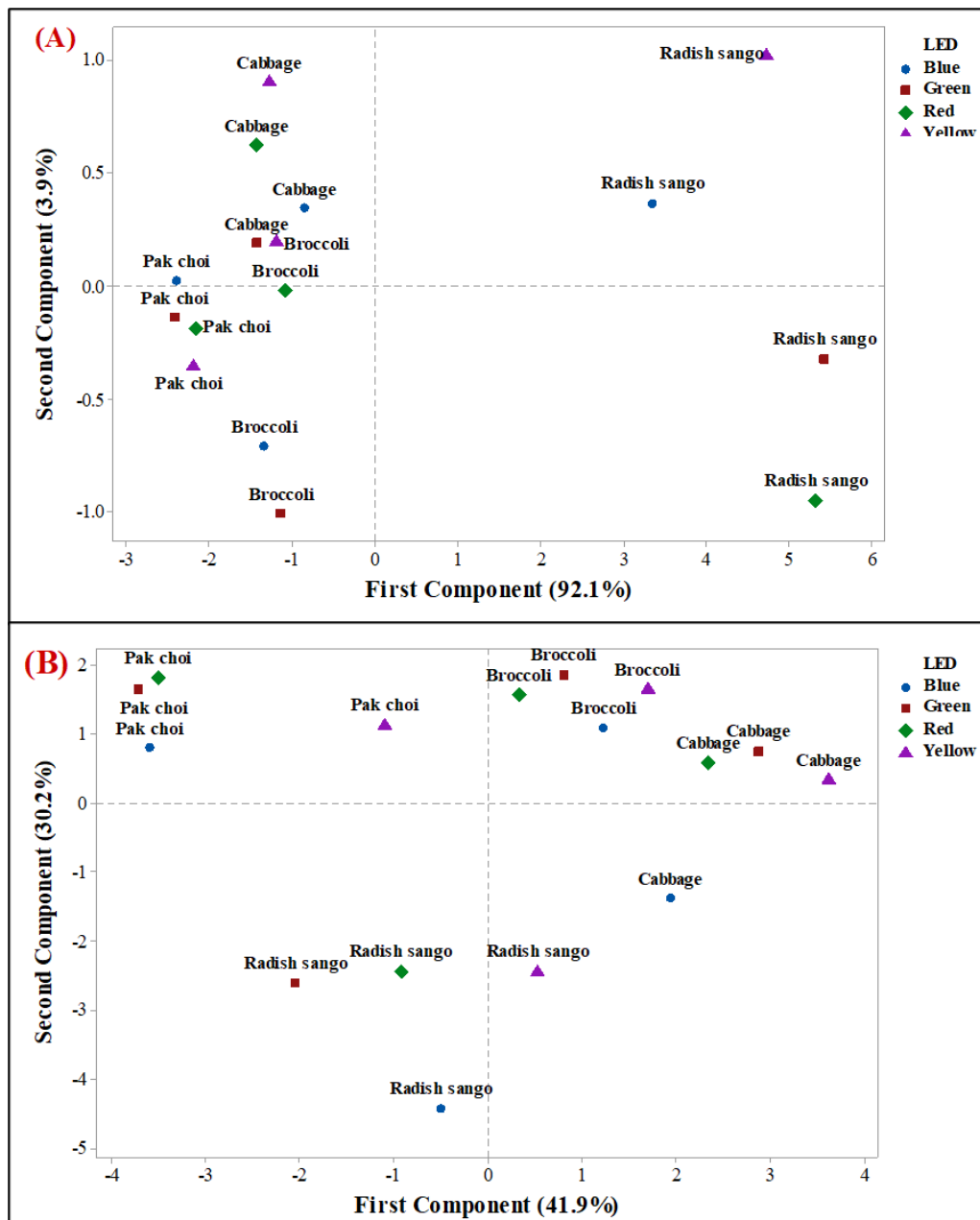


Figure 6.8. Principal component analysis score plot (A and B) describes the relationship among different selected parameters of microgreens obtained from *Brassicaceae* families microgreens grown under various LED-light treatments.

This suggests that PC-2 captures a trade-off between elongation and biomass allocation, also visualized in the score plot (**Figure 6.9**). These patterns reflect that LED light treatments exerted a broad, coordinated influence on morphological development, rather than isolated trait-specific effects. Similar findings were reported by Samuolienė et al. (2012), who observed that light spectral composition significantly influenced biomass accumulation and plant stature in microgreens and leafy vegetables such as lettuce and basil. Although PC-2 contributed only

3.9 % of the variance, it provided biologically meaningful insights into the internal allocation of resources. Specifically, the strong negative loading of shoot height suggests elongation without corresponding biomass gain, a phenomenon that could arise under specific light spectra. Johkan et al. (2010) explained that certain light qualities, such as red light, promote stem elongation at the cost of structural biomass, whereas blue light enhances leaf expansion and dry weight (Bian et al., 2015). This observation aligns with our finding that elongation is not always positively correlated with overall yield, especially in microgreens where compact growth and high dry matter content are often desirable (Pinho et al., 2012). Thus, PCA not only quantified the total morphological variation but also clarified how different growth traits interact under varying LED treatments, offering a more integrative understanding of morphological adaptation across light environments.

Table 6.16. Loadings of the first two principal components based on factor analysis different morphological parameters of selected microgreens families varieties grown in different LED light treatment.

Factor loadings	Principal components (PC)	
	PC-1	PC-2
Microgreens weight of single shoot (WSS)	0.345	0.095
Microgreens height of shoot (HS)	0.292	-0.875
Microgreens width shoot (SW)	0.329	-0.091
Yield	0.332	-0.109
Microgreens leaf length (LL)	0.335	0.297
Microgreens leaf width (LW)	0.343	0.155
Microgreen total leaf area (LA)	0.341	0.205
Microgreens 25 shoot fresh weight (M25FSW)	0.345	-0.024
Microgreens 25 shoot dry weight (M25DSW)	0.335	0.223
Eigenvalue	8.2888	0.3511
Total variance (%)	92.1	3.9
Cumulative variance (%)	92.1	96.0

Another PCA test was performed on the biochemical and antioxidant parameters, including chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl a+b), carotenoid content, anthocyanin content, total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, tannin content, ferric-reducing antioxidant power (FAPs), ascorbic acid content (AA), protein content (PC), and total glucosinolate (TG) content. The first two principal components (PC1 and PC2) together accounted for approximately 72.1% of the total variance, with PC1 (41.9%) and PC2 (30.2%). These findings suggest that these two components effectively summarize the given biochemical parameters, as shown in **(Figure 6.8)**. The score plot **(Figure 6.9)** showed distinct clustering patterns among the microgreens and growing media combinations. Radish sango in cocopeat scored the highest on PC-1,

indicating that this sample had high levels of the parameters that contributed most positively to this component.

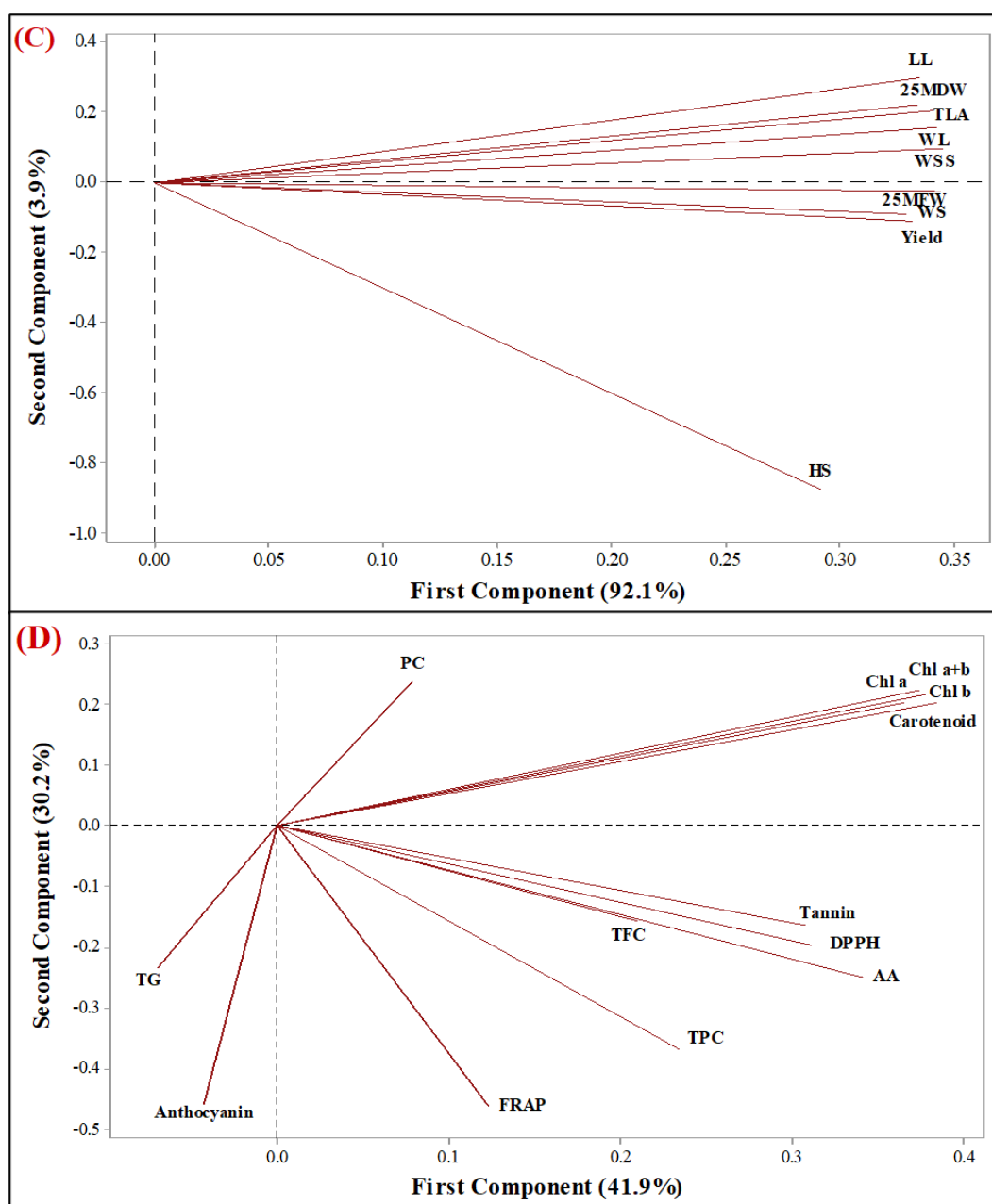


Figure 6.9. Principal component analysis of loading plots (C and D) describes the relationship among different selected parameters of microgreens obtained from *Brassicaceae* families microgreens grown under various LED-light conditions.

In contrast, hydroponic and soil-grown samples showed more moderate and clustered positioning, suggesting less variability in their biochemical responses. The loading plot (**Table 6.17**) revealed that PC-1 was positively driven by chlorophyll, carotenoid, total phenol content, total flavonoid content, tannin content, antioxidant activity (DPPH and FRAP), ascorbic acid, and protein content, suggesting antioxidant pigments and activity played a more dominant role in this axis (**Figure 6.9**).

Table 6.17. Loadings of the first three principal components based on factor analysis different biochemical and antioxidant parameters of selected microgreens families varieties grown in different LED light treatment.

Factor loadings	Principal components (PC)		
	PC-1	PC-2	PC-3
Chlorophyll a (Chl a)	0.374	0.224	-0.111
Chlorophyll b (Chl b)	0.385	0.202	-0.096
Chlorophyll a + b (Chl a+ b)	0.378	0.217	-0.106
Total carotenoid	0.365	0.203	-0.122
Anthocyanin	-0.042	-0.459	0.143
Total phenol content (TPC)	0.235	-0.368	0.180
Total flavonoid content (TFC)	0.210	-0.157	0.127
Antioxidant activity DPPH (DPPH)	0.311	-0.196	0.321
Tannin content	0.308	-0.164	-0.385
Antioxidant activity FRAP (FRAP)	0.123	-0.460	-0.021
Ascorbic acid (AA)	0.341	-0.248	0.112
Protein content (PC)	0.079	0.237	0.553
Total Glucosinolate (TG)	-0.070	-0.234	-0.560
Eigenvalue	5.4507	3.9299	2.1337
Total variance (%)	41.9	30.2	16.4
Cumulative variance (%)	41.9	72.2	88.6

The PCA revealed that PC-1 reflects an axis of high nutritional and antioxidant potential, combining protein content, phenolics, flavonoids, and antioxidant activity (FRAP). Radish sango in cocopeat exhibited the highest PC-1 scores, indicating a superior phytochemical and antioxidant profile compared to other treatments. This is in line with previous findings that microgreens from the *Brassicaceae* family accumulate higher levels of phenolics and antioxidants under moderate stress conditions (Xiao et al., 2012). Importantly, PC-2 helped distinguish samples based on pigment-related antioxidants, including chlorophyll a and carotenoids. These findings highlight that antioxidant responses are multidimensional, with both non-pigment antioxidants (phenolics, ascorbic acid) and pigment-based components (chlorophylls, carotenoids) contributing to the variability across samples. This agrees with the study of Samuolienė et al. (2013), where light spectra modulated antioxidant pigments and ascorbic acid levels in microgreens. These results confirm that PCA is a valuable tool to capture the complexity of biochemical responses, identify high-performing combinations for radish sango microgreens in cocopeat, and dissect how traits co-vary across environmental treatments.

6.1.3. The effect of foliar spray treatments on morphological parameters and bioactive compounds in selected varieties of microgreens

6.1.3.1. Effect on morphological parameters

The morphological parameters of microgreens subjected to various foliar spray treatments revealed significant differences ($P < 0.05$) during the growth of radish sango

microgreens. Each treatment had a unique influence on growth parameters, which is critical for optimizing the cultivation of microgreens. The harvested radish sango microgreens from each treatment on the 9th day after sowing are shown in (Figure 6.10).

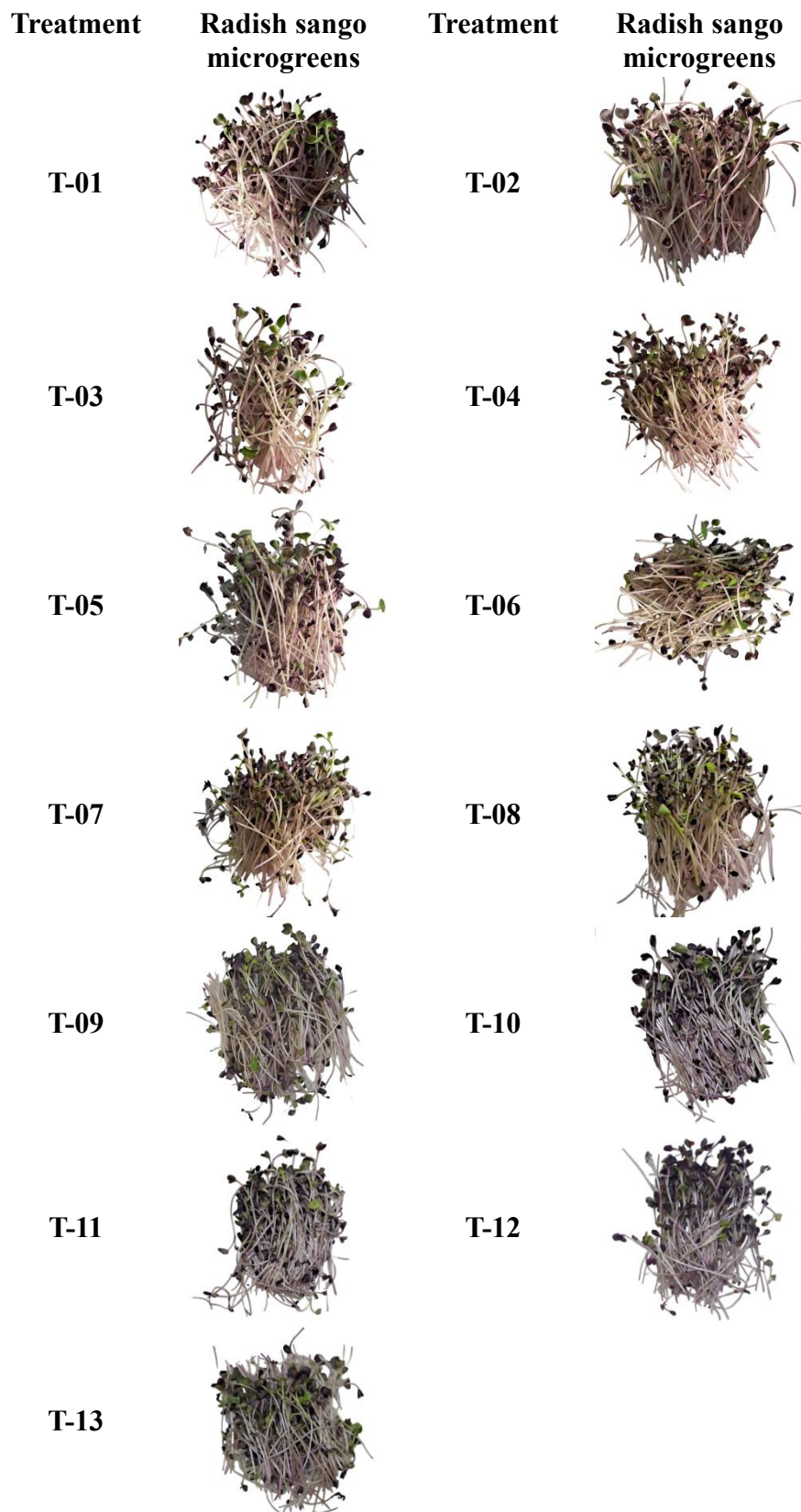


Figure 6.10. The radish sango microgreens were harvested on the 9th day

To determine the values of the variables calcium chloride (A) and potassium nitrate (B) for the study, RSM recommended using Central Composite Rotatable Design (CCRD) and conducting 13 sets of test treatments. The results of the effect of different combinations of foliar spray treatments on the morphological traits in the radish sango microgreens based on the Central Composite Rotatable Design (CCRD) are shown in (Table 6.18 and Table 6.19).

6.1.3.1.1. Shoot height and weight

The maximum microgreens shoot height (9.96 cm) and shoot weight (265 mg) were recorded under the foliar spray treatment combination of 18 mM of calcium chloride (CaCl_2) and 8 mM of potassium nitrate (KNO_3) (Treatment (T) -7), and minimum for (T-6 and T-13) were observed, as shown in (Table 6.18). Similar findings were observed for the red radish microgreens on the 9th day of harvesting stage shoot heights 6 to 9 cm were observed (Dhaka et al., 2023). In another study, the shoot height for radish microgreens was 9.2 cm reported (Li et al., 2021). The fresh weight of a single shoot of radish microgreens in the range 207.2 mg was observed in different biostimulants (TRAINER®, AQUAMIN®, leaf moringa extract, and distilled water (control)), the seedlings having a fresh weight of 38.00% higher than that measured in those control sample (Toscano et al., 2023). The microgreen species variations in their shoot weights and height are due to seeding rate, fertilization, growing medium, pre-sowing seed treatment, harvest stage, and microenvironment including temperature and lighting conditions (Li et al., 2021). In this experiment study work, the different foliar spray treatments showed a significant effect ($P < 0.05$) on the shoot height and weight parameters. The CaCl_2 , KNO_3 , and along with are showed a significant effect ($P < 0.05$) on radish sango microgreens single shoot height and weight morphological parameters as shown in (Table 6.18).

The quadratic equation linking the effect of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) and levels of different foliar sprays on radish sango microgreens single shoot height and weight is given in (Table 6.20). The linear effect of calcium chloride (A) had a positive impact on the radish sango microgreens single height and weight were observed in (Figure 6.11 (A and B)). The linear effect of potassium nitrate (B) had a positive impact on the radish sango microgreens single weight was observed in (Figure 6.12 (A)). In (Figure 6.11 (A)) explains the effect of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) on radish sango microgreens shoot height. The figure shows that increasing levels of both (A and B) improves shoot height. Calcium chloride (CaCl_2) increases shoot height and length in microgreens by stabilizing cell walls, promoting cell elongation, and improving nutrient

uptake. It also regulates growth hormone signaling and helps in maintaining cell turgor, leading to better overall growth. When calcium chloride is applied, it increases calcium availability in the growing environment (Kou et al., 2014). This process enhances the firmness and strength of cell walls, contributing to the overall shoot height and length as plants can grow more reliably and withstand stress (Lu et al., 2018). The results were similar to those of earlier studies, which reported that KNO_3 priming enhanced three *medicago* species (*medicago indicus*, *medicago interexta*, and *medicago polymorpha*) of sprout weight on 10th days of harvesting when compared with a control group of samples (Zrig et al., 2021). The application of potassium nitrate in the cultivation of microgreens enhances shoot height and length primarily through improved nutrient uptake and hormonal regulation. Potassium plays a critical role in osmoregulation and water balance within plant cells, promoting cell expansion and elongation (Choudhary et al., 2024).

6.1.3.1.2. Leaf length, width, and leaf area

Results for leaf length, width, and leaf area of radish microgreens are shown in **(Table 6.18)** of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) foliar spray application using response surface methodology based on the Central Composite Rotatable Design (CCRD). In the foliar spray treatment, microgreen leaf length ranged from (0.82 to 1.08 cm), leaf width ranged from (0.98 to 1.15 cm), and leaf area ranged from (0.65 to 1.06 cm²) were observed. The highest leaf length for treatment (T-07; 18.00 mM CaCl_2 : 8.00 mM KNO_3) and lowest for the treatment (T-13; 04.00 mM CaCl_2 : 8.00 mM KNO_3) were observed. The highest leaf width for treatment (T-08; 11.00 mM CaCl_2 : 9.243 mM KNO_3) and lowest for the treatment (T-02; 04.00 mM CaCl_2 : 2.00 mM KNO_3) were observed and the highest leaf area for treatment (T-08; 11.00 mM CaCl_2 : 9.243 mM KNO_3) and lowest for the treatment (T-06; 01.101 mM CaCl_2 : 5.00 mM KNO_3) were observed. *Brassicaceae* families microgreens were grown in peat substrate for 10 days from sowing to harvest and leaf area for mustard (1.1 to 3.1 cm²), red pak choi (2.0 to 3.4 cm²), tatsoi (1.2 to 2.0 cm²), and kohlrabi (4.5 to 5.6 cm²) were observed under different irradiation levels treatment (Samuolienė et al., 2013). In another study, Di Bella et al. (2020) reported that two distinct types of broccoli microgreens were cultivated for 19 days following seeding, during which time their leaf length and width varied between 25.2 and 17.7 mm were observed. These finding values are greater than ours; this variation is primarily caused by different harvest times; some plants are harvested at the cotyledon stage, while others are harvested at the first true leaves or after a predetermined number of days (Senevirathne et al., 2019). These variations have a major impact on these morphological characteristics, as do microgreen seed sowing density (Signore et al., 2024) and light irradiance (Luo et al., 2024).

The response surface plots for radish sango microgreens leaf length, and leaf area shown in **(Figure 6.11 (B and C))**. depict the effect of different levels of concentration of foliar spray CaCl_2 and KNO_3 . A significant effect ($P < 0.05$) was found across different levels of CaCl_2 concentration for the leaf length, leaf width, and leaf area among all treatments as shown in **(Table 6.21)**. The linear effect of calcium chloride (A) had a positive impact on the radish sango microgreens leaf length, width, and leaf area were shown in **(Figure 6.13 (C, E, and B))**. The potassium nitrate (B) had a positive impact on the leaf area as shown in **(Figure 6.14 (B))**. In **(Table 6.20)** presents the regression coefficients equation for the second-order polynomial equations, indicating the suggested model's adequacy, significant lack of fit, and satisfactory R^2 values; furthermore, adjusted R^2 and predicted R^2 , which is typically lower than R^2 measures the model's predictive performance on leaf length, width, and leaf area. All models were regarded as suitable for analysis because their R^2 values all exceeded 80%. The effect of foliar spray of 20 mM CaCl_2 concentration significantly enhanced the leaf length of the lettuce crop. This increase suggests that CaCl_2 can play a crucial role in promoting leaf elongation, thereby enhancing the overall growth of the plant (Youssef et al., 2017; Hagagg et al., 2020). The chloride ions from CaCl_2 actively regulate leaf cell size and water relations, optimizing conditions for cell expansion and contributing to the enhancement of leaf length, width, and leaf area (Guo et al., 2021). Potassium nitrate (KNO_3) foliar sprays have been observed to increase leaf length and width by improving the nutrient availability and uptake efficiency of plants. Studies have shown that KNO_3 application in crops such as sunflower leads to significant increases in leaf area, which correlates with enhanced leaf length. The nitrogen component in KNO_3 is particularly vital for vegetative growth, enhancing processes such as cell division and elongation (Jabeen and Ahmad, 2011). The combination of foliar sprays of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) has been shown to significantly enhance leaf length, width, and area in various crops (Rathor et al., 2021). The mechanisms of action involve improved nutrient uptake, enhanced physiological processes, and stress regulation, which collectively contribute to better plant growth and productivity (Goud and Kumar, 2021).

Table 6.18. Effect of different combination of foliar spray treatments on the morphological traits in the radish sango microgreens based on the Central Composite Rotatable Design (CCRD).

Treatments	Weight of shoot (mg); (n=25)	Height of shoot (cm); (n=25)	Length of leaf (cm); (n=25)	Width of leaf (cm);(n=25)	Leaf area (cm ²); (n=25)	Fresh weight (g/25 shoots); (n=03)	Dry weight (g/25 shoots); (n=03)	Yield (g/m ²); (n=03)
T-01	230.83	9.11	0.98	1.09	0.83	5.53	0.245	4539.73
T-02	218.22	8.95	0.91	0.98	0.74	5.35	0.242	4589.85
T-03	240.55	9.67	0.99	1.03	0.90	5.45	0.244	4973.13
T-04	237.94	9.46	1.05	1.07	0.92	5.32	0.248	5047.69
T-05	235.20	9.41	0.98	1.02	0.88	5.81	0.264	4527.60
T-06	198.22	8.26	0.80	0.91	0.65	5.23	0.235	4413.85
T-07	265.06	9.96	1.08	1.08	0.99	5.88	0.295	5105.82
T-08	239.66	9.15	1.06	1.15	1.06	5.86	0.293	5142.58
T-09	230.34	9.51	1.02	1.02	0.90	5.34	0.245	4980.20
T-10	221.32	9.12	0.94	1.10	0.92	5.55	0.265	4671.72
T-11	240.30	9.31	0.98	1.04	0.86	5.43	0.252	4913.45
T-12	239.88	9.39	1.06	1.06	0.88	5.42	0.255	4847.58
T-13	201.02	7.97	0.82	1.02	0.72	5.38	0.252	4508.50

All the experimental results values are presented as mean value basis based on each morphological trait. Where, n: number of observations.

6.1.3.1.3. Fresh weight, dry weight and yield

The minimum fresh weight was observed in response to the treatments (T-03) and highest for treatment (T-07), the maximum dry weight was observed for treatment (T-07) and lowest for treatment (T-06) as shown in **(Table 6.18)**. The highest levels of yield (5142.58 g/m²) obtained in response to the treatment (T-08) were presented in **(Table 6.18)**. The fresh weight, dry weight, and yield in radish sango microgreens significantly increased with the addition of calcium chloride (CaCl₂) and potassium nitrate (KNO₃) where both the foliar spray chemicals had the maximum positive impact shown in **(Table 6.20)**. Our findings are consistent with those of previous studies on the fresh and dry weights of radish, cabbage, and rocket microgreens cultivated under different seed densities and growth media (L. L. Ntsoane et al., 2023). The okra, carrot, leek, spinach, and cress vegetables microgreens were sown at two different sowing densities and the fresh yield was in ranged from 719.8 to 9984.6 g/m² observed (Sariyer et al., 2024). In another study, the priming with 1.00% KNO₃ enhanced the fresh weight by 90% as compared with control (pure water) linseed sprouts (Zrig et al., 2023).

The linear effect of calcium chloride (A) had a positive impact on the radish sango microgreens fresh weight, dry weight, and fresh weight were observed in **(Figure 6.13 (D, G, and H))**. The linear effect of potassium nitrate (B) had a positive impact on the radish sango microgreens single weight was observed in **(Figure 6.14 (C, D, and E))**. **(Figure 6.11 (D, E, and F))**. explain the effect of calcium chloride (CaCl₂) and potassium nitrate (KNO₃) on radish sango microgreens fresh weight, dry weight, and yield. The increasing levels of both foliar (A and B) improve the fresh weight, dry weight, and yield. Calcium chloride (CaCl₂) has been shown to significantly enhance the fresh weight of radish sango microgreens. Studies have demonstrated that microgreens treated with 10 mM CaCl₂ contain over 160% more calcium than those sprayed with water alone, leading to improved overall quality and increased freshness. The increase in fresh weight can be attributed to the enhanced structural integrity of the plant cells facilitated by calcium, which improves water retention and cellular functions (Kou et al., 2014). Increased yield in radish sango microgreens due to KNO₃ treatment is a result of improved photosynthesis and metabolic activities. KNO₃ treatments lead to enhanced leaf area, which contributes to a higher photosynthetic rate and greater biomass production in microgreens. The foliar application of KNO₃ has been shown to result in significant yield improvements, likely due to the promotion of growth hormones and improved nutrient translocation within the plant (Zrig et al., 2021; Alotaibi et al., 2024; Choudhary et al., 2024).

6.1.3.1.4. Effect on pigment content

The highest level of total chlorophyll content 42.77 $\mu\text{g/g}$ was observed in treatment (T-05; 20.900 mM CaCl_2 : 5.00 mM KNO_3) and the lowest level of total chlorophyll content 25.42 $\mu\text{g/g}$ was observed in treatment (T-02; 04.00 mM CaCl_2 : 2.00 mM KNO_3) were shown in **(Table 6.19)**. The foliar spray treatments applied on radish sango microgreens showed a significant effect ($P < 0.05$) on total carotenoid content. The total carotenoid content was observed in ranged of 8.07 to 9.66 $\mu\text{g/g}$ among all treatments. The lowest carotenoid content was observed in (T-02; 4.00 mM CaCl_2 : 2.00 mM KNO_3) and the highest was observed in (T-05; 20.90 mM CaCl_2 : 5.00 mM KNO_3) foliar spray treatment, as shown in **(Table 6.19)**. The total chlorophyll and carotenoid content in radish sango microgreens significantly ($P < 0.05$) increased by increasing the concentration of CaCl_2 and KNO_3 , where both had the maximum positive impact observed in **(Table 6.21)**.

The chlorophyll content in mizuna, arugula, green basil, and cress radish microgreens species after fortification with sodium selenate, the chlorophyll a content in range (249.9 to 500 mg/kg) and chlorophyll b content in range (114.5 to 245.8 mg/kg) were observed (Mezeyová et al., 2022). Carotenoid content in microgreens ranged from 2906 to 9107 $\mu\text{g}/100\text{g}$ of three lettuce cultivars grown under different light-emitting diode (LED) lamp spectra reported (Ferrón-Carrillo et al., 2021). The chlorophyll a and b and carotenoid content in a range of (0.036 to 0.047 mg/g, 0.027 to 0.032 mg/g and 0.0307 to 0.0365 mg/g) observed when applied different types of foliar spray treatments including ammonium nitrate, Urea, ammonium sulphate, deionized water, calcium nitrate, potassium nitrate, monosodium glutamate and monoammonium phosphate on sunflower microgreens (Hassama et al., 2022). Additionally, a significant increase in total chlorophyll by 56%, was recorded as compared to control when applying KNO_3 priming affects the sprouting process species on three *Medicago* species (Zrig et al., 2021). The various types of biostimulants such as TRAINER®, AQUAMIN®, leaf moringa extract, and distilled water (control) were applied to the radish and turnip greens, and the total chlorophyll content in a range of 0.192 to 0.283 $\mu\text{g}/\text{mg}$ and 0.270 to 0.495 $\mu\text{g}/\text{mg}$ were observed (Toscano et al., 2023).

The experimental values obtained for total chlorophyll and carotenoid content were subjected to linear regression analyses to fit a reduced second-order polynomial model as shown in **(Table 6.20 and Table 6.21)**. The developed models manifested high significance and satisfactory results with a low probability level (P-value) and high R^2 and F values, which implies that this mathematical model can predict dependent variables resultant from any combination of independent variables **(Table 6.21)**. The linear effect of calcium chloride (A) had a positive impact on the radish sango microgreens total chlorophyll and carotenoid content

were observed in **(Figure 6.15 (A and B))**. The linear effect of potassium nitrate (B) had a positive impact on the radish sango microgreens single weight was observed in **(Figure 6.16 (A and B))**. In **(Figure 6.12 (B and C))** explain the effect of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) on radish sango microgreens total chlorophyll and carotenoid content and the increasing levels of both foliar (A and B) enhanced the total chlorophyll and carotenoid content. Similarly, the foliar spray treatment with KNO_3 enhanced the total chlorophyll and carotenoid content by 86% and 85% as compared with control (pure water) linseed sprouts (Zrig et al., 2023). Calcium plays a crucial role in stabilizing cell walls and membranes, which enhances the structural integrity of chloroplasts. This stabilization helps in maintaining the chlorophyll content by reducing the degradation caused by environmental stress (Valivand and Amooaghaie, 2021). Calcium also aids in the activation of certain enzymes involved in chlorophyll biosynthesis, thereby potentially increasing chlorophyll content (Sadak et al., 2023). Potassium is essential for various physiological processes, including photosynthesis. It regulates stomatal opening, improving CO_2 uptake and consequently enhancing photosynthetic efficiency (Rawat et al., 2022). Nitrate from KNO_3 serves as a nitrogen source, which is a vital component of chlorophyll molecules. The availability of nitrogen boosts chlorophyll synthesis, while potassium supports the overall metabolic processes, leading to an increase in both chlorophyll and carotenoid contents (Zrig et al., 2021). Furthermore, potassium nitrate is a source of nitrate, which stimulates the synthesis of growth-regulating hormones such as auxins, leading to enhanced cell division and elongation responses that contribute to increased shoot growth (Abeed et al., 2023).

6.1.3.1.5. Effect on total phenol content and flavonoid content

The effect of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) on total phenol content and total flavonoid content of radish microgreens in the range of 314.02 to 364.82 mg GAE/100g and 32.40 to 36.49 mg QE/100g were observed as shown in **(Table 6.19)**. The total phenol content in radish and turnip greens microgreens (>159 82 mg GAE/100g) was observed in different biostimulants (TRAINER®, AQUAMIN®, leaf moringa extract, and distilled water (control)), the seedlings microgreens. In both species, slight but significant differences were found for total phenol content ($P < 0.05$) (Toscano et al., 2023). Similarly, in another study, the total phenol content value of the barley sprout was 302.8 to 367.7 mg GAE/100g (Liu et al., 2022; Barbarestani et al., 2024). However, our results findings related to total phenol content were slightly similar to these supportive finding results. The effects of alternative Zn sources (ZnSO_4 , ZnO , and Zn-EDTA) and application rates (0, 5, 10, and 15 mg/L of Zn) on total phenol content and flavonoid content of pea, radish, and sunflower microgreens grown in

a soilless cultivation system and the total phenol and flavonoid content 9.9 to 32.0 mg GAE/g and 2.21 to 9.91 mg CE/g, respectively observed. This study showed that the total phenols and flavonoid content increased with increasing Zn rate with all three Zn sources (Poudel et al., 2024). The application of calcium chloride application caused a significant increase in the total phenolic content of cauliflower crops during harvest time from 712.78 to 1055 mg/100g and this study showed that the foliar spray treatment with calcium chloride (0.4%) spraying enhanced the 48% total phenol content compared to compared with control (Pavithra et al., 2021). Similarly, at the highest doses of CaCl_2 (2%), it increased the total phenolic content from 0.25 to 0.35 mg/g in comparison to lower doses and control tomatoes varieties (MT-1, MT-3, 303, and 105) (Mazumder et al., 2021). The effect of foliar application of 0.25 to 0.50% potassium nitrate (KNO_3) on the fruit quality of pomegranate was investigated. In this study, the total phenol content of 255 to 334 mg/L was observed and the total phenol content at higher concentrations was shown (Safa et al., 2020). The effect of salinity (0 to 50 mM NaCl) and foliar treatments (0 to 2 mg/L) on Aloe vera plants were studied and the phenolics content and flavonoid content in leaf (209 to 295 mg/g and 51.3 to 122 mg/g) and in gel (125 to 208 mg/g and 29.3 to 69 mg/g) were found (Ebrahimzadeh et al., 2022).

Table 6.19. Effect of different combination of foliar spray treatments on the biochemical and antioxidant traits in the radish sango microgreens based on the Central Composite Rotatable Design (CCRD).

Treatments	Total chlorophyll (µg/g)	Carotenoid (µg/g)	Anthocyanin (µmol/100g)	Ascorbic acid (mg/100g)	Total glucosinolate (µmol/g)	TPC (mg GAE/100g)	TFC (mg QE/100g)	DPPH (%)	FRAP (µmol TE/g)
T-01	41.12	9.29	63.91	330.83	40.02	342.20	36.22	72.72	36.24
T-02	25.42	7.88	61.55	318.22	25.42	328.30	31.83	70.11	30.98
T-03	37.46	8.55	63.12	340.55	37.46	314.02	32.40	71.65	32.42
T-04	38.19	8.45	63.14	337.94	38.19	316.12	31.61	71.45	31.61
T-05	42.77	9.66	68.99	335.20	43.77	344.91	36.49	73.24	37.15
T-06	33.64	8.07	61.66	298.22	33.64	343.29	34.32	71.15	34.22
T-07	37.85	9.26	67.44	365.06	38.99	334.46	33.44	73.93	33.40
T-08	41.27	9.39	62.23	358.66	42.27	357.28	35.72	73.76	35.12
T-09	31.31	8.48	61.22	330.34	31.33	323.04	32.30	71.43	32.33
T-10	34.20	8.23	61.01	321.32	34.20	323.59	32.35	71.13	32.44
T-11	31.43	8.24	61.82	340.30	31.33	323.22	32.32	71.86	32.40
T-12	32.17	8.27	61.71	339.88	31.77	325.54	32.55	71.26	32.66
T-13	38.97	8.66	61.35	321.02	39.44	364.82	36.48	73.23	36.55

All the experimental results values are presented as mean value basis based on each biochemical and antioxidant trait. Where, n: number of observations; TPC: Total phenol content; TFC: Total flavonoid content; GAE: Gallic acid; QE: Quercetin; DPPH: DPPH Antioxidant activity; FRAP Antioxidant activity.

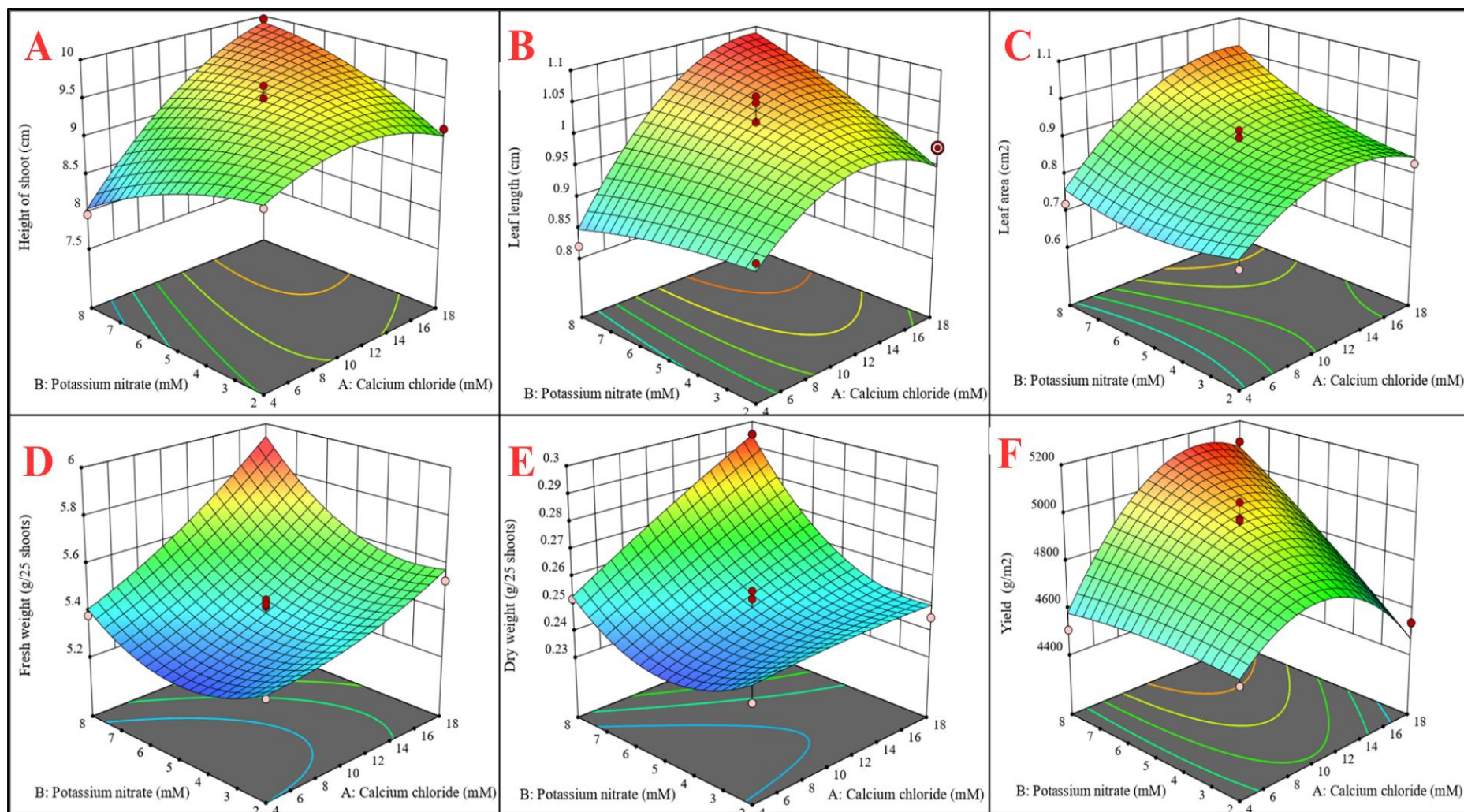


Figure 6.11. Response surface plots showing the effect of calcium chloride (A) and potassium nitrate (B) on morphological traits in radish sango microgreens. Where, A: Height of shoot; B: Leaf length; C: Leaf area; D: Fresh weight; E: Dry weight and; F: Yield.

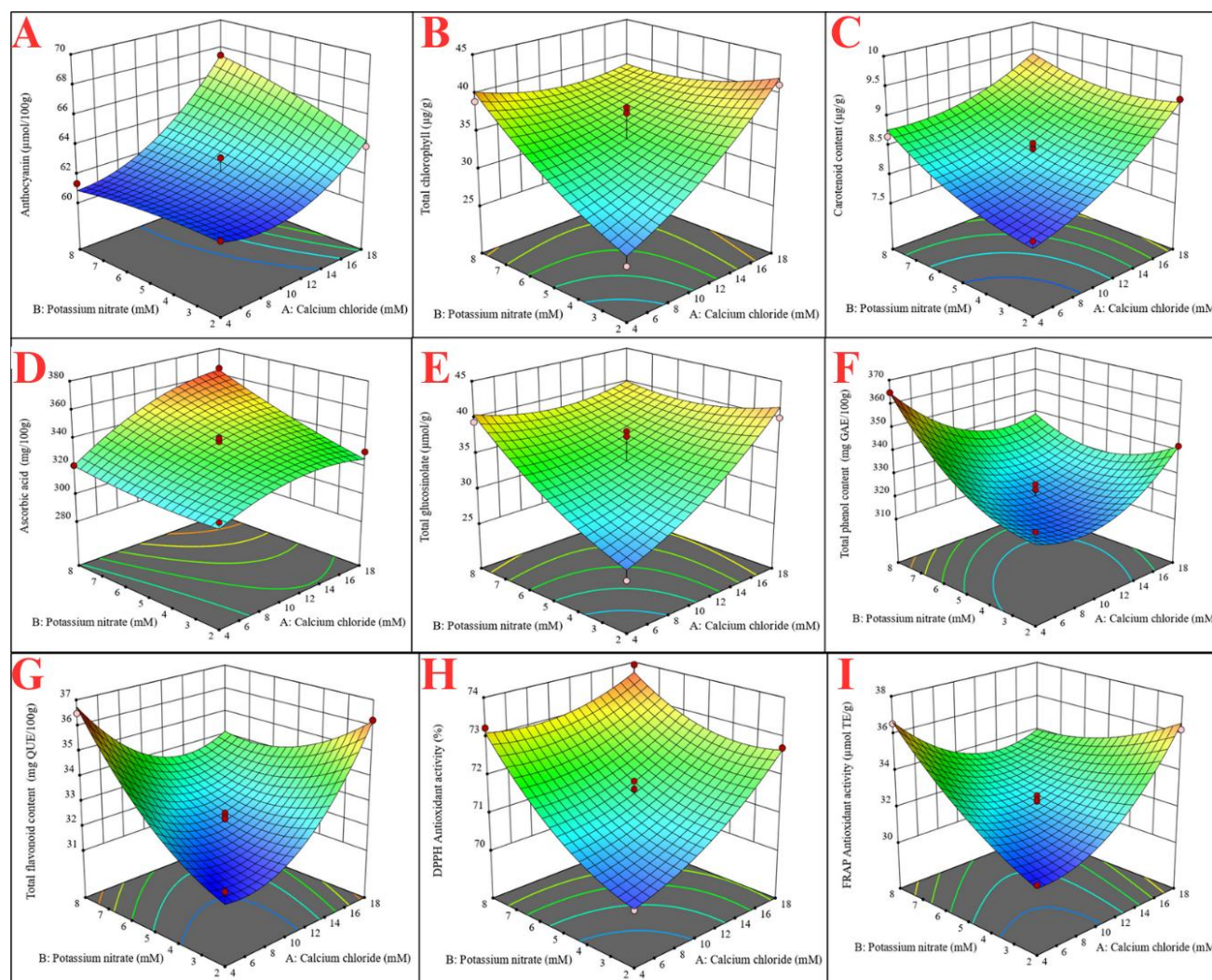


Figure 6.12. Response surface plots showing the effect of calcium chloride (A) and potassium nitrate (B) on biochemical and antioxidant traits in radish sango microgreens. Where, A: Anthocyanin; B: Total chlorophyll; C: Carotenoid content; D: Ascorbic acid; E: Total glucosinolate; F: Total phenol content; G: Total flavonoid content; H: DPPH Antioxidant activity and I: FRAP Antioxidant activity.

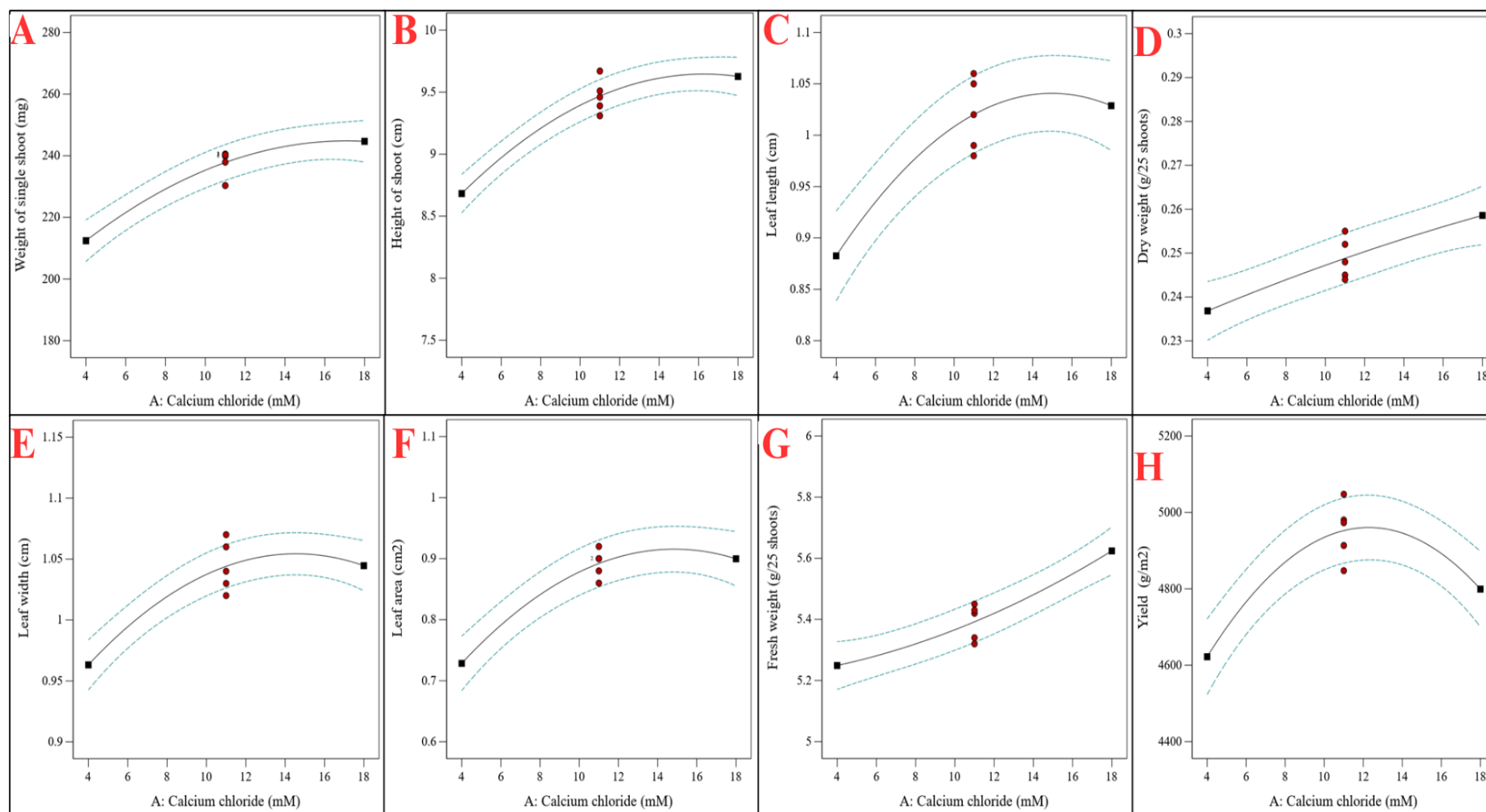


Figure 6.13. Response surface plots showing the effect of calcium chloride (A) on morphological traits in radish sango microgreens. Where, A: Weight of single shoot; B: Height of shoot; C: Leaf length; D: Dry weight; E: Leaf width; F: Leaf area; G: Fresh weight and H: Yield.

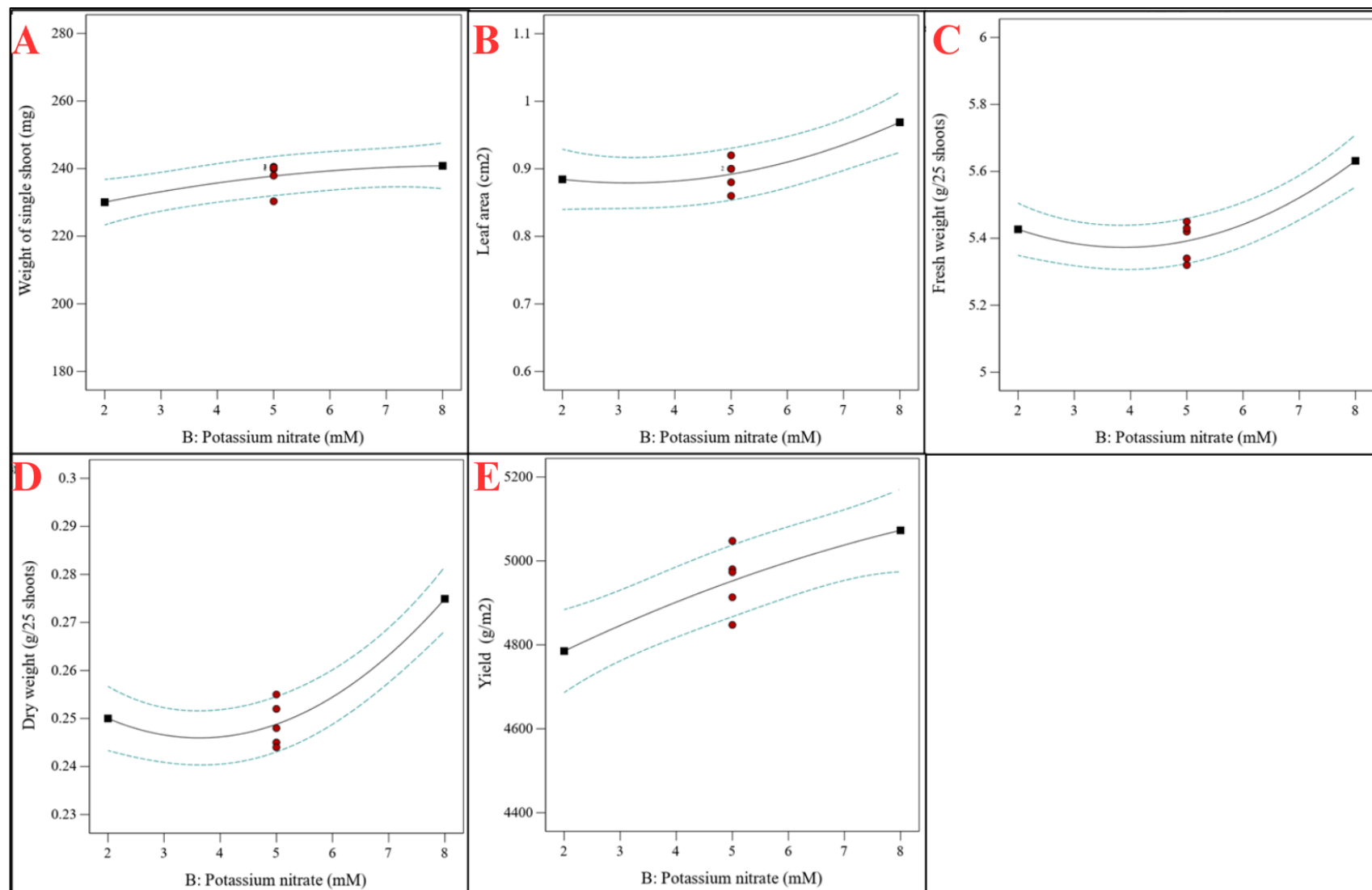


Figure 6.14. Response surface plots showing the effect of potassium nitrate (B) on morphological traits in the radish sango microgreens. Where, A: Weight of single shoot; B: Leaf area; C: Fresh weight; D: Dry weight and E: Yield.

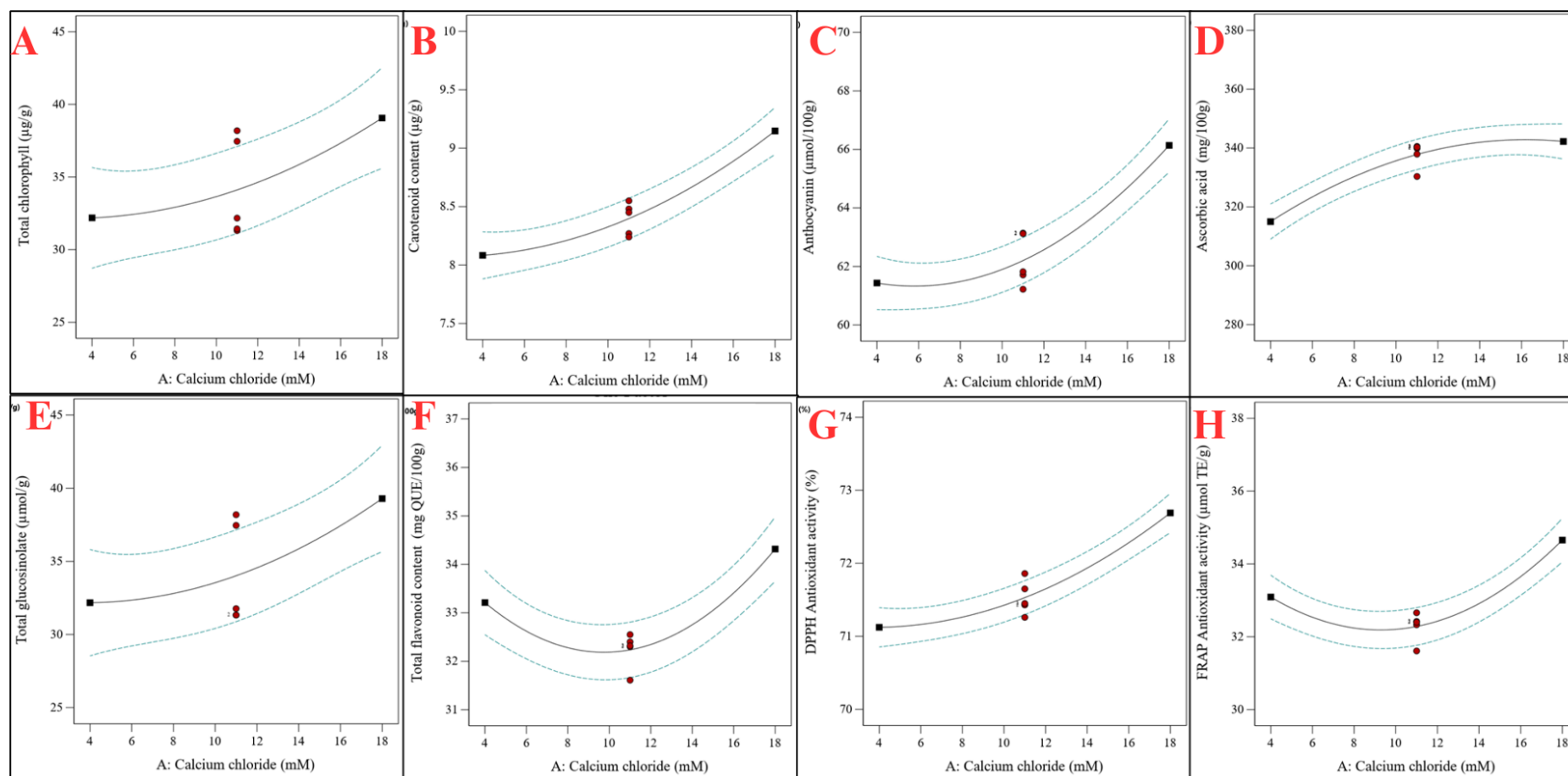


Figure 6.15. Response surface plots showing the effect of calcium chloride (A) on biochemical and antioxidant traits in the radish sango microgreens. Where, A: Total chlorophyll; B: Carotenoid content; C: Anthocyanin; D: Ascorbic acid; E: Total glucosinolate; F: Total flavonoid content; G: DPPH Antioxidant activity and H: FRAP Antioxidant activity.

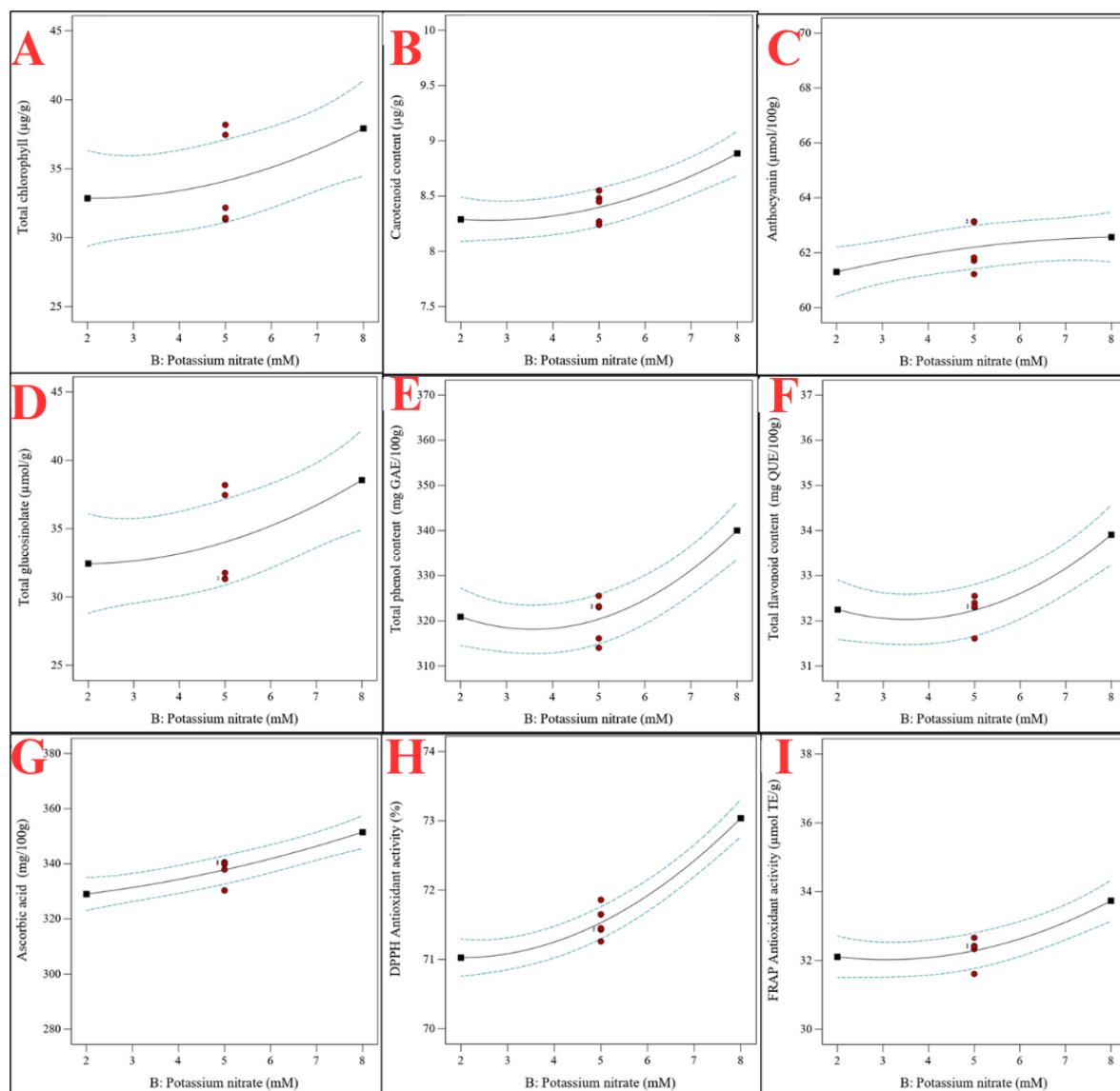


Figure 6.16. Response surface plots showing the effect of potassium nitrate (B) on biochemical and antioxidant traits in the radish sango microgreens. Where, A: Total chlorophyll; B: Carotenoid content; C: Anthocyanin; D: Total glucosinolate; E: Total phenol content; F: Total flavonoid content; G: Ascorbic acid; H: DPPH Antioxidant activity and I: FRAP Antioxidant activity.

Table 6.20. The predicted models to experimental data in CCRD design for the effect of foliar spray treatments on radish sango microgreens selected dependent variables

Variables	Regression equation	Mean±SD	C.V. %	R ²	Adjusted R ²	Predicted R ²	Adeq precision
Weight of single shoot	$237.80+16.12\times A+5.37\times B+12.86\times AB-9.25\times A^2-2.36\times B^2$	230.66±5.50	2.38	0.9441	0.9042	0.7108	17.1426
Height of shoot	$9.47+0.4720\times A-0.0109\times B+0.4575\times AB-0.3134\times A^2-0.1634\times B^2$	9.17±0.12	1.38	0.9682	0.9455	0.8900	21.6353
Leaf length	$1.02+0.0731\times A+0.0225\times B+0.0475\times AB-0.0644\times A^2-0.0094\times B^2$	0.97±0.03	3.66	0.9047	0.8366	0.6190	12.4323
Leaf width	$1.04+0.0407\times A+0.0126\times B-0.0125\times AB-0.0401\times A^2+0.0399\times B^2$	1.04±0.16	1.61	0.9540	0.9211	0.8962	20.6732
Leaf area	$0.8920+0.0857\times A+0.0422\times B+0.0450\times AB-0.0779\times A^2+0.0346\times B^2$	0.86±0.03	4.20	0.9369	0.8918	0.6297	16.4500
Fresh weight	$5.39+0.1875\times A+0.1023\times B+0.0800\times AB+0.0446\times A^2+0.1371\times B^2$	5.50±0.06	1.16	0.9489	0.9123	0.7711	16.8196
Dry weight	$0.2488+0.0109\times A+0.0124\times B+0.0100\times AB-0.0011\times A^2+0.0137\times B^2$	0.25±0.00	2.13	0.9499	0.9141	0.7596	17.1329
Total chlorophyll	$34.11+3.44\times A+2.53\times B-4.20\times AB+1.51\times A^2+1.28\times B^2$	35.83±2.83	7.90	0.9115	0.8984	0.7466	7.9474
Carotenoid content	$8.40+0.5323\times A+0.2988\times B-0.2025\times AB+0.2173\times A^2+0.1898\times B^2$	8.65±0.16	1.90	0.9510	0.9160	0.7580	16.2682
Anthocyanin content	$62.20+2.35\times A+0.6319\times B+0.9325\times AB+1.58\times A^2-0.2685\times B^2$	63.01±0.74	1.18	0.9476	0.9101	0.8582	15.6847
Ascorbic acid	$337.80+13.62\times A+11.23\times B+7.86\times AB-9.19\times A^2+2.45\times B^2$	333.66±4.87	1.46	0.9536	0.9205	0.7845	19.2078
Total glucosinolate	$34.02+3.56\times A+3.05\times B-3.76\times AB+1.72\times A^2+1.48\times B^2$	35.99±2.97	8.24	0.9229	0.8778	0.859	7.7613
Total phenol content	$320.39-1.77\times A+9.55\times B-11.06\times AB+11.90\times A^2+10.07\times B^2$	333.91±5.18	1.56	0.9356	0.8896	0.7319	12.5701
Total flavonoid content	$32.24+0.5524\times A+0.8295\times B-1.86\times AB+1.53\times A^2+0.8426\times B^2$	33.69±0.54	1.61	0.9523	0.9182	0.7290	14.6186
DPPH Antioxidant activity	$71.53+0.7832\times A+1.01\times B-0.4775\times AB+0.3769\times A^2+0.5019\times B^2$	72.07±0.21	0.30	0.9798	0.9653	0.9267	25.5576
FRAP Antioxidant activity	$32.28+0.7817\times A+0.8150\times B-2.10\times AB+1.59\times A^2+0.6380\times B^2$	33.66±0.48	1.45	0.9655	0.9408	0.8264	17.5616

Table 6.21. ANOVA Quadratic model for the different responses

Responses	Source	Sum of Squares	Mean Square	F-value	P-value
Weight of single shoot	Model	3575.57	715.11	23.65	0.0003
	A-Calcium chloride	2078.66	2078.66	68.75	< 0.0001
	B-Potassium nitrate	230.84	230.84	7.64	0.028
	AB	661.44	661.44	21.88	0.0023
	A ²	595.21	595.21	19.69	0.003
	B ²	38.74	38.74	1.28	0.2949
Height of shoot	Model	3.41	0.682	42.63	< 0.0001
	A-Calcium chloride	1.78	1.78	111.43	< 0.0001
	B-Potassium nitrate	0.001	0.001	0.0599	0.8136
	AB	0.8372	0.8372	52.33	0.0002
	A ²	0.6832	0.6832	42.7	0.0003
	B ²	0.1857	0.1857	11.61	0.0113
Leaf length	Model	0.0846	0.0169	13.29	0.0018
	A-Calcium chloride	0.0427	0.0427	33.55	0.0007
	B-Potassium nitrate	0.004	0.004	3.17	0.1182
	AB	0.009	0.009	7.09	0.0324
	A ²	0.0288	0.0288	22.64	0.0021
	B ²	0.0006	0.0006	0.4802	0.5106
Leaf width	Model	0.0407	0.0081	29.02	0.0002
	A-Calcium chloride	0.0132	0.0132	47.18	0.0002
	B-Potassium nitrate	0.0013	0.0013	4.51	0.0712
	AB	0.0006	0.0006	2.23	0.1794
	A ²	0.0112	0.0112	39.88	0.0004
	B ²	0.0111	0.0111	39.39	0.0004
Leaf area	Model	0.1375	0.0275	20.77	0.0005
	A-Calcium chloride	0.0587	0.0587	44.36	0.0003
	B-Potassium nitrate	0.0143	0.0143	10.79	0.0134
	AB	0.0081	0.0081	6.12	0.0426
	A ²	0.0422	0.0422	31.88	0.0008
	B ²	0.0083	0.0083	6.3	0.0404
Fresh weight	Model	0.5265	0.1053	25.98	0.0002
	A-Calcium chloride	0.2813	0.2813	69.4	< 0.0001
	B-Potassium nitrate	0.0837	0.0837	20.65	0.0027
	AB	0.0256	0.0256	6.31	0.0402
	A ²	0.0139	0.0139	3.42	0.107
	B ²	0.1308	0.1308	32.27	0.0008
Dry weight	Model	0.0039	0.0008	26.53	0.0002
	A-Calcium chloride	0.0009	0.0009	31.84	0.0008
	B-Potassium nitrate	0.0012	0.0012	41.72	0.0003
	AB	0.0004	0.0004	13.46	0.008
	A ²	8.23E-06	8.23E-06	0.2768	0.6151
	B ²	0.0013	0.0013	43.69	0.0003
Yield	Model	7.39E+05	1.48E+05	22.72	0.0003

	A-Calcium chloride	62671.38	62671.38	9.63	0.0172
	B-Potassium nitrate	1.66E+05	1.66E+05	25.43	0.0015
	AB	1.05E+05	1.05E+05	16.1	0.0051
	A ²	4.06E+05	4.06E+05	62.38	< 0.0001
	B ²	3799.53	3799.53	0.5837	0.4698
Total chlorophyll	Model	240.83	48.17	6.01	0.0179
	A-Calcium chloride	94.47	94.47	11.79	0.0109
	B-Potassium nitrate	51.4	51.4	6.42	0.0391
	AB	70.73	70.73	8.83	0.0208
	A ²	15.95	15.95	1.99	0.2012
	B ²	11.38	11.38	1.42	0.2722
Carotenoid content	Model	3.66	0.7316	27.16	0.0002
	A-Calcium chloride	2.27	2.27	84.15	< 0.0001
	B-Potassium nitrate	0.7143	0.7143	26.52	0.0013
	AB	0.164	0.164	6.09	0.043
	A ²	0.3283	0.3283	12.19	0.0101
	B ²	0.2505	0.2505	9.3	0.0186
Anthocyanin	Model	69.98	14	25.3	0.0002
	A-Calcium chloride	44.26	44.26	80.01	< 0.0001
	B-Potassium nitrate	3.19	3.19	5.78	0.0472
	AB	3.48	3.48	6.29	0.0405
	A ²	17.45	17.45	31.56	0.0008
	B ²	0.5015	0.5015	0.9067	0.3727
Ascorbic acid	Model	3421.47	684.29	28.8	0.0002
	A-Calcium chloride	1483.89	1483.89	62.45	< 0.0001
	B-Potassium nitrate	1008.99	1008.99	42.46	0.0003
	AB	247.07	247.07	10.4	0.0146
	A ²	587.19	587.19	24.71	0.0016
	B ²	41.84	41.84	1.76	0.2262
Total glucosinolate	Model	264.21	52.84	6	0.018
	A-Calcium chloride	101.36	101.36	11.52	0.0115
	B-Potassium nitrate	74.44	74.44	8.46	0.0227
	AB	56.63	56.63	6.44	0.0388
	A ²	20.55	20.55	2.34	0.1703
	B ²	15.32	15.32	1.74	0.2286
Total phenol content	Model	2743.29	548.66	20.34	0.0005
	A-Calcium chloride	25.09	25.09	0.9301	0.367
	B-Potassium nitrate	730.09	730.09	27.06	0.0013
	AB	489.74	489.74	18.15	0.0037
	A ²	985.18	985.18	36.51	0.0005
	B ²	705.13	705.13	26.13	0.0014
Total flavonoid content	Model	40.91	8.18	27.94	0.0002
	A-Calcium chloride	2.44	2.44	8.34	0.0234
	B-Potassium nitrate	5.5	5.5	18.8	0.0034
	AB	13.8	13.8	47.13	0.0002
	A ²	16.23	16.23	55.44	0.0001

	B ²	4.94	4.94	16.87	0.0045
DPPH Antioxidant activity	Model	16.36	3.27	67.77	< 0.0001
	A-Calcium chloride	4.91	4.91	101.66	< 0.0001
	B-Potassium nitrate	8.1	8.1	167.78	< 0.0001
	AB	0.912	0.912	18.89	0.0034
	A ²	0.9881	0.9881	20.47	0.0027
	B ²	1.75	1.75	36.3	0.0005
FRAP Antioxidant activity	Model	46.79	9.36	39.13	< 0.0001
	A-Calcium chloride	4.89	4.89	20.44	0.0027
	B-Potassium nitrate	5.31	5.31	22.22	0.0022
	AB	17.68	17.68	73.92	< 0.0001
	A ²	17.6	17.6	73.57	< 0.0001
	B ²	2.83	2.83	11.84	0.0108

Similarly, the foliar spray treatment with KNO₃ enhanced the total phenol and flavonoid content by 14 and 45%, respectively as compared with control (pure water) linseed sprouts (Zrig et al., 2023). Differences could arise from variations in genetic backgrounds, germination time, cultivation and environmental factors, agronomic practices, and polarity of solvents used for extraction (Barbarestani et al., 2024).

The quadratic equation linking the effect of calcium chloride (CaCl₂) and potassium nitrate (KNO₃) foliar spray treatments on total phenol content and total flavonoid content is given in **(Table 6.20)**. The linear effect of calcium chloride (CaCl₂) and potassium nitrate (KNO₃) had a negative impact on the flavor of radish sango microgreens on total phenol content and flavonoid content. **(Figure 6.14 (F and G))** explain the effect of calcium chloride (CaCl₂) and potassium nitrate (KNO₃) on total phenol content and flavonoid content. The plot indicates that total phenol and flavonoid content increases with moderate levels of both CaCl₂ and KNO₃, but a decrease is observed at high concentrations (Safa et al., 2020). Phenolic compounds are antioxidants that help in plant defense. Phenolic compounds are synthesized through the shikimic acid pathway, which requires calcium for enzyme activity and potassium for maintaining cellular pH and ionic balance (Adams et al., 2020). These elements indirectly influence the activity of phenylalanine ammonia-lyase (PAL), a key enzyme in phenol biosynthesis (Santos-Sánchez et al., 2019). As shown in **(Figure 6.16 (E))**, the increased phenolic content with KNO₃ application is due to the enhanced activity of PAL and other enzymes involved in the shikimic acid pathway, leading to a higher accumulation of phenolic compounds. Apart from this, calcium aids in cell wall fortification, enhancing phenol stability (Ebrahimzadeh et al., 2022). Furthermore, the enhancement in flavonoid content is due to the upregulation of biosynthetic pathways and improved flavonoid stability within the vacuole,

facilitated by the presence of calcium and potassium, and this effect of shown in **(Figure 6.15 (F))** and **(Figure 6.16 (F))** (Shomali et al., 2022).

6.1.3.1.6. Effect on anthocyanin, ascorbic acid, and total glucosinolate content

Total anthocyanin, ascorbic acid, and total glucosinolate content in radish sango microgreens varied within each foliar spray treatment ($P < 0.05$). The anthocyanin content, ascorbic acid, and total glucosinolate content in the range of 61.01 to 68.99 $\mu\text{mol}/100\text{g}$, 298.22 to 365.06 $\text{mg}/100\text{g}$, and 25.42 to 43.77 $\mu\text{mol}/\text{g}$ were respectively observed in all the foliar spray treatments. The treatments (T-05) showed the highest level of anthocyanin and glucosinolate content and the lowest amount observed in treatment (T-10 and T-02) were observed the anthocyanin and glucosinolate content in radish sango microgreens. The highest level of ascorbic acid was observed in (T-07) and the lowest was observed in (T-06) as shown in **(Table 6.19)**. The foliar spray treatments applied on radish sango microgreens showed a significant effect ($P < 0.05$) on their bioactive compounds. The anthocyanin and ascorbic acid content in the selected microgreens including mungbean, lentil, pearl millet, red radish, mustard, and red cabbage the anthocyanin and ascorbic acid content in ranged from 62.12 to 186.68 $\text{mg}/100\text{g}$ and 16.34 to 48.56 $\text{mg}/100\text{g}$ respectively observed (Dhaka et al., 2023). In another study, the fourteen microgreens belonging to *Brassicaceae*, *Fabaceae*, *Pedaliaceae*, *Polygonaceae*, *Convolvulaceae*, and *Malvaceae* families the anthocyanin and ascorbic acid content in the range of 0.148 to 0.246 $\text{mg CGE}/100\text{g}$ and 16.78 to 128.70 $\text{mg}/100\text{g}$ were observed (Kowitcharoen et al., 2021). Apart from these, in 11 lettuce varieties evaluated in the 3 development stages (microgreen, baby, adult) their anthocyanin and ascorbic acid content, from this study work in microgreens the ascorbic acid 34.26 to 87.01 $\text{mg}/100\text{g}$ and anthocyanin content 36.12 to 72.34 $\mu\text{mol}/100\text{g}$ were observed (Martínez-Ispizua et al., 2022). In another study, the total glucosinolate and ascorbic acid content in 30 varieties of microgreens in the *Brassicaceae* family were in the range of 1.0 to 535.5 $\mu\text{mol}/100\text{g}$ and 18.9 to 89.3 $\text{mg}/100\text{g}$ respectively observed (Xiao et al., 2019). In addition, Pratap et al. (2023) reported that the concentration of glucosinolates was also higher in microgreens (17.15 $\mu\text{mol}/\text{g}$) compared to mature counterparts (8.30 $\mu\text{mol}/\text{g}$). The treatment with CaSO_4 was found highly effective and dramatically enhanced glucosinolate content, especially glucoraphanin, in broccoli sprouts during growth and prevented its loss during storage (Guo et al., 2018). Another study has reported that 10 mM calcium chloride (CaCl_2) treatments during germination and growth in broccoli microgreens increased aliphatic glucosinolates, glucoraphanin, and glucoerucin contents (Lu et al., 2018).

The quadratic equation linking the effect of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) foliar spray treatments on total anthocyanin, ascorbic acid, and total glucosinolate content is given in **(Table 6.20)**. The **(Table 6.20)** represents the regression coefficients equation for the second-order polynomial equations, indicating the suggested model's adequacy, significant lack of fit, and satisfactory R^2 values; furthermore, adjusted R^2 and predicted R^2 , which is typically lower than R^2 measures the model's predictive performance on leaf length, width, and leaf area. All models were regarded as suitable for analysis because their R^2 values all exceeded 80%. In, **(Figure 6.12 (A, D, and E))** explain the effect of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) on total anthocyanin, ascorbic acid, and glucosinolate content. The linear effect of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) on anthocyanin content were shown in **(Figure 6.15 (C))** and **(Figure 6.16 (C))**. **(Figure 6.12 (A))** showed that increasing concentration of CaCl_2 from low to moderate concentrations results in an increase in anthocyanin content. However, the effect of KNO_3 is minimal, with a slight increase in anthocyanin content at moderate KNO_3 levels. Anthocyanins are synthesized through the phenylpropanoid pathway, which is influenced by calcium due to its role in stabilizing cell membranes and protecting against oxidative stress. The slight enhancement by KNO_3 might be due to improved nitrogen availability, which is a precursor for the phenylalanine pathway, indirectly promoting anthocyanin synthesis (Mattioli et al., 2020).

Ascorbic acid content increases with the rise in both CaCl_2 and KNO_3 levels as shown in **(Figure 6.12 (D))**, showing a peak at moderate levels before decreasing slightly at very high concentrations. The linear effect of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) on ascorbic acid content was shown in **(Figure 6.15 (D))** and **(Figure 6.16 (G))**. In this both the figures showed that ascorbic acid content increases with the rise in both CaCl_2 and KNO_3 levels, showing a peak at moderate levels before decreasing slightly at very high concentrations. The enhanced ascorbic acid content with CaCl_2 and KNO_3 foliar spray application is due to increased enzyme activity and substrate availability for ascorbic acid biosynthesis, resulting in higher antioxidant capacity (Rab & Haq, 2012). Calcium helps maintain cellular integrity, reducing the need for ascorbic acid consumption in counteracting oxidative damage. Potassium enhances ascorbate biosynthesis by improving the efficiency of photosynthesis, where ascorbate plays a crucial role. Excess nutrients might stress the plant, causing a slight reduction at very high concentrations (Gallie, 2013). The **(Figure 6.12 (E))** shows the total glucosinolate content increases with moderate CaCl_2 concentration and shows a significant ($P < 0.05$) increase with KNO_3 , particularly at moderate to high concentrations. The

linear effect of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) on ascorbic acid content was shown in **(Figure 6.15 (E))** and **(Figure 6.16 (D))**. Glucosinolates are sulfur-containing compounds with defensive roles. Calcium might influence their synthesis by enhancing cellular structure, while nitrate provides nitrogen, which is crucial for the amino acid derivatives involved in glucosinolate biosynthesis. High nitrate availability particularly drives glucosinolate synthesis, leading to the observed trend (Guo et al., 2018; Lu et al., 2018).

6.1.3.1.7. Effect on antioxidant activities

The highest level of DPPH radical scavenging activity 73.93% was observed in treatment (T-07) and the lowest level of 70.11% was observed in treatment (T-02) as shown in **(Table 6.19)**. The total carotenoid content was observed in a range of 30.98 to 37.15 $\mu\text{mol TE/g}$ among all treatments. The lowest ferric-reducing antioxidant power (FRAP) was observed in (T-02) and the highest was observed in (T-05) foliar spray treatment, as shown in **(Table 6.19)**. The foliar spray treatments applied on radish sango microgreens showed a significant effect ($P < 0.05$) on antioxidant activities. The antioxidant activity in radish sango microgreens significantly ($P < 0.05$) increased by increasing the concentration of CaCl_2 and KNO_3 , where both had the maximum positive impact observed in **(Table 6.21)**. Similar trends for DPPH-based free radical scavenging activity ranging from 62 to 84% were observed in six diverse microgreen species mungbean, lentil, red radish, pearl-millet, mustard, and red cabbage (Dhaka et al., 2023). Furthermore, other studies have reported varying DPPH values for different microgreens spinach, carrot, bathua, and Bengal gram in the range of 46.30 to 90.60% were observed (Sanyukta et al., 2023). Similarly, the FRAP activity in onion microgreens was recorded as 7.0 $\mu\text{mol Fe}^{2+}/\text{g}$, while this was 36.3 $\mu\text{mol Fe}^{2+}/\text{g}$ in roselle (Ghoora et al., 2020).

In another study, reported that the FRAP antioxidant activity for mungbean and lentil microgreens grown under Delhi and Leh conditions in the ranged of 5.70 to 20.44 $\mu\text{mol TE/g}$ and 14.70 to 55.00 $\mu\text{mol TE/g}$ observed (Priti et al., 2021). However, KNO_3 foliar spray resulted in a significant increase in total antioxidant capacity, with increases of 27%, respectively (Zrig et al., 2021). Specifically, a low dose (1%) of CaCl_2 foliar spray resulted in an 8.33% increase in DPPH radical scavenging activity compared to the control group in tomato crops, indicating a potentially similar effect was observed in radish sango microgreens (Mazumder et al., 2021). The application of 0 to 20 mM KNO_3 foliar spray on radish two varieties (Mino and 40 day) has been shown to increase the activities of several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), which contribute to improved DPPH radical scavenging activities (Abeed et al., 2023).

The quadratic equation linking the effect of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) foliar spray treatments on radish sango microgreens antioxidant activities is given in (Table 6.20). The (Figure 6.12 (H and I)) showed the effect of calcium chloride and potassium nitrate on DPPH radical scavenging activity and ferric-reducing antioxidant power, which indicates antioxidant capacity, increases at moderate levels of both CaCl_2 and KNO_3 , with a decline at very high concentrations. The increase in antioxidant activities is linked to the higher content of antioxidant compounds (ascorbic acid, phenols, flavonoids) and the improved stability of antioxidant enzymes, resulting from the synergistic effects of CaCl_2 and KNO_3 as shown in (Figure 6.15 (G and H)) and (Figure 6.16 (H and I)). The increase in antioxidant activity reflects the cumulative effect of increased phenolic and flavonoid contents. Calcium and potassium improve cellular health and metabolic function, boosting antioxidant activity. However, excessive nutrient levels may induce oxidative stress, leading to a reduction in antioxidant efficacy (Mazumder et al., 2021).

6.1.3.1.8. Numerical optimization for foliar spray

The software analysis recommended an optimal combination of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) foliar spray treatments at 18.00 mM and 8.00 mM, respectively, for the radish sango microgreens growing, which resulted in a desirability of 0.809. The (Table 6.22) provides a detailed overview of the optimization criteria used for different processes and response variables of foliar spray treatments for radish sango microgreens. Accurateness of the optimized condition was identified by comparing the average experimental values and the predicted values developed by the model were presented in (Table 6.23). The desirability ramp obtained from optimal points is represented in (Figure 6.17).

Table 6.22. Optimization criteria for different process and response variables of foliar spray treatments for radish sango microgreens

Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
A: Calcium chloride	In range	4	18	1	1	3
B: Potassium nitrate	In range	2	8	1	1	3
Weight of single shoot	Maximize	198.22	265.06	1	1	3
Height of shoot	Maximize	7.97	9.96	1	1	3
Leaf length	Maximize	0.8	1.08	1	1	3
Leaf width	Maximize	0.91	1.15	1	1	3
Leaf area	Maximize	0.65	1.06	1	1	3
Fresh weight	Maximize	5.23	5.88	1	1	3
Dry weight	Maximize	0.235	0.295	1	1	3
Yield	Maximize	4413.85	5142.58	1	1	3
Total chlorophyll	Maximize	25.42	42.77	1	1	3
Carotenoid content	Maximize	7.88	9.66	1	1	3
Anthocyanin	Maximize	61.01	68.99	1	1	3
Ascorbic acid	Maximize	298.22	365.06	1	1	3
Total glucosinolate	Maximize	25.42	43.77	1	1	3
Total phenol content	Maximize	314.02	364.82	1	1	3
Total flavonoid content	Maximize	31.61	36.49	1	1	3
DPPH Antioxidant activity	Maximize	70.11	73.93	1	1	3
FRAP Antioxidant activity	Maximize	30.98	37.15	1	1	3

Table 6.23. Predicted and observed optimum responses for effect of foliar spray treatment on radish sango microgreens

Responses	Predicted values	Observed values
Weight of single shoot (mg)	260.542 ^b	282.07±15.46 ^a
Height of shoot (cm)	9.91 ^b	9.92±0.07 ^a
Leaf length (cm)	1.089 ^a	1.26±0.20 ^a
Leaf width(cm)	1.085 ^b	1.09±0.03 ^a
Leaf area (cm ²)	1.022 ^b	1.05±0.02 ^a
Fresh weight (g/25 shoots)	5.944 ^a	6.10±0.12 ^a
Dry weight (g/25 shoots)	0.295 ^b	0.298±0.002 ^a
Yield (g/m ²)	5081.653 ^b	5095.88±12.00 ^b
Total chlorophyll (µg/g)	38.671 ^a	38.89±0.16 ^a
Carotenoid content (µg/g)	9.434 ^b	9.76±0.23 ^a
Anthocyanin (µmol/100g)	67.434 ^b	67.66±0.19 ^a
Ascorbic acid (mg/100g)	363.776 ^a	363.67±0.33 ^a
Total glucosinolate (µmol/g)	40.066 ^b	41.10±0.19 ^a
Total phenol content (mg GAE/100g)	339.073 ^b	339.98±0.73 ^a
Total flavonoid content (mg QE/100g)	34.131 ^b	34.37±0.22 ^a
DPPH Antioxidant activity (%)	73.721 ^b	73.86±0.57 ^a
FRAP Antioxidant activity (µmol TE/g)	34.007 ^b	34.50±0.41 ^a

The obtained results from experiments are represented in mean±standard deviation values with displayed significant differences (P<0.05).

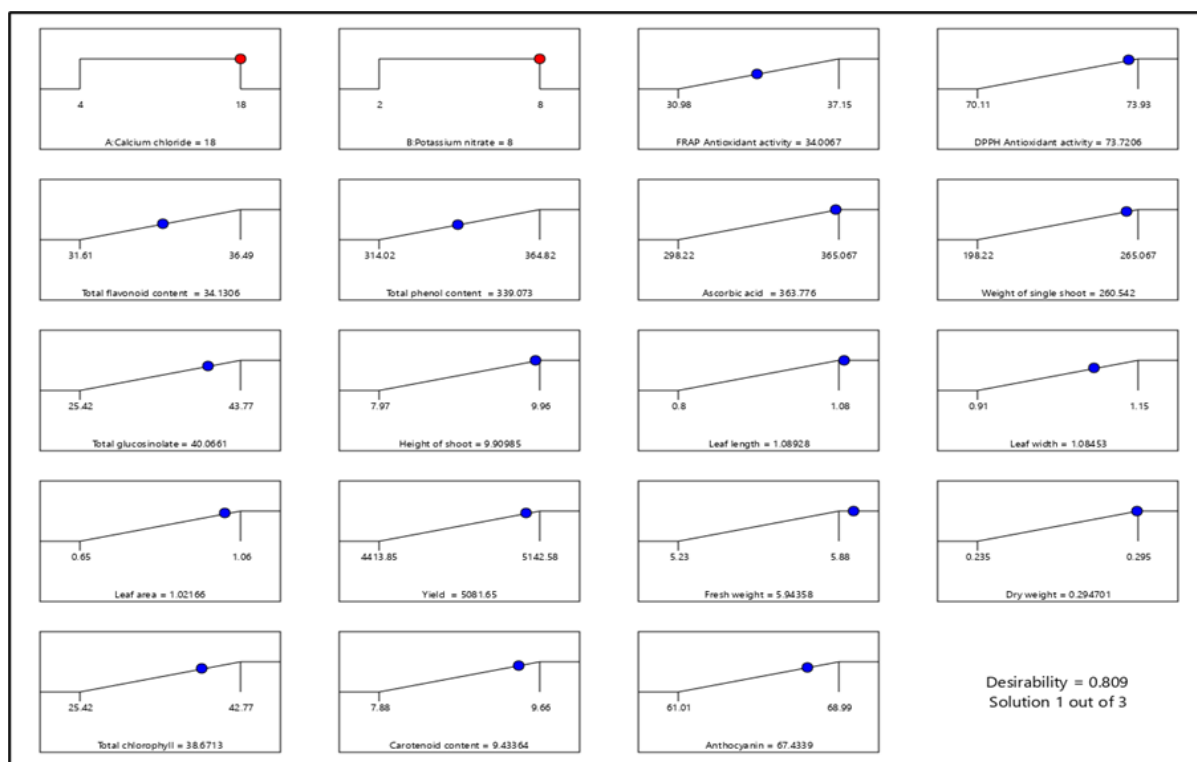


Figure 6.17. Desirability ramp for optimization of foliar spray treatment

6.1.3.1.9. Principal Component Analysis (PCA)

In this study, Principal Component Analysis (PCA) was utilized to evaluate and distinguish the effects of different foliar spray treatments on the morphological, biochemical, and antioxidant traits of Radish Sango microgreens. PCA is a multivariate statistical technique that reduces the complexity of datasets by transforming multiple variables into a few uncorrelated principal components (PCs), which capture the maximum variance in the data. This approach was applied to identify patterns, highlight similarities and differences between treatments, and understand which traits are most responsible for the observed variability in the response of radish sango microgreens to the foliar treatments. The loadings of each variable on the principal components help to clarify the relationships between the measured traits and how they contribute to the differences between treatments. In this study, PCA was conducted on several key morphological parameters such as shoot weight (WS), shoot height (HS), leaf length (LL), leaf width (WL), leaf area (LA), fresh weight (FW), and dry weight (DW). In addition to these morphological traits, biochemical parameters like total phenol content (TPC), total flavonoid content (TFC), ascorbic acid, carotenoid, anthocyanin, and antioxidant activities (DPPH and FRAP) were also included in the analysis were shown in **(Figure 6.18 (B))**.

Based on the Kaiser criterion (eigenvalue > 1), three principal components were retained, explaining a cumulative 83.1% of the total variance (Table 1) for the PC-1 (eigenvalue 8.5922) accounted for 50.5% of the variance, PC-2 (eigenvalue 5.5275) explained 32.5% and

PC-3 (eigenvalue 1.2082) contributed 7.1%. The loading plot (Figure 1B) reveals that PC-1 is characterized by high positive loadings for fresh weight (0.308), dry weight (0.285), carotenoids (0.299), ascorbic acid (0.300), DPPH activity (0.284), total glucosinolates (0.257), and weight of single shoot (0.258). Modest contributions also come from chlorophyll (0.247) and leaf area (0.282). This pattern indicates that PC1 represents a general growth-and-bioactivity axis, where treatments with high PC1 scores exhibit both vigorous biomass accumulation and elevated bioactive compound levels. The PCA model revealed that the first two principal components, PC1 and PC2, accounted for a significant portion of the total variance, explaining 83.1% of the variation among the treatments (PC1 = 50.5%, PC2 = 32.5%) as shown in **(Figure 6.18)**. This high percentage indicates that most of the variability in the data can be attributed to these two components, making them highly informative for interpreting the differences between the treatments. The remaining variance was captured by subsequent components, but these explained only small portions of the total variance, suggesting that PC1 and PC2 provide the most meaningful insights into the dataset.

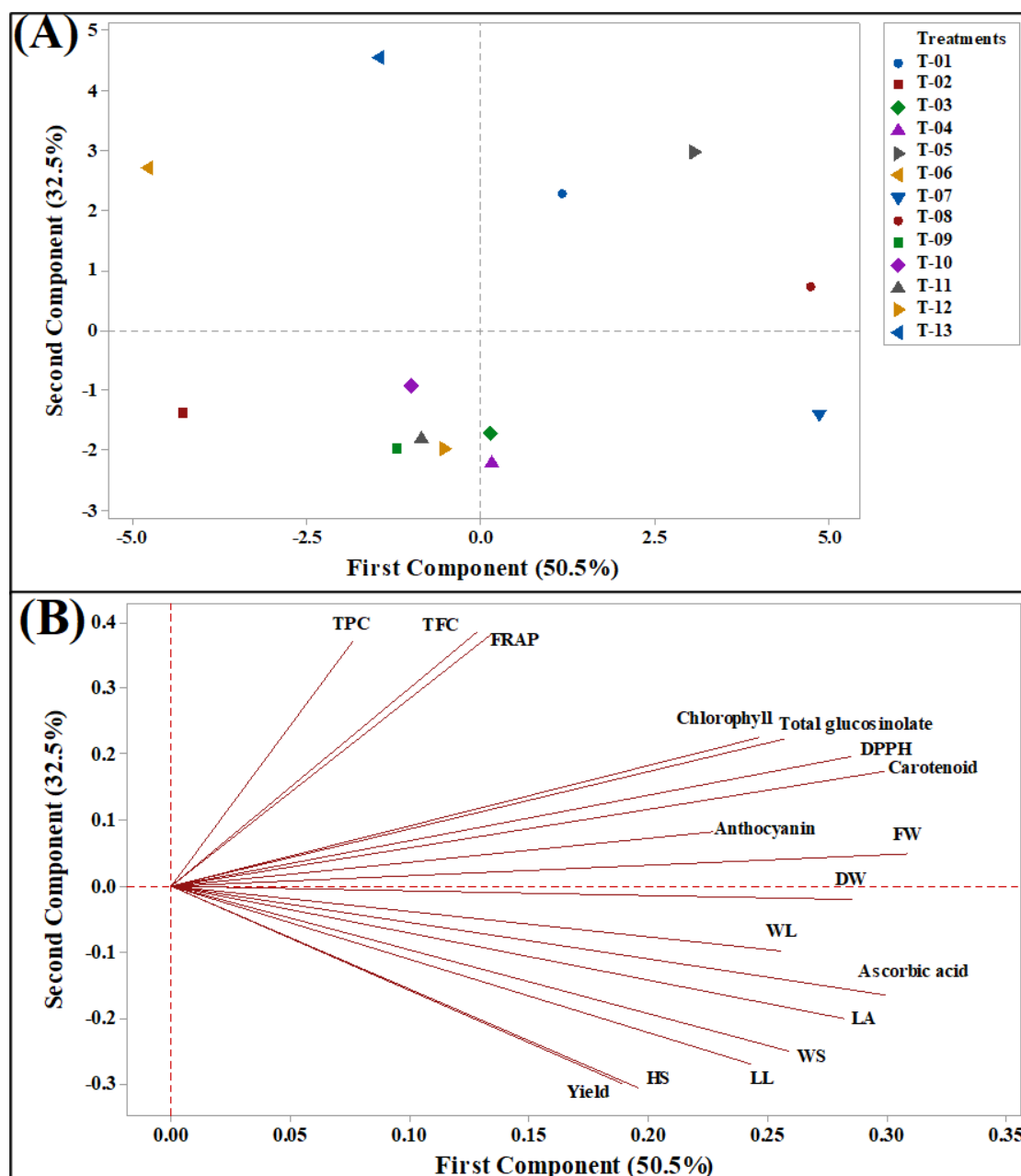


Figure 6.18. Principle component analysis (PCA) of different combination of foliar spray treatments on the morphological, biochemical and antioxidant traits in the radish sango microgreens (A) the combinations of different treatments, (B) the different selected parameters. Where, WS: Weight of shoot, HS: Height of shoot, LL: Length of leaf, WL: Width of leaf, LA: Leaf area, FW: Fresh weight, DW: Dry weight, TPC: Total phenol content, TFC: Total flavonoid content, DPPH and FRAP: Antioxidant activities.

The PC-1 was found to account for 50.5% of the total variance, making it the most dominant component in explaining the differences between treatments. A high positive loading on PC-1 was observed in **(Figure 6.18 (B))** for several important traits, including fresh weight (FW), dry weight (DW), carotenoid content, and the antioxidant capacities measured by DPPH and FRAP. The strong positive loadings indicate that these traits contributed significantly to the variation captured by PC-1, meaning that treatments with higher values for these variables

were positioned towards the positive end of PC-1. Fresh and dry weight are crucial indicators of biomass accumulation in microgreens, which in turn reflect the overall growth performance and vigor of the plants. Higher carotenoid content, along with elevated antioxidant activities (DPPH and FRAP), suggests improved nutritional and bioactive potential, which are desirable traits for microgreens in both commercial and health-promoting applications. These results suggest that treatments with higher scores on PC1 not only supported better growth but also enhanced the accumulation of bioactive compounds with antioxidant properties. Treatments like T1, T2, and T3 were positioned in the positive quadrant of PC-1, indicating that these treatments were associated with superior morphological characteristics, such as greater shoot and leaf development, as well as higher levels of antioxidants and carotenoids. This suggests that these treatments were effective in promoting both growth and the synthesis of health-beneficial compounds in radish sango microgreens.

Another PC-2 accounted for an additional 32.5% of the total variance. The PC-2, that explains 32.5% of the variance, loads most strongly on total flavonoid content (0.386), total phenolic content (0.369), and FRAP activity (0.381), with secondary contributions from chlorophyll (0.225) and glucosinolates (0.222). Negative loadings appear for shoot height (−0.306) and weight of single shoot (−0.249). Thus, PC-2 distinguishes treatments by their capacity to accumulate phenolic and flavonoid antioxidants, somewhat independently of biomass traits. While PC-1 captured the majority of the variation in terms of growth-related traits and antioxidant activity, PC-2 highlighted the role of phenolic and flavonoid compounds, which are known for their strong antioxidant properties and contribution to plant defense mechanisms. Phenolic compounds and flavonoids play critical roles in the nutritional value of microgreens, contributing to their health-promoting properties due to their capacity to neutralize free radicals. The fact that these traits are heavily loaded on PC2 suggests that treatments positioned in the positive direction of PC2 are characterized by higher phenolic and flavonoid content. For instance, treatments such as T6 and T9 were positioned in this quadrant, indicating that these treatments promoted the accumulation of these bioactive compounds as shown in **(Figure 6.18 (A))**. The separation of phenolic and flavonoid content from traits like fresh weight and antioxidant activity on PC-2 implies that these compounds are influenced by different treatment factors compared to the variables loaded on PC-1. While treatments with high scores on PC-1 were characterized by improved growth and general antioxidant capacity, treatments scoring higher on PC-2 had enhanced phenolic and flavonoid profiles, suggesting that the treatments could have differential effects depending on the target traits. The treatments were grouped based on their scores on PC-1 and PC-2, revealing distinct patterns of similarity

and differences in how they influenced the measured traits. Treatments positioned in the positive quadrant of PC-1 and PC-2 were characterized by better overall growth, higher biomass accumulation, and elevated levels of antioxidants, carotenoids, phenolic compounds, and flavonoids. In contrast, treatments in the negative quadrant of PC-1 or PC-2 showed lower values for these desirable traits, indicating less effective foliar treatments.

Table 6.24. Loadings of the first three principal components based on factor analysis of effect of foliar spray treatment on different morphological, biochemical and antioxidant parameters of radish sango microgreens.

Factor loadings	Principal components (PC)		
	PC-1	PC-2	PC-3
Microgreens weight of single shoot (WSS)	0.258	-0.249	-0.187
Microgreens shoot height (HS)	0.196	-0.306	-0.332
Microgreens leaf length (LL)	0.243	-0.269	-0.030
Microgreens width leaf (WL)	0.256	-0.098	0.406
Microgreens leaf area (LA)	0.282	-0.200	0.184
Microgreens fresh weight (FW)	0.308	0.048	-0.002
Microgreens dry weight (DW)	0.285	-0.019	0.280
Yield	0.189	-0.298	0.225
Chlorophyll content	0.247	0.225	-0.082
Carotenoid content	0.299	0.173	-0.155
Anthocyanin content	0.227	0.082	-0.615
Ascorbic acid	0.300	-0.164	0.084
Total glucosinolate content	0.257	0.222	-0.073
Total phenol content (TPC)	0.076	0.369	0.306
Total flavonoid content (TFC)	0.128	0.386	0.030
Antioxidant activity DPPH	0.284	0.195	0.079
Antioxidant activity FRAP	0.134	0.381	-0.055
Eigenvalue	8.5922	5.5275	1.2082
Total variance (%)	50.5	32.5	7.1
Cumulative variance (%)	50.5	83.1	90.2

The use of PCA in this study successfully highlighted the complex interactions between foliar spray treatments and the resulting morphological, biochemical, and antioxidant traits in radish sango microgreens. By reducing the dataset to two principal components, the analysis captured the majority of the variability, allowing for clear distinctions to be made between treatments concerning their effects on growth and bioactive compound accumulation. Treatments such as T1, T2, and T3 emerged as the most effective in promoting both growth and nutritional quality, while treatments like T6 and T9 offered valuable insights into enhancing specific bioactive compounds like phenolics and flavonoids. These findings provide a solid foundation for developing optimized foliar spray strategies to enhance the production of high-quality microgreens with targeted nutritional benefits.

6.2. Post-harvest shelf life of microgreens at different storage condition

The study results showed that washing treatments and storage temperatures had a significant impact on all post-harvest quality parameters of radish sango microgreens. An increase in storage temperature led to a notable reduction in shelf life ($P<0.05$). Specifically, treatment-washed samples stored at 5°C lasted 12 days, whereas those stored at 25°C lasted only 3 days.

6.2.1. Weight loss

The percentage of weight loss increased with rising storage temperatures (**Table 6.25, Appendices Table 1, and Figure 6.19 (A)**). A statistically significant increase in weight loss was observed over time across all washing treatments at both evaluated storage temperatures ($P<0.05$). Notably, after only three days at 25°C, a significant rise in weight loss was recorded ($P<0.05$), underscoring the impact of higher temperatures on microgreen shelf-life. At a storage temperature of 5°C, the percentage of weight loss on day 12 ranged from 1.76% to 3.46%. Over the entire 12 day storage period, the weight loss at this lower 5°C temperature fluctuated between 2.88% and 3.82%. Microgreens packed in various materials, including Low-Density Polyethylene (LDPE), High-Density Polyethylene (HDPE), Metalized Polyester (MP), and Polystyrene Trays (PT), all exhibited weight loss, albeit at varying rates. Specifically, those stored in PT packaging experienced a higher weight loss rate compared to those in MP packaging (**Figure 6.19 (A)**). Among the different washing treatments, sodium hypochlorite demonstrated the most pronounced effect on weight loss, significantly lower than other treatments ($P<0.05$). Furthermore, the highest weight loss was observed in microgreens treated with acetic acid, followed by those subjected to control conditions and treatments with ethanol, calcium chloride, distilled water, and citric acid (**Appendices Table 1**). These findings are consistent with previous studies that examined the storage of mung bean sprouts at temperatures of 4°C and 20°C (Zhang et al., 2018), as well as mustard microgreens stored at 5°C, 10°C, 15°C, 20°C, and 25°C (Dayarathna et al., 2023). The results suggest that low-temperature storage conditions effectively inhibit the respiration rates of microgreens, leading to a reduction in weight loss. Similar patterns were reported in studies on radish microgreens (Xiao et al., 2013) and buckwheat microgreens (Yan et al., 2022).

Table 6.25. Effect of different washing treatments on radish sango microgreens stored in various packaging materials at 25°C on weight loss, electrolyte leakage, overall quality, and off-odor.

Washing treatments	Packaging materials	Days (D)	Overall quality	Off-odor	Weight loss (%)	Electrolyte leakage (%)
Acetic acid	AW	D-0	7.47±0.31 ^b	0.90±0.10 ^a	0.00±0.00 ^a	1.83±0.33 ^a
	HDPE	D-3	5.67±0.58 ^{bA}	4.00±0.00 ^{aA}	29.63±0.45 ^{aBC}	80.87±0.79 ^{aC}
	LDPE	D-3	5.00±0.00 ^{bBC}	4.00±0.00 ^{aA}	29.63±0.45 ^{aC}	81.95±1.12 ^{aB}
	MP	D-3	5.33±0.58 ^{bAB}	4.00±0.00 ^{aA}	27.33±0.76 ^{aB}	81.56±2.09 ^{aBC}
	PT	D-3	5.00±0.00 ^{bC}	4.00±0.00 ^{aA}	29.93±0.35 ^{aA}	84.37±0.30 ^{aA}
Calcium chloride	AW	D-0	8.27±0.12 ^a	0.50±0.10 ^{bc}	0.00±0.00 ^b	1.54±0.22 ^a
	HDPE	D-3	5.67±0.58 ^{aA}	4.00±0.00 ^{bcA}	22.73±0.34 ^{bBC}	80.34±0.97 ^{aC}
	LDPE	D-3	5.00±0.00 ^{aBC}	4.00±0.00 ^{bcA}	22.03±0.25 ^{bC}	81.65±0.32 ^{aB}
	MP	D-3	5.33±0.58 ^{aAB}	4.00±0.00 ^{bcA}	22.93±0.40 ^{bB}	81.08±1.55 ^{aBC}
	PT	D-3	5.00±0.00 ^{aC}	4.00±0.00 ^{bcA}	25.63±0.45 ^{bA}	84.37±0.30 ^{aA}
Citric acid	AW	D-0	8.43±0.06 ^a	0.60±0.17 ^b	0.00±0.00 ^d	2.11±0.13 ^a
	HDPE	D-3	5.67±0.58 ^{aA}	4.00±0.00 ^{bA}	15.03±0.04 ^{dBC}	80.26±0.31 ^{aC}
	LDPE	D-3	5.00±0.00 ^{aBC}	4.00±0.00 ^{bA}	15.33±0.26 ^{dC}	81.27±0.49 ^{aB}
	MP	D-3	5.33±0.58 ^{aAB}	4.00±0.00 ^{bA}	14.93±0.02 ^{dB}	81.41±0.76 ^{aBC}
	PT	D-3	5.00±0.00 ^{aC}	4.00±0.00 ^{bA}	22.83±1.29 ^{dA}	84.60±0.27 ^{aA}
Distilled water	AW	D-0	8.40±0.10 ^a	0.33±0.15 ^d	0.00±0.00 ^d	1.90±0.13 ^b
	HDPE	D-3	5.67±0.58 ^{aA}	4.00±0.00 ^{dA}	14.93±0.07 ^{dB}	77.33±1.03 ^{bC}
	LDPE	D-3	8.40±0.10 ^{aBC}	0.33±0.15 ^{dA}	0.00±0.00 ^{dC}	1.90±0.13 ^{bB}
	LDPE	D-3	5.00±0.00 ^{aBC}	4.00±0.00 ^{dA}	15.23±0.25 ^{dC}	78.77±1.31 ^{bB}
	MP	D-3	5.33±0.58 ^{aAB}	4.00±0.00 ^{dA}	18.93±0.02 ^{dB}	78.28±2.73 ^{bBC}
	PT	D-0	5.00±0.00 ^{aC}	4.00±0.00 ^{dA}	19.73±0.29 ^{dA}	81.67±0.35 ^{bA}
Ethanol	AW	D-0	8.07±0.12 ^a	0.53±0.06 ^{bc}	0.00±0.00 ^b	1.68±0.24 ^a
	HDPE	D-3	5.67±0.58 ^{aA}	4.00±0.00 ^{bcA}	23.33±0.19 ^{bBC}	80.86±0.31 ^{aC}
	LDPE	D-3	5.00±0.00 ^{aBC}	4.00±0.00 ^{bcA}	22.83±0.24 ^{bC}	81.65±0.32 ^{aB}
	MP	D-3	5.33±0.58 ^{aAB}	4.00±0.00 ^{bcA}	23.53±0.21 ^{bB}	81.14±0.58 ^{aBC}
	PT	D-3	5.00±0.00 ^{aC}	4.00±0.00 ^{bcA}	24.73±0.16 ^{bA}	85.08±0.48 ^{aA}
Sodium hypochlorite	AW	D-0	8.30±0.17 ^a	0.57±0.06 ^b	0.00±0.00 ^b	1.97±0.22 ^a
	HDPE	D-3	5.33±0.58 ^{aA}	4.00±0.00 ^{bA}	23.23±0.21 ^{bBC}	80.87±1.71 ^{aC}
	LDPE	D-3	5.00±0.00 ^{aA}	4.00±0.00 ^{bA}	22.63±0.40 ^{bC}	81.15±0.67 ^{aB}
	MP	D-3	5.67±0.58 ^{aAB}	4.00±0.00 ^{bA}	23.43±0.49 ^{bB}	81.14±0.58 ^{aBC}
	PT	D-3	5.00±0.00 ^{aC}	4.00±0.00 ^{bA}	24.73±0.56 ^{bA}	82.68±0.36 ^{aA}
Control	AW	D-0	8.20±0.10 ^a	0.40±0.10 ^{cd}	0.00±0.00 ^c	1.54±0.22 ^c
	HDPE	D-3	5.00±0.00 ^{aA}	4.00±0.00 ^{cdA}	24.23±3.48 ^{cBC}	75.49±1.08 ^{cC}
	LDPE	D-3	5.67±0.58 ^{aA}	4.00±0.00 ^{cdA}	20.83±1.56 ^{cC}	78.31±0.51 ^{cB}
	MP	D-3	5.33±0.58 ^{aAB}	4.00±0.00 ^{cdA}	23.13±0.14 ^{cB}	76.51±2.87 ^{cBC}
	PT	D-3	5.00±0.00 ^{aC}	4.00±0.00 ^{cdA}	19.03±0.43 ^{cA}	79.94±0.35 ^{cA}

Results are expressed as mean ± standard deviation of three replicates. The different lowercase letters within the column indicate significant ($P < 0.05$) differences based on washing treatments and different uppercase letters within the column indicate significant ($p < 0.05$) differences among the packaging materials. Where, D: Days; AW: After wash; HDPE: High-Density Polyethylene; LDPE: Low-Density Polyethylene; MP: Metalized Polyester; PT: Polystyrene Trays.

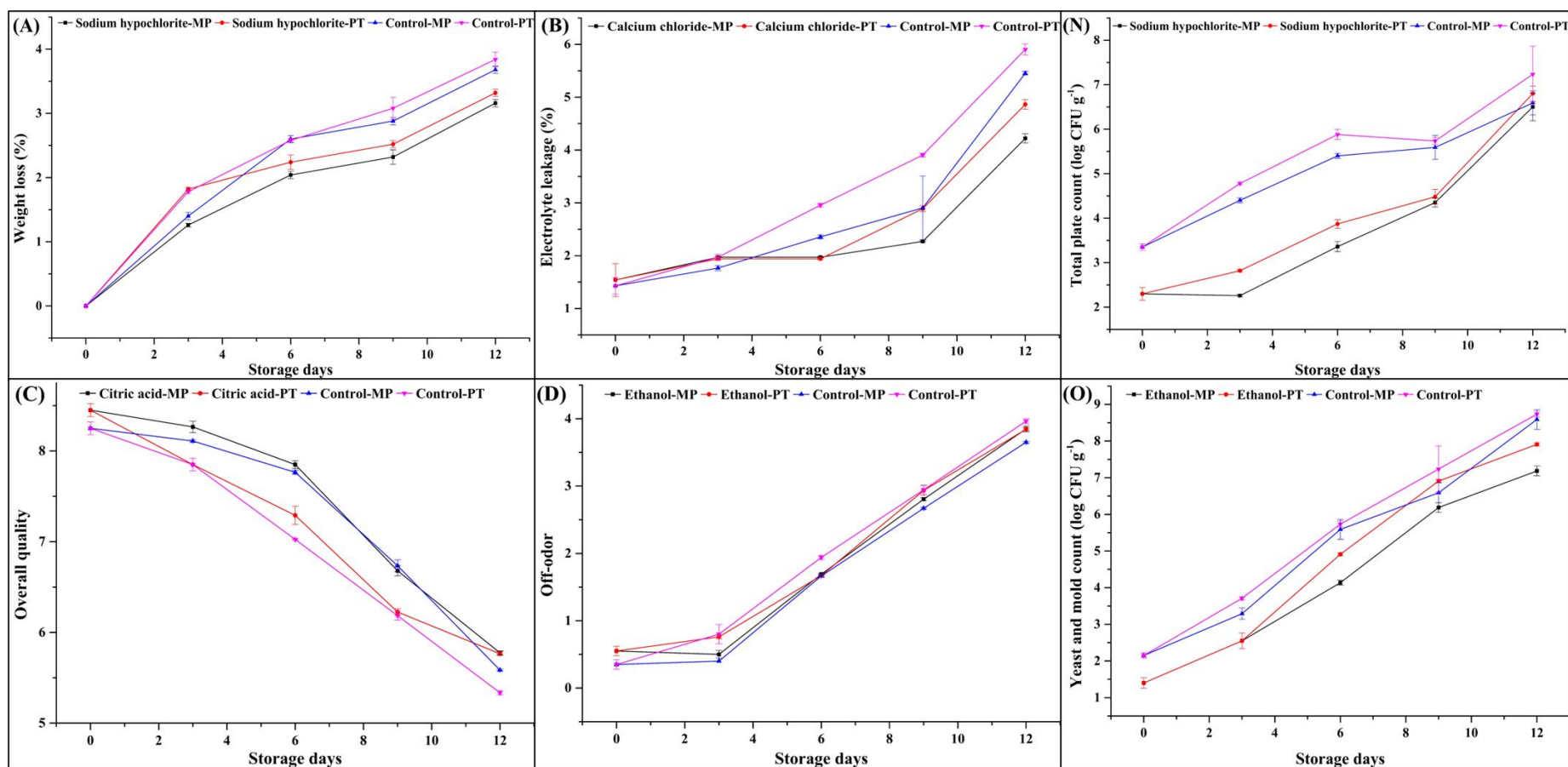


Figure 6.19. Effect of different washing treatments on radish sango microgreens stored in various packaging materials at 5°C on weight loss (A), electrolyte leakage (B), overall quality (C), off-odor (D), total plate count (N), and yeast and mold (O). The figure presents the significant effects ($P < 0.05$) of treatments based on 28 experimental treatments for respective study parameters. Results are expressed as mean $\pm$ standard deviation of three replicates, with error bars indicating the standard deviation from the mean values.

Water loss is a critical factor in the postharvest deterioration of fresh produce, as noted by Ghoola and Srividya (2020). This leads to decreased weight and compromised texture, affecting consumer preference for fresh, crispy microgreens. Our study shows that storing microgreens at 5°C in MP, HDPE, and LDPE bags, combined with postharvest treatments like sodium hypochlorite, calcium chloride, and citric acid washes, effectively reduces water loss, extending shelf life and maintaining high consumer acceptance (Kou et al., 2012; Yan et al., 2022; Sharma et al., 2023). Specifically, radish sango microgreens treated with sodium hypochlorite and packed in MP bags at 5°C demonstrated superior results. effective in limiting weight loss and enhancing the shelf life of these microgreens.

6.2.2. Electrolyte leakage

Electrolyte leakage is a key indicator of plant cell membrane weakening due to ripening or damage caused by physiological stress or mechanical injuries (Dayarathna et al., 2023). In this study, significant differences in electrolyte leakage were observed among the washing treatments and packaging materials. Initial electrolyte leakage across all treatment samples ranged from 1.54% to 1.87% (**Table 6.25**) and (**Appendices Table 1**). When radish sango microgreens, washed with various treatments and the control (unwashed), were packed in different packaging materials and stored at 25°C for 3 days, a high level of electrolyte leakage was observed, ranging from 75.49% to 85.08%. After 12 days of storage at 5°C, electrolyte leakage ranged from 4.09% to 5.94% (**Appendices Table 1**). The highest electrolyte leakage was recorded in calcium chloride-washed radish microgreens stored in PT packaging materials at 5°C on day 12, compared to the control. Additionally, MP packaging treatments had a significant effect compared to other packaging materials (**Figure 6.19 (B)**). Samples stored at 5°C for 12 days exhibited the lowest electrolyte leakage ($P < 0.05$) compared to those stored at 25°C. Among all washing treatments, acetic acid-treated samples showed the highest electrolyte leakage on days 3 and 6 at both storage temperatures (5°C and 25°C) (**Table 6.25**) and (**Appendices Table 1**).

All samples showed minimal increases in electrical conductivity during the entire 12 days storage period at 5°C, indicating that low-temperature storage helped maintain cell membrane integrity. This suggests that radish sango microgreens are not susceptible to chilling injury (Xiao et al., 2013). Similar observations have been made for fresh-cut cilantro (Wang et al., 2004), buckwheat microgreens (Kou et al., 2012), broccoli microgreens (Kou et al., 2013), and radish microgreens (Xiao et al., 2013). Electrolyte leakage in radish sango microgreens stored at 5°C was minimal, while significantly higher leakage was observed at 25°C.

Kou et al. (2013) observed low electrolyte leakage in buckwheat microgreens stored at 5°C, but a significant increase at 1°C after 14 days. Similarly, Ghoola & Srividya (2020) reported higher electrolyte leakage (11% in radish and 16.3% in roselle microgreens) when stored at 5°C in sealed LDPE bags for 8 days. These findings suggest that suboptimal storage conditions may cause chilling injuries, impairing cell membrane function and increasing electrolyte leakage. However, Xiao et al. (2013) noted that the optimal storage temperature for radish microgreens is 1°C, retaining quality for 28 days without chilling injuries. This study, combined with existing literature, highlights that optimal storage conditions and washing treatments for microgreens vary significantly by species.

6.2.3. Overall quality and off-odor

Overall visual quality and off-odor are crucial factors influencing the marketability of fresh products. This experiment demonstrated that various washing treatments, packaging methods, and storage temperatures significantly affected the deterioration of overall quality and the development of off-odor (**Table 6.25**) and (**Appendices Table 1**). Throughout the 12 days storage period, radish microgreens stored at 5°C retained the highest overall quality when treated with citric acid and packed in MP packaging materials, followed by treatments with sodium hypochlorite, calcium chloride, distilled water, ethanol, the control, and acetic acid. Among the packaging materials, MP, HDPE, LDPE, and PT showed similar trends in maintaining off-odor parameters under 5°C conditions (**Appendices Table 1**). Samples stored at 25°C showed low overall quality and developed off-odors within three days, becoming unacceptable for consumption. By day 3, all sensory attributes had degraded, prompting panelists to cease evaluating overall quality and off-odor for radish sango microgreens stored at this temperature. These results highlight the impact of storage temperature on sensory attributes; quality deterioration occurred rapidly at 25°C or higher. In contrast, samples stored at 5°C retained a "good-fair" overall quality after 12 days, though a slight quality decline was observed on day 3, possibly due to sample mishandling.

Considering overall quality and off-odor parameters, radish sango microgreens washed with citric acid or ethanol and stored in MP packaging at 5°C are recommended for maintaining consumer acceptance for up to 12 days (**Figure 6.19 (C)**) and (**Figure 6.19 (D)**). Similar findings have been reported for radish microgreens (Xiao et al., 2013) and mustard microgreens (Dayarathna et al., 2023). Off-odor development correlated positively with increased electrolyte leakage, indicating tissue senescence, cell membrane damage, and the formation of undesirable fermentative volatiles such as ethanol and acetaldehyde (Xiao et al., 2013; Dayarathna et al., 2023). Over a 14-day storage period, radish microgreens stored at 10°C

showed the highest overall quality, followed closely by samples stored at 5°C. Samples at 10°C retained acceptable visual quality for up to 7 days (Xiao et al., 2013). Berba & Uchanski (2012) similarly reported that at 4°C, the visual quality of arugula and red cabbage microgreens was preserved for 14 and 21 days, respectively, while storage at 10°C limited shelf life to 7 and 14 days for these crops. These findings collectively indicate that both storage temperature and pre-storage treatment play a vital role in maintaining the quality and marketability of fresh microgreens.

6.2.4. Total chlorophyll and carotenoid content

The initial total chlorophyll and carotenoid content of radish sango microgreens was approximately 36.30–37.68 µg/g and 8.29–8.54 µg/g, respectively (**Table 6.26**) and (**Appendices Table 2**). Both chlorophyll and carotenoid contents declined across all samples during storage, a reduction associated with cellular degradation and senescence, often used as an indicator of quality loss in leafy vegetables (Lu et al., 2024). Throughout the 12 days storage period, samples stored at 5°C with citric acid wash treatment in MP packaging maintained the highest chlorophyll levels, while sodium hypochlorite and MP packaging helped retain the highest carotenoid content (**Figure 6.20 (E)**) and (**Figure 6.20 (F)**). Notably, no yellowing was observed in these samples by the end of storage. Conversely, samples stored at 25°C showed significant yellowing by day 3, alongside a rapid decline in chlorophyll and carotenoid content (**Table 6.26**).

Similar findings were reported by Paradiso et al. (2018) in lettuce, broccoli, and chicory microgreens stored for 10 days, likely due to organic acids role in controlling chlorophyll degrading enzymes (Albanese et al., 2006). Studies by Polash et al. (2018) observed a decline in β-carotene content in mustard, leaf mustard, radish, and cabbage microgreens after 5 days in airtight polybags at 5°C, underscoring the temperature dependence of carotenoid stability. Lower temperatures appear to inhibit metabolic processes that degrade chlorophyll and carotenoids, thus supporting greater pigment retention (Park et al., 2016; Xiao et al., 2013). Based on the findings of this study, it is advisable to store radish sango microgreens washed with citric acid or sodium hypochlorite and packaged in MP materials at 5°C to ensure high consumer acceptance by preserving their chlorophyll and carotenoid contents effectively. The combination of appropriate washing treatments and optimal storage temperature is crucial in maintaining the quality of these microgreens throughout the storage period.

Table 6.26. Effect of different washing treatments on radish sango microgreens stored in various packaging materials at 25°C on chlorophyll, carotenoid, anthocyanin, and phenol content.

Washing treatments	Packaging materials	Day (D)	Chlorophyll content (µg/g)	Carotenoid content (µg/g)	Anthocyanin content (µmol (µg/g))	Total phenol content (mg GAE/100 g)
Acetic acid	AW	D-0	36.30±0.29 ^b	8.46±0.12 ^{ab}	66.60±0.18 ^{de}	333.84±1.04 ^d
	HDPE	D-3	21.69±0.37 ^{bB}	4.37±0.17 ^{abA}	32.80±0.19 ^{deC}	127.84±0.90 ^{dB}
	LDPE	D-3	22.33±0.15 ^{bA}	4.33±0.08 ^{abA}	32.61±0.10 ^{deB}	126.94±0.90 ^{dB}
	MP	D-3	21.94±0.09 ^{bB}	4.63±0.11 ^{abA}	33.69±0.17 ^{deA}	132.04±0.52 ^{dA}
	PT	D-3	21.45±0.08 ^{bC}	4.72±0.07 ^{abA}	33.30±0.46 ^{deD}	125.74±0.52 ^{dC}
Calcium chloride	AW	D-0	36.54±0.06 ^a	8.42±0.04 ^c	66.80±0.11 ^a	335.35±0.52 ^b
	HDPE	D-3	23.68±0.06 ^{aB}	4.16±0.03 ^{cA}	35.40±0.11 ^{aC}	134.14±0.90 ^{bB}
	LDPE	D-3	23.42±0.06 ^{aA}	4.18±0.05 ^{cA}	35.39±0.41 ^{aB}	132.94±1.04 ^{bB}
	MP	D-3	23.81±0.04 ^{aB}	4.20±0.02 ^{cA}	35.67±0.16 ^{aA}	136.25±0.52 ^{bA}
	PT	D-3	23.25±0.06 ^{aC}	4.29±0.03 ^{cA}	34.19±0.17 ^{aD}	132.64±0.52 ^{bC}
Citric acid	AW	D-0	36.42±0.22 ^{cd}	8.47±0.04 ^{cd}	66.77±0.20 ^{cd}	335.35±1.04 ^b
	HDPE	D-3	20.05±0.09 ^{cdB}	4.14±0.02 ^{cdA}	32.62±0.16 ^{cdC}	130.54±0.90 ^{bB}
	LDPE	D-3	22.30±1.91 ^{cdA}	4.04±0.11 ^{cdA}	34.13±0.26 ^{cdB}	132.64±0.52 ^{bB}
	MP	D-3	20.12±0.01 ^{cdB}	4.14±0.01 ^{cdA}	33.43±0.17 ^{cdA}	136.55±0.52 ^{bA}
	PT	D-3	19.94±0.07 ^{cdC}	4.10±0.01 ^{cdA}	32.53±0.08 ^{cdD}	132.34±0.90 ^{bC}
Distilled water	AW	D-0	37.37±0.21 ^c	8.31±0.11 ^d	67.25±0.32 ^c	338.05±1.04 ^a
	HDPE	D-3	20.16±0.20 ^{cB}	4.20±0.03 ^{dA}	32.54±0.10 ^{cC}	132.04±1.04 ^{aB}
	LDPE	D-3	37.37±0.21 ^{cA}	8.31±0.11 ^{dA}	67.25±0.32 ^{cB}	338.05±1.04 ^{aB}
	LDPE	D-3	20.28±0.30 ^{cA}	4.09±0.08 ^{dA}	34.03±0.17 ^{cB}	132.04±0.52 ^{aB}
	MP	D-3	20.21±0.16 ^{cB}	4.19±0.01 ^{dA}	33.24±0.10 ^{cA}	134.74±1.04 ^{aA}
	PT	D-0	19.84±0.12 ^{cC}	4.15±0.03 ^{dA}	32.50±0.16 ^{cD}	132.94±0.52 ^{aC}
Ethanol	AW	D-0	36.38±0.16 ^c	8.43±0.11 ^a	66.73±0.24 ^b	333.54±1.38 ^c
	HDPE	D-3	21.12±0.06 ^{cB}	4.72±0.10 ^{aA}	34.13±0.16 ^{bC}	127.84±0.90 ^{cB}
	LDPE	D-3	21.45±0.13 ^{cA}	4.76±0.06 ^{aA}	34.35±0.16 ^{bB}	126.64±0.52 ^{cB}
	MP	D-3	21.16±0.06 ^{cB}	4.71±0.05 ^{aA}	35.10±0.28 ^{bA}	126.34±0.52 ^{cA}
	PT	D-3	20.73±0.08 ^{cC}	4.47±0.03 ^{aA}	34.05±0.24 ^{bD}	126.07±0.86 ^{cC}
Sodium hypochlorite	AW	D-0	36.54±0.05 ^d	8.50±0.08 ^b	67.04±0.08 ^d	334.14±0.90 ^c
	HDPE	D-3	20.61±0.08 ^{dB}	4.45±0.03 ^{bA}	32.48±0.16 ^{dC}	130.54±0.90 ^{cB}
	LDPE	D-3	20.48±0.21 ^{dA}	4.40±0.11 ^{bA}	32.71±0.16 ^{dB}	132.04±0.52 ^{cB}
	MP	D-3	20.54±0.13 ^{dB}	4.45±0.01 ^{bA}	33.46±0.28 ^{dA}	128.74±0.90 ^{cA}
	PT	D-3	19.69±0.07 ^{dC}	4.43±0.05 ^{bA}	32.68±0.17 ^{dD}	126.37±0.99 ^{cC}
Control	AW	D-0	37.64±0.09 ^{cd}	8.36±0.17 ^{ab}	67.52±0.39 ^e	339.55±0.90 ^{cd}
	HDPE	D-3	19.36±0.09 ^{cdB}	4.62±0.09 ^{abA}	32.09±0.10 ^{eC}	123.93±0.52 ^{cdB}
	LDPE	D-3	19.56±0.32 ^{cdA}	6.49±2.05 ^{abA}	31.35±0.25 ^{eB}	123.03±0.52 ^{cdB}
	MP	D-3	19.35±0.10 ^{cdB}	4.67±0.12 ^{abA}	32.83±0.16 ^{eA}	123.33±0.90 ^{cdA}
	PT	D-3	19.23±0.08 ^{cdC}	4.63±0.07 ^{aBA}	30.95±0.19 ^{eD}	121.83±0.52 ^{cdC}

Results are expressed as mean ± standard deviation of three replicates. The different lowercase letters within the column indicate significant ($p < 0.05$) differences based on washing treatments and different uppercase letters within the column indicate significant ($P < 0.05$) differences among the packaging materials. Where, D: Days; AW: After wash; HDPE: High-Density Polyethylene; LDPE: Low-Density Polyethylene; MP: Metalized Polyester; PT: Polystyrene Trays.

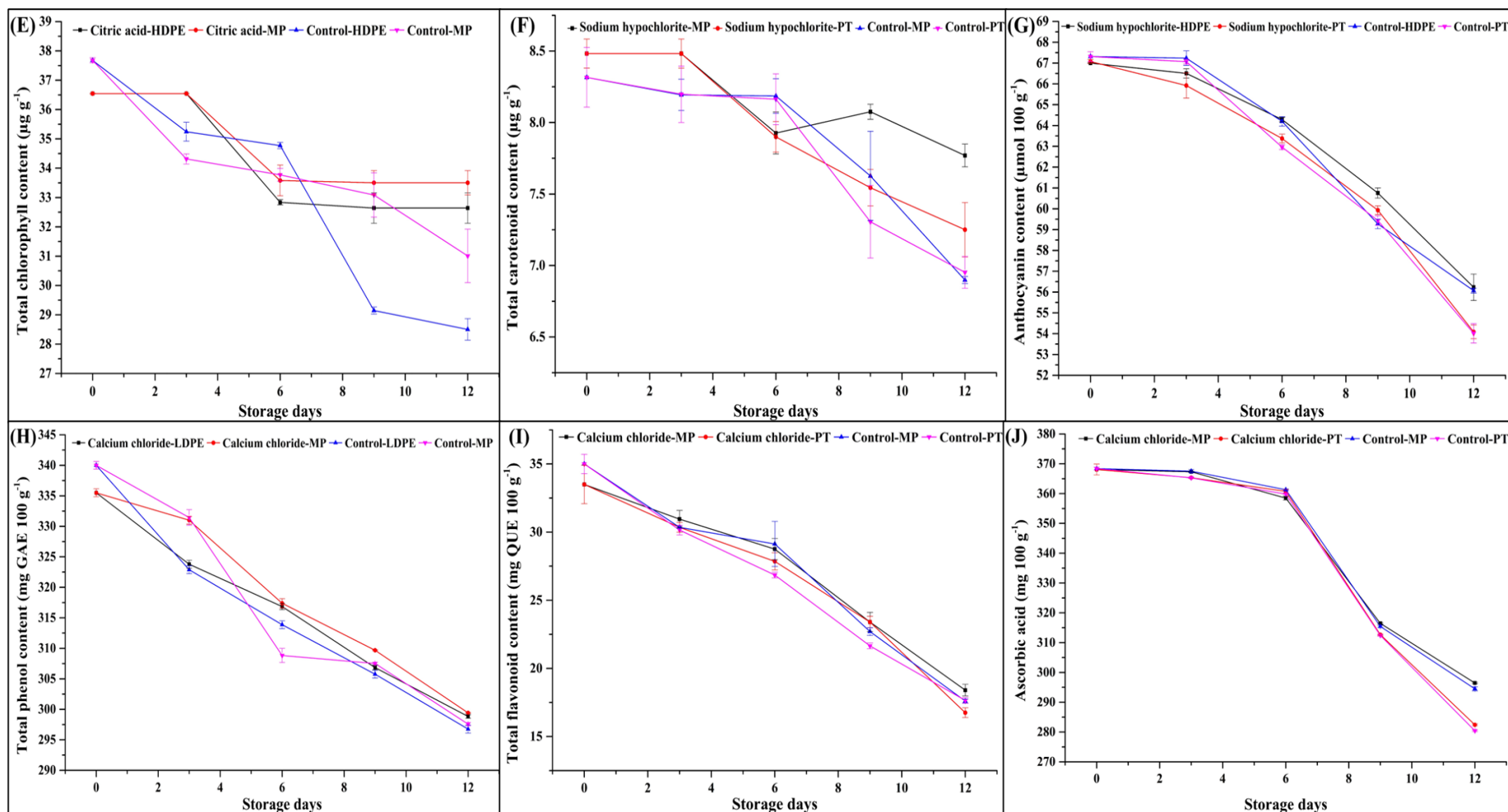


Figure 6.20. Effect of different washing treatments on radish sango microgreens stored in various packaging materials at 5°C storage condition on total chlorophyll content (E), Total carotenoid content (F), Anthocyanin content (C), total phenol content (H), total flavonoid content (I), and ascorbic acid (J). The figure presents the significant effects ($P < 0.05$) of treatments based on 28 experimental treatments for respective study parameters. Results are expressed as mean $\pm$ standard deviation of three replicates, with error bars indicating the standard deviation from the mean values.

6.2.5. Anthocyanin content

A time-dependent decrease in anthocyanin content was observed in radish sango microgreens over the storage period (**Table 6.26**) and (**Appendices Table 2**). Acetic acid wash treatments showed the highest reduction in anthocyanin content, with levels ranging from 61.61 to 61.86 $\mu\text{mol}/100\text{ g}$ at 5°C and from 32.61 to 33.30 $\mu\text{mol}/100\text{ g}$ at 25°C across all four packaging types (HDPE, LDPE, MP, and PT). Significant differences were noted between treatments and the control at both 5°C and 25°C, especially by day 12, when sodium hypochlorite-treated samples in HDPE retained the highest anthocyanin levels compared to the control (**Figure 6.20 (G)**).

The stability of anthocyanins is influenced by multiple factors, including oxygen, pH, storage temperature, light exposure, and the chemical nature of anthocyanins (acylation, glycosylation), as well as enzymatic activity and interactions with other ingredients (Muhamad et al., 2023). While sodium hypochlorite effectively controls microbes, its mild oxidative effect may contribute to slight anthocyanin loss. Overall, sodium hypochlorite, citric acid, and ethanol washes were more effective at retaining anthocyanins, likely due to reduced oxidative stress on the microgreen cells (Zhang et al., 2023). Storage temperature and duration play crucial roles in anthocyanin retention; low-temperature storage at 5°C significantly slows enzymatic degradation, thereby preserving both color and anthocyanin content. In contrast, higher temperatures (25°C) increase metabolic activity, accelerating anthocyanin breakdown and leading to quality loss and discoloration within a few days (Muche et al., 2018). Therefore, a combination of sodium hypochlorite or citric acid washes, MP packaging, and storage at 5°C provides optimal conditions for preserving anthocyanin content in radish sango microgreens.

6.2.6. Total phenol content

In this study, the total phenol content of radish sango microgreens decreased over time, particularly under the higher storage temperature of 25°C. Initial total phenol content levels ranged from 333.84 to 339.55 mg GAE/100 g across treatments (**Table 6.26**). After 3 days at 25°C, the phenol content in acetic acid-treated samples stored in PT packaging dropped to approximately 125.74 mg GAE/100 g, reflecting a significant reduction. Conversely, at 5°C, samples treated with calcium chloride and stored in MP packaging retained up to 299.41 mg GAE/100 g (**Figure 6.20 (H)**). This retention highlights the role of controlled temperature and optimized packaging in stabilizing phenolic compounds during storage. Throughout the 12-day storage period, radish microgreens stored at 5°C retained the highest total phenol content when treated with calcium chloride and packed in MP packaging materials, followed by treatments with sodium hypochlorite, citric acid, distilled water, ethanol, the control, and acetic acid.

Among the packaging materials, MP, HDPE, PT, and LDPE showed significant trends in parameters under 5°C conditions (**Appendices Table 2**).

Time-dependent degradation of phenolic compounds has been observed in sunflower microgreens (Dalal et al., 2020), buckwheat microgreens (Yan et al., 2022), and lettuce (Altunkaya and Gökmen, 2009). The degradation of phenolic compounds is primarily attributed to oxidative enzymes, particularly polyphenol oxidase (PPO) and peroxidase (POD), which catalyze the oxidation of phenols, leading to the formation of quinones and subsequent browning in plant tissues. These enzymes are highly temperature sensitive, with increased activity at higher storage temperatures like 25°C (Yan et al., 2022). Elevated temperatures can disrupt cellular integrity, allowing enzymes greater access to phenolic compounds and thus accelerating oxidation. Additionally, exposure to oxygen in certain packaging materials, such as PT, can enhance oxidative degradation, given that oxygen is a key substrate for PPO and POD activity (Rho et al., 2021). Lowering the storage temperature to 5°C mitigates enzymatic activity, as metabolic reactions generally decrease with reduced thermal energy. This effectively slows the enzymatic oxidation of phenols, preserving their antioxidant properties (Yan et al., 2022). MP packaging plays an essential role here by limiting oxygen permeability, reducing the availability of oxygen necessary for oxidative reactions (Singh et al., 2024). Furthermore, washing treatments, such as citric acid and sodium hypochlorite, contribute to phenolic retention by creating an environment less conducive to microbial growth and enzyme activity. Citric acid's mild acidity may further inhibit PPO by altering the pH on the surface of microgreens, while sodium hypochlorite may have an indirect antioxidative effect through its microbial inhibition properties (Dalal et al., 2020). Together, these treatments provide a synergistic effect, combining low oxygen exposure, controlled temperature, and enzyme inhibition to effectively stabilize phenolic content, extending the antioxidant potential of radish sango microgreens during storage.

6.2.7. Total flavonoid content

Flavonoid content in radish sango microgreens also declined over time, with temperature and packaging materials showing a significant influence on the rate of degradation. Initially, flavonoid levels ranged from 30.83 to 34.83 mg QE/100 g across treatments (**Table 6.27**). By day 3 at 25°C, acetic acid-treated samples stored in PT packaging had dropped to 15.75 mg QE/100 g, indicating considerable loss. However, samples stored at 5°C showed a more gradual reduction in flavonoid content, with citric acid and sodium hypochlorite treatments in MP packaging maintaining up to 22.75 mg QE/100 g by day 12. Conversely, at 5°C, samples treated with calcium chloride and citric acid and stored in MP packaging retained

up to 31.62 to 33.50 mg QE/100 g (**Appendices Table 3**). Among all the washing treatments and packaging methods, the calcium chloride and MP packaging materials showed a significant effect ($P < 0.05$) compared to other treatments (**Figure 6.20 (I)**). This retention highlights the role of controlled temperature and optimized packaging with washing treatment in stabilizing flavonoids during storage.

A similar finding for flavonoid content has been observed in buckwheat microgreens (Yan et al., 2022). Calcium chloride and citric acid-washed buckwheat microgreens exhibited higher total flavonoid content compared to those washed with water throughout the storage period. Initially, the flavonoid content was maintained for the first 4 days of storage, then decreased towards the end (Yan et al., 2022). However, limited studies are available on the effect of storage temperature, packaging material, and wash treatment on the flavonoid content of microgreens. Flavonoids, like phenols, are susceptible to oxidative degradation, especially in the presence of reactive oxygen species (ROS) generated during storage. Higher temperatures increase cellular respiration and metabolic activity, leading to elevated ROS production, which can degrade flavonoids. While beneficial in living tissues, flavonoids act as scavengers of free radicals, leading to their depletion postharvest (Grzegorzewski et al., 2009). At 25°C, the combination of heightened metabolic activity and ROS accelerates flavonoid degradation, reducing their bioavailability and antioxidant properties (Huchzermeyer et al., 2022). Storing at 5°C mitigates ROS formation by slowing cellular metabolism, reducing the rate of flavonoid degradation (Yan et al., 2022). MP packaging supports flavonoid retention by minimizing oxygen ingress, which is crucial as oxygen exposure enhances ROS production and oxidative reactions. This protection is advantageous for flavonoids, preventing rapid quality loss in the presence of light, heat, and oxygen (Hassan & El-Gendy, 2023). These results highlight the importance of integrated postharvest strategies to protect flavonoid content in radish sango microgreens, enhancing their nutritional value and consumer acceptance (Jones-Baumgardt et al., 2020; Sharma et al., 2023).

6.2.8. Ascorbic acid

Ascorbic acid (vitamin C) content in radish sango microgreens decreased significantly during storage, with temperature and packaging playing crucial roles in the rate of decline. Initially, ascorbic acid levels ranged from 366.13 to 368.92 mg/100 g across treatments (**Table 6.27**). The retention of ascorbic acid was consistently higher in samples treated with citric acid and sodium hypochlorite, particularly when stored in MP packaging at 5°C. These samples retained over 170 mg/100 g by day 12, whereas samples treated with acetic acid and stored in PT packaging at 25°C dropped significantly to around 169 mg/100 g (**Appendices Table 3**).

Among all washing treatments, the calcium chloride washing treatment showed a significant effect on ascorbic acid content (**Figure 6.20 (J)**). These results demonstrate that lower temperatures and oxygen-restrictive packaging significantly mitigate ascorbic acid degradation.

Ascorbic acid is highly susceptible to oxidation, especially at elevated temperatures and in the presence of oxygen. During storage at 25°C, oxidative reactions increase as ascorbic acid reacts with ROS and oxygen, leading to dehydroascorbic acid formation, a non-functional form with reduced antioxidant activity (Patil et al., 2024). Storing at 5°C slows metabolic processes, including the enzymatic and non-enzymatic oxidation of ascorbic acid, thereby preserving its concentration (Tsaniklidis et al., 2014). Additionally, MP packaging, with low oxygen permeability, helps to minimize ascorbic acid degradation by reducing the substrate (oxygen) available for oxidation reactions (Yin et al., 2022).

Washing treatments also influence ascorbic acid stability. Sodium hypochlorite and citric acid washes help limit microbial growth on the surface of the microgreens (Dalal et al., 2020). Microbial activity can deplete ascorbic acid as microbes utilize it during their metabolic processes, leading to faster degradation. Sodium hypochlorite may further preserve ascorbic acid by reducing the initial microbial load and, consequently, the metabolic demand for ascorbic acid (Patil et al., 2024). These findings emphasize the importance of integrating low temperatures, oxygen-limiting packaging, and microbial-limiting washes to maintain ascorbic acid content in stored microgreens.

Table 6.27. Effect of different washing treatments on radish sango microgreens stored in various packaging materials at 25°C on total flavonoid content, ascorbic acid, total glucosinolate, and antioxidant activities (DPPH and FRAP).

Washing treatments	Packaging materials	Days	TFC (mg QE/100 g)	AA (mg /100 g)	TG (μmol/g)	Antioxidant activity DPPH (%)	Antioxidant activity FRAP (μmol TE/g)
Acetic acid	AW	D-0	30.83±0.5 _{8^c}	366.13±1.12 _d	39.85±0.14 _a	73.08±0.19 _b	33.48±0.33 _c
	HDPE	D-3	20.40±0.7 _{2^{cB}}	174.73±0.37 _{dB}	27.58±0.66 _{aB}	33.78±0.28 _{bB}	18.03±0.44 _{cA}
	LDPE	D-3	20.87±0.5 _{5^{cB}}	172.58±0.65 _{dC}	27.54±0.73 _{aC}	34.85±0.23 _{bA}	16.40±0.58 _{cB}
	MP	D-3	18.77±0.2 _{1^{cA}}	176.52±0.11 _{dA}	27.82±0.76 _{aA}	34.63±0.36 _{bB}	19.18±0.60 _{cA}
	PT	D-3	15.75±0.2 _{8^{cC}}	169.96±0.39 _{dD}	26.73±0.03 _{aC}	31.59±0.33 _{bC}	16.02±0.33 _{cC}
Calcium chloride	AW	D-0	33.50±1.0 _{0^b}	368.06±1.29 _{abc}	40.13±0.18 _a	72.75±0.19 _{ab}	34.15±0.44 _{ab}
	HDPE	D-3	20.50±0.5 _{6^{bB}}	174.47±0.20 _{abcB}	27.30±0.24 _{aB}	33.89±0.17 _{abB}	18.99±0.58 _{abA}
	LDPE	D-3	21.64±0.2 _{4^{bB}}	172.73±0.69 _{abcC}	26.88±0.06 _{aC}	35.78±0.23 _{abA}	18.70±1.15 _{abB}
	MP	D-3	19.77±0.8 _{6^{bA}}	176.26±0.51 _{abcA}	27.86±0.18 _{aA}	34.52±0.34 _{abB}	19.38±0.60 _{abA}
	PT	D-3	15.77±0.0 _{6^{bC}}	169.70±0.56 _{abcD}	26.34±0.12 _{aC}	32.70±0.11 _{abC}	15.35±0.44 _{abC}
Citric acid	AW	D-0	33.20±0.6 _{2^b}	368.06±0.65 _{abc}	40.09±0.14 _{ab}	73.01±0.17 _{ab}	34.72±0.44 _a
	HDPE	D-3	20.83±0.2 _{1^{bB}}	174.04±0.54 _{abcB}	27.10±0.18 _{abB}	33.67±0.17 _{abB}	19.18±0.44 _{aA}
	LDPE	D-3	21.03±0.7 _{6^{bB}}	172.84±0.68 _{abcC}	26.70±0.12 _{abC}	36.34±0.28 _{abA}	18.61±0.44 _{abB}
	MP	D-3	19.43±0.3 _{8^{bA}}	176.04±0.39 _{abcA}	27.34±0.07 _{abA}	34.63±0.72 _{abB}	19.66±0.17 _{aA}
	PT	D-3	15.73±0.0 _{6^{bC}}	169.05±0.48 _{abc}	26.50±0.07 _{abC}	32.41±0.17 _{abC}	15.54±0.29 _{aC}
Distilled water	AW	D-0	33.87±0.4 _{7^b}	368.49±0.37 _a	40.09±0.07 _{ab}	73.01±0.06 _{ab}	34.53±0.29 _a
	HDPE	D-3	19.83±0.2 _{1^{bB}}	174.83±0.24 _{aB}	27.06±0.12 _{abB}	35.15±0.29 _{abB}	19.47±0.17 _{aA}
	LDPE	D-3	18.37±0.7 _{6^{bB}}	173.27±0.75 _{aC}	26.66±0.07 _{abC}	36.26±0.11 _{abA}	18.32±0.33 _{abB}
	MP	D-3	20.43±0.6 _{7^{bA}}	176.43±0.49 _{aA}	27.26±0.30 _{abA}	34.15±0.87 _{abB}	19.47±0.17 _{aA}
	PT	D-3	16.00±0.1 _{0^{bC}}	168.84±0.51 _{aD}	26.78±0.57 _{abC}	31.33±0.23 _{abC}	16.21±0.44 _{aC}
Ethanol	AW	D-0	31.90±0.5 _{3^b}	367.42±0.65 _{cd}	39.81±0.12 _c	72.90±0.17 _a	32.81±0.86 _{bc}
	HDPE	D-3	19.20±0.6 _{6^{bB}}	174.40±0.38 _{cdB}	26.82±0.21 _{cB}	35.15±0.19 _{aB}	19.66±0.33 _{bcA}

Sodium hypochlorite	LDPE	D-3	18.82±0.1 9 ^{bB}	172.62±0.43 cdC	26.30±0.18 cC	36.41±0.17 aA	18.13±0.29 bcB
	MP	D-3	22.75±0.9 3 ^{bA}	175.66±0.36 cdA	27.18±0.21 cA	34.26±0.88 aB	19.66±0.44 bcA
	PT	D-3	20.07±1.0 1 ^{bC}	168.41±0.73 cdD	26.30±0.07 cC	31.85±0.28 aC	17.27±0.29 bcC
	AW	D-0	32.83±0.5 8 ^b	368.06±0.65 bcd	40.13±0.14 bc	72.78±0.13 ab	34.05±0.88 a
	HDPE	D-3	18.83±0.5 8 ^{bB}	174.09±0.37 bcdB	27.18±0.24 bcB	34.82±0.19 abB	19.57±0.29 aA
Control	LDPE	D-3	18.17±0.5 8 ^{bB}	172.37±0.37 bcdC	26.10±0.12 bcC	36.15±0.22 abA	18.23±0.17 aB
	MP	D-3	22.17±1.1 5 ^{bA}	175.38±0.37 bcdA	27.38±0.37 bcA	34.04±0.40 abB	19.95±0.44 aA
	PT	D-3	19.83±1.1 5 ^{bC}	168.06±0.65 bcdD	25.82±0.49 bcC	31.15±0.67 abC	17.46±0.44 aC
	AW	D-0	34.83±0.5 8 ^a	368.92±0.99 ab	40.05±0.12 bc	72.86±0.11 c	34.34±0.44 a
	HDPE	D-3	19.23±0.6 4 ^{aB}	174.24±0.26 abB	27.22±0.18 bcB	33.67±0.17 cB	19.28±0.29 aA
	LDPE	D-3	17.83±0.5 8 ^{aB}	172.58±0.65 abC	25.74±0.12 bcC	34.96±0.13 cA	17.84±0.29 aB
	MP	D-3	21.83±0.2 5 ^{aA}	175.57±0.21 abA	27.58±0.07 bcA	34.04±0.11 cB	19.95±0.44 aA
	PT	D-3	18.77±0.2 3 ^{aC}	168.26±0.49 abD	25.86±0.32 bcC	30.37±0.29 cC	17.75±0.44 aC

Results are expressed as mean ± standard deviation of three replicates. The different lowercase letters within the column indicate significant ($P<0.05$) differences based on washing treatments and different uppercase letters within the column indicate significant ($p<0.05$) differences among the packaging materials. Where, D: Days; AW: After wash; HDPE: High-Density Polyethylene; LDPE: Low-Density Polyethylene; MP: Metalized Polyester; PT: Polystyrene Trays; TFC: Total flavonoid content; AA: Ascorbic acid; TG: total glucosinolate.

6.2.9. Total glucosinolate content

Total glucosinolate content in radish sango microgreens demonstrated a significant decline over the storage period, with storage temperature, packaging materials, and washing treatments influencing retention levels. Initially, glucosinolate levels ranged between 39.81 and 40.13 $\mu\text{mol/g}$ across treatments (**Table 6.27**). By day 3, samples stored at 25°C with acetic acid wash and PT packaging showed a marked reduction, retaining only 25.82 $\mu\text{mol/g}$. In contrast, microgreens stored at 5°C with calcium chloride treatment in MP packaging retained up to 27.90 $\mu\text{mol/g}$ by day 12 (**Appendices Table 3**) and (**Figure 6.21 (K)**). The retention of glucosinolates varied across treatments, with sodium hypochlorite and citric acid-treated samples in MP packaging at 5°C showing the best retention (27.38 $\mu\text{mol/g}$) by day 12. Comparatively, acetic acid-treated samples in PT packaging stored at 25°C retained only 25.82 $\mu\text{mol/g}$ (**Table 6.27**). These findings indicate that controlled storage conditions, particularly

lower temperatures, and selective packaging, significantly affect glucosinolate stability in radish sango microgreens.

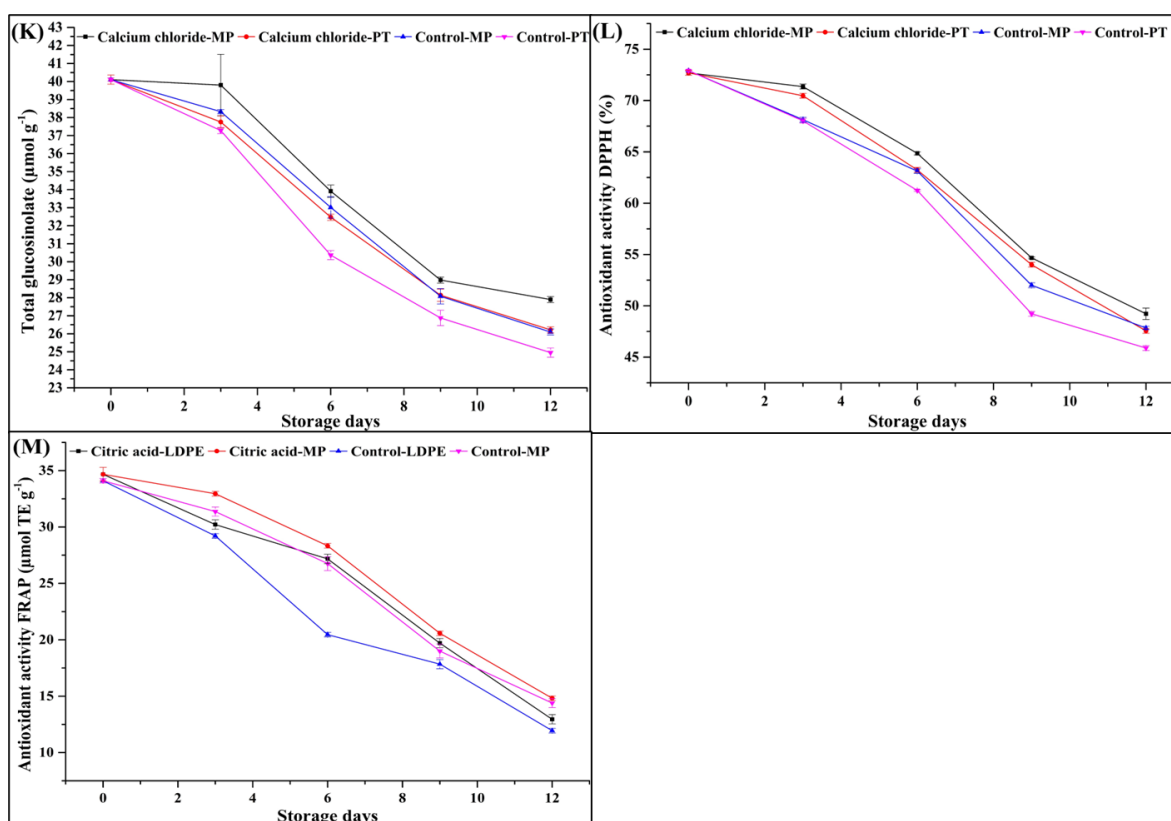


Figure 6.21. Effect of different washing treatments on radish sango microgreens stored in various packaging materials at 5°C storage condition on total glucosinolate content (K), antioxidant activity DPPH (L), and antioxidant activity FRAP (M). The figure presents the significant effects ($P < 0.05$) of treatments based on 28 experimental treatments for respective study parameters. Results are expressed as mean $\pm$ standard deviation of three replicates, with error bars indicating the standard deviation from the mean values.

Glucosinolates are sulfur-rich compounds that provide both defense against pathogens and potential health benefits for consumers, but they are sensitive to enzymatic degradation. Myrosinase, the enzyme responsible for hydrolyzing glucosinolates into active compounds such as isothiocyanates, is highly temperature-sensitive. Higher storage temperatures (25°C) increase cellular metabolic activity, leading to enhanced myrosinase action and rapid glucosinolate loss (Abdel-Massih et al., 2023). This enzymatic breakdown is further accelerated in oxygen-permeable packaging like PT, where greater oxygen exposure facilitates oxidative reactions that destabilize glucosinolate structures (Dunbar et al., 2017). Storage at 5°C slows myrosinase activity, significantly reducing glucosinolate degradation. MP packaging, with its low oxygen permeability, further contributes to glucosinolate preservation by limiting oxygen exposure, thus reducing oxidative stress and enzymatic degradation (Abdel-Massih et al., 2023; Cheng et al., 2024). These integrated storage practices effectively preserve

glucosinolates, retaining the bioactive compounds responsible for the health-promoting properties of radish sango microgreens.

6.2.10. Antioxidant activity (DPPH and FRAP)

The antioxidant activity in radish sango microgreens, measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and FRAP (Ferric Reducing Antioxidant Power), displayed significant changes during the storage period. Both DPPH and FRAP values declined over time, with the rate of decrease influenced by temperature, packaging type, and washing treatments (**Table 6.27**) and (**Appendices Table 3**). Initially, DPPH activity ranged from 85.72% to 87.24% across treatments, while FRAP values were between 6.48 and 6.85 $\mu\text{mol TE/g}$ (**Table 6.27**). After 3 days at 25°C, acetic acid-treated samples stored in PT packaging showed the greatest reduction, with DPPH and FRAP values dropping to 51.42% and 3.12 $\mu\text{mol TE/g}$, respectively. In contrast, samples stored at 5°C with calcium chloride wash in MP packaging retained the highest DPPH activity (**Figure 6.21 (L)**), and citric acid wash in MP packaging retained high-level FRAP values (**Figure 6.21 (M)**).

The decline in antioxidant activity over storage can be attributed to the degradation of bioactive compounds responsible for free radical scavenging and ferric ion reduction, primarily phenolic compounds, flavonoids, and ascorbic acid (Dalal et al., 2020; Sharma et al., 2023; Patil et al., 2024). These compounds are susceptible to oxidative degradation, particularly at higher temperatures and in the presence of oxygen (Singh et al., 2024). At 25°C, increased metabolic activity in microgreens enhances reactive oxygen species (ROS) production, leading to the oxidation of antioxidant molecules and reducing DPPH and FRAP values (Sharma et al., 2023). This effect is further accelerated in PT packaging, which has higher oxygen permeability, allowing greater oxygen exposure and thus higher oxidative stress. Storage at 5°C significantly reduces metabolic and oxidative degradation rates by slowing down enzymatic activity and ROS formation (Chandra et al., 2012; Yan et al., 2022). MP packaging also aids in antioxidant retention by limiting oxygen permeability and reducing exposure to oxidative conditions. Washing treatments play a supportive role, such as sodium hypochlorite and citric acid washes, helping to stabilize antioxidant compounds by reducing microbial load on the surface, which can otherwise accelerate ROS production and degradation of antioxidant molecules (Dalal et al., 2020). Together, these postharvest conditions effectively preserve DPPH and FRAP antioxidant values, maintaining the antioxidant potential of radish sango microgreens over an extended storage period. For optimal antioxidant retention in radish sango microgreens, a combination of MP packaging, low-temperature storage at 5°C, and washing treatments with calcium chloride and citric acid is recommended. This strategy effectively

slows down oxidative and enzymatic degradation, enhancing the storage stability of bioactive compounds that contribute to DPPH and FRAP activity.

6.2.11. Microbial load

The microbial load in radish sango microgreens, measured by total plate count (TPC) and yeast and mold count, increased over the storage period, with storage temperature, packaging type, and washing treatments significantly affecting microbial growth rates. Initially, TPC ranged from 2.30 to 3.35 log CFU/g, and yeast and mold counts were between 1.40 and 2.15 log CFU/g across treatments (**Table 6.28**) and (**Appendices Table 4**). By day 3, samples stored at 25°C with acetic acid wash in PT packaging showed the highest microbial load, with TPC reaching 7.32 log CFU/g and yeast and mold counts at 4.65 log CFU/g. In contrast, samples stored at 5°C in all the treatments had TPC and yeast and mold count of 6.19 to 7.77 log CFU/g and 7.17 to 8.77 log CFU/g, respectively, observed on day 12, demonstrating a slower microbial growth rate under these conditions (**Appendices Table 4**). Among the washing treatments, the sodium hypochlorite and ethanol treatments showed a significant effect compared to other washing treatments along with the control sample for TPC and yeast and mold count (**Figure 6.19 (N)**) and (**Figure 6.19 (O)**).

Similar findings were reported regarding the effect of storage temperature, packaging film, and chlorine wash treatment on radish microgreens. The study demonstrated that chlorine wash treatment (100 mg/L) significantly reduced initial microbial populations by 0.5 log CFU/g, including aerobic mesophilic bacteria and yeast & mold count. However, microbial populations rebounded after 7 days (Xiao et al., 2014). In another study, chlorine (100 mg/L) wash significantly ($P < 0.05$) reduced microbial populations in buckwheat microgreens initially; however, all washed microgreens experienced accelerated microbial populations after 7 days of storage (Kou et al., 2013). Storage temperature and duration significantly impacted the growth of total coliforms and yeast and mold in microgreens (Xiao et al., 2014). Coliform counts increased more rapidly in buckwheat microgreens stored at 25°C, reaching around 7.3 log CFU/g within 2 days, compared to 6.9 and 7.2 log CFU/g after 8 days at 5°C and 10°C, respectively (Yan et al., 2022). These findings indicate that lower storage temperatures effectively inhibit coliform and yeast and mold growth, aligning with results observed in our radish sango microgreen studies.

Microbial growth in fresh produce is influenced by environmental factors such as temperature, oxygen availability, and surface hygiene. At 25°C, warmer temperatures promote faster microbial metabolism and replication, leading to rapid increases in total plate count and yeast and mold counts (Alegbeleye et al., 2018).

Table 6.28. Effect of different washing treatments on radish sango microgreens stored in various packaging materials at 25°C on microbial parameters.

Washing treatments	Packaging materials	Days	Yeast and mould count (log CFU/g)	Total plate count (log CFU/g)
Acetic acid	AW	Day-0	1.67±0.15 ^{bc}	2.47±0.12 ^b
	HDPE	Day-3	7.20±0.10 ^{bcA}	6.40±0.10 ^{bA}
	LDPE	Day-3	7.17±0.06 ^{bcA}	6.77±0.15 ^{bA}
	MP	Day-3	6.80±0.10 ^{bcB}	5.90±0.17 ^{bB}
	PT	Day-3	6.50±0.10 ^{bcB}	6.40±0.10 ^{bA}
Calcium chloride	AW	Day-0	1.63±0.15 ^{bc}	2.70±0.26 ^b
	HDPE	Day-3	7.23±0.06 ^{bcA}	6.43±0.15 ^{bA}
	LDPE	Day-3	7.27±0.12 ^{bcA}	6.67±0.15 ^{bA}
	MP	Day-3	6.67±0.06 ^{bcB}	5.83±0.25 ^{bB}
	PT	Day-3	6.67±0.12 ^{bcB}	6.30±0.10 ^{bA}
Citric acid	AW	Day-0	1.63±0.06 ^{bc}	2.47±0.06 ^b
	HDPE	Day-3	7.30±0.10 ^{bcA}	6.47±0.15 ^{bA}
	LDPE	Day-3	7.07±0.12 ^{bcA}	6.83±0.06 ^{bA}
	MP	Day-3	6.70±0.10 ^{bcB}	5.77±0.15 ^{bB}
	PT	Day-3	6.83±0.06 ^{bcB}	6.10±0.20 ^{bA}
Distilled water	AW	Day-0	1.77±0.15 ^b	2.57±0.21 ^b
	HDPE	Day-3	7.33±0.15 ^{bA}	6.43±0.32 ^{bA}
	LDPE	Day-3	7.30±0.10 ^{bA}	6.60±0.20 ^{bA}
	MP	Day-3	6.80±0.10 ^{bB}	5.83±0.06 ^{bB}
	PT	Day-3	6.60±0.20 ^{bB}	6.40±0.20 ^{bA}
Ethanol	AW	Day-0	1.43±0.12 ^c	2.60±0.17 ^b
	HDPE	Day-3	7.17±0.42 ^{cA}	6.50±0.36 ^{bA}
	LDPE	Day-3	7.00±0.26 ^{cA}	6.03±0.40 ^{bA}
	MP	Day-3	6.87±0.15 ^{cB}	6.77±0.06 ^{bB}
	PT	Day-3	6.73±0.12 ^{cB}	6.63±0.25 ^{bA}
Sodium hypochlorite	AW	Day-0	1.53±0.06 ^c	2.27±0.12 ^b
	HDPE	Day-3	6.97±0.47 ^{cA}	6.70±0.17 ^{bA}
	LDPE	Day-3	6.80±0.10 ^{cA}	6.63±0.21 ^{bA}
	MP	Day-3	7.07±0.21 ^{cB}	6.80±0.00 ^{bB}
	PT	Day-3	6.87±0.21 ^{cB}	6.47±0.21 ^{bA}
Control	AW	Day-0	2.23±0.15 ^a	3.27±0.15 ^a
	HDPE	Day-3	6.97±0.25 ^{aA}	6.53±0.55 ^{aA}
	LDPE	Day-3	7.00±0.26 ^{aA}	6.13±0.58 ^{aA}
	MP	Day-3	6.70±0.10 ^{aB}	5.77±0.15 ^{aB}
	PT	Day-3	7.03±0.15 ^{aB}	6.57±0.49 ^{aA}

Results are expressed as mean ± standard deviation of three replicates. The different lowercase letters within the column indicate significant ($p < 0.05$) differences based on washing treatments and different uppercase letters within the column indicate significant ($p < 0.05$) differences among the packaging materials. Where, AW: After wash; HDPE: High-Density Polyethylene; LDPE: Low-Density Polyethylene; MP: Metalized Polyester; PT: Polystyrene Trays

PT packaging, with higher oxygen permeability, facilitates microbial growth by allowing aerobic microorganisms to thrive (San et al., 2022). MP packaging, with lower oxygen permeability, further limits microbial growth by reducing oxygen availability for aerobic microorganisms, commonly responsible for spoilage (Singh et al., 2024). Washing treatments, particularly sodium hypochlorite, initially reduce microbial load on microgreen surfaces through antimicrobial effects. Sodium hypochlorite disrupts cell walls and enzymes, reducing initial microbial populations and curtailing their growth during storage (Sun et al., 2012). Citric acid wash, with its acidic pH, exerts a mild antimicrobial effect, creating a less favorable environment for microbial proliferation, though it is less effective than sodium hypochlorite (Dalal et al., 2020).

6.2.12. Principal Component Analysis (PCA)

The Principal Component Analysis (PCA) was conducted on radish sango microgreens stored at 5°C to analyze the effect of different treatments on preserving the quality, antioxidant content, and microbial stability of these microgreens. PCA identified two principal components (PC-1 and PC-2), which explained a significant portion of the data variability of 84.5% and 9.2%, respectively (**Table 6.29**). The PC-1 represented a strong quality-retention axis and was positively associated with most beneficial quality indicators such as FRAP (0.279), DPPH (0.274), total phenol content (0.270), flavonoids (0.270), glucosinolates (0.272), ascorbic acid (0.256), anthocyanins (0.268), total chlorophyll (0.247), and carotenoids (0.245). Additionally, it showed positive loading for overall quality (0.270) and negative loadings for microbial spoilage related variables such as total plate count (−0.262), yeast and mold count (−0.272), off-odor (−0.277), weight loss (−0.238), and electrolyte leakage (−0.145). These associations clearly define PC-1 as a component representing both nutritional quality and microbial safety. In another PC-2, which explained 9.2% of the variance, was primarily associated with membrane integrity and microbial instability. It exhibited strong negative loadings for electrolyte leakage (−0.701) and weight loss (−0.379), and moderate positive loadings for total chlorophyll (0.326) and yeast and mold count (0.093). This indicates that PC-2 distinguishes treatments based on their effect on physiological deterioration and microbial spoilage, potentially separating treatments that retain visual appearance from those with better microbial quality. Positive loadings on PC1 are associated with storage at 5°C and the use of MP packaging,

which are effective in preserving these bioactive and nutritional attributes (Singh et al., 2024). Among washing treatments, citric acid and sodium hypochlorite are particularly effective, as indicated by their positive contribution to PC1. This clustering along PC1 suggests that low-temperature storage combined with MP packaging and selected washing treatments (citric acid or sodium hypochlorite) effectively stabilizes antioxidant content and pigmentation, helping to retain both the nutritional quality and visual appeal of the radish sango microgreens (**Figure 6.23 (A)**). PC-2 is primarily associated with microbial parameters, such as total plate count (TPC) and yeast and mold counts, as well as weight loss. These variables show positive loadings on PC2 when associated with PT packaging, facilitating faster microbial growth and spoilage. Samples treated with acetic acid and stored at 5°C in PT packaging exhibited lower microbial stability, leading to increased microbial loads and weight loss. This clustering along PC2 highlights the impact of oxygen-permeable PT packaging and higher temperatures on microbial proliferation, which accelerates spoilage and reduces shelf life (**Figure 6.23 (B)**). The score plot in (**Figure 6.23**) illustrates distinct clustering patterns among the treatment groups, with samples stored at 5°C in MP packaging and treated with citric acid or sodium hypochlorite clustering closer to higher quality retention. In contrast, samples stored in PT packaging at higher temperatures clustered along PC2, indicating increased microbial load and reduced quality stability. These findings emphasize the importance of an integrated storage approach that combines low-temperature storage, oxygen-restrictive packaging, and antimicrobial washing treatments to maximize the shelf life and quality retention of radish sango microgreens (Yan et al., 2022).

Based on the PCA results (**Figure 6.23**), the most effective treatment for preserving quality and extending shelf life is storage at 5°C in MP packaging, with a citric acid or sodium hypochlorite wash. This treatment combination was consistently associated with high antioxidant retention, bioactive stability, and minimized microbial growth, making it ideal for preserving the sensory, nutritional, and microbial quality of radish sango microgreens during storage. These findings support the application of optimized postharvest practices to enhance product quality and meet consumer expectations for fresh, minimally processed produce.

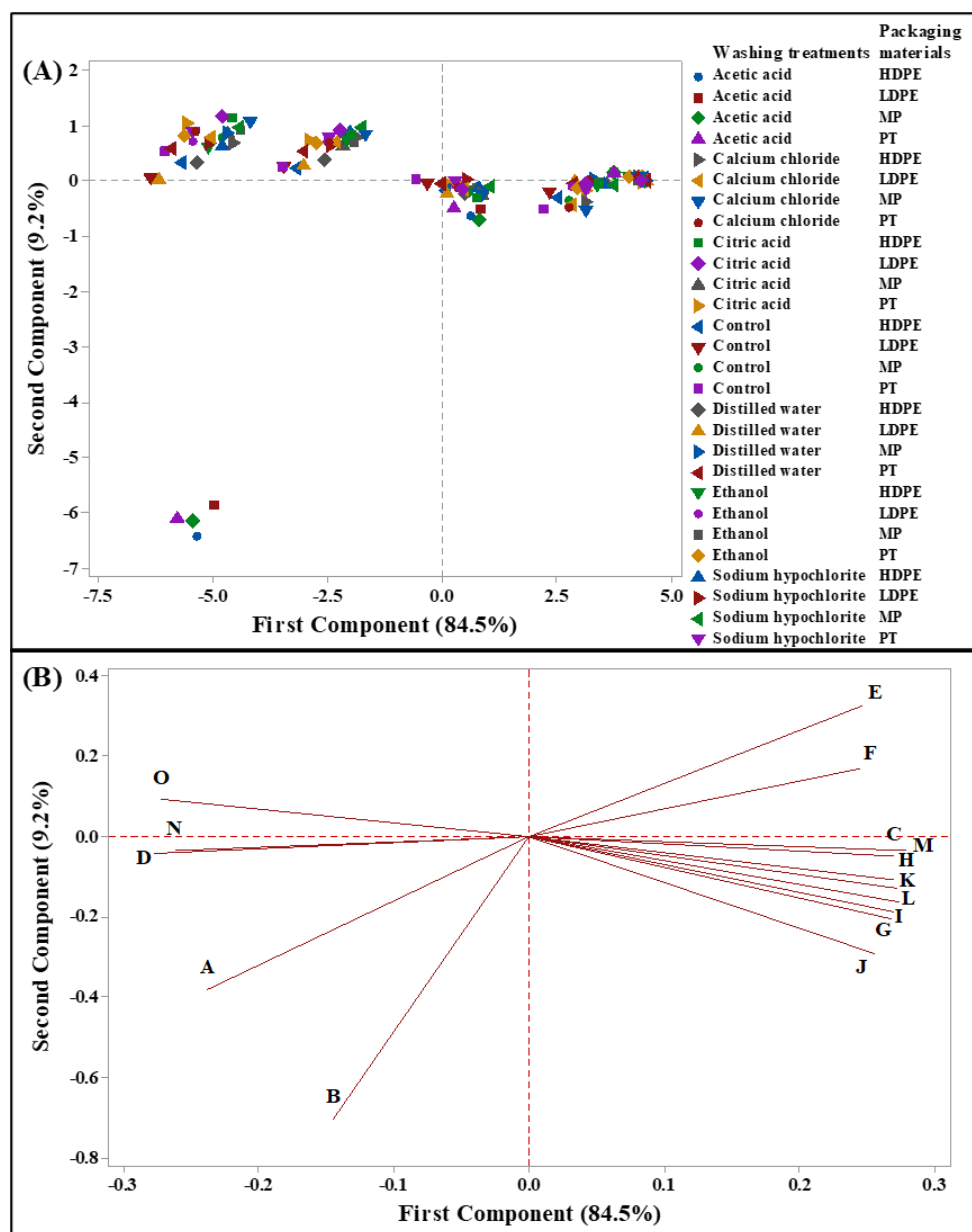


Figure 6.23. Score plot (A) and loading plot (B) of the principal components analysis carried out on the selected parameters of radish sango microgreens during storage 5°C storage condition, under different washing treatments and packaging systems. Where, A: weight loss, B: electrolyte leakage, C: overall quality, D: off odor; E: total chlorophyll content, F: total carotenoid content, G: anthocyanin content, H: total phenol content, I: total flavonoid content, J: ascorbic acid content, K: total glucosinolate content, L: antioxidant activity DPPH, M: antioxidant activity FRAP, N: total plate count, O: yeast and mold count

Table 6.29. Loadings of the first two principal components based on the selected parameters of radish sango microgreens during different storage condition, under different washing treatments and packaging systems.

Factor loadings	Principal components (PC)	
	PC-1	PC-2
Weight loss (A)	-0.238	-0.379
Electrolyte leakage (B)	-0.145	-0.701
Overall quality (C)	0.270	-0.047
Off odor (D)	-0.277	-0.041
Total chlorophyll content (E)	0.247	0.326
Total carotenoid content (F)	0.245	0.171
Anthocyanin content (G)	0.268	-0.204
Total phenol content (H)	0.270	-0.107
Total flavonoid content (I)	0.270	-0.187
Ascorbic acid content (J)	0.256	-0.290
Total glucosinolate content (K)	0.272	-0.127
Antioxidant activity DPPH (L)	0.274	-0.161
Antioxidant activity FRAP (M)	0.279	-0.034
Total plate count (N)	-0.262	-0.034
Yeast and mold count (O)	-0.272	0.093
Eigenvalue	12.670	1.375
Total variance (%)	84.5	9.2
Cumulative variance (%)	84.5	93.6

6.3. Effect of drying condition on radish sango microgreens

6.3.1. Drying curves

Two different loading densities, such as 0.057 g/cm² (**Figure 6.24 (A)**) and 0.113 g/cm² (**Figure 6.24 (B)**), are shown in the moisture ratio (MR) profiles of radish sango microgreens during convective tray drying at various temperature ranges (40°C, 50°C, and 60°C). The drying kinetics with a lower loading density of 0.057 g/cm² are shown in (**Figure 6.24 (A)**). At higher temperatures, the moisture ratio drops more quickly; the drying curve at 60°C exhibits the steepest MR loss, followed by the curves at 50°C and 40°C. This is to be expected because greater temperatures hasten the water evaporation, which lowers the moisture content faster. The high moisture content at the outset of drying is indicated by the initial MR at all temperatures, which starts near 1 and gradually drops. Another illustrates drying kinetics at a greater loading density of 0.113 g/cm² (**Figure 6.24 (B)**). Although the moisture ratio profiles show a similar pattern here, the higher loading density causes MR to decrease more slowly. Because of the higher mass of material per unit area, which restricts the rate of heat transfer and moisture evaporation, the moisture content drops more gradually at all temperatures. The curves nevertheless demonstrate that faster drying rates are associated with greater drying temperatures. The moisture ratio in both loading densities approaches zero as drying time increases, indicating that drying is complete. Temperature and loading density variations in MR

offer important information on how these variables affect drying kinetics and the amount of energy needed for each phase. In particular, drying at higher temperatures (60°C) is more effective and uses less energy, drying at lower temperatures (40°C) necessitates longer drying times and uses more energy.

The moisture ratio of radish sango microgreens was significantly impacted by the drying temperature. This is due to the fact that drying at a higher temperature increases the pushing power for heat transmission. For comparable horticulture crops, the drying curves are normal. One of the factors that drives drying is the water vapor pressure deficit, which is the difference between the saturated and partial pressures of water vapor in air at a specific temperature and is greater at higher drying temperatures (Srikanth et al., 2018). Additionally, this study's findings are consistent with earlier research on the convection drying method of various food products other than radish sango microgreens, including mangoes (Ampah et al., 2022), pomegranates (De Oliveira Sousa Wanderley et al., 2023), peels of potatoes (Brahmi et al., 2023), and wheatgrass (Chakraborty et al., 2023). Therefore, the drying conditions most notably the temperature (the longest drying time is at 40°C and the shortest at 60°C) and loading densities (the shortest drying time is at 0.057 g/cm² and the longer drying time is at 0.113 g/cm²) determine the amount of time needed to reduce the initial moisture content to any given level.

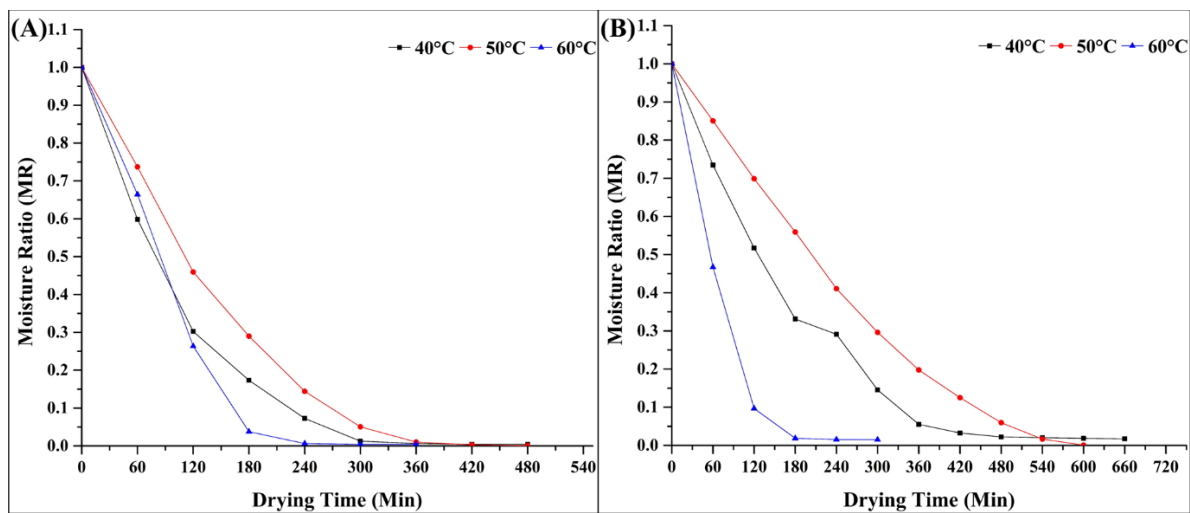


Figure 6.22. Moisture ratio profiles of radish sango microgreens during convective tray drying at various temperature ranges (40, 50, and 60°C) drying at (A) loading density tray-0.057 g/cm² and (B) loading density tray-0.113 g/cm².

Table 6.30. The different parameters of mathematical models fitted for drying data of radish sango microgreens at loading density tray (0.057 g/cm²) and coefficient of determination (R²), root mean square error (RMSE), and chi-square test (χ^2)

Models loading density tray-0.057 g/cm ²	T (°C)	The drying constants and coefficients						Statistical parameters					
		k	n	a	c	g	b	RMSE	Average	R ²	Average	χ^2	Average
Newton	40	0.0099						0.0229	0.0484	0.9918	0.9581	0.0676	0.1574
	50	0.0073						0.0494		0.9552		0.1849	
	60	0.0109						0.0730		0.9274		0.2196	
Page	40	0.0041	1.1804					0.0104	0.0147	0.9983	0.9958	0.0212	0.3108
	50	0.0010	1.3803					0.0140		0.9964		0.0354	
	60	0.0002	1.8293					0.0197		0.9927		0.5861	
Logarithmic	40	0.0099		1.0169	0.00003			0.0221	0.0463	0.9924	0.9618	0.0637	0.1451
	50	0.0075		1.0478	0.00012			0.0461		0.9610		0.1668	
	60	0.0112		1.0465	0.00002			0.0706		0.9321		0.2048	
Handerson and pabis	40	0.0100		1.0169				0.0221	0.0463	0.9924	0.9619	0.0635	0.1449
	50	0.0076		1.0478				0.0461		0.9611		0.1664	
	60	0.0113		1.0465				0.0706		0.9321		0.2047	
Two terms	40	0.0099		1.0099			0.0071	0.0221	0.0463	0.9924	0.9619	0.0635	0.1449
	50	0.0075		1.0408			0.0070	0.0461		0.9611		0.1664	
	60	0.0113		0.1254			0.9211	0.0706		0.9321		0.2047	
Two term expo.	40	0.0130		1.7115				0.0109	0.0266	0.9981	0.9827	0.0239	0.0826
	50	0.0073		0.9997				0.0494		0.9552		0.1849	
	60	0.0192		2.3920				0.0195		0.9948		0.0390	
Verma et al	40	0.0152		8.2732		0.0164		0.0106	0.0147	0.9983	0.9965	0.0215	0.0356
	50	0.0133		14.161		0.0142		0.0165		0.9950		0.0514	
	60	0.0236		7.3924		0.0289		0.0169		0.9961		0.0338	
Approximation of diffusion mode	40	0.0066		9.1086			0.9534	0.0136	0.0347	0.9971	0.9778	0.0252	0.0206
	50	0.0057		1.1141			0.0150	0.0299		0.9863		0.0036	
	60	0.0087		1.1065			0.0332	0.0607		0.9499		0.0331	
Diffusion	40			1.0169			0.0100	0.0221	0.0463	0.9924	0.9619	0.0635	0.1449
	50			1.0478			0.0075	0.0461		0.9611		0.1664	
	60			1.0465			0.0113	0.0706		0.9321		0.2047	
Midillis et al.	40	0.0041	1.1900	0.9991	6.38415×10^{-06}			0.0137	0.0143	0.9971	0.9963	0.0239	0.0240
	50	0.0010	1.4008	0.9958	5.94268×10^{-06}			0.0194		0.9931		0.0373	
	60	0.0002	1.8089	0.9996	5.52114×10^{-06}			0.0099		0.9987		0.0106	

Table 6.31. The different parameters of mathematical models fitted for drying data of radish sango microgreens at loading density tray (0.113 g/cm²) and coefficient of determination (R²), root mean square error (RMSE), and chi-square test (χ^2)

Models loading density tray-0.113 g/cm ²	T (°C)	The drying constants and coefficients						Statistical parameters					
		k	n	a	c	g	b	RMSE	Average	R ²	Average	x ²	Average
Newton	40	0.006						0.0332	0.0468	0.9755	0.9259	0.1088	0.1678
	50	0.0042						0.0649		0.8222		0.3216	
	60	0.0154						0.0423		0.98		0.073	
Page	40	0.0024	1.1761					0.0238	0.0176	0.9874	0.9898	0.0633	0.264
	50	0.0003	1.467					0.0201		0.983		0.0758	
	60	0.0011	1.5949					0.0089		0.9991		0.653	
Logarithmic	40	0.0062		1.0279	0.0003			0.0318	0.04393	0.9775	0.9383	0.0993	0.1487
	50	0.0045		1.0779	0.0005			0.0582		0.8571		0.2755	
	60	0.0156		1.0146	0.0004			0.0418		0.9804		0.0713	
Handerson and pabis	40	0.0062		1.0279				0.0318	0.04373	0.9775	0.9892	0.0993	0.144
	50	0.0045		1.0783				0.0576		0.8598		0.2613	
	60	0.0156		1.0149				0.0418		0.9804		0.0713	
Two term	40	0.0062		0.8725	0.1554	0.0062		0.0318	0.04393	0.9775	0.9383	0.0993	0.1487
	50	0.0045		0.9339	0.1444	0.0045		0.0582		0.8571		0.2755	
	60	0.0156		0.8802	0.1347	0.0156		0.0418		0.9804		0.0713	
Two term expo.	40	0.0079		1.6934				0.0241	0.0201	0.9871	0.9838	0.0608	0.1609
	50	0.0063		1.9617				0.0288		0.965		0.1311	
	60	0.0269		2.4811				0.0075		0.9994		0.2909	
Verma et al	40	0.0044		1.8607		0.0031		0.0256	0.029	0.9855	0.981	0.2833	0.1492
	50	0.0018		3.1183		0.0012		0.026		0.9715		0.023	
	60	0.0105		2.9706		0.0088		0.0353		0.986		0.1414	
Approximation of diffusion mode	40	0.0094		9.7123			1.063	0.0236	0.0301	0.9876	0.9793	0.0618	0.0461
	50	0.0025		1.2926			0.0012	0.0279		0.9673		0.0266	
	60	0.0142		1.0328			0.0089	0.0388		0.9831		0.05	
Diffusion	40			1.0279			0.0062	0.0318	0.0439	0.9775	0.9383	0.0993	0.1487
	50			1.0783			0.0045	0.0582		0.8571		0.2755	
	60			1.0149			0.0156	0.0418		0.9804		0.0713	
Midillis et al	40	0.0017	1.2359	0.9839	2.654×10^{-08}			0.0243	0.0148	0.9869	0.9906	0.0711	0.0483
	50	0.0003	1.4896	0.9851	5.465×10^{-08}			0.019		0.9849		0.0734	
	60	0.0009	1.6477	0.9999	5.376×10^{-05}			0.001		0.9999		0.0004	

6.3.2. Experimental data modelling and fitting of curves

Mathematical modelling of the drying process is an essential aspect of drying technology, especially in the postharvest treatment of agricultural materials (Harish et al., 2014). Accurate mathematical modelling of drying kinetics is crucial to translating experimental research data into industrial applications effectively (Ashtiani et al., 2017). Numerous mathematical models have been documented in the literature, while new models continue to be proposed to improve predictions and applications (Khalangre et al., 2024).

The mathematical modelling of drying kinetics for radish sango microgreens was performed using several models to assess their suitability under varying drying conditions, specifically at two loading densities (0.057 g/cm² and 0.113 g/cm²), and temperatures of 40°C, 50°C, and 60°C. The models performance was evaluated using statistical parameters such as coefficient of determination (R^2), root mean square error (RMSE), and chi-square (χ^2) values, which reflect how well the model predicts the experimental data. The results, presented in **(Table 6.30)** and **(Tables 6.31)**, reveal that the Midilli et al model consistently outperformed the other models, offering the best fit across all temperature and loading density conditions. The Midilli et al model achieved an average R^2 of 0.9963 at a lower loading density (0.057 g/cm²) and 0.9906 at a higher loading density (0.113 g/cm²), with exceptionally low χ^2 (0.0240 and 0.0483, respectively) and RMSE (0.0143 and 0.0148, respectively) values, indicating its superior predictive accuracy.

The Newton model, while simpler, exhibited lower R^2 values at both loading densities (0.9581 and 0.9259, respectively) and higher RMSE values (0.0484 and 0.0468, respectively), making it less suitable for capturing the nonlinear drying behavior of microgreens. These models provided accurate predictions of moisture loss and were consistent with findings in previous studies on drying kinetics for similar matrices (Izli et al., 2017). This aligned with trends reported for drying processes of herbs like basil and parsley (Sledz et al., 2015; Mouhoubi et al., 2020).

The Midilli et al model emerged as the most reliable model for predicting the drying behavior of radish sango microgreens under the experimental conditions tested, offering minimal deviation from experimental data as reflected by its high R^2 and low RMSE and χ^2 values. The Page, Logarithmic and Two-term exponential models also showed strong predictive capability, especially at higher temperatures, but the Midilli model remained the most consistent across both loading densities and temperatures. While the Midilli et al and page models were optimal for radish sango microgreens in this study, other models such as the

Newton model might perform better for drying other matrices like mango or coconut pith (Izli et al., 2017). These findings highlight the importance of selecting appropriate models to accurately represent drying behavior in microgreens and underscore the need to consider factors such as loading density when designing drying processes.

The fitting of curves is a crucial component in understanding the drying kinetics of radish sango microgreens. The data presented in **(Figure 6.25)** and **(Figure 6.26)** provide an in-depth analysis of the moisture ratio (MR) predictions and their comparison with experimental values, as well as the determination of key parameters such as effective moisture diffusivity and activation energy. These figures offer a clear insight into the drying behavior of microgreens under varying conditions, including different loading densities and temperature ranges.

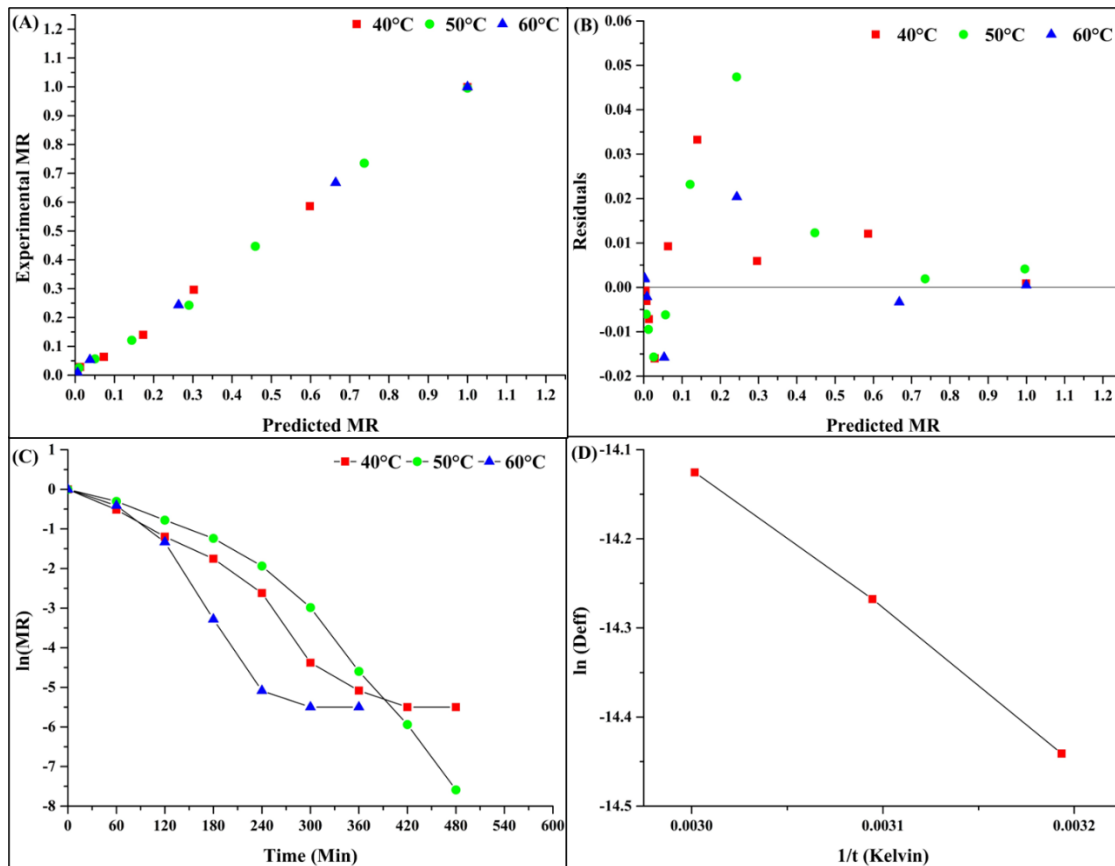


Figure 6.25. (A) MR experimental vs MR predicted, (B) Residuals vs MR predicted, (C) Plot of the natural logarithm of MR vs time for the determination of effective moisture diffusivity, (D) Plot of the natural logarithm of effective diffusivity vs inverse of temperature in kelvin for determination of activation energy for the radish sango microgreens dried at temperatures ranges (40, 50, and 60°C) drying at loading density tray (0.057 g/cm²).

The **(Figure 6.25)** and **(Figure 6.26 (A and B))** display the comparison between the experimental and predicted moisture ratios, highlighting the accuracy of the fitted models. In **(Figure 6.25)** and **(Figure 6.26 (A and B))**, the MR experimental vs. MR predicted plots show

a close alignment between the observed and model-predicted data, confirming the models' reliability in capturing the drying process. Small residuals indicate that the models' error is negligible, reinforcing their suitability for describing the moisture loss dynamics of radish sango microgreens under the given drying conditions.

Overall, the fitting of these curves demonstrates that the selected models successfully describe the drying kinetics of radish Sango microgreens, while providing insights into the key physical properties of the drying process, such as moisture diffusivity and activation energy. The consistency between experimental and predicted values, coupled with the determination of effective diffusivity and activation energy, further validates the mathematical models for optimizing drying conditions and improving energy efficiency in industrial drying applications.

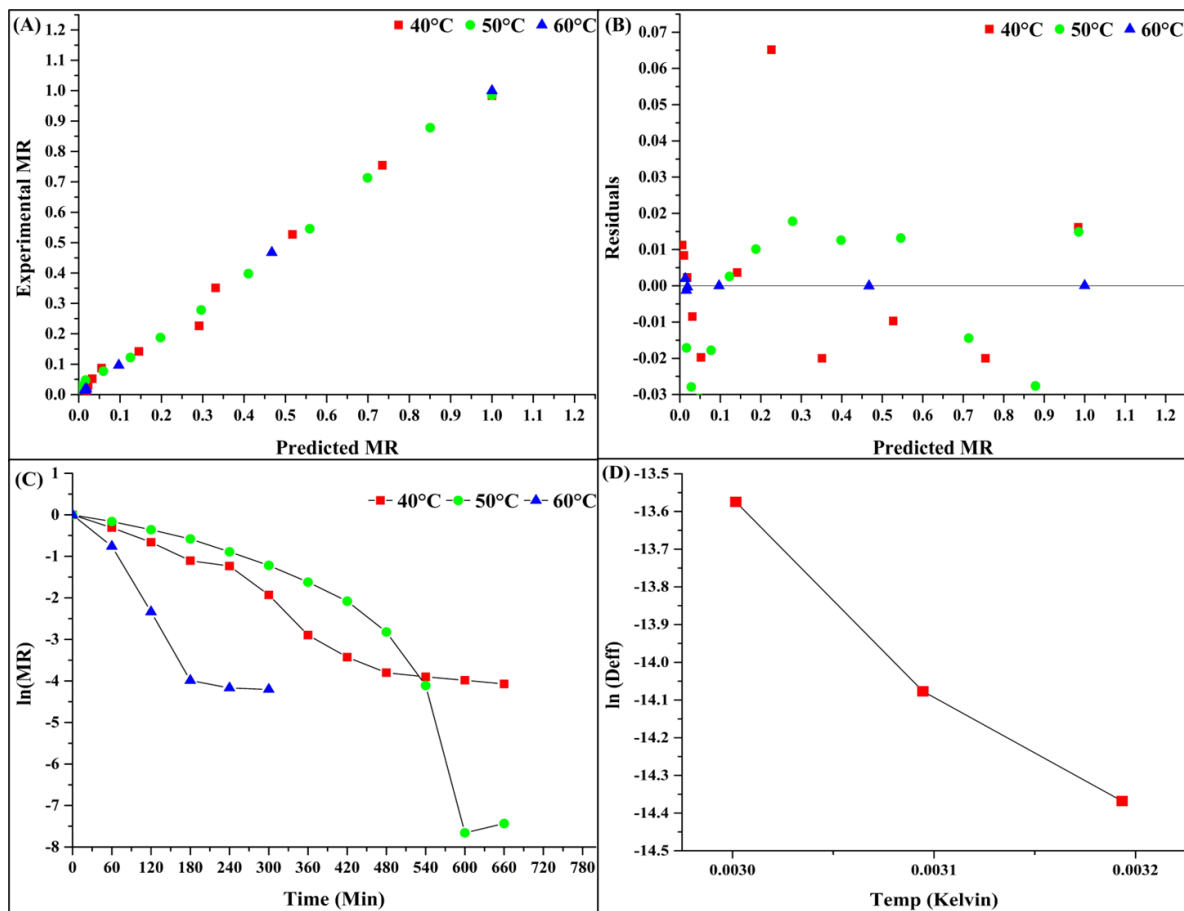


Figure 6.26. (A) MR experimental vs MR predicted, (B) Residuals vs MR predicted, (C) Plot of the natural logarithm of MR vs time for the determination of effective moisture diffusivity, (D) Plot of the natural logarithm of effective diffusivity vs inverse of temperature in kelvin for determination of activation energy for the radish sango microgreens dried at temperatures ranges (40, 50, and 60°C) drying at loading density tray (0.113 g/cm²).

6.3.3. Effective moisture diffusivity and activation energy

The effective moisture diffusivity (D_{eff}) was calculated using Equation (02), and the values are shown in (Table 6.32). As indicated, the D_{eff} increases with increasing drying

temperature, a proportional relationship was noted between this parameter and the used temperature. The effective moisture diffusivity was measured by plotting the natural logarithm of MR versus drying time as shown in **(Figure 6.25 (C))** for the loading density tray (0.057 g/cm²) and in **(Figure 6.26 (C))** for the loading density tray (0.113 g/cm²) for radish sango microgreens under different drying temperatures. The values for D_{eff} for 40 to 60°C ranged from 1.273×10^{-6} to 7.700×10^{-7} m²/s were observed at different loading densities **(Table 6.32 (a))**, and all the observed values are positive. A similar trend in the increase in diffusivity with drying temperature was observed by other researchers for different horticulture crops (Guemouni et al., 2022; Chakraborty et al., 2023; Khalangre et al., 2024). The differences between these results can be explained by the effect of the type, shape, size, and initial and final moisture of the cultivated matrix, the composition and characteristics of the microgreens tissues, and the proposed model used for the calculations (Guemouni et al., 2022).

Table 6.32. The specific electric energy consumption (SECe), electrical energy (EE), effective moisture diffusivity (Deff), and activation energy (Ea) for radish sango microgreens at different temperatures and loading density trays

Loading density tray (g/cm ²)	Temperature (°C)	SECe (MJ/kg H ₂ O)	EE (%)	Deff (m ² /s)	Ea (kJ/mol)
0.057	40	30.56×10^{-6}	0.79×10^{-2}	5.350×10^{-7}	13.7
	50	29.56×10^{-6}	0.72×10^{-2}	6.323×10^{-7}	
	60	15.35×10^{-6}	1.53×10^{-2}	7.336×10^{-7}	
0.113	40	30.25×10^{-6}	0.74×10^{-2}	5.760×10^{-7}	34.31
	50	28.56×10^{-6}	1.45×10^{-2}	7.700×10^{-7}	
	60	13.25×10^{-6}	1.53×10^{-2}	1.273×10^{-6}	

The activation energy (Ea) is nothing but the energy required to displace one mole of water from the sample and is expressed as kJ/mol. The Ea of radish sango microgreens at different temperatures and loading density trays were calculated by Equations 20 and 21. The acquired values were a loading density of 0.057 g/cm² was 13.7 KJ/mol and a loading density of 0.113 g/cm² 34.31 KJ/mol observed at different temperatures **(Table 6.32)**, respectively. The obtained Ea values in this study were within the recommended range (12.7–110 kJ/mol) for agricultural materials (Guemouni et al., 2022). Similar values of activation energy have been mentioned in previous studies for red grape pomace dried at temperatures between 40 and 80°C, values of 42.79 kJ/mol were obtained (Khalangre et al., 2024), and for wheatgrass 34.535 kJ/mol dried at temperatures (50 to 70°C), the values were reported 34.535 kJ/mol (Chakraborty et al., 2023).

6.3.4. Energy consumption and electrical energy

The data presented in **(Table 6.32)** provides insights into the specific electric energy consumption (SECe) and electrical energy efficiency (EE) for radish sango microgreens dried at different temperatures (40°C, 50°C, and 60°C) and loading densities (0.057 g/cm² and 0.113 g/cm²). At the lower loading density of 0.057 g/cm², SECe decreases significantly as the drying temperature increases, with values reducing from 30.56×10^{-6} at 40°C to 15.35×10^{-6} at 60°C. This trend indicates that higher drying temperatures improve energy utilization due to shorter drying times. The electrical energy efficiency (EE) follows a similar pattern, improving from 0.79×10^{-2} at 40°C to 1.53×10^{-2} at 60°C, showing a higher proportion of electrical energy being converted effectively for moisture removal at elevated temperatures. It should be noted that such an opposite relationship observed for SECe and EE has also been noted by researchers (Mouhoubi et al., 2020; Mouhoubi et al., 2021). The decrease in SECe values has already been reported by authors (EL-Mesery et al., 2021; Mouhoubi et al., 2020). The possible explanation for the decrease in SECe during convective tray drying is that when increasing the temperature, the thermal gradient and the acceleration of moisture extraction were improved which implies a reduction in drying time and SECe (Kaveh et al., 2020).

6.3.5. Effect of drying temperatures and loading densities on bioactive compounds degradations

Radish sango microgreens are a rich source of different phenols, flavonoids, and anthocyanins and the presence of these compounds in radish sango microgreens provides many health-benefitting characteristics (Singh et al., 2024). These compounds, which provide significant health benefits, are considered valuable quality components in radish sango microgreens. However, they are highly sensitive to various external environmental factors that can trigger their degradation. Temperature and loading densities, in particular, are major factors during the drying process, having a substantial impact on these quality parameters. The degradation of bioactive compounds and antioxidant activity such as total phenol content, anthocyanin content, ascorbic acid, and DPPH antioxidant activity in radish sango microgreens under different drying temperatures (40, 50, and 60°C) and loading densities (0.057 and 0.113 g/cm²) were comprehensively analyzed. These parameters exhibited a temperature and density-dependent decline during drying. These changes are effectively shown in **(Figure 6.27)** and **(Figure 6.28)**.

The radish sango microgreens dried at 40, 50, and 60°C with a loading density of 0.057 g/cm² exhibited a reduction in total anthocyanin content from 67.65 to 34.24, 68.52 to 43.23, and 65.26 to 25.26 µmol/100g, respectively **(Figure 6.27 (A))**. Similarly, at a loading density of 0.113 g/cm², the total anthocyanin content decreased from 68.37 to 26.56, 69.35 to 34.56,

and 68.43 to 18.71 $\mu\text{mol}/100\text{g}$, respectively (**Figure 6.28 (A)**). The ascorbic acid content in radish sango microgreens decreased across drying temperatures of 40, 50, and 60°C with a loading density of 0.057 g/cm^2 , declining from 363.52 to 182.62, 364.32 to 210.55, and 361.36 to 139.36 $\text{mg}/100\text{g}$, respectively (**Figure 6.27 (B)**). Likewise, for a loading density of 0.113 g/cm^2 , the ascorbic acid content reduced from 371.54 to 213.35, 367.36 to 239.18, and 373.38 to 193.34 $\text{mg}/100\text{g}$, respectively (**Figure 6.28 (B)**). The radish sango microgreens dried at 40, 50, and 60°C for loading density (0.057 g/cm^2) showed the degradation of total phenol content from 338.36 to 158.46, 339.67 to 189.45, and 335.38 to 139.34 $\text{mg GAE}/100\text{g}$, respectively (**Figure 6.27 (C)**). For the loading density (0.113 g/cm^2) showed the degradation of total phenol content from 342.63 to 142.19, 341.60 to 176.23, and 340.07 to 152.16 $\text{mg GAE}/100\text{g}$, respectively (**Figure 6.28 (C)**). The radish sango microgreens dried at 40, 50, and 60°C with a loading density of 0.057 g/cm^2 exhibited a reduction in antioxidant activity DPPH from 74.48 to 40.64, 75.46 to 43.13, and 74.26 to 26.49 %, respectively (**Figure 6.27 (D)**). Similarly, at a loading density of 0.113 g/cm^2 , the total anthocyanin content decreased from 75.58 to 22.10, 76.42 to 38.43, and 76.33 to 17.75 $\mu\text{mol}/100\text{g}$, respectively (**Figure 6.28 (D)**).

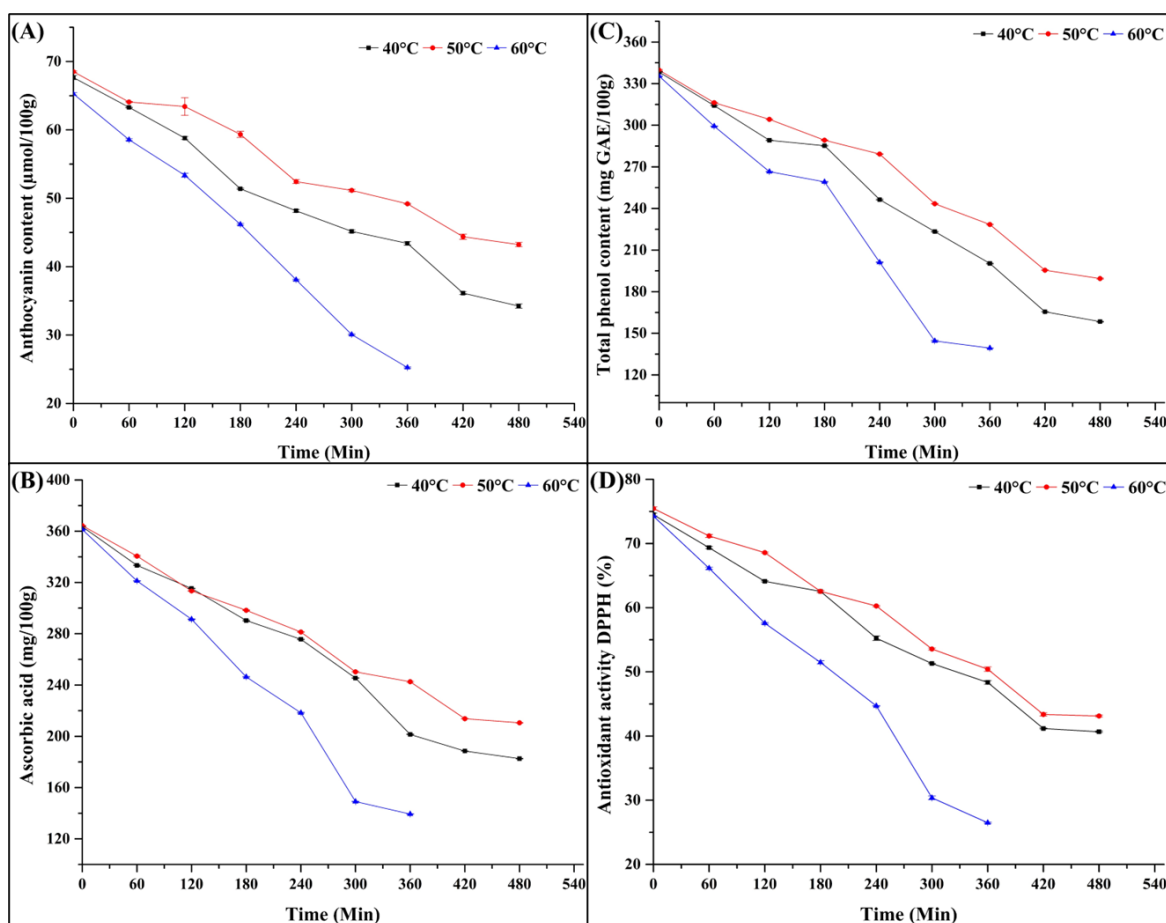


Figure 6.27. The degradation of (A) anthocyanin content, (B) ascorbic acid, (C) total phenol content, and (D) antioxidant activity during drying at 40°C, 50°C and 60°C at 0.057 g/cm^2 loading density in the tray dryer.

The total phenol content, anthocyanin content, ascorbic acid, and DPPH antioxidant activity of radish sango microgreens was significantly degraded with the increase in drying temperature and with lower loading densities. This shows the strong correlation between total phenolic contents and DPPH free radical scavenging activity of radish sango microgreens. Higher drying temperature and lower tray loading density led to greater degradation of bioactive compounds such as anthocyanin content, ascorbic acid, and total phenol content causing a reduction in their ability to neutralize the free radicals. The concentration of all parameters decreases with an increase in drying time and a lower tray loading density. The values for k , R^2 , and E_a are presented in **(Table 6.33)** and **(Table 6.34)**. The degradation of total phenolic content followed a first-order kinetic pattern, with a rate constant (k) increasing with temperature. For a loading density of 0.057 g/cm², k values ranged from 0.3912 at 40°C to 0.5731 at 60°C. Similarly, at a higher density (0.113 g/cm²), k ranged from 0.2942 to 0.5792. These results indicate accelerated degradation at higher temperatures, attributable to the phenolic compound's thermal instability. The activation energy (E_a) for degradation was higher at 0.113 g/cm² (29.09 kJ/mol) compared to 0.057 g/cm² (16.21 kJ/mol), suggesting greater resistance to thermal degradation at higher densities, possibly due to reduced heat penetration and slower moisture removal. Anthocyanin degradation exhibited sensitivity to temperature, with k values for 0.057 g/cm² ranging from 0.0699 (40°C) to 0.1145 (60°C), and for 0.113 g/cm² from 0.0655 to 0.1614. The higher E_a (38.65 kJ/mol) at 0.113 g/cm² indicates a stronger temperature dependence compared to 0.057 g/cm² (20.95 kJ/mol). This trend aligns with anthocyanins susceptibility to degradation under oxidative and thermal conditions, which is exacerbated at elevated drying temperatures.

The degradation rate of ascorbic acid was most pronounced among the studied parameters, with k values reaching 0.6452 at 60°C (0.057 g/cm²) and 0.6122 at the same temperature (0.113 g/cm²). The E_a was slightly higher at 0.113 g/cm² (37.99 kJ/mol) compared to 0.057 g/cm² (20.57 kJ/mol), which indicated that higher loading densities provided minimal protective effects against thermal degradation. These results are consistent with ascorbic acid well known sensitivity to heat and its rapid degradation under oxidative drying conditions. The DPPH antioxidant activity demonstrated a degradation pattern consistent with bioactive compound loss. The rate constant increased from 0.0731 (40°C) to 0.1361 (60°C) for 0.057 g/cm² and from 0.0739 to 0.2038 for 0.113 g/cm². The higher E_a at 0.113 g/cm² (43.50 kJ/mol) compared to 0.057 g/cm² (26.65 kJ/mol) highlights a pronounced temperature dependency, reflecting the collective thermal instability of antioxidant compounds. The first-order kinetic model accurately described the degradation behavior, as evidenced by R^2 values exceeding

0.95 in most cases. This approach aligns with the general degradation mechanisms of bioactive compounds, involving exponential decay governed by temperature and exposure time. The loading density significantly influenced degradation kinetics. Higher densities (0.113 g/cm²) generally exhibited slower degradation rates at lower temperatures but not at 60°C, where the elevated thermal gradient overcame the insulating effects of increased mass. This phenomenon emphasizes the need to optimize drying conditions to balance bioactive retention with drying efficiency. The Arrhenius equation provided insights into the temperature dependence of degradation. Higher E_a values for anthocyanins and DPPH activity signify stronger thermal sensitivity, while lower E_a for phenolic content reflects relatively greater thermal stability.

Table 6.33. The parameters for degradation kinetics for total phenol content, anthocyanin content, ascorbic acid, and antioxidant activity in radish sango microgreens during drying at temperatures 40, 50, and 60°C at loading density 0.057 g/cm²

Parameters	Temperature (°C)	k	R ²	t _{1/2}	E _a (kJ/mol)
Total phenol content	40	0.3912	0.9877	1.771849	16.21396
	50	0.3235	0.9813	2.14265	
	60	0.5731	0.9667	1.20947	
Anthocyanin content	40	0.0699	0.9821	9.916269	20.94629
	50	0.0541	0.9813	12.81233	
	60	0.1145	0.9964	6.053687	
Ascorbic acid	40	0.3979	0.9831	1.742014	20.57133
	50	0.3298	0.9869	2.10172	
	60	0.6452	0.9852	1.074314	
Antioxidant activity DPPH	40	0.0731	0.9889	9.482178	26.65219
	50	0.0717	0.9866	9.667325	
	60	0.1361	0.9877	5.092926	

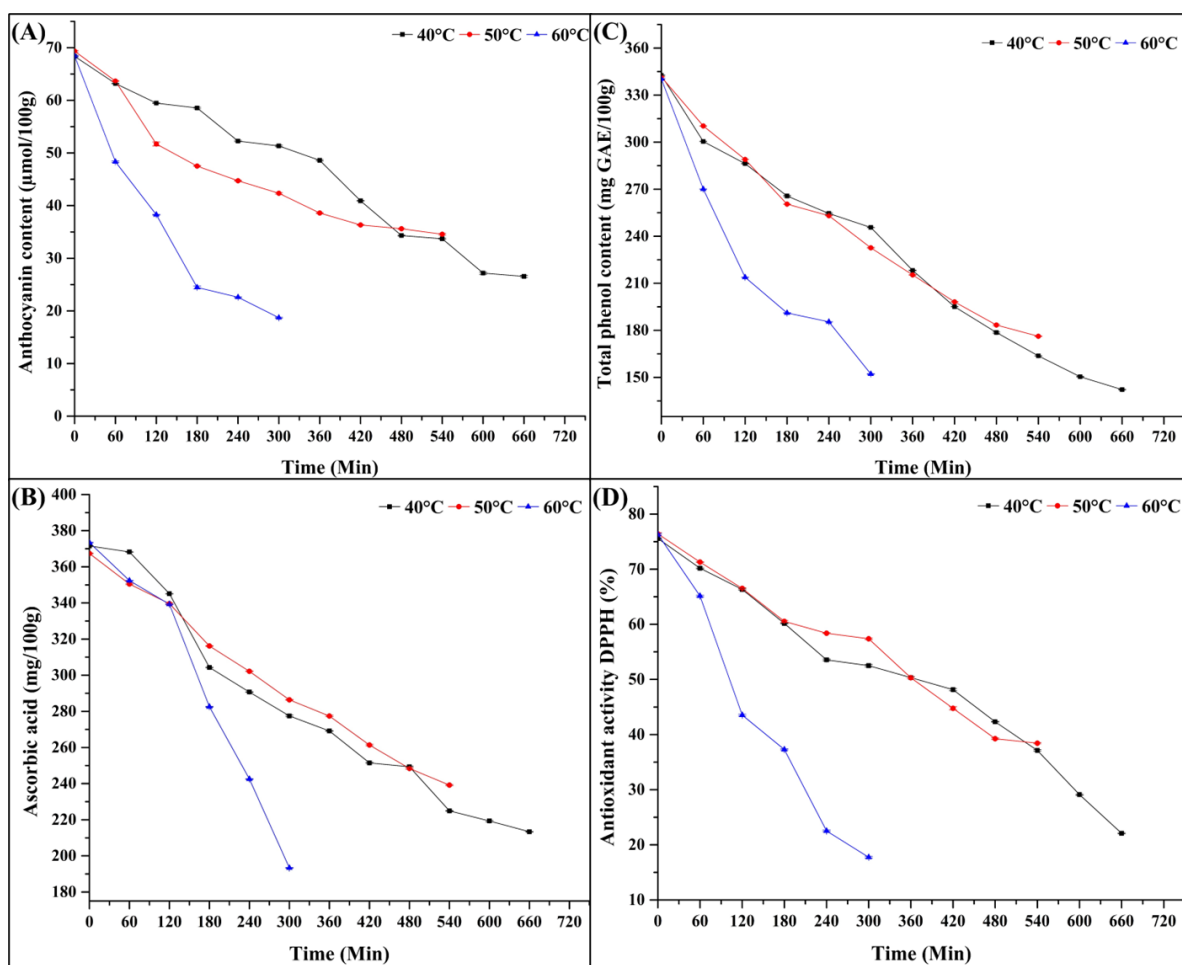


Figure 6.28. The degradation of (A) anthocyanin content, (B) ascorbic acid, (C) total phenol content, and (D) antioxidant activity during drying at 40°C, 50°C and 60°C at 0.113 g/cm² loading density in the tray dryer.

Table 6.34. The parameters for degradation kinetics for total phenol content, anthocyanin content, ascorbic acid, and antioxidant activity in radish sango microgreens during drying at temperatures 40, 50, and 60°C at loading density 0.113 g/cm²

Parameters	Temperature (°C)	k	R ²	t _{1/2}	E _a (kJ/mol)
Total phenol content	40	0.2942	0.9849	2.356041	29.0857
	50	0.301	0.9801	2.302815	
	60	0.5792	0.9674	1.196732	
Anthocyanin content	40	0.0655	0.9824	10.5824	38.65179
	50	0.0624	0.8915	11.10813	
	60	0.1614	0.9066	4.294592	
Ascorbic acid	40	0.2523	0.9582	2.747313	37.99747
	50	0.242	0.956	2.864245	
	60	0.6122	0.9606	1.132223	
Antioxidant activity DPPH	40	0.0739	0.9751	9.379529	43.49552
	50	0.0714	0.9846	9.707944	
	60	0.2038	0.969	3.401115	

The highest activation energy (E_a) was observed for DPPH antioxidant activity, followed by anthocyanin content, ascorbic acid, and total phenolic content. This indicated that the degradation of DPPH antioxidant activity is more sensitive to temperature changes compared to the other parameters. In contrast, the lowest E_a was recorded for total phenolic content, suggesting that it plays a significant role in antioxidant activity degradation during the drying of radish sango microgreens. Additional factors, including system temperature, airflow, light exposure, and oxygen levels, may also contribute to the observed degradation. These findings align with reported E_a values for the drying of pomegranate arils under varying temperatures (Vardin & Yilmaz, 2017) and are consistent with studies on total phenolic content in blanched eggplant, beetroot, green pepper, and green pea (Nambi et al., 2016), black rice (Bolea et al., 2016), sweet cherries (Ursache et al., 2017), and dried banana (Sarpong et al., 2018). For instance, drying at temperatures above 50°C can precipitate rapid losses in total phenols, with studies reporting reductions of approximately 50% within 120 min of drying (Patrón-Vázquez et al., 2019). Anthocyanins, known for their antioxidant properties, are particularly sensitive to thermal processing. Researchers show that the degradation rates of anthocyanins increase exponentially with temperature. The drying conditions of 60°C can cause substantial reductions in anthocyanin concentrations (Nawawi et al., 2023). Studies on other fruits indicate that drying at higher temperatures results in color shifts from red to brown, indicating chemical changes and degradation of anthocyanin content (Routray & Orsat, 2011).

Ascorbic acid is critically prone to degradation during drying, primarily due to oxidative processes highlighted by the presence of oxygen and heat (Giannakourou & Taoukis, 2021). Research findings showed that ascorbic acid degrades significantly at high temperatures and lower densities, with losses noted to be exacerbated by prolonged drying time (ElGamal et al., 2023). However, for ascorbic acid, contrasting results have been noted, with its degradation following first-order kinetics in orange juice (Vikram et al., 2004). Similar patterns of free-radical scavenging activity (% inhibition) were reported by Zahir et al. (2015). Reduction in DPPH antioxidant activity is closely correlated with the degradation patterns of phenolic content and ascorbic acid levels (Liu et al., 2022). The higher E_a values observed in this study emphasize the temperature dependence of phytochemical degradation, highlighting the need for careful optimization of drying conditions. Drying at 50°C with lower loading densities was identified as the optimal condition for maximizing bioactive compound retention, making it suitable for processing radish sango microgreens for functional food applications.

6.3.6. Effect on bioactive compounds

6.3.6.1. Anthocyanin content

The highest anthocyanin content retention was observed at 50°C with 0.057 g/cm² loading density (43.23 µmol/100 g), followed by 50°C with 0.113 g/cm² (34.56 µmol/100 g) as shown in (Table 6.35). A significant reduction occurred at 60°C, with the lowest levels recorded at 0.057 g/cm² (25.26 µmol/100 g) and 0.113 g/cm² (26.56 µmol/100 g). Moderate anthocyanin levels were seen at 40°C, with values between 33.70 and 34.24 µmol/100 g. Anthocyanins, water-soluble pigments responsible for the purple color in radish microgreens, are highly sensitive to heat, oxygen, and light. A similar effect of pretreatment and drying temperature on the total anthocyanin content of black carrots has been reported. In that study, the highest anthocyanin retention was observed for the blanched treatment at 50°C for 6 min, yielding 282.6 mg/100 g, followed by 278.8 mg/100 g at 60°C and 247.0 mg/100 g at 40°C (Garba et al., 2015). A similar trend was observed in another study on the drying of red grape pomace at temperatures ranging from 40 to 80°C. The total anthocyanin content in the fresh sample was 32,269.24 mg/kg C3GE, which degraded to 16,120.23, 24,604.20, 22,452.34, 10,381.87, and 9,336.80 mg/kg C3GE after drying at 40, 50, 60, 70, and 80°C, respectively (Khalangre et al., 2024). The stability of anthocyanins is influenced by multiple factors, including processing conditions, the intrinsic structure of anthocyanins, and structural damage caused during sample preparation and drying, all of which contribute to degradation reactions. Moreover, reduced water content tends to facilitate oxidative processes and alter the structure of anthocyanin pigments. In radish sango microgreens, enzymatic degradation of anthocyanins is often linked to polyphenol oxidase (PPO) activity. The retention of anthocyanins at lower drying temperatures can be attributed to the denaturation of PPO. Although PPO is relatively resistant to heat, its activity diminishes when exposed to temperatures exceeding 50°C for an extended period (Méndez-Lagunas et al., 2017). At higher temperatures (60°C), degradation occurs due to thermal cleavage of the glycosidic bonds, leading to pigment oxidation and subsequent color loss (Li et al., 2023). At 50°C, optimal drying conditions likely reduced thermal stress and oxidative damage, retaining more anthocyanins. Lower temperature drying (40°C) might have required longer drying durations, exposing anthocyanins to prolonged oxidative and enzymatic degradation (Koop et al., 2022). The loading density impacts drying kinetics a lower density (0.057 g/cm²) allows faster moisture removal, with more preservation of anthocyanins.

Table 6.35. Effect of drying temperatures and loading densities on bioactive compounds of dried radish sango microgreens powder

T (°C)	TL (g/cm ²)	Anthocyanin (μmol/100g)	TPC (mg GAE/100g)	TFC (mg QE/100g)	AA (mg/100g)	Total glucosinolate (μmol/g)
40	0.057	34.24±0.31 ^b	158.46±0.28 ^d	4.99±0.11 ^c	182.78±0.00 ^e	46.86±0.59 ^a
40	0.113	33.70±0.11 ^b	163.71±0.06 ^c	5.26±0.07 ^{bc}	224.88±0.14 ^b	42.46±0.25 ^b
50	0.057	43.23±0.33 ^a	189.45±0.30 ^a	4.98±0.07 ^c	210.55±0.16 ^d	41.25±0.28 ^b
50	0.113	34.56±0.15 ^b	176.23±0.16 ^b	5.86±0.21 ^a	239.18±0.23 ^a	41.93±0.17 ^b
60	0.057	25.26±0.11 ^d	139.34±0.13 ^f	5.36±0.07 ^{bc}	139.36±0.11 ^f	33.91±0.59 ^c
60	0.113	26.56±0.18 ^c	142.19±0.23 ^e	5.72±0.13 ^{ab}	213.35±0.13 ^c	32.37±0.08 ^d

Values are presented in mean ± SD with different superscript letters in a column being significantly different (P<0.05). Where; T: Temperature; TL: Tray load; TPC: Total phenol content; AA: Ascorbic acid; GAE: Gallic acid equivalent; QE: Quercetin equivalent

6.3.6.2. Total phenolic and flavonoid content

The total phenolic and flavonoid content of radish sango microgreen powder is shown in (Table 6.35). The highest total phenol content was observed at 50°C and 0.057 g/cm² (189.45 mg GAE/100 g) and lower content was observed at 60°C, majorly at 0.057 g/cm² loading density (139.34 mg GAE/100 g). At 40°C, total phenol content was moderately retained, with higher values (163.71 mg GAE/100 g) seen at 0.113 g/cm² compared to 0.057 g/cm². The maximum total flavonoid content was observed at 50°C with 0.113 g/cm² loading of 5.86 mg QE/100 g, while the lowest retention occurred at 40°C and 0.057 g/cm² of 4.99 mg QE/100 g. At 60°C, total flavonoid content showed slight variation, with values ranging between 5.36 and 5.72 mg QE/100 g.

A similar observation was proved by Khalangre et al. (2024) in their total phenol and flavonoid content kinetic degradation study of bananas respective to drying temperatures. Higher temperatures allowed shorter drying times and reduced the exposure of the product, but eventually produced a lower total phenol and flavonoid content. The highest retention of total phenol content is often observed in flours obtained from lemon wastes. For instance, a study indicates that lemon waste dried at 40 to 110°C temperatures range from this experiment at 50°C lemon waste flour maintains about 189.45 mg GAE/100 g of total phenols exhibited superior phenolic retention due to a favorable balance between moisture removal and thermal degradation effects (Patrón-Vázquez et al., 2019). Flavonoids are moderately heat-sensitive and can degrade during prolonged and high-temperature drying (Negi et al., 2024). The total flavonoid content of dried powder was strongly influenced by the prolonged and high-temperature drying, and drying air conditions. The variation in flavonoid content may result from interactions between enzymatic and non-enzymatic reactions. It is evident that different

enzymes like glycosidase, polyphenol oxidase as well and peroxidase are liable for the mortification of flavonoids (Plabon et al., 2024). Phenolic compounds are secondary metabolites with antioxidant activity. Drying conditions influenced their retention, as cell wall breakdown during drying can enhance phenolic extractability (Kayacan et al., 2019). At 50°C, the balance between moisture removal and thermal degradation was optimal, resulting in higher total phenol content. During 60°C, excessive heat likely caused oxidative degradation of phenolic compounds. This reduction is attributed to the accelerated oxidative degradation of phenolic compounds caused by increased thermal energy, leading to bond disruption and loss of antioxidant properties of radish sango microgreens obtained powder (ElGamal et al., 2023). Furthermore, longer drying times at 40°C might have led to enzymatic oxidation of phenolic compounds due to incomplete enzyme inactivation. A higher loading density (0.113 g/cm²) likely slowed drying, allowing phenolics to remain intact longer, though this effect diminishes under extreme conditions at 60°C. Apart from this, the total phenol and flavonoid content can fluctuate based on various factors such as extraction times, temperatures, solvents, and methods employed in the extraction process (Plabon et al., 2024).

6.3.6.3. Ascorbic acid

The highest ascorbic acid content in radish sango microgreens powder was observed at 50°C and 0.113 g/cm² (239.18 mg/100 g) and the lowest was at 60°C and 0.057 g/cm² (139.36 mg/100 g). Ascorbic acid retention was higher at 40°C compared to 60°C but was consistently lower than at 50°C as shown in **(Table 6.35)**. The selected drying temperature and loading density showed a significant effect ($P < 0.05$) on the ascorbic acid content of radish sango microgreens powder. Ascorbic acid is highly thermolabile and sensitive to oxidative degradation (Akbar et al., 2023). At 50°C, moderate heat likely minimized prolonged exposure to oxidizing agents, preserving ascorbic acid better than at 40°C or 60°C. At 60°C, higher temperatures accelerate ascorbic acid degradation through both thermal and oxidative pathways (Nzimand et al., 2024). In another study drying temperatures were significantly influenced by the ascorbic acid content of wheatgrass powder were observed. The (50°C) lower temperature showed the highest ascorbic acid content (7.36 mg/100g) retention as compared to (60°C) higher temperature showed a 4.60 mg/100g ascorbic content (Pallavi et al., 2016). The longer exposure to oxygen at lower temperatures (40°C) also contributed to reduced retention. The lower density facilitated faster drying, reducing oxidative losses of ascorbic acid. Similar results were reported for the drying of dried curry leaf powder at temperatures (40, 60, and 80°C) drying on ascorbic acid content (Negi et al., 2024).

6.3.6.4. Total glucosinolate

The highest glucosinolate content retention occurred at a drying temperature of 40°C and 0.057 g/cm² loading density of 46.86 µmol/g glucosinolate content and the lowest was observed at 60°C and 0.113 g/cm² loading density of 32.37 µmol/g glucosinolate content as showed in **(Table 6.35)**. Glucosinolates are sulfur-containing compounds known for their health-promoting properties. They degrade into isothiocyanates under high temperatures or during enzymatic hydrolysis (Mrkic et al., 2010). During low temperature (40°C) reduced enzymatic activity while preserving glucosinolates content (Tetteh et al., 2018). However, prolonged drying times might have led to partial degradation. At 50°C, moderate thermal stress balanced enzymatic inactivation and glucosinolate stability. At 60°C, thermal decomposition dominated, leading to the lowest retention (Bas-Bellver et al., 2022).

6.3.6.5. Effect on antioxidant activities

The results for the effect of drying temperatures (40, 50, and 60°C) and loading densities (0.057 and 0.113 g/cm²) on antioxidant activities (DPPH and FRAP) of dried radish sango microgreens powder were shown in **(Table 6.36)**. The highest DPPH and FRAP antioxidant activity was observed for lower tray load density (0.057 g/cm²) resulting in DPPH scavenging activity (43.13%) and FRAP (10.80 µmol TE/g). Both the drying conditions (temperature and loading densities) showed a significant effect ($P < 0.05$) on these parameters. These values suggest that optimal retention of antioxidant compounds under these conditions is due to moderate thermal exposure and enhanced airflow reducing oxidative degradation (Khalangre et al., 2024). The significant reduction at 60°C both densities (0.057 and 0.113 g/cm²) for DPPH and FRAP antioxidant activity was observed. This reduction is attributed to the higher thermal degradation rates of ascorbic acid, anthocyanin, phenolic, and flavonoid compounds responsible for antioxidant properties (Akbar et al., 2023; Negi et al., 2024).

Table 6.36. Effect of drying temperatures and loading densities on antioxidant activities of dried radish sango microgreens powder

Temperature (°C)	Tray load (g/cm ²)	Antioxidant activity DPPH (%)	Antioxidant activity FRAP (µmol TE/g)
40	0.057	40.67±0.11 ^b	10.10±0.21 ^a
40	0.113	37.12±0.13 ^d	10.02±0.48 ^a
50	0.057	43.13±0.16 ^a	10.80±0.19 ^a
50	0.113	38.43±0.09 ^c	9.92±0.14 ^a
60	0.057	26.49±0.08 ^e	8.46±0.14 ^b
60	0.113	22.10±0.09 ^f	8.08±0.06 ^b

Values are presented in mean ± SD with different superscript letters in a column being significantly different ($P < 0.05$).

Similar findings were reported for DPPH and FRAP assays during the drying of plums into powders (Michalska et al., 2016), as well as in studies on dried blueberries (Zielinska & Markowski, 2015) and red grape pomace (Khalangre et al., 2024). Radish sango microgreens are a rich source of ascorbic acid, anthocyanins, glucosinolates, and polyphenols, demonstrating a correlation between antioxidant activity and ferric-reducing antioxidant power (Mlinarić et al., 2023; Paglialunga et al., 2023). Additionally, antioxidant activity may result from the formation of novel compounds, such as Maillard reaction products, which exhibit antioxidant properties (Chakraborty et al., 2023). Antioxidant compounds such as phenolics, flavonoids, and ascorbic acid are highly sensitive to heat (Motegaonkar et al., 2024). At moderate temperatures (40 to 50°C), drying minimizes enzymatic oxidation and chemical degradation, preserving bioactive compounds. However, at 60°C, the increased thermal exposure accelerates degradation, reducing antioxidant activities (DPPH and FRAP) (Mphahlele et al., 2016). The lower tray density enhanced airflow and heat transfer allowing uniform drying, limiting oxidative damage, hence retaining higher antioxidant activities. Apart from this, the high tray loading density denser packing restricts air circulation and prolongs moisture retention, resulting in non-uniform drying (Akbar et al., 2023; Yang et al., 2024). From this study was observed that moderate drying temperature (50°C) and low tray loading density (0.057 g/cm²) are optimal for retaining antioxidant properties in radish sango microgreens powder.

6.3.7. Effect on functional properties

Functional properties represent key quality attributes of food products, reflecting the complexity of their physicochemical characteristics, structures, molecular conformations, and compositions during processing. Various studies have suggested that different food processing methods can influence these functional properties, and the drying process is no exception (Chakraborty et al., 2023). The results of various functional properties are presented in **(Figure 6.29)** and **(Figure 6.30)**.

6.3.7.1. Water activity (a_w), moisture content, and dry matter

Water activity (a_w), which measures the quantity of free water available for microbial development growth and chemical reactions and ultimately determines the stability of food shelf life, is the most important characteristic among the several functional parameters (Thakur et al., 2024). The dried radish sango microgreen water activity (a_w) is displayed in **(Figure 6.29 (A))**. After radish sango microgreens were dried at different temperatures and sample loading densities, a statistically significant change was observed ($P < 0.05$). The water activity of radish sango microgreen powder showed a significant decrease as the drying temperature

increased. The drying condition at 40°C, 0.34 for (0.057 g/cm²) and 0.29 for (0.113 g/cm²) loading density water activity was observed. As the drying temperature increased to 50°C, dropped to 0.31 (0.057 g/cm²) and 0.36 (0.113 g/cm²). The lowest values were observed at 60°C, with 0.19 (0.057 g/cm²) and 0.19 (0.113 g/cm²). This trend is attributed to the higher temperatures promoting faster moisture evaporation, reducing water content, and ultimately lowering. Additionally, lower tray loading densities (0.057 g/cm²) allow for better airflow and more uniform drying, which further decreases the water activity compared to higher loading densities (0.113 g/cm²) (Chakraborty et al., 2023).

The moisture content of radish sango microgreen powder was influenced by varying drying temperatures (40, 50, and 60°C) and loading densities (0.057 and 0.113 g/cm²). Significant changes in moisture content were observed with increasing drying temperatures ($P < 0.05$), as depicted in **(Figure 6.29 (B))**. As the drying temperature increased from 40°C to 60°C, the moisture content of the powdered samples decreased notably. This reduction can be attributed to the higher thermal energy at elevated temperatures, which accelerates moisture vaporization. The increased air-drying temperature lowers the relative humidity, enhancing the removal of moisture from the samples (Chakraborty et al., 2023). The percentage of dry matter increased with drying temperature as depicted in **(Figure 6.29 (C))**. The dry matter (%) value ranged from 5.07 to 7.40 observed in all dried samples. The increase in dry matter (%) is due to the reduction in moisture content, concentrating the solid content of the radish sango microgreen powder. Additionally, lower tray loading densities enable more efficient drying, resulting in higher dry matter values compared to higher tray densities.

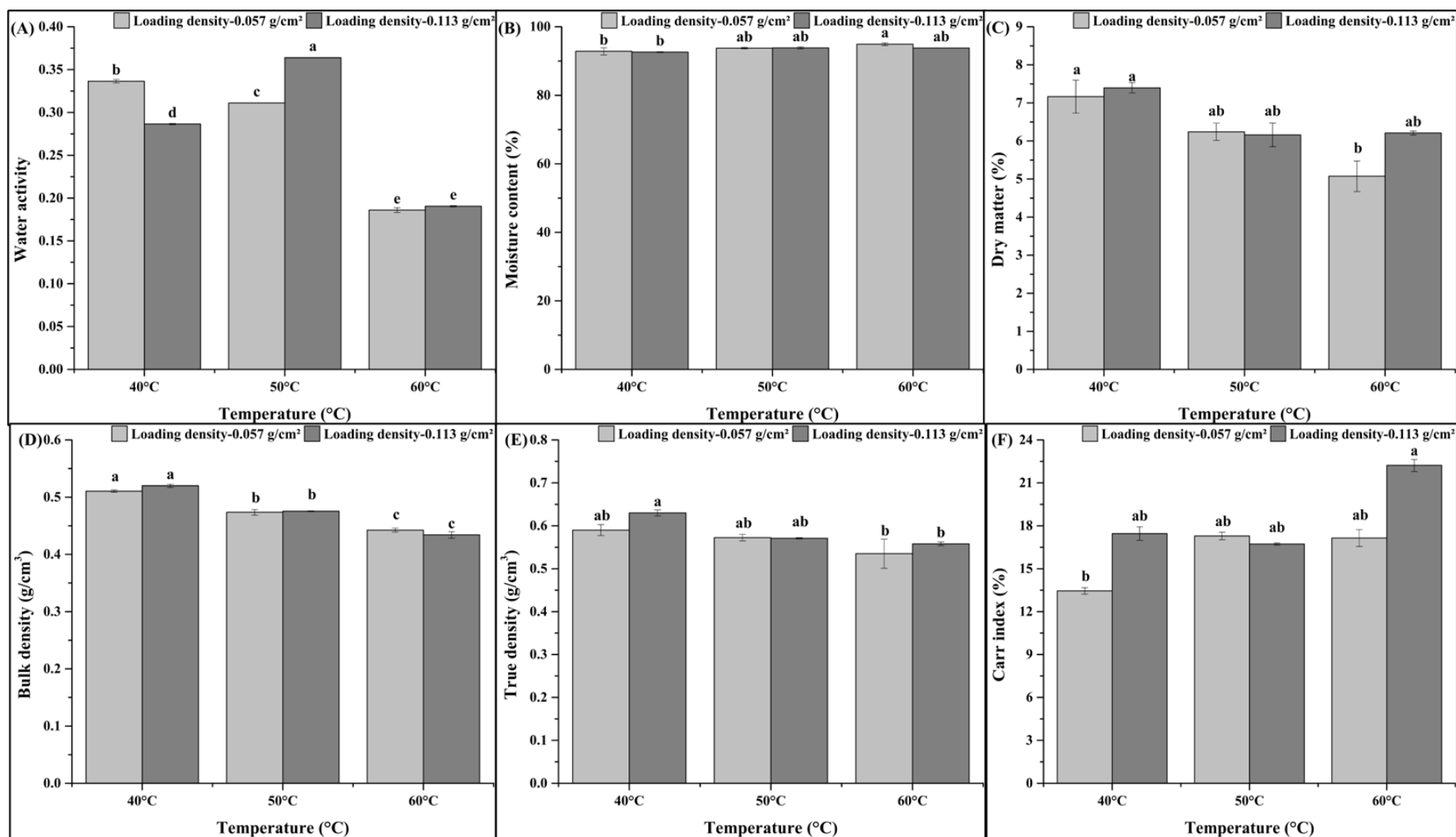


Figure 6.29. The effect of different drying temperatures and loading density on functional properties of radish sango microgreens powder. The results were presented as mean $\pm$ standard deviations. The different lowercase letters within the bar indicate significant ($P<0.05$) differences among the treatments. Where, (A) Water activity; (B) Moisture content; (C) Dry matter; (D) Bulk density; (E) True density; (F) Carr index.

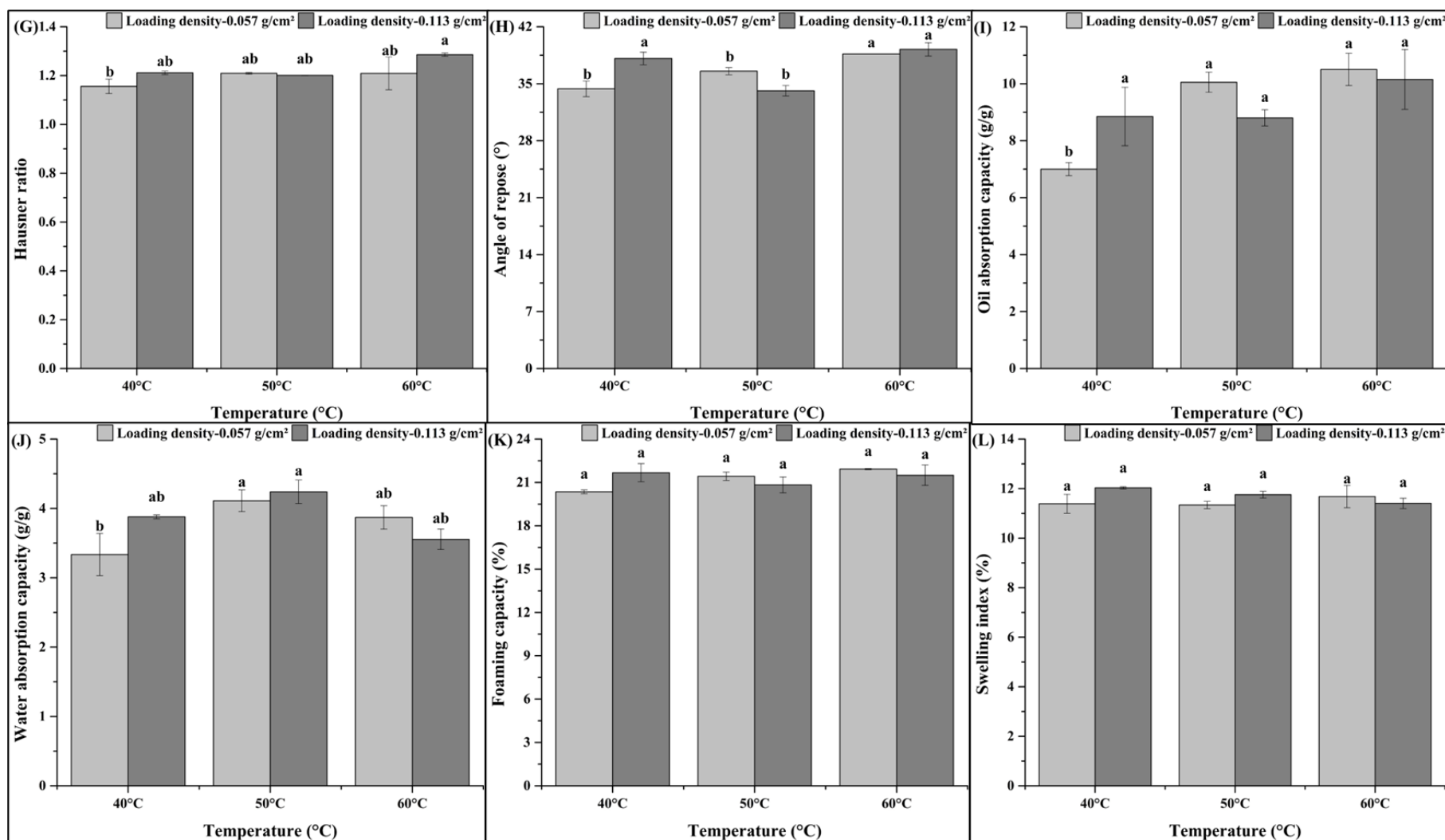


Figure 6.30. The effect of different drying temperatures and loading density on functional properties of radish sango microgreens powder. The results were presented as mean $\pm$ standard deviations. The different lowercase letters within the bar indicate significant ($P < 0.05$) differences among the treatments. Where, (G) Hausner ratio; (H) Angle of repose; (I) Oil absorption capacity; (J) Water absorption capacity; (K) Foaming capacity; (L) Swelling index.

6.3.7.2. Bulk density and true density

The results for the effect of drying temperatures (40, 50, and 60°C) and loading densities (0.057 and 0.113 g/cm²) on bulk density and true density of dried radish sango microgreens powder were shown in **(Figure 6.29 (D and E))**. The highest bulk density and true density were observed for tray load density (0.113 g/cm²) resulting in bulk density (0.43 to 0.52 g/cm³) and true density (0.56 to 0.63 g/cm³). Both the drying conditions (temperature and loading densities) showed a significant effect ($P < 0.05$) on these parameters. Similar findings were reported for bulk density (0.62 to 0.91 g/ml) during the drying of pumpkin powders prepared by hot air-drying at different temperatures (40, 50, and 60°C). The reduction in bulk density is a consequence of the shrinkage of the particles as moisture is removed during drying (Roongruangsri et al., 2016). Higher tray-loading densities result in slightly higher bulk density due to less space between particles, leading to some compaction (Yemmireddy et al., 2012). High drying temperatures cause the microgreens to compact and lose moisture, resulting in an increase in true density (Rodríguez-Ramírez et al., 2012). The high tray loading densities also contributed to higher true density due to the reduced airspace between the particles.

6.3.7.3. Carr index and Hausner ratio

The Carr index (%) value increased significantly with the increase in drying temperature, indicating a deterioration in the flowability of the powder as shown in **(Figure 6.29 (F))**. The highest Carr index values were recorded at 60°C, with 22.22% for loading density (0.057 g/cm²) and the lowest 13.45% for loading density (0.113 g/cm²) observed. The increase in the Carr index reflects the decreased flowability of the powder, which is likely caused by thermal-induced irregularities in particle shape and surface texture, as well as compaction at higher loading densities (Plabon et al., 2024).

The Hausner ratio, which is indicative of powder cohesiveness, showed a similar increasing trend with drying temperature. The highest Hausner ratio values were recorded at 60°C, with 1.29 (0.113 g/cm²) loading density and lowest for the 1.16 (0.057 g/cm²) as depicted in **(Figure 6.30 (G))**. The Hausner ratio increased with drying temperature, reflecting a higher tendency for the powder to compact and form cohesive structures, which is consistent with the increased Carr index values (Negi et al., 2024).

6.3.7.4. Oil and water absorption capacity

Oil absorption capacity reflects the ability of the protein to bind and retain fat, while water absorption capacity is a key factor influencing water retention, swelling, solubility, and gelation properties of proteins. Both attributes play a significant role in determining the texture and quality of food products (Shen et al., 2020). The results of oil and water absorption capacity

of radish sango microgreen powder are depicted in **(Figure 6.30)**. The oil absorption capacity decreased as the drying temperature increased. The highest 10.50 g/g oil absorption capacity at temperature (60°C) for the loading density (0.057 g/cm²) and the lowest oil absorption was recorded at 40°C, with values of 7.0 g/g for the loading density (0.057 g/cm²) respectively observed. Similar kind of results were reported for the drying of pumpkin powder at 50 to 70°C. The oil adsorption capacities were found to be in ranges of 3.87 to 4.42 g/g (Roongruangsri et al., 2016). The decrease in oil absorption capacity is due to the denaturation of hydrophobic compounds and proteins that contribute to oil binding, which is exacerbated by higher drying temperatures (Roongruangsri et al., 2016; Shen et al., 2020).

The water absorption capacity followed a similar trend to the oil absorption capacity, decreasing with increasing drying temperature. The lowest water absorption was observed at 50°C, with 3.34 g/g loading density (0.057 g/cm²) and highest 4.24 g/g (0.113 g/cm²). The water absorption capacity of mallow powder dried using different drying (sun, tray drying, shade drying) ranged from 1.46 to 1.63 g/g, these finding slightly lower than our results (Plabon et al., 2024). The reduction in water absorption is a result of the thermal degradation of hydrophilic functional groups, which are essential for water retention, especially at higher temperatures (Chakraborty et al., 2023).

6.3.7.5. Foaming capacity, swelling capacity, and angle of repose

The results for the effect of drying temperatures (40, 50, and 60°C) and loading densities (0.057 and 0.113 g/cm²) on the foaming capacity of dried radish sango microgreens powder are shown in **(Figure 6.30 (K))**. The foaming capacity of radish sango microgreen powder is 20.35 to 21.92% observed at drying temperature and loading density. There is no significant effect ($P > 0.05$) observed in the foaming capacity of radish sango microgreens foaming capacity. The slight variation in these values may be due to its high positive correlation with surface hydrophobicity. With the temperature rise, the wheatgrass protein dispersions had greater surface hydrophobicity and lower surface tension, giving a better foaming capacity (Chakraborty et al., 2023).

The angle of repose, which indicates flowability, showed a decreasing trend with increasing temperature **(Figure 6.30 (H))**. The lowest water absorption was observed at 50°C, with 34.14° loading density (0.113 g/cm²), and highest at 60°C, with 38.65° loading density (0.113 g/cm²). The increased compaction at higher loading densities likely restricted particle movement, reducing flowability despite higher drying temperatures. The various factors influence the angle of repose functional properties such as moisture content, particle size, and shape of food materials (Kalman, 2021; Xu et al., 2024). The swelling capacity, a measure of

the food powder ability to absorb water, remained relatively consistent across all conditions as depicted in **(Figure 6.30 (L))**, the values ranging from 11.34 to 12.03%. There is no significant effect ($P>0.05$) observed by drying temperature and loading density on the swelling capacity of radish sango microgreens. This stability suggests that the structural integrity of the polysaccharides and hydrophilic components was not significantly altered by the drying temperature and loading density, maintaining the radish sango microgreens powder functional water absorption capacity (Chakraborty et al., 2023). These obtained results highlight the effect of drying temperature and tray loading density on the functional properties of radish sango microgreen powder. At high-temperature conditions generally enhance drying efficiency but may compromise certain functional properties such as oil and water absorption, flowability, and particle integrity properties. Apart from this the tray loading density also plays a crucial role in determining how efficiently the microgreens dry, affecting properties like bulk density, true density, and moisture content. The optimal balance between drying temperature and loading density should be carefully considered to preserve the quality and functional characteristics of the microgreen powder.

6.3.8. Functional group determination

The FTIR spectrum of dried radish sango microgreen powder, as shown in **(Figure 6.31 (A))**, reveals the presence of various functional groups. The broad absorption band at 3208.64 cm^{-1} corresponds to O–H stretching vibrations, indicating the presence of hydroxyl groups, which are typically associated with moisture content, polysaccharides, or phenolic compounds. The peak at 2918.88 cm^{-1} is attributed to C–H stretching vibrations, suggesting the presence of aliphatic hydrocarbons. The peak at 1628.94 cm^{-1} corresponds to C=O stretching vibrations, likely due to the presence of carboxylic acids or amides. The band at 1409.42 cm^{-1} may be related to C–H bending vibrations of methyl or methylene groups. The absorption peaks at 1102.64 cm^{-1} and 1052.19 cm^{-1} are characteristic of C–O stretching vibrations, which are commonly associated with carbohydrates or polysaccharides. The peaks at 616.53 cm^{-1} and 536.29 cm^{-1} correspond to bending vibrations of aromatic rings or C–X bonds (where X represents halogens such as chlorine). These findings suggested a complex chemical composition of the radish sango microgreen powder, including polysaccharides, phenolics, and other bioactive compounds (Mohamed et al., 2023; Drygaś et al., 2021). The identified functional groups justify the potential health benefits of the microgreens, as these compounds are often associated with antioxidant, antimicrobial, and other functional properties.

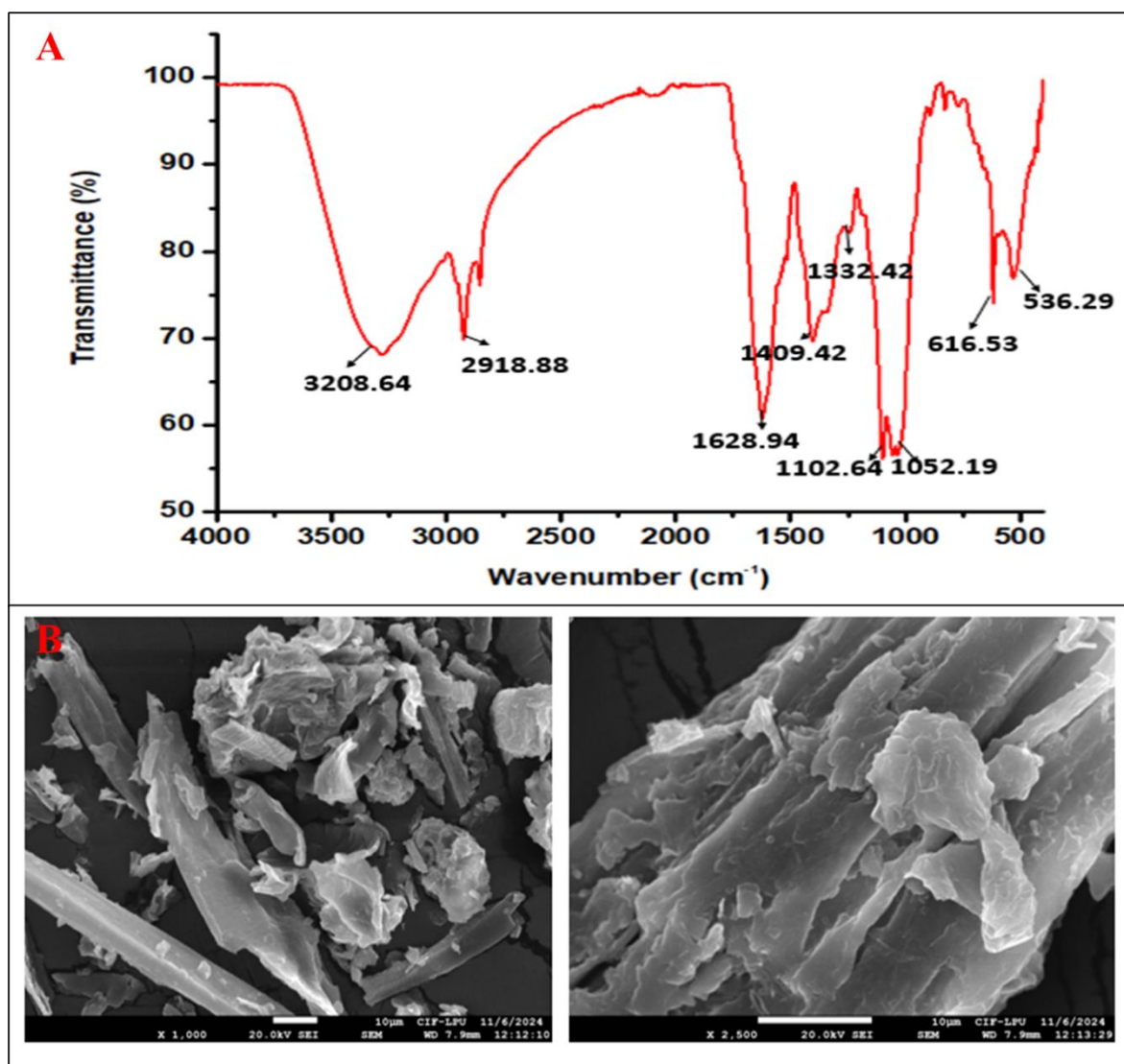


Figure 6.31. A: FTIR spectra and B: SEM micrographs of dried radish sango microgreens powder

6.3.9. Surface morphology

The SEM micrographs of dried radish sango microgreen powder, captured at magnifications of 1000 $\times$ and 2500 $\times$, reveal the morphological characteristics and surface structure of the sample (**Figure 6.31 (B)**). At 1000 $\times$ magnification, the particles exhibit an irregular, flake-like morphology with sharp edges and a heterogeneous size distribution. The presence of layered structures suggests the aggregation of smaller particles, which is typical in dried plant-based powders due to the drying process and subsequent compaction. At a higher magnification of 2500 $\times$, the surface texture is more clearly visible, showing rough and uneven surfaces with multiple folds and cracks. This rough morphology is indicative of the natural fibrous structure of plant materials and could be attributed to the presence of cellulose, hemicellulose, and other structural polysaccharides. The cracked edges and layered appearance might also result from the mechanical forces applied during sample preparation or drying

processes. The structural features observed in the SEM images are consistent with the microstructure of dried plant powders, which play a critical role in determining their functional properties, such as water retention, solubility, and rehydration capacity (Sanyukta et al., 2023). Furthermore, the rough surface and high surface area may enhance the powder's interaction with solvents or other ingredients, making it suitable for applications in food or nutraceutical formulations (Khoja et al., 2020).

6.3.10. Differential scanning calorimetry

The DSC thermogram (**Figure 6.32 (A)**) of the dried radish sango microgreens powder demonstrates a significant thermal transition at a peak temperature of 303.64°C, accompanied by an enthalpy change (ΔH) of 647.8093 J/g. This exothermic peak indicates a major decomposition or thermal event, likely associated with the degradation of organic components such as polysaccharides, proteins, or other bioactive compounds present in the microgreen powder. The total area under the curve, measured as 259.1237 mJ, reflects the energy required for the thermal transition. The onset of thermal decomposition begins at approximately 280°C, suggesting the material's good thermal stability up to this temperature, which is indicative of a robust molecular structure and resistance to early thermal degradation. The thermal stability of the sample is crucial for its potential application in food or pharmaceutical industries, where processing at elevated temperatures may be required. The observed transition at a relatively high temperature aligns with the typical behavior of plant-based powders rich in carbohydrates and bioactive components (Reddy et al., 2024). The exothermic nature of the peak suggested the breakdown of complex molecular bonds, potentially involving dehydration and the decomposition of polysaccharides or phenolic compounds.

6.3.11. Thermogravimetric analysis

The Thermogravimetric analysis (TGA) results (**Figure 6.32 (B)**) of radish sango microgreen powder exhibit distinct thermal decomposition patterns, indicating the materials compositional and thermal stability properties. The analysis demonstrates an initial weight loss of 9.27 mg at an onset temperature of 25.89°C, which corresponds to the evaporation of moisture and volatile components. This initial phase reflects the removal of surface-bound water or physically adsorbed moisture in the sample. The second significant weight loss, observed at 8.16 mg with an onset temperature of 165.25°C, marks the degradation of more thermally stable organic components such as carbohydrates and proteins. Following this, a third decomposition stage is noted at 7.14 mg at an onset temperature of 226.95°C, likely associated with the thermal breakdown of hemicelluloses and pectin-like substances. A notable transition occurs at 5.66 mg at 302.92°C, where lignocellulosic materials such as cellulose and lignin

undergo decomposition. This phase signifies the main thermal degradation of the organic matrix. Finally, a residual weight of 2.89 mg is observed at an onset temperature of 552.9°C, which is attributed to the pyrolysis of more thermally resistant compounds, possibly including inorganic residues or ash content. The multi-stage decomposition profile indicated the heterogeneous composition of radish sango microgreen powder, characterized by a mix of volatile, organic, and inorganic constituents (Sanyukta et al., 2023).

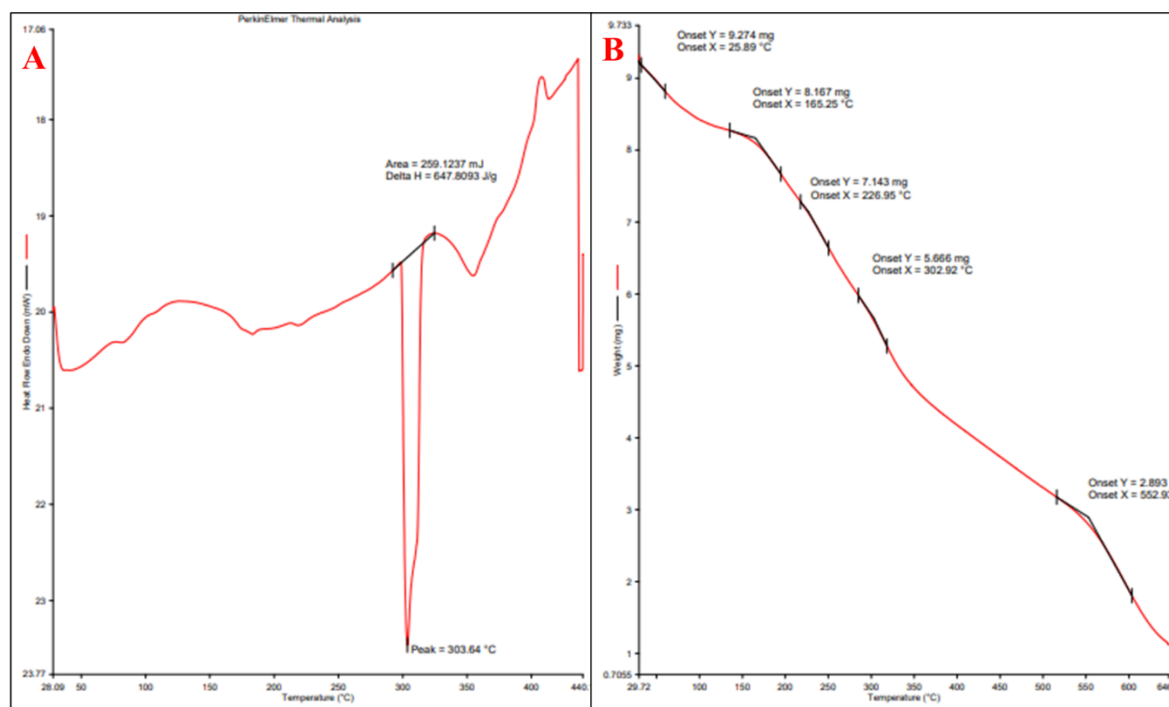


Figure 6.32. A: DSC thermogram and B: Thermogravimetric analysis of dried radish sango microgreens powder

6.3.12. Particle size and zeta potential

The results for the particle and zeta potential for radish sango microgreens are shown in **(Figure 6.33 (A and B))**. The particle size distribution analysis of the dried radish sango microgreens powder reveals a Z-average size of 1403 nm, indicating the average hydrodynamic diameter of particles in the sample. The particle size distribution is bimodal, with two distinct peaks: Peak 1 at 997.1 nm (95.3% intensity) and Peak 2 at 5469 nm (4.7% intensity). The predominant peak at 997.1 nm indicates that most particles are within the nanometer scale, whereas the minor peak at 5469 nm suggests the presence of larger aggregates or clusters. The polydispersity index (PDI) of 0.389 indicates moderate heterogeneity in the particle size distribution, suggesting a partially uniform dispersion. The zeta potential of the particles was measured at -11.0 mV, indicating a low negative surface charge. This value suggested limited electrostatic repulsion between particles, which could lead to aggregation over time. The moderately low zeta potential may impact the colloidal stability of the powder suspension,

making it less stable under certain conditions. However, the presence of stabilizers or optimization of dispersion methods (e.g., sonication) could improve stability (Sanyukta et al., 2023). These results highlight the microstructure and stability characteristics of the powder, which are critical for its potential applications in food, pharmaceuticals, and nutraceutical formulations. The observed particle size, particularly the dominance of smaller particles, contributes to higher surface area, which may enhance interactions with solvents or other components in formulation (Thepsilvisut et al., 2023).

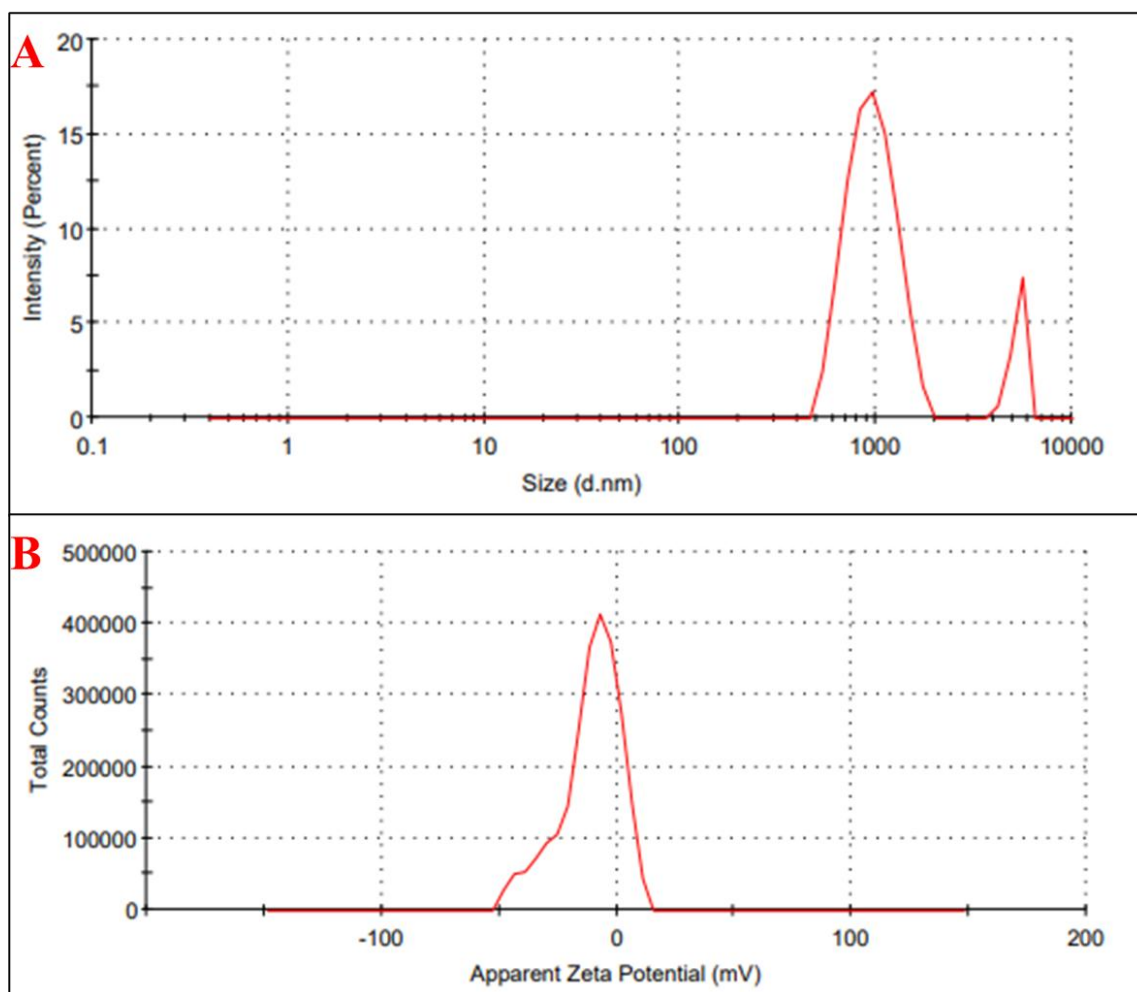


Figure 6.33. A: Particle size distribution and B: Zeta potential of dried radish sango microgreens powder

6.4. Effect particle size distribution on physical and functional properties

The radish sango microgreens particle size distribution significantly ($P < 0.05$) influenced the physical properties and functional properties of radish sango microgreens powder, as shown in (Table 6.37) and (Table 6.38).

6.4.1. Water activity and moisture content

Water activity (a_w) is a key parameter for determining the shelf life, microbial stability, and chemical stability of the food powder materials (Singh et al., 2024). The highest water

activity value for the 600 μm particle size is 0.33 and the lowest for the 75 μm particle size is 0.31 observed, respectively. Similar findings were reported for the water activity of infant food powder ranging from 0.12 to 0.32 (Nugroho et al., 2020). The reduction in water activity at small particle sizes is likely due to their larger surface area, which facilitates more efficient moisture removal during drying (Pérez-Reyes et al., 2021). This lower water activity value helps to improve storage stability by minimizing microbial growth and enzymatic activities, thus extending the shelf life of radish sango microgreen powder (Budnimath et al., 2023).

Table 6.37. The effect of particle size distribution of radish sango microgreens powder on physical properties

Particle sizes (μm)	Water activity	Moisture content (%)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Hausner ratio	Carr index (%)
600	0.33 $\pm$ 0.00 ^a	7.19 $\pm$ 0.09 ^a	0.55 $\pm$ 0.01 ^a	0.73 $\pm$ 0.01 ^a	1.33 $\pm$ 0.04 ^a	24.47 $\pm$ 2.09 ^a
425	0.32 $\pm$ 0.00 ^{ab}	7.11 $\pm$ 0.07 ^a	0.54 $\pm$ 0.02 ^a	0.70 $\pm$ 0.01 ^a	1.30 $\pm$ 0.02 ^a	22.73 $\pm$ 1.52 ^a
300	0.32 $\pm$ 0.00 ^b	6.40 $\pm$ 0.23 ^b	0.51 $\pm$ 0.00 ^{ab}	0.68 $\pm$ 0.01 ^{ab}	1.34 $\pm$ 0.01 ^a	25.38 $\pm$ 0.44 ^a
150	0.31 $\pm$ 0.00 ^b	6.33 $\pm$ 0.16 ^b	0.48 $\pm$ 0.01 ^b	0.64 $\pm$ 0.02 ^b	1.34 $\pm$ 0.08 ^a	25.02 $\pm$ 4.60 ^a
75	0.31 $\pm$ 0.00 ^b	6.38 $\pm$ 0.18 ^b	0.47 $\pm$ 0.01 ^b	0.59 $\pm$ 0.01 ^c	1.25 $\pm$ 0.01 ^a	19.65 $\pm$ 0.16 ^a

Values are presented in mean $\pm$ standard deviation ($n = 3$) with different superscript letters in a column being significantly different ($P < 0.05$).

The moisture content of food powders is an important physical parameter it affects the physical and chemical properties of food products as well as its effects on the shelf life and product stability (Mathlouthi 2001). In this study, all particle size fractions exhibited moisture content within safe storage limits (6.33–7.19%), as shown in **(Table 6.37)**. However, significant variations ($P < 0.05$) were observed among the fractions, except for the smallest particle size range (75–300 μm), which showed a decrease due to reduced specific surface area. The slight reduction in moisture content with decreasing particle size can be attributed to the heat generated during grinding, which may also negatively affect the bioactive compound content of radish sango microgreen powder. Comparable findings were reported by Nabil et al. (2019) for eight granulometric classes (45–500 μm) of cladode powder, where moisture content ranged from 9.09% to 10.18%. Similarly, Becker et al. (2017) observed that smaller particles experienced a more pronounced reduction in moisture content due to the heat generated during the grinding process. Moisture content directly affects powder properties such as flowability, compressibility, and the tendency to form clumps during storage. Lower moisture levels in smaller particles enhance their suitability for long-term storage and high-temperature processing (Nabil et al., 2019).

6.4.2. Bulk and tapped density

The bulk and tapped density are the important indicator parameters of powder compressibility, flow behavior, and suitability for packaging. As presented in **(Table 6.37)**, the bulk density of radish sango microgreens powder varied between 0.55 g/cm³ for 600 µm particles and 0.47 g/cm³ for 75 µm particles, while the tapped density ranged from 0.73 g/cm³ to 0.59 g/cm³. Similarly, for corn silk powder different particle sizes of 750 to 75 µm, bulk density was reported to range from 0.385 to 0.595 g/cm³, and tapped density from 0.580 to 0.725 g/cm³ (Singh et al., 2022). Consistent findings were reported by Nabil et al. (2019) for cladode powder across eight granulometric classes (45 to 500 µm), where bulk density ranged from 0.65 to 0.83 g/cm³. The lower densities observed in smaller microgreen powder particles reflect higher porosity and reduced packing efficiency under gravity alone (Ma & Mu, 2015).

Hausner ratios and Carr index, derived from bulk and tapped densities, provide valuable insights into the flowability of microgreen powders. The Hausner ratio ranged from 1.25 to 1.34, and the Carr index ranged between 19.65% and 25.38%. Comparable values were observed for cladode powder (Nabil et al., 2019) and corn silk powder (Singh et al., 2022). These results suggest that all particle sizes exhibit acceptable flow properties.

6.4.3. Angle of repose

The angle of repose serves as a key indicator of powder flowability. The measured angle of repose for radish Sango microgreens powder across various particle sizes is presented in **(Table 6.38)**, with values ranging from 38.65° for 600 µm, 37.26° for 425 µm, 33.61° for 300 µm, 33.39° for 150 µm, and 34.46° for 75 µm. A statistically significant difference ($P < 0.05$) was observed among the samples, with a decreasing trend in the angle of repose from coarser to finer particles. These results align with previously reported values for ginger powder (300 to 8.34 µm (Zhao et al., 2008), corn silk powder 25.77° to 38.29° (Singh et al., 2022), Arabic gum powder 20.44° to 26.28° (Abel et al., 2020), orange powder 40.9 to 43.5° (Camacho et al., 2022), and instant drink mix 28.81 to 48.33° (Budnimath et al., 2023) of angle of repose. The improved flowability of smaller particles can be attributed to their reduced surface irregularities and enhanced packing behavior. Conversely, an increase in the angle of repose indicates lower flowability in granular materials, as noted by Ileleji and Zhou (2008).

6.4.4. Foaming capacity and swelling index

Foam capacity refers to the ability of proteins to interact with air, forming a stable surface where air is entrapped, thereby inhibiting gas exchange (Agbemaflé, 2019). The protein content, particularly surface proteins, plays a pivotal role in foam formation during agitation, as they are the most active in creating and stabilizing foam. In the current study, foaming capacity and swelling index were influenced by particle size, despite the protein content

remaining constant across samples. The foaming capacity and swelling index of radish Sango microgreen powder with varying particle sizes are presented in **(Table 6.38)**. The foaming capacity was highest in 600 μm particles (26.49%) and declined to 21.61% for 75 μm particles, as larger particles can trap and retain more air during whipping, enhancing foam stability. Conversely, the swelling index, indicative of water-holding capacity, increased from 9.92% for 600 μm particles to 11.62% for 75 μm particles. Similar foaming capacities have been reported for spinach (26.5%), carrot (14.89%), bathua (18.08%), and Bengal gram (33.7%) microgreen powders (Sanyukta et al., 2023). For corn silk powder, Singh et al. (2022) observed foaming capacities ranging from 27.50% to 30.21%. Additionally, Bala et al. (2020) demonstrated a significant reduction in foaming capacity with decreasing particle size (249–74 μm). Foam stability, a critical parameter, requires the whipping agent to sustain the foam over an extended period, emphasizing its importance in practical applications.

Table 6.38. The effect of particle size distribution of radish sango microgreens powder on physical properties

Particle sizes (μm)	Angle of repose ($^{\circ}$)	Foaming capacity (%)	Swelling index (%)	Water absorption capacity (%)	Oil absorption capacity (%)
600	38.65 $\pm$ 0.40 ^a	26.49 $\pm$ 0.23 ^a	9.92 $\pm$ 0.05 ^c	3.06 $\pm$ 0.08 ^b	9.79 $\pm$ 0.01 ^a
425	37.26 $\pm$ 0.42 ^b	26.31 $\pm$ 0.08 ^{ab}	9.86 $\pm$ 0.07 ^c	3.24 $\pm$ 0.16 ^b	9.69 $\pm$ 0.04 ^a
300	33.61 $\pm$ 0.11 ^c	25.15 $\pm$ 0.26 ^{bc}	10.18 $\pm$ 0.10 ^{bc}	3.35 $\pm$ 0.14 ^b	9.40 $\pm$ 0.06 ^a
150	33.39 $\pm$ 0.09 ^c	24.76 $\pm$ 0.28 ^c	10.93 $\pm$ 0.39 ^{ab}	3.48 $\pm$ 0.06 ^b	9.32 $\pm$ 0.29 ^a
75	34.46 $\pm$ 0.33 ^c	21.61 $\pm$ 0.49 ^d	11.62 $\pm$ 0.10 ^a	4.21 $\pm$ 0.13 ^a	8.37 $\pm$ 0.11 ^b

Values are presented in mean $\pm$ standard deviation (n = 3) with different superscript letters in a column being significantly different ($P < 0.05$).

6.4.5. Water and oil absorption capacity

The water absorption capacity increased significantly ($P < 0.05$) with decreasing particle size, ranging from 3.06% for 600 μm particles to 4.21% for 75 μm particles, as shown in **(Table 6.31)**. These values are notably higher than those reported for spinach (1.23%), carrot (1.20%), bathua (1.58%), and Bengal gram (1.38%) microgreen powders (Sanyukta et al., 2023). Similarly, in another study, water absorption capacity for grass pea flour samples varied between 1.75 and 2.12 g/g for particle sizes ranging from 249 to 74 μm (Bala et al., 2020). Such variations may stem from differences in the morphological, physicochemical, and processing characteristics of plant food powders (Fenn et al., 2024). Smaller particles exhibit greater surface area and porosity, which enhance water retention and hydration (Singh et al., 2022). This property is particularly crucial in bakery applications, such as doughs and emulsions, where efficient water binding is essential (Kumar & Singh, 2024).

Conversely, oil absorption capacity decreased as particle size reduced, ranging from 9.79% for 600 μm particles to 8.37% for 75 μm particles. Factors such as particle size, shape, surface area, and chemical composition influence oil absorption capacity (Heras et al., 2017). These findings align with Singh et al. (2022), who observed a similar decrease in oil absorption capacity for corn silk powder as particle size reduced from 750 to 75 μm . Comparable trends were also reported for grass pea flour, where oil absorption capacity decreased with particle size reduction from 249 to 74 μm (Bala et al., 2020). This reduction is attributed to lower protein content and limited accessibility of hydrophobic chains in finer flour fractions, as oil is primarily retained in the nonpolar side chains of proteins (Wang et al., 2020). The oil absorption capacity is a critical parameter for flavor retention and texture enhancement in fried and savory food products (Mah & Brannan, 2009).

6.4.6. Effect of particle size on bioactive compounds

6.4.6.1. Total phenolic and flavonoid content

The particle size distribution had a significant ($P < 0.05$) impact on the total phenolic and flavonoid content, as presented in (Table 6.39). A slight increase in total phenolic content was observed with decreasing particle size, ranging from 176.22 mg GAE/100g for 600 μm particles to 180.44 mg GAE/100g for 75 μm particles in the microgreen powders. These findings are consistent with those reported by Nabil et al. (2019) for cladode powder with particle sizes of 45–500 μm . Similarly, Chen et al. (2015) observed a comparable trend for Okra powder. The enhanced phenolic content in smaller particles can be attributed to the improved disruption of cell walls during grinding, which facilitates the release and efficient extraction of phenolic compounds (Gil-Martín et al., 2021). In contrast, total flavonoid content decreased as particle size reduced, declining from 5.65 mg QE/100g for 600 μm particles to 5.05 ± 0.14 mg QE/100g for 75 μm particles. This reduction is likely due to the oxidative degradation of flavonoids caused by intense grinding, particularly under conditions of heat and oxygen exposure (Zaiter et al., 2016; Nabil et al., 2019). Current results corresponded well with those reported by Nabil et al. (2019) for cladode powder.

6.4.6.2. Glucosinolate, ascorbic acid, and anthocyanin content

The influence of particle size distribution on the glucosinolate, ascorbic acid, and anthocyanin content of radish sango microgreens powder is presented in (Table 6.39). A slight reduction in glucosinolate and ascorbic acid content was observed with decreasing particle size, declining from 42.18 $\mu\text{mol/g}$ and 239.54 mg/100g in 600 μm particles to 39.94 $\mu\text{mol/g}$ and 209.48 mg/100g in 75 μm particles, respectively. Glucosinolates, which are sulfur-containing compounds, are susceptible to mechanical and thermal stresses, leading to partial degradation

during fine grinding (Narra et al., 2024). Preserving glucosinolate levels is crucial to retaining the health benefits of microgreens, particularly their anti-carcinogenic properties. Similar observations have been reported for ascorbic acid content in orange powders of varying particle sizes, ranging from 269 to 273 mg/100g for fine, medium, and coarse powders (Camacho et al., 2022). Ascorbic acid, a water-soluble and highly labile vitamin, is prone to oxidative degradation during grinding (Narra et al., 2024). Larger particle sizes minimize surface exposure to oxidative stress, thereby preserving ascorbic acid content more effectively (Ionescu et al., 2024). Conversely, anthocyanin content increased significantly with smaller particle sizes, reaching 40.32 $\mu\text{mol}/100\text{g}$ in 75 μm particles compared to 32.59 $\mu\text{mol}/100\text{g}$ in 600 μm particles. This enhancement is attributed to the efficient release of anthocyanins from cellular structures during fine grinding. Anthocyanins serve as potent antioxidants and natural colorants, improving both the functional and sensory attributes of the powder (Cid-Ortega & Guerrero-Beltran, 2014; Ionescu et al., 2024).

Table 6.39. The effect of particle size distribution of radish sango microgreens powder on bioactive compounds

Particle sizes (μm)	Total phenolic content (mg GAE/100g)	Total flavonoid content (mg QE/100g)	Glucosinolate content ($\mu\text{mol}/\text{g}$)	Ascorbic acid content (mg/100g)	Anthocyanin content ($\mu\text{mol}/100\text{g}$)
600	176.22 $\pm$ 0.94 ^b	5.65 $\pm$ 0.13 ^a	42.18 $\pm$ 0.52 ^a	239.54 $\pm$ 0.45 ^a	32.59 $\pm$ 0.37 ^d
425	175.54 $\pm$ 0.45 ^b	5.36 $\pm$ 0.15 ^{ab}	40.42 $\pm$ 0.09 ^b	240.45 $\pm$ 0.30 ^a	32.54 $\pm$ 0.45 ^d
300	177.44 $\pm$ 0.30 ^b	5.30 $\pm$ 0.08 ^{ab}	41.29 $\pm$ 0.10 ^{ab}	210.60 $\pm$ 0.37 ^c	35.38 $\pm$ 0.36 ^c
150	176.35 $\pm$ 0.18 ^b	5.09 $\pm$ 0.11 ^b	40.73 $\pm$ 0.23 ^b	215.65 $\pm$ 0.45 ^b	38.44 $\pm$ 0.31 ^b
75	180.44 $\pm$ 0.31 ^a	5.05 $\pm$ 0.14 ^b	39.94 $\pm$ 0.55 ^b	209.48 $\pm$ 0.21 ^c	40.32 $\pm$ 0.29 ^a

Values are presented in mean $\pm$ standard deviation (n = 3) with different superscript letters in a column being significantly different (P<0.05).

6.4.7. Effect of particle size on antioxidant properties

The effect of particle size distribution on the antioxidant activities of DPPH and FRAP in radish sango microgreen powder is illustrated in **(Figure 6.34)**. The highest DPPH radical scavenging activity was observed in the 75 μm particle size (44.27%), whereas the lowest activity was recorded for the 600 μm particle size (38.44%). This enhanced radical scavenging capacity in finer particles can be attributed to their higher phenolic and anthocyanin content, which serve as efficient hydrogen donors, neutralizing free radicals and mitigating oxidative stress. These findings align with those of Ahmed et al. (2018), who reported a significant increase in DPPH scavenging efficacy with reduced particle size in quinoa powder. Similarly, Zhao et al. (2017) observed higher extractability of phenolics and antioxidants in superfine rice bran powder compared to its coarse counterpart. Nabil et al. (2019) also reported a decrease in

DPPH antioxidant activity with particle size reduction in cladode powder, ranging from 67.77% to 54.51%.

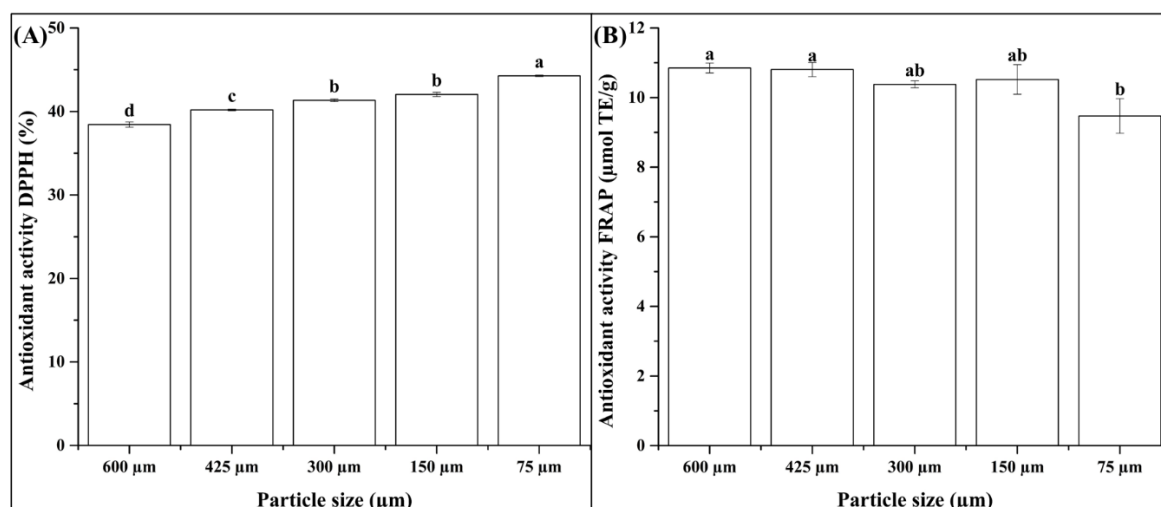


Figure 6.34. The effect particle size distribution of radish sango microgreens powder on antioxidant activities. Values are presented in means $\pm$ standard deviation ($n = 3$) with different superscript letters in a bar being significantly different ($P < 0.05$).

The antioxidant activity FRAP also increased with decreasing particle size, ranging from 10.85 $\mu\text{mol TE/g}$ for 600 μm particles to 9.47 $\mu\text{mol TE/g}$ for 75 μm particles. Comparable trends were observed by Nabil et al. (2019) for cladode powder and by Botella-Martíne et al. (2021) for Ghanaian cocoa, where antioxidant activity (DPPH and FRAP) was enhanced with particle size reduction. This phenomenon is explained by the greater release of bioactive compounds in smaller particles due to their larger surface area, enhancing antioxidant potential. Smaller particles also exhibit improved solubility and availability of antioxidant compounds, thereby increasing their capacity to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) (Singh et al., 2022).

6.4.8. Characterization of microgreens powder

6.4.8.1. Functional group determination

The FTIR spectrum of dried radish sango microgreen powder shows the characteristic fingerprint of a plant-based material composed primarily of proteins, carbohydrates, and lipids (**Figure 6.35 (A)**). The broad band centered around 3279 cm^{-1} is indicative of O–H or N–H stretching, often attributed to hydroxyl groups in polysaccharides and/or amide groups in proteins. The notable absorption peaks at 2922 and 2852 cm^{-1} correspond to asymmetric and symmetric stretching of C–H bonds, suggesting the presence of aliphatic chains typically found in lipids and certain protein side chains. The prominent band near 1622 cm^{-1} can be ascribed to amide I vibrations (C=O stretching in the protein backbone) or to C=C stretching in certain phenolic compounds. Additional features in the 1400–1200 cm^{-1} region (1403 cm^{-1}) point to

C–H bending or C–O stretching modes often associated with carbohydrate structures. The intense signals around 1098 cm^{-1} and 1034 cm^{-1} are also characteristic of C–O stretching modes in polysaccharides (cellulose or other dietary fibers) (Sanyukta et al., 2023). Finally, weaker peaks below 1000 cm^{-1} (827 and 616 cm^{-1}) showed the bending or skeletal vibrations, which are commonly seen in complex plant matrices. These results confirmed that the dried radish sango microgreens powder contains a mix of proteins, lipids, and carbohydrates, all of which are consistent with the expected biochemical composition of a dried plant-derived material.

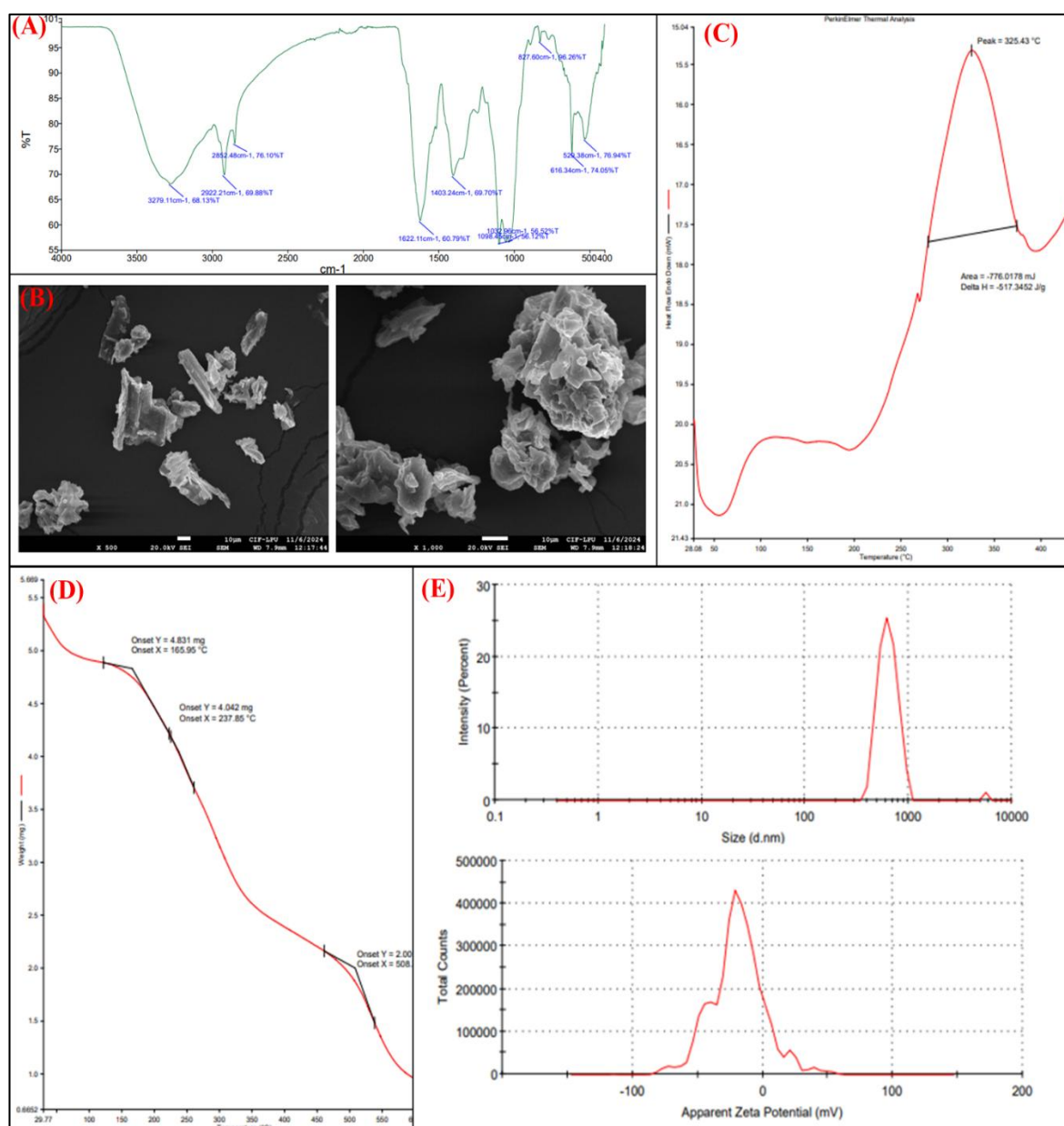


Figure 6.35. Characterization of microgreens powder, A: FTIR spectra, B: SEM micrographs, C: DSC thermogram, D: Thermogravimetric analysis, and E: Particle size distribution and Zeta potential.

6.4.8.2. Surface morphology

The SEM micrographs of dried radish sango microgreen powder reveal a heterogeneous and flake-like morphology characteristic of plant-based powders subjected to dehydration. At lower magnification ($\times 500$), large, irregularly shaped platelets with layered or sheet-like appearances are observed, indicating that the drying process likely led to partial collapse of the cellular surface (**Figure 6.35 (B)**). At higher magnification ($\times 1000$) showed that these flakes tend to agglomerate into loosely bound clusters with rough, wrinkled surfaces. This layered structure and the folds visible on the particle surfaces suggest the presence of residual cell wall materials (cellulose), proteins, and other bio-macromolecules compacted during drying. Such morphology is typical for dried plant cells and may influence properties like bulk density, flow behavior, and rehydration capacity (Mansouri et al., 2024). Overall, the SEM images confirmed that the radish sango microgreens powder is composed of aggregated, plate-like particles derived from the collapsed plant tissue, consistent with the expected structural features of a freeze-dried or air-dried plant material.

6.4.8.3. Differential scanning calorimetry (DSC)

The DSC thermogram of the dried radish sango microgreens powder exhibits one dominant exothermic event, with a peak at approximately $325.43\text{ }^{\circ}\text{C}$ and an associated enthalpy change (ΔH) of about -517 J/g . This pronounced exothermic peak most likely arises from the decomposition or oxidation of the organic constituents (polysaccharides, proteins, and other cellular components) as their structures break down at elevated temperatures, releasing heat in the process (**Figure 6.35 (C)**). A minor endothermic shift may also be observed at lower temperatures (below $150\text{--}200\text{ }^{\circ}\text{C}$), which can be attributed to the loss of residual moisture or other volatile species. Overall, the relatively high temperature at which the major exothermic reaction occurs indicates a moderate degree of thermal stability for the dried powder; in other words, the robust plant cellular architecture in radish sango microgreens can tolerate moderate heating before significant thermal degradation takes place (Reddy et al., 2024).

6.4.8.4. Thermogravimetric analysis (TGA)

The TGA curve of the dried radish sango microgreens powder shows a multistep weight loss profile, reflecting the sequential breakdown of various plant components. An initial, relatively minor drop in mass occurs around $165\text{--}170\text{ }^{\circ}\text{C}$, which can be attributed to the evaporation of physically adsorbed water and any low-molecular-weight volatiles as shown in (**Figure 6.35 (D)**). The more pronounced decomposition step begins near $237\text{--}240\text{ }^{\circ}\text{C}$, likely associated with the degradation of hemicelluloses, proteins, and other thermally labile constituents. As the temperature increases further, a larger mass loss occurs around $500\text{ }^{\circ}\text{C}$, indicated the thermal decomposition and oxidation of more stable organic fractions, such as

cellulose and lignin-rich structures. Beyond about 600 °C, a residual mass remains, attributable to inorganic minerals (ash content) (Sanyukta et al., 2023). The stepwise weight loss profile is characteristic of plant materials undergoing dehydration, volatilization of labile organics, and eventual decomposition of structural biopolymers, followed by the formation of a mineral residue.

6.4.8.5. Particle size and Zeta potential

Particle size analysis of the dried radish sango microgreens powder dispersion reveals a primary particle (agglomerate) size peak centered around 771 nm with a minor population extending toward larger diameters (**Figure 6.36 (E)**). This suggested that the plant-derived powder tends to form relatively large aggregates in aqueous media, likely due to interactions among hydrophobic and hydrophilic components. The zeta potential measurements show a predominantly negative charge (−20 to −30 mV), reflecting the presence of ionizable functional groups (carboxyl, phenolic, or other acidic residues) on the surface of the particles. While not highly negative, this level of zeta potential can still provide moderate electrostatic repulsion in suspension (Kumar et al., 2024), thereby offering some colloidal stability. Overall, the combination of a broad size distribution and a moderately negative surface charge indicates that the plant material possesses both a propensity to cluster and a moderate degree of dispersion in aqueous systems, which can be relevant for formulating products involving radish sango microgreen powder.

6.5. Optimization of variables microgreens-based dairy (lassi) beverage

To determine the values of the variables Dahi, and microgreen powder for the study, response surface methodology (RSM) was recommended using Central Composite Rotatable Design (CCRD) and conducting 13 runs (n=3) treatments of tests. The (**Table 6.40, Table 6.41, and Table 6.42**) presents the results of sensory, physicochemical, biochemical, and antioxidant parameters scores of average mean values (n=3) which were influenced by Dahi and microgreen powder. According to the results, the optimal composition for various selected parameters for the functional fermented microgreens-based dairy (lassi) beverage with microgreen powder was determined to be 70% Dahi, 2.5% microgreen powder mixed with 12% sugar, 0.25% CMC, and 15.25% of drinking water. The optimization criteria for a functional fermented microgreens-based dairy (lassi) beverage using two main process variables including Dahi (70 to 75%, minimize), and microgreen powder (1 to 2.5%, maximize), each with set goals and importance levels as shown in (**Table 6.43**). It also specifies response variables of different parameters.

Table 6.40. The effect of constituents on sensory parameters of microgreen-based dairy (lassi) beverage

Treatments	Dahi (%)	Microgreen powder (%)	Colour & Appearance	Flavour & Sweetness	Consistency	Mouthfeel	Overall Acceptability
1	72.50	1.75	8.20	7.02	8.00	7.11	7.45
2	72.50	1.75	8.19	7.15	7.90	7.15	7.35
3	68.96	1.75	7.95	7.22	7.27	7.21	7.21
4	76.04	1.75	8.05	7.45	7.30	7.49	7.55
5	70.00	1.00	8.22	7.86	7.65	7.88	7.86
6	72.50	2.81	7.07	7.03	7.85	6.03	6.43
7	70.00	2.50	7.25	6.85	7.45	6.55	6.85
8	75.00	2.50	7.31	6.78	7.80	6.48	6.74
9	72.50	1.75	8.08	7.70	7.60	7.71	7.35
10	72.50	1.75	7.89	7.10	7.85	7.61	7.22
11	75.00	1.00	8.35	7.90	7.70	7.98	7.92
12	72.50	0.69	8.69	8.55	8.20	8.78	8.01
13	72.50	1.75	8.14	7.18	8.00	7.15	7.68

Note: All the experimental results values are presented as mean value (n=3) basis based on each sensory parameter

Table 6.41. The effect of constituents on physicochemical parameters of microgreen-based dairy (lassi) beverage

Treatments	Dahi (%)	Microgreen powder (%)	pH	Acidity (% lactic acid)	TSS (°Brix)	Water holding capacity (%)	Syneresis (%)
1	72.50	1.75	4.34	0.54	22.3	42.77	43.4
2	72.50	1.75	4.37	0.57	22.4	42.57	43.7
3	68.96	1.75	4.29	0.59	22.3	50.87	42.9
4	76.04	1.75	4.36	0.56	22.7	50.68	43.6
5	70.00	1.00	4.34	0.55	17.9	55.09	43.4
6	72.50	2.81	4.39	0.59	22.7	41.03	43.9
7	70.00	2.50	4.32	0.57	22.6	42.28	43.2
8	75.00	2.50	4.29	0.59	22.6	43.28	42.9
9	72.50	1.75	4.33	0.53	21.6	42.77	43.3
10	72.50	1.75	4.34	0.54	22.4	42.86	43.4
11	75.00	1.00	4.52	0.52	18.1	54.91	45.2
12	72.50	0.69	4.50	0.50	16.4	55.78	45.2
13	72.50	1.75	4.35	0.55	22.6	41.06	43.5

Note: All the experimental results values are presented as mean value (n=3) basis based on each physicochemical parameter.

Table 6.42. The effect of constituents on biochemical and antioxidant parameters of microgreen-based dairy (lassi) beverage

Treatments	Dahi (%)	Microgreen powder (%)	Anthocyanin content (μmol/100g)	Total phenol content (mg GAE/100g)	Total flavonoid content (mg QE/100g)	Antioxidant activity DPPH (%)	Antioxidant activity FRAP (μmol TE/g)	Ascorbic acid (mg/100g)
1	72.50	1.75	36.21	79.44	44.70	77.48	14.27	79.03
2	72.50	1.75	41.65	78.36	44.50	77.57	13.52	106.77
3	68.96	1.75	30.20	85.20	45.08	75.92	12.37	108.71
4	76.04	1.75	39.25	97.09	45.16	81.57	13.23	110.00
5	70.00	1.00	21.07	65.38	22.02	69.51	8.63	76.45
6	72.50	2.81	56.71	120.523	65.09	86.76	19.41	162.90
7	70.00	2.50	49.98	118.23	67.68	82.43	19.32	153.22
8	75.00	2.50	51.74	113.22	51.77	85.79	16.97	146.77
9	72.50	1.75	38.77	82.86	44.70	76.80	14.42	122.25
10	72.50	1.75	44.09	65.38	44.71	77.23	15.82	119.67
11	75.00	1.00	21.91	60.70	38.04	76.39	12.66	95.16
12	72.50	0.69	17.08	46.64	22.66	69.81	7.90	67.41
13	72.50	1.75	35.84	78.27	45.50	77.32	14.1	102.90

All the experimental results values are presented as mean value (n=3) basis based on each biochemical and antioxidant parameter. Where, GAE: Gallic acid equivalent; QE: Quercetin equivalent; TE: Trolox equivalent.

Table 6.43. Optimization criteria for different process and response variables for microgreen-based dairy (lassi) beverage

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Dahi	Minimize	70	75	1	1	3
B: Microgreen powder	Maximize	1	2.5	1	1	5
Colour & Appearance	In range	7.07	8.69	1	1	3
Flavour & Sweetness	In range	6.78	8.55	1	1	3
Consistency	In range	7.27	8.2	1	1	3
Mouthfeel	In range	6.03	8.78	1	1	3
Overall Acceptability	Maximize	6.43	8.01	1	1	3
pH	Minimize	4.29	4.52	1	1	5
Acidity	In range	0.5	0.59	1	1	3
TSS	Maximize	16.4	22.7	1	1	5
Water holding capacity	In range	41.03	55.78	1	1	3
Syneresis	Minimize	42.9	45.2	1	1	5
Anthocyanin content	Maximize	17.08	56.71	1	1	5
Total phenol content	Maximize	46.64	120.523	1	1	5
Total flavonoid content	Maximize	22.02	67.68	1	1	5
Antioxidant activity DPPH	Maximize	69.51	86.76	1	1	5
Antioxidant activity FRAP	Maximize	7.9	19.41	1	1	5
Ascorbic acid	Maximize	67.4194	162.903	1	1	5

These criteria are designed to balance various selected sensory, physicochemical, biochemical, and antioxidant parameters to create an optimal functional fermented microgreens-based dairy (lassi) beverage formulation. The **(Figure 6.36)** shows that this combination had a desirability score of 0.818, indicating that it was the best combination for the study. In **(Table 6.44)**, both the observed and predicted responses are presented. The table clearly shows that there is no significant difference ($P>0.05$) between the observed and predicted values, with the exception of overall acceptability.

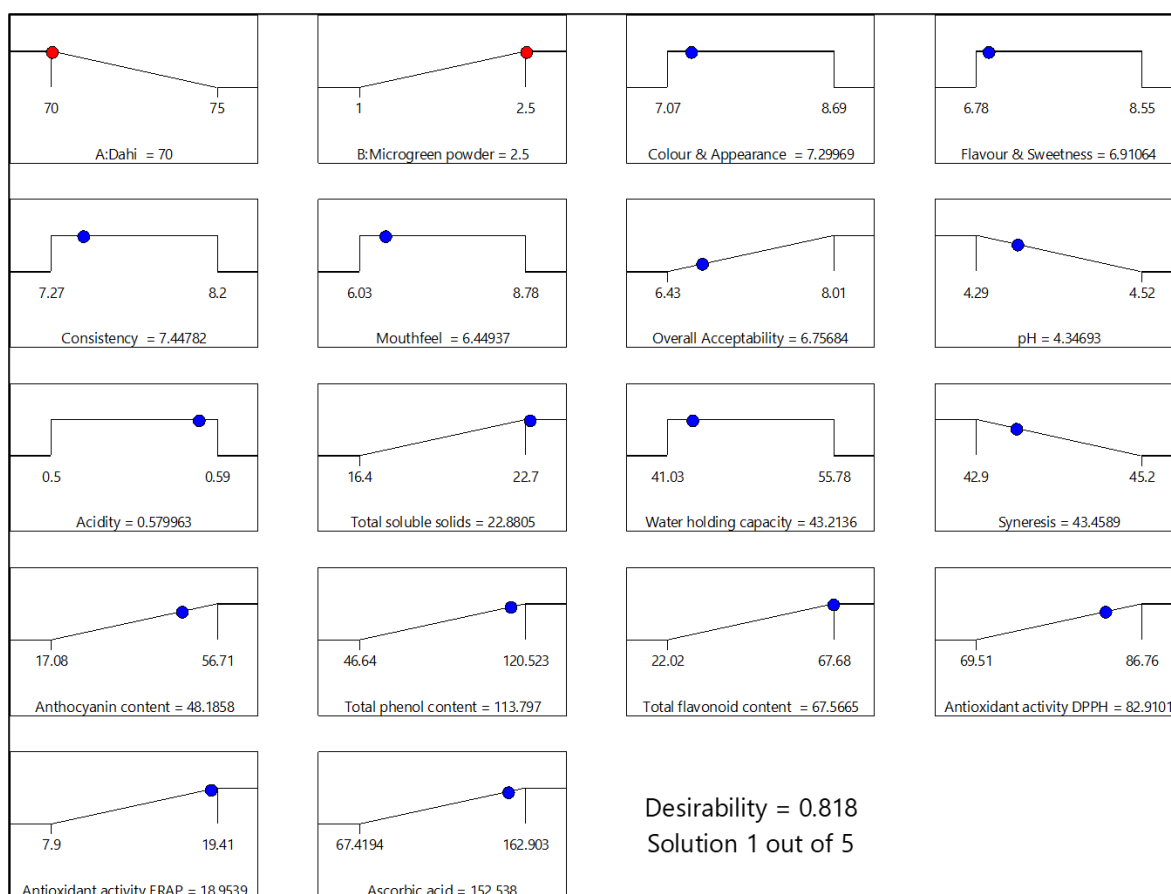


Figure 6.36. Desirability ramp for optimization of microgreen-based dairy (lassi) beverage

Table 6.44. Predicted and observed optimum responses for microgreen-based dairy (lassi) beverage

Responses	Predicted values	Observed values
Colour & Appearance	7.30	7.35±0.05
Flavour & Sweetness	6.91	6.90±0.05
Consistency	7.44	7.72±0.28
Mouthfeel	6.44	6.44±0.11
Overall acceptability	6.75	8.60±0.38
pH	4.34	4.37±0.02
Acidity (% lactic acid)	0.58	0.65±0.01
TSS (°Brix)	22.88	22.88±0.10
Water holding capacity (%)	43.21	43.45±0.24
Syneresis (%)	43.45	43.41±0.09
Anthocyanin content (µmol/100g)	48.18	48.23±0.13
Total phenol content (mg GAE/100g)	113.79	114.00±0.34
Total flavonoid content (mg QE/100g)	67.56	67.77±0.11
Antioxidant activity DPPH (%)	82.91	83.19±0.17
Antioxidant activity FRAP (µmol TE/g)	18.95	19.12±0.14
Ascorbic acid (mg/100g)	152.53	152.77±0.11

Note: The obtained results from experiments are represented in mean±standard deviation values with displayed significant differences ($P < 0.05$).

6.5.1. Effect of processing variables of sensory parameters of developed beverage

Sensory evaluation is a critical aspect of consumer acceptability, encompassing parameters like color and appearance, flavor and sweetness, consistency, mouthfeel, and overall acceptability. This study employed a semi-trained panel (n=30) to assess the impact of Dahi (A) and microgreens powder (B) on developed functional fermented microgreens-based dairy (lassi) beverages in selected sensory study parameters. The obtained results of sensory parameters are shown in **(Table 6.40)**. The **(Table 6.45)** and **(Table 6.46)** represents the regression coefficients and F-values for the second-order polynomial equations, indicating the suggested model's adequacy, significant lack of fit, and satisfactory R^2 values; furthermore, R^2 -pred, which is typically lower than R^2 measures the model's predictive performance on future observations. All models were regarded as suitable for analysis because their R^2 values were all above 80%.

The sensory parameters reveal that when the percentage of microgreen powder in the beverage increased, it significantly ($P<0.05$) affected the sensory parameter of color and appearance of the prepared beverage. The color and appearance scores ranged from 7.07 to 8.69, with formulations containing moderate levels of microgreen powder scoring the highest **(Figure 6.37 (A))**. The addition of excessive microgreen powder (%) resulted in darker hues, which some panel members found less appealing. This can be attributed to the high anthocyanin content (28.06 to 45.87 $\mu\text{mol}/100\text{g}$, fresh weight basis) and pigments in radish sango microgreens, which intensify coloration. Similarly, the effect of tomato pomace powder addition affects the color and appearance parameters of developed lassi beverage, while in tomato pomace added lassi, 1% tomato pomace powder added lassi had better color and appearance sensory parameters (Kumar et al., 2023). In another study, different levels of carrot extract (10 to 25%), ginger extract (1 to 4%), and turmeric extract (1 to 4%) were incorporated into herbal lassi, from this study showed that the increase in the concentration of extract significantly reduces the color and appearance of prepared lassi beverage. From this study, the maximum concentration of herbal extracts added during the processing of herbal lassi was optimized with ginger (2%), turmeric (1%), and carrot extracts (15%) are suggested (Maji et al., 2020).

Another sensory parameter is flavor and sweetness, the sensory score for flavor and sweetness parameters of the functionally prepared beverage ranged from 6.78 to 8.55 **(Table 6.40)**, with balanced formulations achieving the highest ratings **(Figure 6.38 (B))**. Excessive amounts of radish sango microgreen powder (%) introduced an earthy and slightly bitter taste, lowering flavor and sweetness sensory scores (Rozali & Rahim, 2024). Sweetness levels were

primarily influenced by the total soluble solids (TSS) content, with higher Dahi levels providing a complementary sweetness (Kumar et al., 2017). For the mouthfeel sensory scores prepared beverage score ranged from 7.27 to 8.20 as shown in (Table 6.40). The quadratic equation linking the effect of Dahi and microgreen powder on mouthfeel sensory parameters is given in (Table 6.45). The linear effect of microgreen powder was found negative on the mouthfeel, while the effects of the Dahi variable were positive. The (Figure 6.37 (E)) illustrates the effect of different level concentrations (%) on the mouthfeel and shows that as levels of microgreen powder increase, the mouthfeel score decreases. Optimal formulations achieved a creamy texture, while excessive microgreen powder resulted in a grainy consistency, reducing scores. This is due to the particulate nature of microgreen powder, which disrupts the smooth gel matrix formed by Dahi proteins (Hussain et al., 2015). A similar effect for mouthfeel parameters was reported for the different types of herbal lassi (ginger lassi, carrot lassi, turmeric lassi) (Maji et al., 2020).

Similar color and appearance, flavor and sweetness, consistency, mouthfeel, the addition of microgreen powder (%) at the linear level and quadratic level significantly ($P < 0.05$) influenced the overall acceptability of the functional fermented microgreens-based dairy (lassi) beverage (Table 6.45) and (Figure 6.37 (F)). The overall acceptability scores ranged from 6.43 to 8.01 on the 9-point hedonic scale (Table 6.40). The regression equation (R^2) accounted for 94.16% of the total variation in the overall acceptability of the lassi beverage. The quadratic models for sensory parameters (Table 6.45) exhibited significant effects of both Dahi and microgreen powder. The interaction terms highlight the importance of balancing these ingredients to optimize sensory appeal. The high R^2 values across sensory parameters validate the models predictive reliability. Similar trends have been reported in functional dairy (lassi) beverages such as ginger lassi, carrot lassi, turmeric lassi (Maji et al., 2020), tomato pomace lassi (Kumar et al., 2023), Aloe-probiotic lassi (Kumar et al., 2017), and Aloe *barbadensis* Miller lassi (Hussain et al., 2015). The plant-based additives enhanced visual and functional appeal but required optimization to maintain palatability.

6.5.2. Effect of processing variables of physicochemical parameters of developed beverage

The pH value of the prepared functional fermented microgreens-based dairy (lassi) beverage ranged from 4.29 to 4.52, as shown in (Table 6.41). The addition of microgreens at the linear level (Figure 6.37 (C)) and the quadratic level (Table 6.45) significantly ($P < 0.05$) influenced the pH value of the beverage, as indicated in (Table 6.46).

Table 6.45. The predicted models to experimental data in CCRD design for the effect of microgreen-based dairy (lassi) beverage selected dependent variables

Variables	Regression equation	Mean±SD	C.V. %	R ²	Adjusted R ²	Predicted R ²	Adeq precision
Colour & Appearance	$8.10+0.0414\times A-0.5376\times B-0.0175\times AB-0.0894\times A^2-0.1494\times B^2$	7.95±0.13	1.67	0.9531	0.9195	0.8011	16.8189
Flavour & Sweetness	$7.23+0.0369\times A-0.5350\times B-0.0275\times AB-0.0013\times A^2-0.2263\times B^2$	7.37±0.23	3.24	0.8698	0.7768	0.6006	9.4222
Consistency	$7.87+0.0553\times A-0.0744\times B-0.0750\times AB-0.2938\times A^2-0.0763\times B^2$	7.74±0.14	1.85	0.8453	0.7348	0.5458	9.4919
Mouthfeel	$7.35+0.0532\times A-0.8399\times B-0.0425\times AB-0.0368\times A^2-0.0092\times B^2$	7.32±0.27	3.79	0.9133	0.8513	0.6817	12.5929
Overall Acceptability	$7.41+0.0539\times A-0.5531\times B-0.0425\times AB-0.0044\times A^2-0.0844\times B^2$	7.36±0.14	2.04	0.9416	0.8998	0.8279	15.3806
pH	$4.35+0.00311\times A-0.0507\times B-0.0525\times AB-0.0149\times A^2-0.0451\times B^2$	4.36±0.02	0.47	0.9501	0.9144	0.7306	17.0246
Acidity	$0.5460+0.0066\times A-0.0272\times B-0.0125\times AB-0.0139\times A^2-0.0011\times B^2$	0.55±0.01	2.40	0.8698	0.7768	0.6112	9.5773
TSS	$22.26+0.0957\times A+2.26\times B-0.0500\times AB-0.0613\times A^2-1.54\times B^2$	21.28±0.48	2.30	0.9718	0.9517	0.8551	21.0491
WHC	$42.41+0.0689\times A-5.66\times B+0.2950\times AB+4.01\times A^2-2.82\times B^2$	46.61±0.84	1.82	0.9877	0.9790	0.9440	27.7824
Syneresis	$43.46+0.3112\times A-0.5423\times B+0.5250\times AB-0.1613\times A^2+0.4888\times B^2$	43.66±0.20	0.45	0.9571	0.9264	0.7728	18.4363
Anthocyanin content	$39.31+1.92\times A+14.35\times B+0.2300\times AB-2.20\times A^2-1.12\times B^2$	37.27±3.03	8.14	0.9638	0.9379	0.8992	19.6880
TPC	$76.86+0.8906\times A+26.23\times B-0.0825\times AB+7.65\times A^2+3.86\times B^2$	83.95±6.27	7.46	0.9560	0.9247	0.8460	17.4321
TFC	$44.82+0.0279\times A+14.92\times B-7.98\times AB+0.2440\times A^2-0.3785\times B^2$	44.74±0.36	0.81	0.9995	0.9992	0.9984	183.8520
Antioxidant activity DPPH	$77.28+2.28\times A+5.79\times B-0.8800\times AB+0.7363\times A^2+0.5063\times B^2$	78.04±0.43	0.55	0.9958	0.9928	0.9765	57.0130
Antioxidant activity FRAP	$14.43+0.3620\times A+3.91\times B-1.59\times AB-0.5211\times A^2-0.0936\times B^2$	14.05±0.91	6.51	0.9586	0.9290	0.8191	19.0346
Ascorbic acid	$106.13+1.76\times A+32.93\times B-6.29\times AB+3.02\times A^2+5.93\times B^2$	111.64±13.48	12.07	0.8778	0.7906	0.7649	10.1712

Where, TSS: Total soluble solids; TPC: Total phenol content; TFC: Total flavonoid content; WHC: Water holding capacity

Table 6.46. ANOVA Quadratic model for the different responses

Responses	Source	Sum of Squares	df	Mean Square	F-value	P-value	
Colour & Appearance	Model	2.52	5	0.5034	28.42	0.0002	<0.05
	A-Dahi	0.0137	1	0.0137	0.7752	0.4078	
	B-MG	2.31	1	2.31	130.56	< 0.0001	
	AB	0.0012	1	0.0012	0.0692	0.8001	
	A ²	0.0556	1	0.0556	3.14	0.1198	
	B ²	0.1552	1	0.1552	8.76	0.0211	
	Residual	0.124	7	0.0177			
	LF	0.0598	3	0.0199	1.24	0.4051	>0.05
Flavour & Sweetness	Model	2.67	5	0.5332	9.35	0.0053	<0.05
	A-Dahi	0.0109	1	0.0109	0.1912	0.6751	
	B-MG	2.29	1	2.29	40.16	0.0004	
	AB	0.003	1	0.003	0.0531	0.8244	
	A ²	0	1	0	0.0002	0.9894	
	B ²	0.3561	1	0.3561	6.25	0.041	
	Residual	0.3991	7	0.057			
	LF	0.1083	3	0.0361	0.4964	0.7042	>0.05
Consistency	Model	0.7844	5	0.1569	7.65	0.0093	<0.05
	A-Dahi	0.0245	1	0.0245	1.19	0.3108	
	B-MG	0.0442	1	0.0442	2.16	0.1853	
	AB	0.0225	1	0.0225	1.1	0.3297	
	A ²	0.6003	1	0.6003	29.27	0.001	
	B ²	0.0404	1	0.0404	1.97	0.203	
	Residual	0.1435	7	0.0205			
	LF	0.0355	3	0.0118	0.4387	0.7378	>0.05
Mouthfeel	Model	5.68	5	1.14	14.74	0.0013	<0.05
	A-Dahi	0.0227	1	0.0227	0.2942	0.6044	
	B-MG	5.64	1	5.64	73.19	< 0.0001	
	AB	0.0072	1	0.0072	0.0937	0.7684	
	A ²	0.0094	1	0.0094	0.1219	0.7373	
	B ²	0.0006	1	0.0006	0.0077	0.9324	
	Residual	0.5397	7	0.0771			
	LF	0.205	3	0.0683	0.8166	0.5481	>0.05
Overall Acceptability	Model	2.53	5	0.5054	22.55	0.0003	<0.05
	A-Dahi	0.0232	1	0.0232	1.04	0.3428	
	B-MG	2.45	1	2.45	109.18	< 0.0001	
	AB	0.0072	1	0.0072	0.3224	0.5879	
	A ²	0.0001	1	0.0001	0.0059	0.9407	
	B ²	0.0495	1	0.0495	2.21	0.1807	
	Residual	0.1569	7	0.0224			
	LF	0.0391	3	0.013	0.4424	0.7356	>0.05

pH	Model	0.0566	5	0.0113	26.63	0.0002	<0.05
	A-Dahi	0.0077	1	0.0077	18.25	0.0037	
	B-MG	0.0206	1	0.0206	48.42	0.0002	
	AB	0.011	1	0.011	25.96	0.0014	
	A ²	0.0015	1	0.0015	3.62	0.0986	
	B ²	0.0142	1	0.0142	33.36	0.0007	
	Residual	0.003	7	0.0004			
	LF	0.0021	3	0.0007	2.97	0.1598	>0.05
Acidity	Model	0.0083	5	0.0017	9.35	0.0053	<0.05
	A-Dahi	0.0003	1	0.0003	1.94	0.206	
	B-MG	0.0059	1	0.0059	33.38	0.0007	
	AB	0.0006	1	0.0006	3.53	0.1021	
	A ²	0.0013	1	0.0013	7.57	0.0284	
	B ²	8.80E-06	1	8.80E-06	0.0498	0.8298	
	Residual	0.0012	7	0.0002			
	LF	0.0003	3	0.0001	0.4604	0.725	>0.05
Total soluble solids	Model	57.63	5	11.53	48.3	< 0.0001	<0.05
	A-Dahi	0.0733	1	0.0733	0.3071	0.5967	
	B-MG	40.99	1	40.99	171.78	< 0.0001	
	AB	0.01	1	0.01	0.0419	0.8436	
	A ²	0.0261	1	0.0261	0.1094	0.7506	
	B ²	16.42	1	16.42	68.8	< 0.0001	
	Residual	1.67	7	0.2386			
	LF	1.08	3	0.3595	2.43	0.2055	>0.05
Water holding capacity	Model	406.21	5	81.24	112.83	< 0.0001	<0.05
	A-Dahi	0.038	1	0.038	0.0528	0.8249	
	B-MG	256.51	1	256.51	356.24	< 0.0001	
	AB	0.3481	1	0.3481	0.4834	0.5093	
	A ²	111.83	1	111.83	155.32	< 0.0001	
	B ²	55.5	1	55.5	77.08	< 0.0001	
	Residual	5.04	7	0.72			
	LF	2.73	3	0.9102	1.58	0.3272	>0.05
Syneresis	Model	6.25	5	1.25	31.2	0.0001	<0.05
	A-Dahi	0.775	1	0.775	19.34	0.0032	
	B-MG	2.35	1	2.35	58.72	0.0001	
	AB	1.1	1	1.1	27.52	0.0012	
	A ²	0.1809	1	0.1809	4.51	0.0712	
	B ²	1.66	1	1.66	41.47	0.0004	
	Residual	0.2805	7	0.0401			
	LF	0.1885	3	0.0628	2.73	0.1781	>0.05
Anthocyanin content	Model	1715.42	5	343.08	37.27	< 0.0001	<0.05
	A-Dahi	29.64	1	29.64	3.22	0.1158	

	B-MG	1646.96	1	1646.9	178.9	< 0.0001	
	AB	0.2116	1	0.2116	0.023	0.8838	
	A ²	33.74	1	33.74	3.66	0.0971	
	B ²	8.68	1	8.68	0.9432	0.3638	
	Residual	64.44	7	9.21			
	LF	14.18	3	4.73	0.376	0.7761	>0.05
Total phenol content	Model	5976.2	5	1195.2	30.45	0.0001	<0.05
	A-Dahi	6.35	1	6.35	0.1617	0.6996	
	B-MG	5504.93	1	5504.9	140.25	< 0.0001	
	AB	0.0272	1	0.0272	0.0007	0.9797	
	A ²	406.72	1	406.72	10.36	0.0147	
	B ²	103.89	1	103.89	2.65	0.1478	
	Residual	274.75	7	39.25			
	LF	96.07	3	32.02	0.7169	0.5916	>0.05
Total flavonoid content	Model	2038.39	5	407.68	3030.1	< 0.0001	<0.05
	A-Dahi	0.0062	1	0.0062	0.0463	0.8358	
	B-MG	1781.9	1	1781.9	13244.49	< 0.0001	
	AB	254.88	1	254.88	1894.4	< 0.0001	
	A ²	0.4142	1	0.4142	3.08	0.1228	
	B ²	0.9966	1	0.9966	7.41	0.0297	
	Residual	0.9418	7	0.1345			
	LF	0.3361	3	0.112	0.7399	0.5811	>0.05
Antioxidant activity DPPH	Model	317.46	5	63.49	333.57	< 0.0001	<0.05
	A-Dahi	41.54	1	41.54	218.26	< 0.0001	
	B-MG	267.86	1	267.86	1407.2	< 0.0001	
	AB	3.1	1	3.1	16.27	0.005	
	A ²	3.77	1	3.77	19.81	0.003	
	B ²	1.78	1	1.78	9.37	0.0183	
	Residual	1.33	7	0.1903			
	LF	0.9738	3	0.3246	3.62	0.1229	>0.05
Antioxidant activity FRAP	Model	135.4	5	27.08	32.39	0.0001	<0.05
	A-Dahi	1.05	1	1.05	1.25	0.2997	
	B-MG	122.29	1	122.29	146.28	< 0.0001	
	AB	10.18	1	10.18	12.17	0.0101	
	A ²	1.89	1	1.89	2.26	0.1765	
	B ²	0.061	1	0.061	0.0729	0.7949	
	Residual	5.85	7	0.836			
	LF	2.96	3	0.9857	1.36	0.3742	>0.05
Ascorbic acid	Model	9137.21	5	1827.4	10.06	0.0043	<0.05
	A-Dahi	24.79	1	24.79	0.1365	0.7227	
	B-MG	8673.87	1	8673.8	47.75	0.0002	
	AB	158.27	1	158.27	0.8713	0.3817	

	A ²	63.62	1	63.62	0.3502	0.5726	
	B ²	244.41	1	244.41	1.35	0.2841	
	Residual	1271.63	7	181.66			
	LF	82.87	3	27.62	0.0929	0.96	>0.05

Where, MG: Microgreen powder; LF: Lack of fit; <0.05: Significant; >0.05: Not significant

RSM analysis (**Figure 6.40 (a)**) also supports this finding, and demonstrates a significant interaction between the concentration of Dahi (A) and microgreens powder (B), with increasing microgreens powder (%) causing a slight decrease in the pH value of beverage. This decrease is primarily due to the presence of acidic phenolic and organic compounds inherent in microgreens, which contribute to the overall acidic profile of the beverage (Kaur et al., 2018). The regression model (**Table 6.45**) exhibited a strong fit ($R^2 = 0.9501$), indicating the reliability of the quadratic model in predicting pH behavior. The optimized pH of around 4.35 ensures not only microbial stability but also supports the activity of lactic acid bacteria (LAB) during fermentation (Kumar et al., 2017). The observed pH values align with previous studies on functional dairy (lassi) beverages enriched with plant-based powder/extract additives (Hussain et al., 2015; Kumar et al., 2017; Kumar et al., 2023).

The acidity (% lactic acid) levels in the beverage formulations ranged from 0.50% to 0.59% (**Table 6.41**), reflecting the degree of lactic acid production during the fermentation process. The direct correlation between microgreen powder concentration and acidity, with higher levels resulting in increased acidity as shown in (**Figure 6.37 (G)**) and (**Figure 6.35 (A)**). This trend underscores the role of microgreen powder in enhancing microbial activity, as its bioactive compounds stimulate the metabolic functions of LAB. The ANOVA results (**Table 6.46**) showed significant ($P < 0.05$) linear and quadratic effects of microgreen powder on acidity. The quadratic model's fit ($R^2 = 0.9246$) validates the predictive accuracy of the experimental design, emphasizing the critical role of ingredient ratios in modulating acidity. These findings are consistent with studies on the development of tomato pomace in lassi the acidity (0.65 to 0.78%) (Kumar et al., 2023) and moringa pod powder added to lassi the acidity (0.24 to 0.65%) (Mistry et al., 2018), where tomato pomace powder and moringa pod powder enhanced microbial activity and acid production.

The Total Soluble Solids (TSS) values varied between 16.4 to 22.7°Brix (**Table 6.41**), with a strong dependency on the concentration of Dahi. The microgreen powder (%) slightly significant effect on the TSS content of beverage (**Figure 6.37 (D)**).

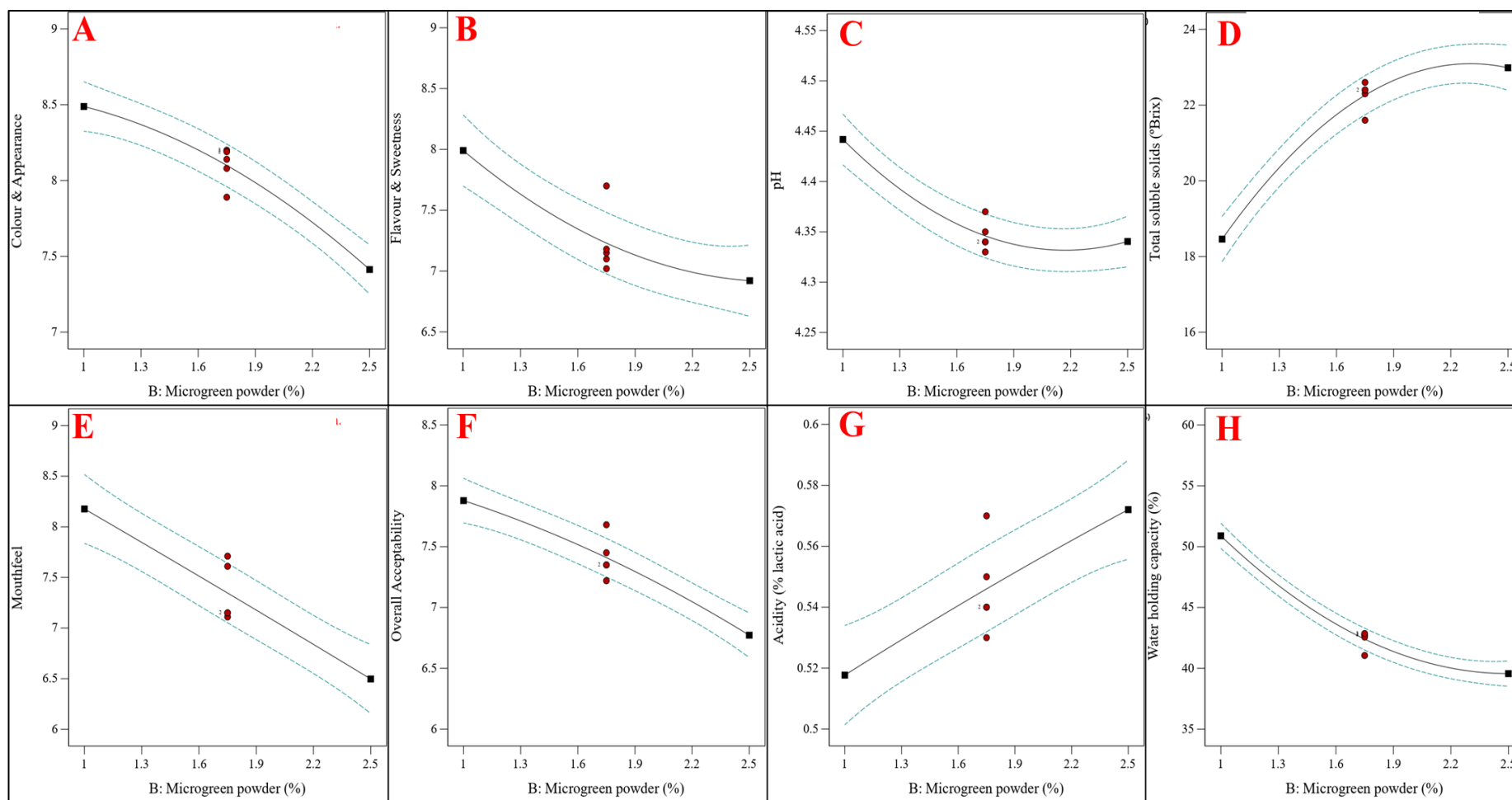


Figure 6.37. Response surface plots showing the effect of microgreen powder (B) on microgreen-based dairy (lassi) beverage selected study parameters. Where, A: Colour & Appearance; B: Flavour & Sweetness; C: pH; D: Total soluble solids; E: Mouthfeel; F: Overall Acceptability; G: Acidity; H: Water holding capacity.

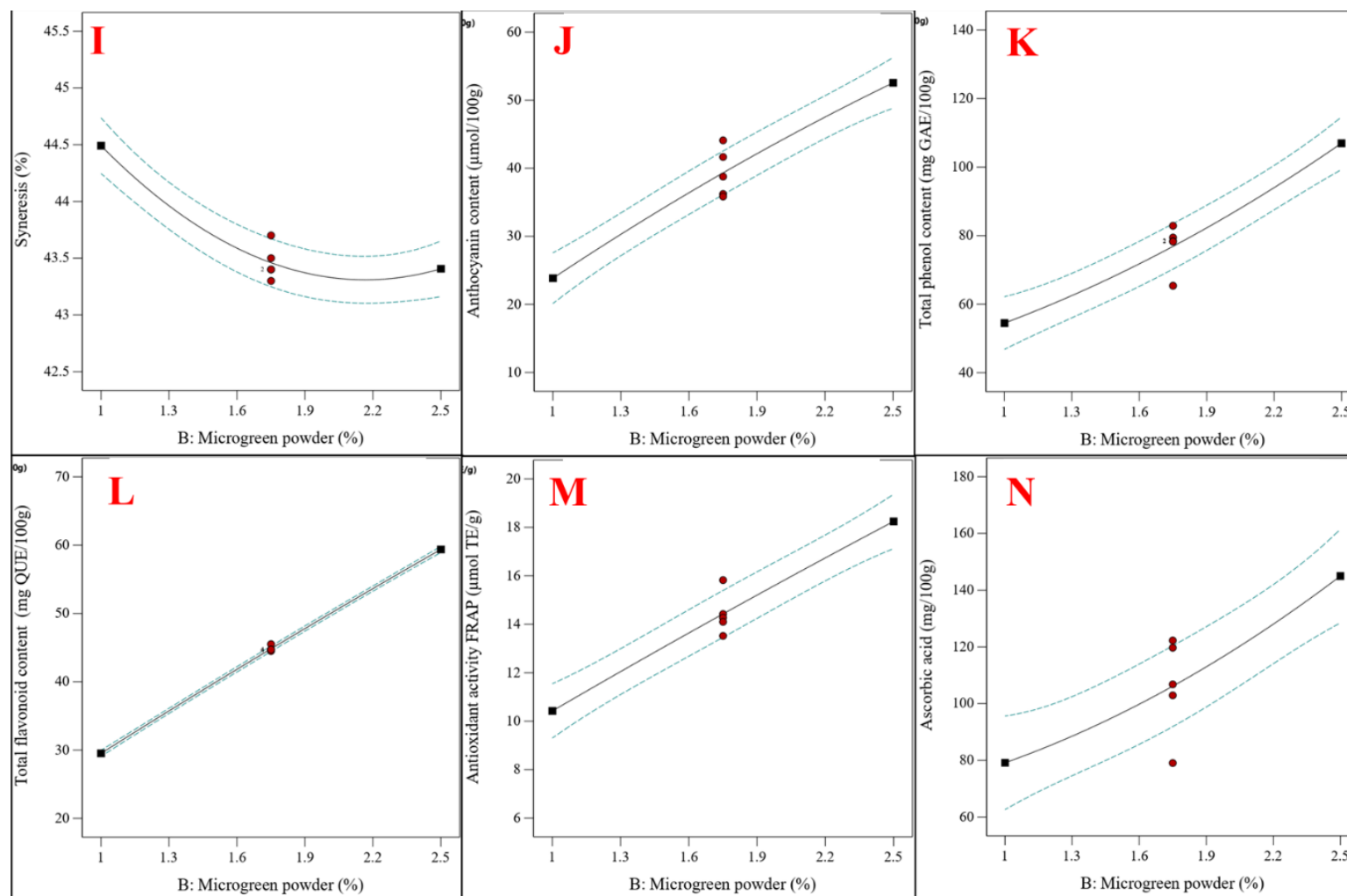


Figure 6.38. Response surface plots showing the effect of microgreen powder (B) on microgreen-based dairy (lassi) beverage selected study parameters. Where, I: Syneresis; J: Anthocyanin content; K: Total phenol content; L: Total flavonoid content; M: Antioxidant activity DPPH; N: Ascorbic acid

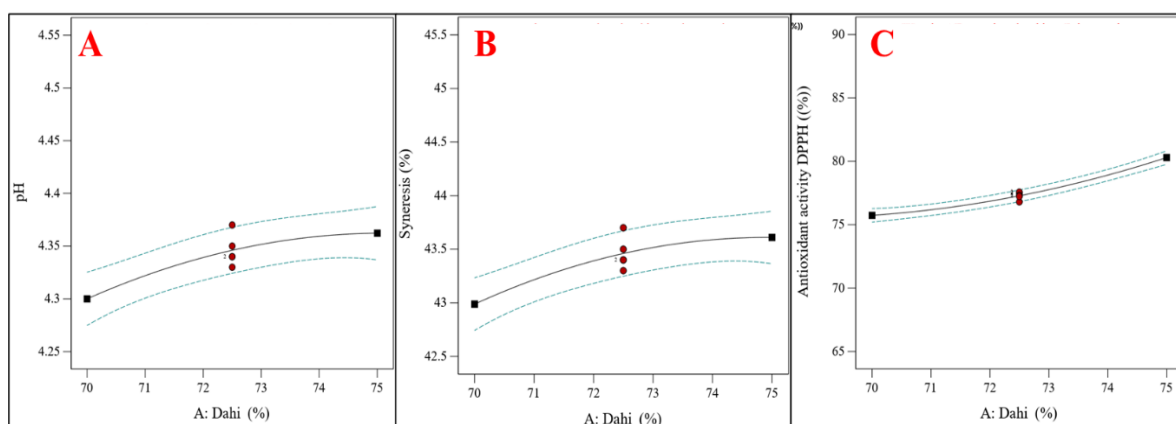


Figure 6.39. Response surface plots showing the effect of Dahi (A) on microgreen-based dairy (lassi) beverage selected study parameters. Where, A: pH; B: Syneresis; C: Antioxidant activity DPPH.

In the quadratic model (**Table 6.45**), the R^2 value (0.9718) captured the significant interaction effects, emphasizing the importance of balancing Dahi and microgreens powder concentrations to achieve the desired TSS. Similar results were reported for the fermented dairy (lassi) product prepared with varying levels of mango powder (2.5 to 7.5%) and beetroot powder (1 to 4%). The TSS values for mango powder lassi (9.80 to 17%) and beetroot powder lassi (9.80 to 11.30%) were observed (Chawla & Sivakumar, 2017).

Water holding capacity (WHC) measures the ability of the lassi matrix to retain water, directly influencing texture, mouthfeel, and consumer acceptability. The WHC in prepared beverage formulations ranged from 41.03 to 55.78% were observed (**Table 6.41**). This variation underscores the interplay between Dahi (A) and microgreen powder (B), as captured in (**Figure 6.37 (H)**). WHC decreased with increasing microgreen powder concentration due to potential disruptions in the protein network. The (**Table 6.46**) shows significant ($P < 0.05$) linear and quadratic effects of microgreens powder on WHC. The regression model high R^2 value (0.9458) indicates strong predictive reliability. The response surface plot demonstrates that higher levels of Dahi mitigate the negative impact of microgreen powder on WHC, likely due to enhanced protein availability (Hussain et al., 2015).

Syneresis refers to the separation of whey or liquid from the gel matrix of the lassi, directly affecting its texture, appearance, and overall consumer acceptability. In this study, syneresis ranged from 42.9% to 45.2%. The (**Figure 6.38 (I)**) and (**Figure 6.40 (B)**) highlight the significant impact of microgreen powder (B) and its interaction with Dahi (A) on syneresis. Higher concentrations of microgreen powder generally increased syneresis, reflecting a compromise in gel stability. The regression model (**Table 6.45**) captured significant linear and quadratic effects of microgreen powder on syneresis ($P < 0.05$).

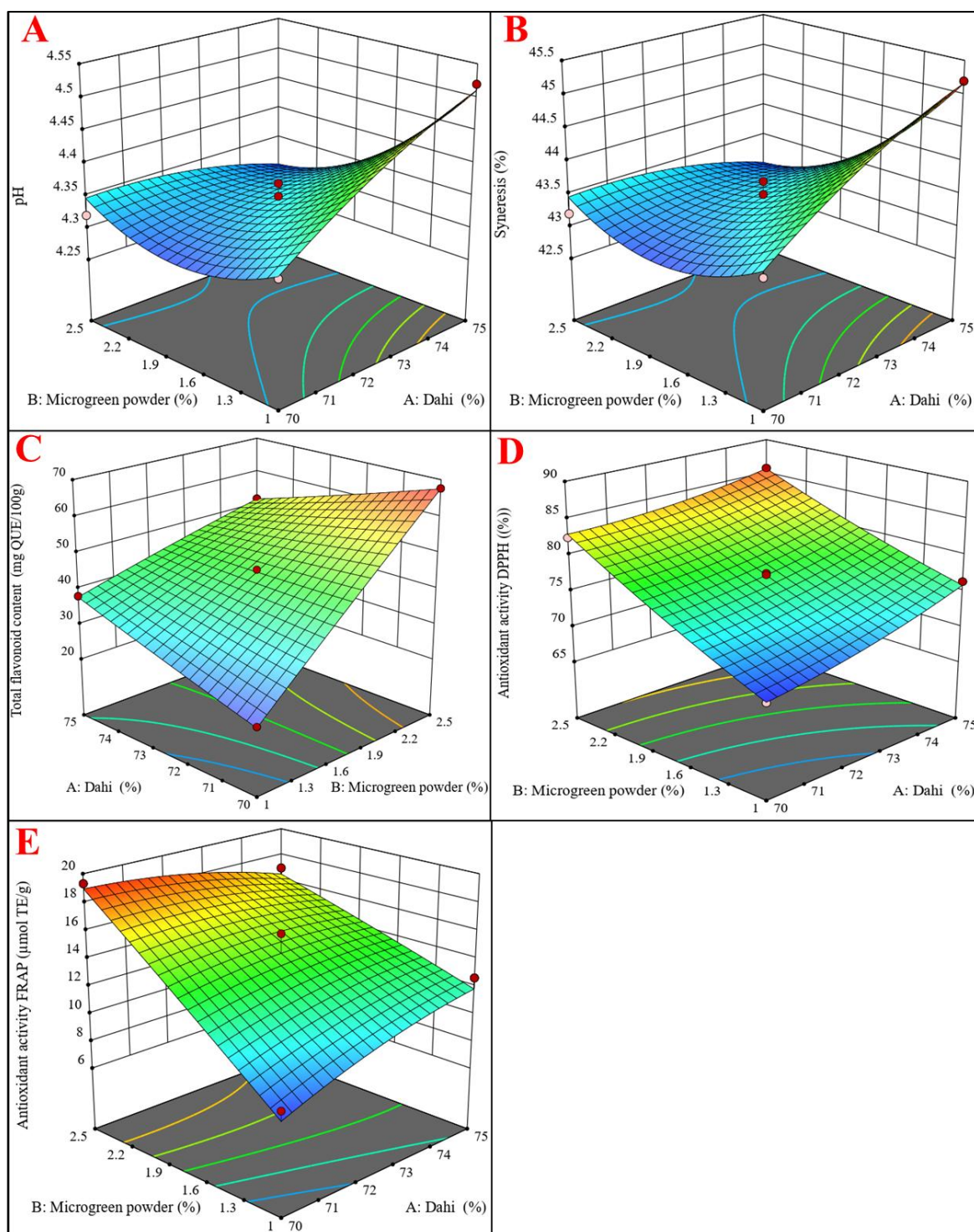


Figure 6.46. Response surface plots showing the effect of Dahi (A) and microgreen powder (B) on various study parameters of microgreen-based dairy (lassi) beverage. Where, A: pH; B: Syneresis; C: Total flavonoid content; D: Antioxidant activity DPPH; E: Antioxidant activity FRAP.

The response surface plot (**Figure 6.37 (I)**) and (**Figure 6.40 (B)**) shows that while increasing Dahi concentration reduces syneresis, excessive microgreen powder levels counteract this effect. The models R^2 value (0.9321) confirms its predictive accuracy. The observed syneresis trends align with studies on lassi enriched with tomato pomace powder,

where excessive fiber and bioactive composition high content ingredients weakened gel structures (Kumar et al., 2023)

6.5.3. Effect of processing variables of biochemical and antioxidant parameters of developed beverage

6.5.3.1. Total phenol and flavonoid content

The microgreen powder showed a significant effect on the microgreens-based dairy (lassi) beverage, with total phenol content ranging from 46.64 mg GAE/100g to 120.52 mg GAE/100g (**Table 6.42**). Total flavonoid content ranged from 22.02 mg QE/100g to 67.68 mg QE/100g was observed in microgreens-based dairy (lassi) beverage (**Table 6.42**), reflecting the substantial impact of microgreens powder. The higher flavonoid content in radish sango microgreens augmented the overall antioxidant capacity, creating a synergistic effect with phenolic compounds (Kowitcharoen et al., 2021; Dhaka et al., 2023). In (**Figure 6.38 (L)**) and (**Table 6.45**) illustrate the strong positive correlation between microgreen powder concentration and total flavonoid content, with the regression model achieving an R^2 value of 0.9968. Radish sango microgreens, known for their good source of phenolic and flavonoid compounds, substantially enhanced the phenolic and flavonoid content of the lassi. Phenolics, which act as primary antioxidants, contribute to free radical scavenging and overall oxidative stability. The fermentation process, facilitated by Dahi, played a crucial role in stabilizing these compounds, ensuring their bioavailability in the final product (Kumar et al., 2023). Our finding results align with Varnaite et al. (2022) and Alqahtani et al. (2020) reported a similar trend of total phenol content for cranberry and tomato pomace-incorporated yogurt. In another study, total phenol content range from 0.124 to 0.226 mg GAE/gm, was reported for the different types of herbal lassi (ginger lassi, carrot lassi, turmeric lassi), these findings were lower than our results (Maji et al., 2020). Many studies showed that microgreens contain higher amounts of bioactive compounds than their counterparts. As shown in (**Table 6.45**) and (**Figure 6.37 (K)**) and (**Figure 6.40 (C)**), the regression model highlighted strong linear and quadratic effects ($P < 0.05$) of microgreen powder on total phenol content, with an R^2 value of 0.9892, indicating high predictive reliability. While increasing microgreens powder concentration improved total phenolic and flavonoid content, excessive amounts led to diminishing returns, possibly due to saturation effects or interactions that limit these compounds solubility.

6.5.3.2. Ascorbic acid and anthocyanin content

The amount of ascorbic acid and anthocyanin content in the microgreens-based dairy (lassi) beverage is shown in (**Table 6.42**). Ascorbic acid, ranging from 67.41 mg/100g to 162.90 mg/100g, is an important water-soluble antioxidant compound that protects against oxidative

degradation while enhancing the shelf life of the product. On the other hand, anthocyanin content varied from 17.08 $\mu\text{mol}/100\text{g}$ to 56.71 $\mu\text{mol}/100\text{g}$, contributing to both the visual appeal and antioxidant potential of the beverage. The regression analysis for ascorbic acid and anthocyanin content (**Table 6.45**) indicated significant linear and quadratic effects ($P < 0.05$) of microgreens powder, with R^2 values of 0.9786 and 0.9642, respectively. The response surface plots for both compounds (**Figure 6.37 (J and N)**) revealed a direct correlation between microgreen powder concentration and these bioactive compounds. The increase in anthocyanin levels reflects the pigmentation properties of radish sango microgreens, which are rich in these natural antioxidants. Meanwhile, the stability of ascorbic acid during the fermentation process highlights the protective environment provided by the acidic matrix of the lassi. The findings for ascorbic acid are consistent with studies on, 2.5% Orange flavedo incorporated functional lassi were the 136 mg/100g reported in functional beverage (Ananthakumar & Narayanan, 2021). In another study, the different concentrations of mulberry pomace powder (1 to 3%) were incorporated into the preparation of fortified stirred yogurt, from this study the anthocyanin content of 23.29 to 79.09 g cyanidin-3glucoside/g was observed (Du et al., 2021).

6.5.3.3. Antioxidant properties (DPPH and FRAP)

The antioxidant activity of the microgreens-based dairy (lassi) beverage was evaluated using DPPH free radical scavenging activity and FRAP (Ferric Reducing Antioxidant Power). Both parameters showed significant effect ($P < 0.05$) and revealed the strong antioxidant potential of the dairy beverage, attributed to the combined effects of phenolics, flavonoids, ascorbic acid, and anthocyanins.

The DPPH scavenging activity ranged from 69.51% to 86.76% was observed (**Table 6.42**), showcasing the lassi beverage ability to neutralize free radicals effectively. This activity is primarily driven by the electron-donating capacity of phenolic and flavonoid compounds, as well as the synergistic contribution of ascorbic acid (Kumar et al., 2023). The response surface plots (**Figure 6.40 (D)**), highlight the significant ($P < 0.05$) influence of microgreens powder on DPPH activity. The regression model (**Table 6.45**) demonstrated a high R^2 value of 0.9934, affirming the reliability of the results. Another antioxidant parameter FRAP, ranged from 7.90 $\mu\text{mol TE/g}$ to 19.41 $\mu\text{mol TE/g}$, reflecting the electron-donating properties of the lassi bioactive components. Phenolics and flavonoids from microgreen powder interact synergistically with ascorbic acid, enhancing the reducing power of the beverage. In (**Figure 6.40 (E)**) illustrates the response surface plot for FRAP, which, like DPPH, showed a significant dependence on microgreen powder concentration. The regression model's R^2 value of 0.9861 validates the strong relationship between microgreen powder and antioxidant capacity. The symbiotic lassi

with honey, the DPPH scavenging activity of fresh product was found to be 28.43 % (Shah et al., 2016) and the lassi prepared from the addition of tomato pomace powder was DPPH antioxidant activity found in a range of 13.11 to 28.12% (Kumar et al., 2023), these values are slightly lower than our finding values.

6.5.4. Effect of storage conditions on optimized microgreen-based dairy (lassi) beverage

6.5.4.1. Physicochemical properties

Storage conditions had a significant ($P < 0.05$) impact on the physicochemical properties of the optimized microgreen-based dairy (lassi) beverage, including pH, titratable acidity, total soluble solids, water holding capacity, and syneresis parameters. These parameters are critical for maintaining the products sensory, structural, and microbiological stability over time period.

The pH of the dairy (lassi) beverage steadily declined across all storage conditions (5°C, 15°C, and 25°C) indicative of the progressive acidification caused by the metabolic activity of residual lactic acid bacteria. The pH of the optimized microgreen-based dairy (lassi) beverage on the initial day of preparation was 4.37 and decreased to 5°C (3.73), 15°C (3.76), and 25°C (3.00) on the 15th day of storage (**Table 6.47**). The analysis of variance showed that storage days and temperature conditions showed a significant ($P < 0.05$) effect on the pH of the optimized microgreen-based dairy (lassi) beverage. Initially, the pH of the beverage was recorded as 4.34. Over 15 days of storage at 5°C, the pH decreased, reflecting minimal microbial activity due to the low-temperature storage environment condition (Kumar et al., 2017). However, at 25°C, the pH showed a marked reduction, a significant drop attributed to enhanced microbial proliferation and metabolic processes in high-temperature conditions (Pandhi & Poonia, 2021). This decline in pH correlates with an increase in titratable acidity (lactic acid), which increased from an initial value of 0.54% to 0.68% at 25°C, while at 5°C, the increase was much less, reaching 0.57% by 15th storage day (**Table 6.47**). The production of lactic acid during fermentation by LAB is the primary cause of these changes. Higher storage temperatures accelerated these processes, leading to greater acid accumulation and a sharper decline in pH value (Shah et al., 2016). The observed trends align with previous studies on fermented dairy products the increase in acidity and reduction in pH value of lassi beverage with increasing temperature ($>10^{\circ}\text{C}$) which was in agreement with the current observation (George et al., 2010; Shah et al., 2016; Kumar et al., 2017).

The total soluble solid (TSS) content of the dairy (lassi) beverage displayed gradually increasing trends over the storage period with storage temperatures, influenced by microbial activity and biochemical reactions.

Table 6.47. Effect of storage temperature conditions on physicochemical parameters of optimized microgreen-based dairy (lassi) beverage

Days	Temperature (°C)	Overall acceptability	pH	Acidity (% lactic acid)	Total soluble solid (°Brix)	Water holding capacity (%)	Syneresis (%)
Day-0	5	8.60±0.53 ^{aA}	4.37±0.02 ^{aA}	0.65±0.01 ^{iC}	22.88±0.14 ^{gB}	43.45±0.34 ^{aA}	43.41±0.13 ^{hC}
	15	8.60±0.53 ^{aB}	4.37±0.02 ^{aB}	0.65±0.01 ^{iB}	22.88±0.14 ^{gA}	43.45±0.34 ^{aB}	43.41±0.13 ^{hB}
	25	8.60±0.53 ^{aB}	4.37±0.02 ^{aC}	0.65±0.01 ^{iA}	22.88±0.14 ^{gA}	43.45±0.34 ^{aC}	43.41±0.13 ^{hA}
Day-3	5	8.35±0.18 ^{aA}	4.26±0.01 ^{bA}	0.68±0.01 ^{hiC}	23.08±0.08 ^{fgB}	43.18±0.01 ^{abA}	43.64±0.02 ^{ghC}
	15	8.10±0.11 ^{abB}	4.23±0.01 ^{bB}	0.75±0.04 ^{hiB}	23.13±0.01 ^{fgA}	43.03±0.03 ^{abcB}	43.90±0.07 ^{efgB}
	25	8.00±0.11 ^{abB}	4.03±0.01 ^{bC}	0.95±0.02 ^{defA}	23.27±0.02 ^{efA}	42.68±0.04 ^{bcd^{ef}C}	44.10±0.06 ^{cdefgA}
Day-6	5	7.90±0.18 ^{abA}	4.21±0.01 ^{bA}	0.76±0.05 ^{ghC}	23.23±0.01 ^{efB}	43.10±0.03 ^{abA}	43.77±0.03 ^{ghC}
	15	7.90±0.25 ^{abB}	4.14±0.01 ^{cB}	0.84±0.02 ^{fgB}	23.48±0.06 ^{cdeA}	42.78±0.04 ^{bcd^eB}	43.97±0.03 ^{defgB}
	25	7.80±0.25 ^{abB}	3.04±0.01 ^{gC}	1.02±0.01 ^{cdA}	23.62±0.09 ^{bcdA}	42.19±0.05 ^{fgC}	44.35±0.13 ^{abcdA}
Day-9	5	7.80±0.04 ^{abA}	4.03±0.01 ^{dA}	0.84±0.02 ^{fgC}	23.34±0.12 ^{defB}	42.90±0.03 ^{abcdA}	43.92±0.10 ^{efgC}
	15	7.09±0.11 ^{bcdB}	4.02±0.02 ^{dB}	0.93±0.01 ^{defB}	23.68±0.06 ^{abcA}	42.47±0.03 ^{cdefgB}	44.12±0.10 ^{cdefB}
	25	7.07±0.07 ^{bcdB}	3.04±0.01 ^{gC}	1.06±0.03 ^{bcA}	23.72±0.09 ^{abcA}	42.27±0.03 ^{efgC}	44.45±0.13 ^{abcA}
Day-12	5	7.25±0.18 ^{bcA}	3.83±0.01 ^{eA}	0.91±0.03 ^{efC}	23.84±0.02 ^{abB}	42.66±0.04 ^{bcd^eA}	43.97±0.03 ^{defgC}
	15	6.32±0.15 ^{cdB}	3.76±0.02 ^{fB}	1.00±0.03 ^{cdeB}	23.84±0.01 ^{abA}	42.23±0.04 ^{efgB}	44.24±0.07 ^{bcd^eB}
	25	6.27±0.07 ^{cdB}	3.01±0.00 ^{gC}	1.17±0.02 ^{cdA}	23.87±0.02 ^{abA}	41.89±0.08 ^{gC}	44.53±0.09 ^{abA}
Day-15	5	6.70±0.32 ^{cdA}	3.73±0.01 ^{fA}	1.03±0.01 ^{cdC}	23.87±0.02 ^{abB}	42.39±0.08 ^{defgA}	44.08±0.04 ^{cdefC}
	15	6.18±0.08 ^{dB}	3.76±0.02 ^{fB}	1.15±0.04 ^{abB}	23.87±0.02 ^{abA}	42.14±0.01 ^{fgB}	44.34±0.07 ^{abcdB}
	25	6.07±0.07 ^{dB}	3.00±0.02 ^{gC}	1.25±0.04 ^{aA}	23.97±0.02 ^{aA}	41.99±0.08 ^{gC}	44.64±0.20 ^{aA}

Note: The results were presented as mean±standard deviations (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments and different uppercase letters within the column indicate significant (P<0.05) differences in temperature condition for the parameters.

The TSS content on the initial day (0 day) was recorded at 22.88°Brix, the TSS increased to 23.87°Brix at 5°C, and 23.87°Brix at 15°C to 23.97°Brix at 25°C by Day 15, showed in **(Table 6.47)**. This increasing trend can be attributed to the microgreen powder soluble particle and the effect was more pronounced at higher temperatures due to enhanced microbial activity (Birwal et al., 2024).

The effect of storage temperature conditions on water holding capacity and syneresis on optimized microgreen-based dairy (lassi) beverage are presented in **(Table 6.47)**. The water holding capacity is a measure of the ability of the lassi beverage matrix to retain water, which is critical for texture and mouthfeel sensory parameters. The water holding of the beverage declined significantly during the selected storage period, from an initial value of 43.45% to 42.39% at 5°C, 42.14% at 15°C, and 41.99% at 25°C by Day 15 **(Table 6.47)**. A similar decline trend was observed in strawberry polyphenol extract–fortified stirred dahi in water holding capacity from 91.99% to 84.92% during storage at 7-8°C for 21 days storage period (Singh et al., 2012). This decreasing trend indicates a structural weakening in the protein network, likely due to enzymatic degradation and microbial activity that disrupts the gel matrix formed by the interaction of proteins and stabilizers (Hussain et al., 2015). On the other side, syneresis is the separation of whey or liquid (water) from the gel and increases over time, further underscoring the destabilization of the dairy beverage structural integrity. The initial syneresis value was recorded at 43.41% and it progressively increased to 44.64% at 25°C by Day 15, while the lowest value was observed at 5°C as shown in **(Table 6.47)**. The storage temperature 25°C, exacerbated this phenomenon, likely due to the degradation of stabilizing components and the destabilizing effects of microbial activity. With the advancement of the storage period, a significant increase ($P < 0.05$) in syneresis was observed across all treatments compared to the initial values. This trend aligns with the findings of Koksoy & Kilic (2003) and Hussain et al. (2015), who reported elevated whey syneresis during storage in stirred yogurt and probiotic lassi, respectively. The authors attributed these changes to increased bacterial growth and a reduction in pH, which promoted whey separation. The higher acidity levels led to the aggregation of protein micelles, thereby enhancing whey syneresis over the storage duration. The observed rise in whey syneresis in the optimized microgreen-based dairy (lassi) beverage during storage can be ascribed to continuous post-acidification.

6.5.4.2. Biochemical parameters

6.5.4.2.1. Total phenolic and flavonoid content

Variations in the total phenolic and flavonoid content throughout storage: The total phenol and flavonoid content of the optimized microgreen-based dairy (lassi) beverage sample

was 114.00 mg GAE/100mg and 67.77 mg QE/100mg, which significantly reduced with the increase in storage time and temperature condition. The highest total phenolic and flavonoid content retention were observed in lower temperature conditions at 5°C>15°C >25°C during the storage period of 15 days has been represented in **(Table 6.48)**.

The initial increase in total phenol and flavonoid content can be attributed to the enzymatic hydrolysis of bound phenolic compounds during early storage, releasing free phenolics into the matrix. However, prolonged storage led to oxidative degradation of these bioactive compounds, exacerbated by higher temperatures. This trend underscores the protective effect of lower temperature conditions, which slows oxidative reactions and preserves phenolic content. These findings are consistent with studies on functional dairy products enriched with herbal lassi fortified with turmeric extract (Maji et al., 2018), pea milk-fortified dahi incorporated with jamun seed extract (Pandhi & Poonia, 2021), and yogurt fortified with goji berries (Taneva & Zlatev, 2020), where phenolic and flavonoid compounds stability was found to be temperature dependent at selected storage days.

6.5.4.2.2. Ascorbic acid and anthocyanin content

Ascorbic acid, a water-soluble vitamin and powerful antioxidant, plays a vital role in preventing oxidative spoilage and enhancing the beverage shelf life. During the selected storage period the ascorbic acid content decreased steadily, with the rate of decline significantly influenced by temperature conditions. The ascorbic acid content was reduced from 5°C (152.77 to 131.95 mg/100g), 15°C (125.20 mg/100g), and 25°C (117.71 mg/100g) by Day 15. At a storage temperature of 25°C, the reduction was more pronounced as compared to 5°C and 15°C storage conditions **(Table 6.48)**. The degradation of ascorbic acid is primarily due to its sensitivity to oxidation, which is accelerated by higher temperatures and prolonged storage. At lower temperature (5°C to 15°C) slows this process by reducing the rate of oxidative reactions, thereby preserving the nutritional quality of the dairy (lassi) beverage. These observations align with previous studies on fermented dairy beverages which reported significant temperature-dependent losses of ascorbic acid during storage (Znamirowska et al., 2021; Ahmed et al., 2023).

Anthocyanins are responsible for the vibrant color and additional antioxidant properties of the beverages. In the optimized microgreen-based dairy (lassi) beverage initially anthocyanin content was 48.23 µmol/100g were observed **(Table 6.48)**. The storage temperature and storage duration significantly influenced anthocyanin content retention in dairy (lassi) beverages.

Table 6.48. Effect of storage temperature conditions on biochemical parameters of optimized microgreen-based dairy (lassi) beverage

Days	Temperature (°C)	Anthocyanin content (μmol/100g)	Total phenol content (mg GAE/100g)	Total flavonoid content (mg QE/100g)	Ascorbic acid (mg/100g)	Free fatty acid content (μg/g)	Thiobarbituric acid content (mg MDA/kg)
Day-0	5	48.23±0.18 ^{aC}	114.00±0.47 ^{aA}	67.77±0.15 ^{aA}	152.77±0.16 ^{aA}	0.53±0.01 ^{jA}	0.03±0.00 ^{iC}
	15	48.23±0.18 ^{aB}	114.00±0.47 ^{aB}	67.77±0.15 ^{aB}	152.77±0.16 ^{aB}	0.53±0.01 ^{jB}	0.03±0.00 ^{iB}
	25	48.23±0.18 ^{aA}	114.00±0.47 ^{aB}	67.77±0.15 ^{aC}	152.77±0.16 ^{aC}	0.53±0.01 ^{jC}	0.03±0.00 ^{iA}
Day-3	5	48.26±0.07 ^{aC}	113.20±0.19 ^{abA}	65.57±0.43 ^{bA}	150.57±0.13 ^{bA}	0.57±0.01 ^{ijA}	0.03±0.00 ^{hiC}
	15	47.96±0.07 ^{abcB}	112.65±0.59 ^{abcB}	65.17±0.08 ^{qbcB}	148.50±0.23 ^{cB}	0.59±0.01 ^{iB}	0.03±0.00 ^{ghiB}
	25	47.86±0.07 ^{abcA}	112.07±0.08 ^{abcdeB}	65.17±0.22 ^{cdC}	146.45±0.16 ^{dC}	0.61±0.01 ^{hiC}	0.04±0.00 ^{fghiA}
Day-6	5	48.14±0.04 ^{abC}	112.26±0.28 ^{abcdA}	64.42±0.36 ^{cdA}	148.77±0.16 ^{cA}	0.60±0.01 ^{hiA}	0.04±0.00 ^{fghiC}
	15	47.81±0.14 ^{abB}	110.25±0.26 ^{defB}	64.02±0.12 ^{deB}	144.85±0.27 ^{eB}	0.67±0.02 ^{efgB}	0.04±0.00 ^{fghB}
	25	47.28±0.24 ^{cdA}	110.22±0.30 ^{efB}	62.39±0.24 ^{fgC}	143.35±0.30 ^{fC}	0.70±0.01 ^{efC}	0.04±0.00 ^{efghA}
Day-9	5	47.97±0.13 ^{abcC}	111.71±0.36 ^{bcdeA}	63.34±0.17 ^{efA}	146.10±0.31 ^{dA}	0.63±0.01 ^{ghiA}	0.04±0.00 ^{fghC}
	15	47.12±0.15 ^{dcB}	105.53±0.13 ^{ghB}	63.20±0.13 ^{fgB}	134.95±0.13 ^{hB}	0.70±0.01 ^{efB}	0.04±0.00 ^{cdefB}
	25	46.53±0.11 ^{efA}	106.72±1.00 ^{gB}	62.96±0.09 ^{fcC}	133.40±0.23 ^{iC}	0.77±0.03 ^{cdC}	0.05±0.00 ^{bcA}
Day-12	5	47.37±0.44 ^{cdC}	110.66±0.71 ^{cdefA}	61.59±0.52 ^{gA}	141.70±0.54 ^{ghA}	0.66±0.01 ^{fghA}	0.04±0.00 ^{cdefgC}
	15	46.29±0.09 ^{fB}	104.38±0.50 ^{hiB}	60.31±0.12 ^{hB}	130.30±0.20 ^{kB}	0.78±0.02 ^{cdB}	0.05±0.00 ^{bcdB}
	25	46.13±0.17 ^{fA}	105.97±0.65 ^{ghB}	60.12±0.16 ^{hC}	123.46±0.14 ^{mC}	0.84±0.02 ^{bC}	0.06±0.00 ^{bA}
Day-15	5	46.12±0.08 ^{fcC}	109.68±0.60 ^{fA}	60.38±0.07 ^{hA}	131.95±0.10 ^{jA}	0.72±0.01 ^{deA}	0.05±0.00 ^{bcdC}
	15	45.34±0.15 ^{gB}	103.33±0.28 ^{ijB}	59.80±0.23 ^{hB}	125.20±0.06 ^{iB}	0.81±0.01 ^{bcB}	0.06±0.00 ^{bB}
	25	45.08±0.10 ^{gA}	101.92±0.69 ^{jB}	58.65±0.45 ^{iC}	117.71±0.49 ^{hC}	0.94±0.02 ^{aC}	0.07±0.00 ^{aA}

Note: The results were presented as mean±standard deviations (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments and different uppercase letters within the column indicate significant (P<0.05) differences in temperature condition for the parameters.

The highest anthocyanin content retention was observed under 5°C (46.12 µmol/100g), followed by 15°C (45.34 µmol/100g) and 25°C (45.08 µmol/100g), respectively. The loss of anthocyanins not only affects the dairy (lassi) beverage antioxidant activities but also its visual appeal, as color intensity diminishes over time (Mefleh et al., 2022). Refrigeration significantly reduced the rate of degradation, preserving both the functional and sensory attributes of the optimized product. This finding is consistent with studies on anthocyanin enriched dairy beverages, where low-temperature storage was shown to enhance pigment stability (Ścibisz et al., 2019; Hapuarachchi et al., 2023).

6.5.4.2.3. Free fatty acid and thiobarbituric acid

Free fatty acid (FFA) content is indicative of lipid hydrolysis and is closely associated with the oxidative stability of the food products (Singh et al., 2024). During storage, FFA content increased progressively, reflecting enzymatic and microbial activity. At 5°C, FFA content from an initial value of 0.53 to increased up to 0.72 to 0.94%, and at 25°C, the highest FFA content was observed (**Table 6.48**). The higher rate of FFA formation at elevated temperatures is attributed to the activity of lipases and other hydrolytic enzymes, which are more active under warmer conditions (Yadav et al., 2007; Singh et al., 2024). The controlled environment of lower temperature effectively slowed down lipid hydrolysis, maintaining lower FFA levels and ensuring better sensory quality by minimizing off-flavors and rancidity development (Vaseji et al., 2012). A similar trend was observed in free fatty acid content increased from 0.56 to 0.85 µg/g in synbiotic lassi samples respectively during storage of 28 days (Sharma et al., 2015).

Thiobarbituric acid (TBA) value is a measure of secondary lipid oxidation products, particularly malondialdehyde, which contributes to rancidity and off-flavors. Over the storage period, TBA values increased across all selected storage conditions, with the rate of increase being temperature-dependent. The highest TBA value was observed under 25°C (0.07 mg MDA/kg) followed by the 15°C (0.05 mg MDA/kg) and lowest observed 5°C (0.05 mg MDA/kg), respectively (**Table 6.48**). Our findings values align with the long-life kheer ranging from 0.073 to 0.081 stored under 37°C (Jha et al., 2011). The accelerated increase in TBA values at higher temperatures highlights the role of thermal stress in promoting lipid oxidation (Jain et al., 2014; Shiby et al., 2017). The lower temperature condition effectively minimized this process, preserving the sensory and oxidative stability of the beverage-optimized product (Jain et al., 2014).

6.5.4.3. Antioxidant activities

The antioxidant activity such as (DPPH and FRAP) measured during storage, demonstrated significant ($P<0.05$) changes during the storage period in optimized microgreen-based dairi (lassi) beverage (**Table 6.49**). DPPH activity, indicative of free radical scavenging potential, initially antioxidant activity DPPH was 83.19% were observed and end of the day 15 declining to 74.30% (5°C) > 71.84% (15°C) and > 70.14% (25°C) by Day 15. A similar trend was observed for antioxidant activity FRAP, at 25°C , FRAP activity dropped at 8.67 $\mu\text{mol TE/g}$, and at 5°C dropped to 10.52 $\mu\text{mol TE/g}$ by the end of storage (**Table 6.49**). The dahi were prepared from pea milk and jamun seed extract, were the antioxidant activity DPPH 80.1 to declined up to 54.3% under refrigeration temperature (4 to 7°C) for 21 days (Pandhi & Poonia, 2021). In fruit-fortified probiotic buttermilk (lassi), DPPH radical scavenging, and FRAP assay the scavenging decreased from 64.22 to 38.93%, and in FRAP assay the optical density decreased from 0.657 ± 0.02 to 0.423 ± 0.01 during storage up to 15 days were observed (Kumar & Kumar, 2016). The more rapid decline at 25°C temperature highlights the thermal sensitivity of antioxidant compounds (ascorbic acid, total phenols, and flavonoids), which undergo degradation under oxidative stress (Birwal et al., 2024). The correlation between DPPH and FRAP values and phenolic contents further underscores the importance of bioactive compounds in maintaining antioxidant capacity.

6.5.4.4. Microbial parameters

Microbial quality is an important factor influencing the safety, shelf life, and sensory attributes of dairy-based beverages. In this study, microbial parameters were assessed through total plate count (TPC) and yeast and mold counts. Storage conditions, particularly temperature (5°C to 25°C , significantly ($P<0.05$) affected microbial growth as shown in (**Table 6.49**). The TPC progressively increased during the storage period. At 5°C , TPC rose modestly from 3.38 to 5.69 log CFU/mL on day 15, at 15°C from 3.38 to 6.18 log CFU/mL, and at 25°C from 3.38 to 6.44 log CFU/mL by Day 15. In contrast, at 15°C and 25°C , TPC increased more rapidly as compared to the 5°C storage temperature condition. The slower growth rate at 5°C reflects the inhibitory effect of refrigeration on microbial proliferation, as low temperatures suppress metabolic activity and replication of spoilage microorganisms. The addition of different levels of orange juice significantly ($P<0.05$) influenced the standard plate count (SPC) in the lassi samples during all the days storage period were observed, the SPC count of 5.99 to 7.34×10^7 cfu/g was observed under 7 days storage period (Jadhav et al., 2014). In another study, the SPC count of turmeric lassi in storage showed an increased from the initial day to the 9th day of storage whereas 4.78 to 8.60 log₁₀cfu/ml during 9 days storage period (Maji et al., 2018).

Somewhat similar findings were also reported by Shekh et al. (2009) in Lassi sold in Kolhapur city, from 6.75 to 13.17 x10⁷ cfu/ml observed in six different lassi samples (Munde et al., 2017).

Table 6.49. Effect of storage temperature conditions on antioxidant and microbial parameters of optimized microgreen-based dairy (lassi) beverage

Days	Temperature (°C)	Antioxidant activity DPPH (%)	Antioxidant activity FRAP (μmol TE/g)	Total plate count (log cfu/ml)	Yeast and mold (log cfu/ml)
Day-0	5	83.19±0.24 ^{aA}	19.12±0.19 ^{aA}	3.38±0.07 ^{fA}	2.17±0.08 ^{hB}
	15	83.19±0.24 ^{aB}	19.12±0.19 ^{aB}	3.38±0.07 ^{fB}	2.17±0.08 ^{hB}
	25	83.19±0.24 ^{aC}	19.12±0.19 ^{aC}	3.38±0.07 ^{fB}	2.17±0.08 ^{hA}
Day-3	5	81.14±0.17 ^{bA}	18.32±0.19 ^{bA}	3.91±0.11 ^{efA}	2.17±0.08 ^{hB}
	15	79.84±0.54 ^{bcdB}	18.12±0.09 ^{bcB}	4.03±0.13 ^{efB}	2.20±0.07 ^{hB}
	25	79.54±0.17 ^{cdC}	17.22±0.23 ^{cC}	4.08±0.13 ^{efB}	2.50±0.07 ^{hA}
Day-6	5	80.59±0.81 ^{bcA}	15.92±0.37 ^{dA}	4.04±0.21 ^{efA}	2.22±0.05 ^{ghB}
	15	77.94±0.45 ^{deB}	16.17±0.16 ^{dB}	4.98±0.06 ^{cdB}	2.30±0.07 ^{ghB}
	25	77.29±0.18 ^{efC}	15.57±0.26 ^{dC}	4.93±0.08 ^{dB}	2.80±0.07 ^{efgA}
Day-9	5	79.24±0.31 ^{cdA}	15.42±0.33 ^{dA}	4.54±0.50 ^{deA}	2.91±0.04 ^{efB}
	15	77.09±0.10 ^{efB}	12.42±0.19 ^{eB}	5.68±0.22 ^{bcB}	2.90±0.07 ^{efB}
	25	75.79±0.52 ^{fgC}	11.52±0.19 ^{eC}	5.89±0.15 ^{abB}	3.30±0.21 ^{deA}
Day-12	5	77.29±0.38 ^{efA}	12.32±0.19 ^{eA}	5.09±0.28 ^{cdA}	3.86±0.11 ^{bB}
	15	73.74±0.40 ^{hB}	10.47±0.26 ^{hB}	5.88±0.08 ^{abB}	3.90±0.07 ^{cdB}
	25	73.24±0.11 ^{hiC}	9.57±0.12 ^{ghC}	6.14±0.08 ^{abB}	4.66±0.29 ^{cA}
Day-15	5	74.30±0.25 ^{ghA}	10.52±0.19 ^{fA}	5.69±0.29 ^{bcA}	4.69±0.42 ^{bB}
	15	71.84±0.25 ^{ijB}	9.52±0.33 ^{hB}	6.18±0.06 ^{abB}	4.90±0.07 ^{bB}
	25	70.74±0.82 ^{jC}	8.67±0.30 ^{hC}	6.44±0.06 ^{aB}	5.91±0.06 ^{aA}

Note: The results were presented as mean±standard deviations (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments and different uppercase letters within the column indicate significant (P<0.05) differences in temperature condition for the parameters

The observed differences in TPC between storage conditions highlight the importance of temperature control in extending shelf life. At 5°C, the microbial load remained within acceptable limits for dairy beverages, preserving both safety and sensory quality (Kumar et al., 2024). The values of the total count in lassi beverage were lower than 10⁶ cell/mL established by microbiological quality guidelines for ready-to-eat food (Fikry et al., 2020), suggesting that optimized lassi are microbiologically stable during the shelf-life period. It might be due to the high content of antioxidants in the lassi which can be used as a preservative material.

Yeast and mold counts, indicative of fungal contamination, also increased during storage. The results for yeast and mold counts are presented in (Table 6.49), the highest yeast and mold count was observed at 25°C, counts rose from an initial 2.17 log CFU/mL to 5.91 log CFU/mL by day 15. However, at 5°C, yeast and mold counts were least observed, reaching 4.69 log CFU/mL by the end of the storage period (Table 6.49). The accelerated growth at higher temperatures is consistent with the favorable conditions provided by warm

environments for fungal proliferation. Yeast and molds contribute to off-flavors, texture degradation, and potential spoilage of dairy beverages. The lower counts observed under refrigerated conditions indicate the efficacy of low-temperature storage in mitigating fungal contamination, thereby preserving the product sensory and microbiological quality. Similar kind of results were reported for the different types of lassi beverage, including lassi prepared from orange juice (Jadhav et al., 2014), sweetened lassi (George et al., 2010), and symbiotic lassi containing honey (Sharma et al., 2015).

6.5.5. Kinetic modeling of different parameters and shelf-life model

The kinetic modeling of quality parameters provides a mathematical framework to understand the rate and mechanisms of changes occurring in the microgreen-based dairy (lassi) beverage during storage. This section explores the kinetics of key parameters, including overall acceptability, acidity (lactic acid %), free fatty acid content, thiobarbituric acid (TBA) value, and microbial parameter (total plate count), varying with storage period and temperature were determined by fitting the experimental data to zero, first, and second-order reaction models. The best-fitted model was identified based on the R^2 value as shown in (Table 6.50). A best-fitted model would have the maximum R^2 value. After analyzing the R^2 values, the zero-order model got the maximum ($R^2 = 0.951$ to 0.997), followed by the first-order model ($R^2 = 0.955$ to 0.991) and the second-order reaction model got the minimum ($R^2 = 0.883$ to 0.981) value observed for selected parameters. Therefore, it is inferred that the change in quality parameters was best represented by the zero-order model and poorly by the second-order model. Similarly, numerous prior investigations employed the zero-order reaction model to characterize the deterioration of food quality (Singh et al., 2024; Kumar et al., 2024).

The overall acceptability, acidity, free fatty acid, thiobarbituric acid, and total plate count value increased while storage with temperature, which explains the positive k of selected parameters for the zero-order reaction. The temperature significantly affected the reaction rate, suggesting that it's essential to store the optimized microgreen-based dairy (lassi) beverage. It was consistent with the earlier research findings that the absolute k values enhanced with temperature for acidity and sensory evaluation (Zhi et al., 2017), free fatty acid, and thiobarbituric acid (Kumar & Mishra, 2004), it can also be inferred that the quality degradation was rapid at 25°C compared to 5°C storage conditions. The degradation of selected parameters at 25°C was 3 to 6 times higher than at 5°C .

Table 6.50. Reaction order estimation of quality indices based on the coefficient of determination (R^2) from the zero-, first- and second-order reactions.

Quality indices	T (°C)	Zero order			First order		Second order	
		k	R^2	$t_{1/2}$	k	R^2	k	R^2
Overall acceptability	5	0.012	0.975	21.875	0.019	0.981	0.031	0.981
	10	0.019	0.985	13.815	0.028	0.974	0.044	0.957
	25	0.026	0.997	9.83	0.037	0.989	0.053	0.966
Acidity	5	0.025	0.979	12.696	0.016	0.955	0.002	0.941
	10	0.032	0.991	10.015	0.023	0.960	0.003	0.952
	25	0.038	0.951	8.442	0.024	0.969	0.003	0.960
Free fatty acid	5	0.012	0.975	21.875	0.019	0.981	0.031	0.981
	10	0.019	0.985	13.815	0.028	0.974	0.044	0.957
	25	0.026	0.997	9.831	0.037	0.989	0.053	0.966
Thiobarbaturic acid	5	0.001	0.985	10.576	0.036	0.985	0.998	0.968
	10	0.001	0.983	7.638	0.045	0.991	1.176	0.972
	25	0.002	0.985	5.5	0.056	0.984	1.345	0.947
Total plate count	5	0.148	0.975	11.403	0.016	0.955	0.007	0.982
	10	0.192	0.946	8.774	0.023	0.960	0.008	0.883
	25	0.213	0.957	7.915	0.024	0.969	0.009	0.898

Note: R^2 , coefficient of determination; k, rate of reaction; T: temperature

The decline in sensory quality followed zero-order kinetics, with R^2 values ranging between 0.975 to 0.997, indicating a strong correlation between time, storage temperature, and sensory degradation. The Arrhenius model revealed an activation energy of 9.913 kJ/mol for sensory decline, underscoring the temperature sensitivity of overall acceptability. The findings emphasize that refrigeration significantly slows sensory degradation, preserving consumer acceptability for a longer duration. The zero-order kinetic model provided an excellent fit for acidity data, with R^2 values exceeding 0.97. The rate constant (k) for acidity lower was 0.025 day⁻¹ at 5°C compared to 0.038 day⁻¹ at 25°C. The activation energy for acidity changes was calculated as 12.755 kJ/mol, indicating moderate temperature sensitivity. The temperature condition (5°C) effectively moderated the rate of acidification, ensuring that acidity levels remained within acceptable sensory limits. Excessive acidification, as observed at 25°C, negatively impacted sensory balance and shelf life. The zero-order kinetic model accurately described FFA formation, with R^2 values ranging from 0.975 to 0.997. The activation energy for FFA generation was calculated as 25.049 kJ/mol, reflecting high sensitivity to temperature. Excessive accumulation of FFA contributes to off-flavors and compromises sensory quality. The TBA value, which measures malondialdehyde and other secondary lipid oxidation products, is a critical parameter for assessing oxidative stability. TBA values increased during storage, reflecting the progression of lipid peroxidation. The zero-order kinetic model

demonstrated high predictive accuracy for TBA formation, with R^2 values exceeding 0.95. The rate constant for TBA increase was 0.007 day^{-1} at 5°C compared to 0.016 day^{-1} at 25°C . The activation energy for TBA generation was calculated as 20.991 kJ/mol , highlighting the temperature-sensitive nature of oxidative spoilage. Elevated TBA levels result in the development of off-flavors and reduced overall acceptability. The refrigeration temperature (5°C) significantly reduced TBA formation, thereby preserving oxidative stability and sensory quality. Another parameter, the total plate count increase was 0.213 day^{-1} at 25°C compared to 0.148 day^{-1} at 5°C . The activation energy for TBA generation was calculated as 10.905 kJ/mol .

Table 6.51. Kinetic parameters of quality indices during storage obtained by the Arrhenius and Eyring models.

Parameters	Arrhenius model		Eyring model		
	Ea (kJ/mol)	R ²	ΔH (kJ/mol)	ΔS (J/(mol.k))	R ²
Overall acceptability	9.913	0.5312	7.514	-201.9	0.3935
Acidity	12.755	0.8756	10.356	-202.304	0.8217
Free fatty acid	25.049	0.8806	22.651	-197.683	0.8573
Thiobarbaturic acid	20.991	0.9318	18.593	-201.7084	0.9142
Total plate count	10.905	0.7589	8.507	-201.323	0.6557

Note: R^2 , coefficient of determination; ΔH , enthalpy of activation; ΔS , entropy of activation

The quality parameters free fatty acid content obtained the maximum value of Ea. The values of ΔH closely approximated those of Ea. Further, the entropy (ΔS) and activation enthalpy (ΔH) determined from the Eyring model for various quality parameters have been shown in (Table 6.51). At 5°C , by using the Arrhenius equation (Table 6.50), the shelf-life prediction of microgreen-based dairy (lassi) beverage was 21.875, 12.696, 21.875, 10.576, and 11.403 days based on Overall acceptability, acidity, FFA, TBA, and total plate count, respectively. The kinetic modeling of quality indices, including overall acceptability, acidity, FFA content, TBA values, and microbial parameters, highlighted the pivotal role of storage temperature in determining the rate of changes. Refrigeration at 5°C effectively minimized degradation processes and microbial proliferation, extending the shelf life of the microgreen-based dairy beverage. These findings underscore the critical importance of temperature control in preserving the functional, sensory, and microbial quality of innovative dairy products.

6.5.6. Optimized product cost

The production cost of the developed product was estimated to be ₹36.10 per 200 mL, making it highly competitive with commercial lassi products available in the market, which are typically priced at ₹30 to ₹40 per 200 mL in glass bottles (Table 6.52). Therefore, from an economic perspective, the product is highly feasible.

Table 6.52. The optimized microgreen-based dairy (lassi) beverage cost estimation

Ingredients	Amount/ 1 kg	Cost (₹)
Microgreen powder	25.00 g	35.38
Dahi	700.00 g	49.00
CMC	2.50 g	8.47
Sugar	120.00 g	10.20
Water	152.50 mL	1.20
Packaging cost		15.00
Processing cost		119.2
Marketing and distribution expenses at 25% of product		119.25+29.81=149.06
Profit margin at 30% of cost of product		149.06+31.45=180.51
Total		180.51
Production cost for 200 mL		36.10

6.6. Optimization of variables for the microgreen-based RTS beverage

The optimization of variables for the microgreen-based RTS beverage was carried out using the D-optimal design model, which focused on different components including pomegranate juice (A), microgreen powder (B), and water (C) alongside 5% sugar and 0.5% CMC and 17 runs (n=3) treatments of tests. These were evaluated for their combined effects on sensory attributes, physicochemical and antioxidant properties, and the obtained results were presented in (Table 6.53, Table 6.54, and Table 6.55). The optimal formulation was predicted and validated through experiments. The optimized formulation included 50% pomegranate juice, 2% microgreen powder, and 42.5% water. This combination provided the highest desirability ramp (Figure 6.41) indicating an optimization score of 0.893, affirming the model reliability (Table 6.59). If the desirability value is below 0.37, the product is considered unacceptable. A desirability score of 0.893 demonstrated that the optimized product met 89.3% of the optimization target. In optimization, the aim is not to achieve a desirability value of 1, but to identify the optimal solution among the interactive variables (Putradamni & Pramitasari, 2024). In (Table 6.56), both the observed and predicted responses are presented. This approach highlights the effective integration of statistical modeling with biochemical and sensory parameters to develop a functional beverage with optimized attributes.

6.6.1. Effect of processing variables on sensory parameters

Sensory evaluation is an important parameter of consumer acceptability. In this study, sensory parameters (color & appearance, flavor & sweetness, consistency, mouthfeel, and overall acceptability) were evaluated for microgreen-based RTS beverages prepared using varying proportions of pomegranate juice (A), microgreen powder (B), and water (C).

Table 6.53. The effect of constituents on biochemical and antioxidant parameters of microgreen-based RTS beverage

Treatments	Pomegranate juice (%)	Microgreen powder (%)	Water (%)	Anthocyanin content ($\mu\text{mol}/100\text{g}$)	Total phenol content (mg GAE/100g)	Total flavonoid content (mg QE/100g)	Antioxidant activity DPPH (%)	Antioxidant activity FRAP ($\mu\text{mol TE/g}$)	Ascorbic acid (mg/100g)
1	50.00	2.00	42.50	48.63	55.82	101.33	86.89	18.41	210.46
2	48.31	1.63	44.56	43.2	54.78	89.18	85.17	16.76	207.32
3	42.50	2.00	50.00	41.22	47.66	93.77	82.92	18.79	213.39
4	42.50	2.00	50.00	41.22	47.96	93.98	82.83	18.67	212.93
5	50.00	1.25	43.25	44.12	57.94	97.11	84.73	16.09	199.78
6	46.63	1.25	46.63	38.92	57.24	78.72	83.82	14.48	207.59
7	45.31	0.88	48.31	36.57	56.48	74.19	82.13	12.99	208.51
8	44.00	0.50	50.00	36.11	55.01	69.25	79.68	13.32	209.05
9	46.25	2.00	46.25	43.26	56.33	83.59	83.63	19.92	212.21
10	44.56	1.63	48.31	39.69	55.25	85.45	83.94	16.84	210.75
11	50.00	2.00	42.50	48.63	55.82	102.12	86.71	18.41	210.46
12	43.25	1.25	50.00	38.12	55.21	90.75	83.78	15.12	209.52
13	46.25	2.00	46.25	43.26	56.33	85.89	83.69	19.98	212.21
14	47.00	0.50	47.00	35.74	54.18	57.48	78.98	12.37	205.02
15	50.00	0.50	44.00	40.69	53.56	75.21	78.25	13.23	195.78
16	44.00	0.50	50.00	39.8	52.01	69.95	78.68	12.66	209.16
17	50.00	0.50	44.00	40.6	53.56	76.66	78.35	13.81	193.71

Note: All the experimental results values are presented as mean value (n=3) basis based on each biochemical and antioxidant parameter. Where, GAE: Gallic acid equivalent; QE: Quercetin equivalent

The obtained results, as shown in (Table 6.46), reflect how processing variables significantly influenced these parameters. For the given study work, a special cubic mixture model was utilized to evaluate the combined effect of three interdependent variables (A, B, and C), along with any potential third-order effects that linear or quadratic models. The (Table 6.50) and (Table 6.51) represent the regression coefficients and F-values for the polynomial equations, indicating the suggested model adequacy, significant lack of fit, and satisfactory R^2 values; furthermore, R^2 -pred, which is typically lower than R^2 measures the model predictive performance on future observations. All models were regarded as suitable for analysis because their R^2 values were all above 90%.

Table 6.54. The effect of constituents on sensory parameters of microgreen-based RTS beverage

Treatments	Pomegranate juice (%)	Microgreen powder (%)	Water (%)	Colour & Appearance	Flavour & Sweetness	Consistency	Mouthfeel	Overall Acceptability
1	50.00	2.00	42.50	6.93	7.08	7.19	6.81	6.87
2	48.31	1.63	44.56	6.99	7.26	7.32	6.79	6.79
3	42.50	2.00	50.00	6.53	7.14	7.02	6.48	6.57
4	42.50	2.00	50.00	6.68	7.14	7.12	6.55	6.57
5	50.00	1.25	43.25	7.28	7.6	7.19	7.07	7.03
6	46.63	1.25	46.63	7.21	7.42	7.43	6.88	6.82
7	45.31	0.88	48.31	7.45	7.64	7.45	7.16	7.09
8	44.00	0.50	50.00	7.66	7.95	7.33	7.68	7.66
9	46.25	2.00	46.25	6.54	7.01	7.39	6.63	6.78
10	44.56	1.63	48.31	6.83	7.27	7.19	6.66	6.64
11	50.00	2.00	42.50	6.93	7.08	7.25	6.77	6.87
12	43.25	1.25	50.00	7.06	7.6	6.88	6.91	6.78
13	46.25	2.00	46.25	6.54	6.96	7.39	6.63	6.88
14	47.00	0.50	47.00	7.78	7.85	7.96	7.53	7.58
15	50.00	0.50	44.00	7.64	7.95	7.74	7.5	7.87
16	44.00	0.50	50.00	7.56	8.02	7.69	7.66	8.16
17	50.00	0.50	44.00	7.68	8.06	8.00	7.83	8.33

Note: All the experimental results values are presented as mean value (n=3) basis based on each sensory parameter

The colour and appearance scores for microgreens-based RTS beverage, ranging from 6.53 to 7.78 were observed in different formulations (Table 6.58). The response surface plot (Figure 6.42 (A)) indicated that higher levels of pomegranate juice (%) significantly enhanced the redness and vibrancy of the beverage due to the abundant anthocyanins present in pomegranate (Yue et al., 2024). Radish sango microgreen powder also contributed to the visual attributes by imparting a slight greenish hue, indicative of chlorophyll and carotenoid pigments

(Vučetić et al., 2024). However, excessive microgreen powder (>1.5%) led to a darker and less appealing color due to particle aggregation and reduced light reflectance. Apart from these, the water proportion played a moderating role by diluting the color intensity, especially in formulations with higher water content ($\geq 50\%$) used during RTS beverage preparation. The interaction between pomegranate juice and microgreen powder was significant ($P < 0.05$), as confirmed by ANOVA (**Table 6.58**). The regression model in (**Table 6.57**) confirms that the linear and interaction effects of microgreen powder and water significantly ($P < 0.05$) impacted the beverage color and appearance parameter. Similarly, in the different formulations of the novel drinks powder produced with different concentrations of pumpkin pulp and seed powder, the colour score within the range of 7.5 to 8.7 was observed (Halim et al., 2024). In another study, the whey-fruit-based beverages prepared from the red beetroot peel (1 to 6%) showed a similar trend for the colour and appearance prepared product (Stoica et al., 2024).

Table 6.55. The effect of constituents on physicochemical parameters of microgreen-based RTS beverage

Treatments	Pomegranate juice (%)	Microgreen powder (%)	Water (%)	TSS (°Brix)	pH	Acidity (%)
1	50.00	2.00	42.50	12.6	3.68	1.13
2	48.31	1.63	44.56	11.84	3.76	1.08
3	42.50	2.00	50.00	11.77	3.85	1.14
4	42.50	2.00	50.00	11.79	3.85	1.14
5	50.00	1.25	43.25	11.19	3.63	1.14
6	46.63	1.25	46.63	11.39	3.83	1.09
7	45.31	0.88	48.31	11.07	3.83	1.04
8	44.00	0.50	50.00	10.7	3.73	1.02
9	46.25	2.00	46.25	12.43	3.83	1.15
10	44.56	1.63	48.31	11.45	3.84	1.09
11	50.00	2.00	42.50	12.69	3.68	1.16
12	43.25	1.25	50.00	10.56	3.76	1.11
13	46.25	2.00	46.25	12.43	3.83	1.13
14	47.00	0.50	47.00	11.59	3.83	1.02
15	50.00	0.50	44.00	10.9	3.64	1.06
16	44.00	0.50	50.00	11	3.75	1.01
17	50.00	0.50	44.00	11.7	3.64	1.07

Note: All the experimental results values are presented as mean value (n=3) basis based on each sensory parameter.

Flavour and sweetness scores ranging from 6.96 to 8.06 were obtained from the prepared microgreen-based RTS beverage as shown in (**Table 6.54**), with the highest scores observed in formulations containing 50% pomegranate juice and 0.5% microgreen powder. Pomegranate juice contributed natural sweetness and a fruity flavor profile (Shahkoomahally et al., 2023), while microgreen powder added a subtle earthy and peppery flavor (Xiao et al.,

2015). Excessive amounts of microgreen powder resulted in bitterness, which negatively impacted flavor scores. The response surface plot (**Figure 6.42 (B)**) revealed that pomegranate juice had a predominant effect on sweetness, masking the inherent bitterness of microgreens. ANOVA results (**Table 6.58**) showed significant interactions between pomegranate juice and microgreen powder ($P < 0.05$), emphasizing the importance of balance in the formulation. Lower water content further enhanced the flavor intensity by reducing dilution. The addition of sugar (5%) and CMC stabilizer (0.5%) also played crucial roles in enhancing flavor.

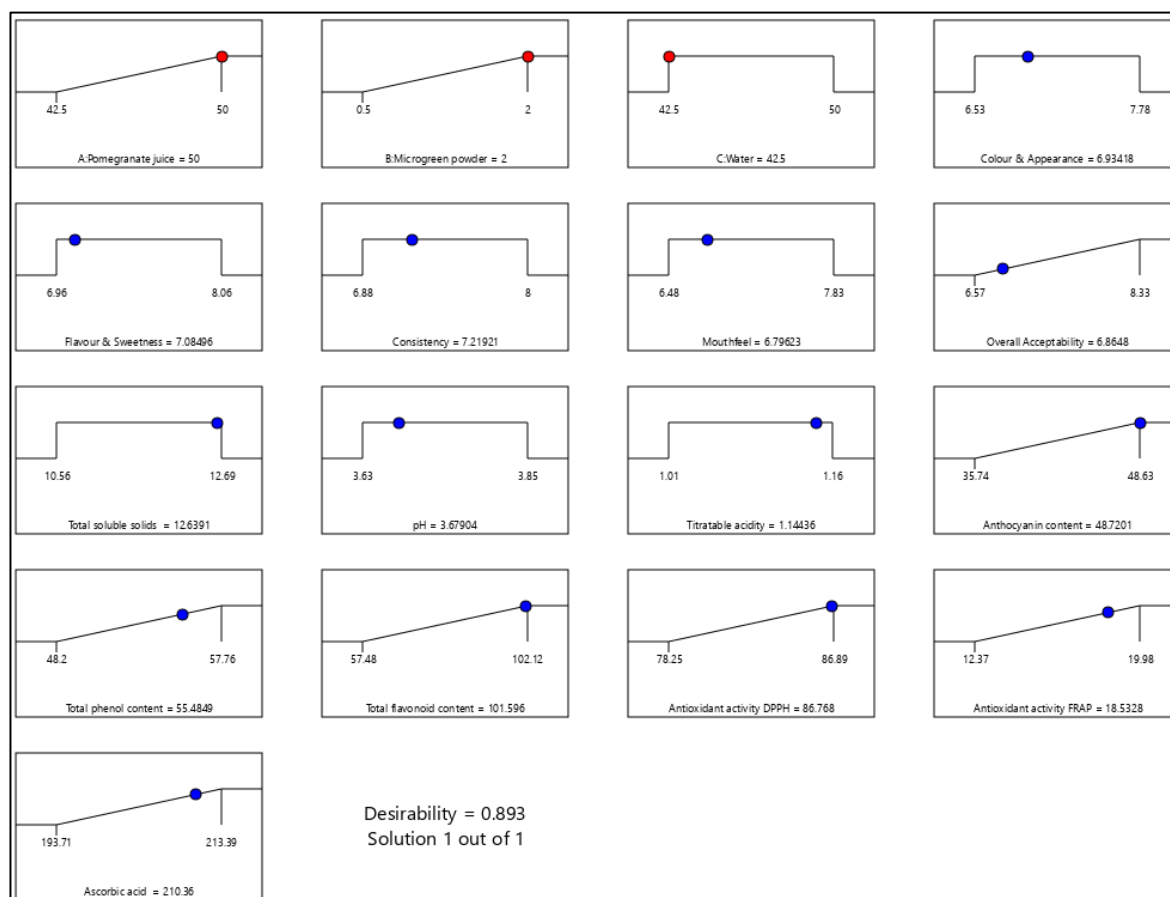


Figure 6.41. Desirability ramp for optimization of microgreen-based RTS beverage

Another sensory parameter is consistency and scores for microgreen-based RTS beverage ranged from 6.88 to 8.00 were observed (**Table 6.54**). CMC stabilizer was critical in achieving desirable consistency by increasing the viscosity of the beverage, thus preventing sedimentation of microgreen powder particles (Liu et al., 2020). The response surface plot (**Figure 6.42 (C)**) highlighted the synergistic effect of pomegranate juice and water on consistency. While pomegranate juice contributed natural pectin, enhancing viscosity, excessive water ($>47\%$) diluted the beverage, reducing its perceived thickness. On the other hand, microgreen powder at moderate levels provided a smooth texture, while higher concentrations resulted in grittiness due to undissolved particles. ANOVA results (**Table 6.58**)

indicated significant interactions ($P<0.05$) between pomegranate juice, microgreen powder, and water. The Ready-to-Drink (RTD) beverage was prepared from the whey and beetroot juice and the consistency score was 7.8 to 8.3 observed, this trend was similar to our finding results (Theba et al., 2024).

Table 6.56. Predicted and observed optimum responses for microgreen-based RTS beverage

Responses	Predicted values	Observed values
Pomegranate juice (%)	50	
Microgreen powder (%)	2	
Water (%)	42.5	
Colour & Appearance	6.934	7.17±0.22
Flavour & Sweetness	7.085	7.26±0.13
Consistency	7.219	7.36±0.34
Mouthfeel	6.796	7.04±0.28
Overall acceptability	6.865	7.27±0.21
Total soluble solids (°Brix)	12.639	12.65±0.01
pH	3.679	3.65±0.01
Titrateable acidity (%)	1.144	1.15±0.00
Anthocyanin content (µmol/100g)	48.72	48.87±0.11
Total phenol content (mg GAE/100g)	55.515	55.93±0.11
Total flavonoid content (mg GAE/100g)	101.596	102.09±0.34
Antioxidant activity DPPH (%)	86.768	86.92±0.08
Antioxidant activity FRAP (µmol TE/g)	18.533	18.56±0.15
Ascorbic acid (mg/100g)	210.36	210.63±0.53

The obtained results from experiments are represented in mean±standard deviation values with displayed significant differences ($P<0.05$).

Mouthfeel scores ranged from 6.48 to 7.83 were observed among the prepared formulation of microgreen-based RTS beverage (**Table 6.54**), with the highest scores observed in formulations containing 50% pomegranate juice, 0.5% microgreen powder, and 42.5% water. Excessive microgreen powder percentage ($>1.5\%$) negatively impacted mouthfeel by introducing grittiness and an astringent sensation. Similarly, lower water content ($<42\%$) resulted in an overly thick and heavy beverage, which reduced consumer preference. The interactions between pomegranate juice, microgreen powder, and water were statistically significant ($P<0.05$) (**Table 6.58**).

Overall acceptability reflects the cumulative impact of sensory attributes and serves as a holistic measure of consumer preference. As seen in (**Table 6.54**), the overall acceptability scores ranged from 6.57 to 8.33 on the 9-point hedonic scale. The regression equation (R^2) accounted for 95.15% of the total variation in the overall acceptability of the microgreen-based RTS beverage. The quadratic models for sensory parameters (**Table 6.57**) exhibited significant effects of pomegranate juice, microgreen powder, and water on overall acceptability. The

addition of the CMC stabilizer further enhanced the overall acceptability by maintaining the physical stability and consistency of the RTS beverage. A similar trend was also found by Halim et al. (2024) in the preparation of novel drinks powder produced with different concentrations of pumpkin pulp and seed powder, the overall acceptability score within the range of 7.00–8.30 was observed.

6.6.2. Effect of processing variables on physicochemical parameters

The physicochemical parameters, including pH, acidity, and total soluble solids (TSS), were significantly influenced by processing variables such as the proportions of pomegranate juice, microgreen powder, and water. The pH value of the microgreen-based RTS beverage ranged from 3.63 to 3.85 across different formulations were observed, respectively (**Table 6.55**). Pomegranate juice had a dominant influence on pH due to its inherent acidic nature, which is attributed to the presence of organic acids, primarily citric acid (Miguel et al., 2004). Higher proportions of pomegranate juice resulted in lower pH values, enhancing the acidic profile of the beverage. This acidic environment is beneficial for microbial stability, as it inhibits the growth of spoilage organisms and pathogens (Atasoy et al., 2023). The response surface plot (**Figure 6.42 (C)**) illustrates the interactive effects of these variables on pH. ANOVA results (**Table 6.58**) confirmed significant interactions between pomegranate juice and water ($P < 0.05$). Titratable acidity, expressed as a percentage of citric acid, ranged from 1.01% to 1.16% across the RTS beverage formulations (**Table 6.55**). Pomegranate juice was the primary contributor to acidity, with its organic acid content imparting a tangy and refreshing taste to the beverage. Higher levels of pomegranate juice significantly increased titratable acidity, enhancing the flavor intensity (Hasnaoui et al., 2010). The addition of microgreen powder at moderate levels did not significantly alter acidity. However, excessive microgreen powder introduced a slight buffering effect, reducing the perceived acidity. Water content influenced titratable acidity by diluting the acidic components, resulting in lower values in formulations with higher water content. ANOVA results (**Table 6.58**) indicated that the interaction between pomegranate juice and water was statistically significant ($P < 0.05$).

The TSS content of microgreen-based RTS beverage, measured in °Brix, ranged from 10.56 to 12.69 °Brix across the formulations (**Table 6.55**). Pomegranate juice was the primary contributor to TSS due to its natural sugar content, including glucose and fructose (Miguel et al., 2004). Higher proportions of pomegranate juice ($\geq 50\%$) resulted in increased TSS values, enhancing the perceived sweetness of the beverage. Microgreen powder had a negligible effect on TSS, as its contribution to soluble solids was minimal. Water content played a diluting role, with higher water added RTS beverage samples reducing TSS values.

Table 6.57. The predicted models to experimental data in D-optimal design for the effect of microgreen-based RTS beverage

Variables	Regression equation	Mean±S D	C.V. %	R ²	Adjusted R ²	Predicted R ²	Adeq precision
Colour & Appearance	$7.48 \times A - 5.91 \times B + 7.38 \times C + 12.17 \times AB + 1.45 \times AC + 10.29 \times BC - 16.29 \times ABC$	7.13±0.61	0.61	0.9937	0.9899	0.9987	44.47
Flavour & Sweetness	$8.23 \times A + 0.67 \times B + 8.19 \times C + 0.8207 \times AB - 1.52 \times AC + 1.48 \times BC + 4.75 \times ABC$	7.47±0.03	0.46	0.9921	0.9921	0.9829	46.35
Consistency	$7.38 \times A + 41.55 \times B + 7.01 \times C - 43.26 \times AB + 2.85 \times AC - 41.07 \times BC - 7.75 \times ABC$	7.38±0.10	1.45	0.9251	0.8801	0.9480	15.47
Mouthfeel	$7.86 \times A + 26.65 \times B + 7.85 \times C - 30.18 \times AB - 1.31 \times AC + 32.18 \times BC + 7.02 \times ABC$	7.03±0.07	1.09	0.9813	0.9700	0.9381	22.64
Overall Acceptability	$8.60 \times A + 64.21 \times B + 8.31 \times C - 79.22 \times AB - 3.33 \times AC - 79.67 \times 24.69 \times ABC$	7.13±0.15	2.20	0.9515	0.9223	0.8388	15.17
pH	$3.43 \times A + 4.07 \times B + 3.58 \times C + 1.05 \times AB - 1.35 \times AC + 1.37 \times BC - 5.73 \times ABC$	3.76±0.006	0.17	0.9957	0.9932	0.9820	49.20
Acidity	$1.12 \times A + 0.6851 \times B + 1.05 \times C + 0.6695 \times AB - 0.3121 \times AC + 1.10 \times BC + 1.35 \times ABC$	1.09±0.01	1.75	0.9073	0.8518	0.7573	10.99
Total soluble solids	$10.71 \times A + 94.22 \times B + 10.04 \times C - 86.32 \times AB + 4.98 \times AC - 88.50 \times BC - 21.71 \times ABC$	11.59±0.19	1.67	0.9461	0.9138	0.8216	16.53
Anthocyanin content	$45.64 \times A + 231.01 \times B + 41.17 \times C - 200.27 \times AB - 30.00 \times AC - 228.51 \times BC + 127.46 \times ABC$	41.16±0.87	2.13	0.9664	0.9462	0.8907	22.80
Total phenol content	$52.72 \times A - 247.43 \times B + 52.11 \times C + 379.90 \times AB + 6.43 \times AC + 331.90 \times BC + 89.80 \times ABC$	54.32±0.46	0.86	0.9817	0.9707	0.9396	31.82
Total flavonoid content	$95.71 \times A + 673.79 \times B + 86.87 \times C + 86.87 \times AB + 965.80 \times AC + 963.68 \times BC + 340.23 \times ABC$	83.80±0.93	1.11	0.9964	0.9943	0.9896	72.75
Antioxidant activity DPPH	$77.75 \times A - 211.65 \times B + 79.20 \times C + 412.20 \times AB + 1.98 \times AC + 375.75 \times BC - 53.66 \times ABC$	82.60±0.24	0.29	0.9954	0.9926	0.9851	54.64
Antioxidant activity FRAP	$15.68 \times A + 207.24 \times B + 14.57 \times C - 209.33 \times AB - 12.76 \times AC - 201.31 \times BC + 116.77 \times ABC$	15.99±0.37	2.37	0.9876	0.9801	0.9498	32.34
Ascorbic acid	$186.55 \times A + 392.14 \times B + 208.33 \times C - 75.28 \times AB + 31.83 \times AC + 185.74 \times BC - 172.79 \times ABC$	207.52±0.53	0.25	0.9948	0.9916	0.9812	54.44

Table 6.58. ANOVA special cubic model for the different responses

Responses	Source	Sum of squares	df	Mean square	F-value	P-value	
Colour & appearance	Model	3.02	6	0.503	263.420	< 0.0001	significant
	⁽¹⁾ Linear Mixture	2.90	2	1.450	758.980	< 0.0001	
	AB	0.02	1	0.019	9.670	0.011	
	AC	0.03	1	0.031	16.160	0.002	
	BC	0.01	1	0.013	6.860	0.026	
	ABC	0.07	1	0.072	37.520	0.000	
	Residual	0.02	10	0.002			not significant
	Lack of Fit	0.00	5	0.000	0.119	0.982	
	Pure Error	0.02	5	0.003			
	Cor Total	3.04	16				
Flavour & sweetness	Model	2.39	6	0.398	334.330	< 0.0001	significant
	⁽¹⁾ Linear Mixture	2.31	2	1.160	972.020	< 0.0001	
	AB	0.00	1	0.000	0.071	0.796	
	AC	0.03	1	0.034	28.600	0.000	
	BC	0.00	1	0.000	0.227	0.644	
	ABC	0.01	1	0.006	5.120	0.047	
	Residual	0.01	10	0.001			not significant
	Lack of Fit	0.00	5	0.000	0.220	0.939	
	Pure Error	0.01	5	0.002			
	Cor Total	2.40	16				
Consistency	Model	1.42	6	0.236	20.580	< 0.0001	significant
	⁽¹⁾ Linear Mixture	0.91	2	0.453	39.430	< 0.0001	
	AB	0.23	1	0.233	20.320	0.001	
	AC	0.12	1	0.119	10.340	0.009	
	BC	0.21	1	0.209	18.170	0.002	
	ABC	0.02	1	0.016	1.410	0.263	
	Residual	0.11	10	0.012			not significant
	Lack of Fit	0.01	5	0.002	0.089	0.991	
	Pure Error	0.11	5	0.021			
	Cor Total	1.53	16				
Mouthfeel	Model	3.09	6	0.515	87.360	< 0.0001	significant
	⁽¹⁾ Linear Mixture	2.90	2	1.450	246.310	< 0.0001	
	AB	0.11	1	0.114	19.260	0.001	
	AC	0.03	1	0.025	4.280	0.065	
	BC	0.13	1	0.128	21.730	0.001	
	ABC	0.01	1	0.013	2.250	0.164	

	Residual	0.06	10	0.006			
	Lack of Fit	0.00	5	0.000	0.018	1.000	not significant
	Pure Error	0.06	5	0.012			
	Cor Total	3.15	16				
Overall acceptability	Model	4.82	6	0.803	32.670	< 0.0001	significant
	⁽¹⁾ Linear Mixture	3.95	2	1.970	80.320	< 0.0001	
	AB	0.78	1	0.782	31.830	0.000	
	AC	0.16	1	0.163	6.620	0.028	
	BC	0.79	1	0.785	31.930	0.000	
	ABC	0.16	1	0.164	6.690	0.027	
	Residual	0.25	10	0.025			not significant
	Lack of Fit	0.01	5	0.002	0.042	0.998	
	Pure Error	0.24	5	0.047			
	Cor Total	5.06	16				
Total soluble solids	Model	6.58	6	1.100	29.260	< 0.0001	significant
	⁽¹⁾ Linear Mixture	4.91	2	2.450	65.450	< 0.0001	
	AB	0.93	1	0.929	24.780	0.001	
	AC	0.36	1	0.364	9.710	0.011	
	BC	0.97	1	0.969	25.850	0.001	
	ABC	0.13	1	0.127	3.390	0.095	
	Residual	0.37	10	0.038			not significant
	Lack of Fit	0.01	5	0.001	0.015	1.000	
	Pure Error	0.37	5	0.074			
	Cor Total	6.96	16				
pH	Model	0.11	6	0.018	388.450	< 0.0001	significant
	⁽¹⁾ Linear Mixture	0.07	2	0.033	726.010	< 0.0001	
	AB	0.00	1	0.000	3.030	0.112	
	AC	0.03	1	0.027	586.460	< 0.0001	
	BC	0.00	1	0.000	5.110	0.047	
	ABC	0.01	1	0.009	194.670	< 0.0001	
	Residual	0.00	10	0.000			not significant
	Lack of Fit	0.00	5	0.000	1.280	0.398	
	Pure Error	0.00	5	0.000			
	Cor Total	0.11	16				
Titrateable acidity	Model	0.04	6	0.006	16.320	0.000	significant
	⁽¹⁾ Linear Mixture	0.03	2	0.016	43.930	< 0.0001	
	AB	0.00	1	0.000	0.153	0.704	
	AC	0.00	1	0.001	3.890	0.077	

	BC	0.00	1	0.000	0.411	0.536	not significant
	ABC	0.00	1	0.001	1.340	0.274	
	Residual	0.00	10	0.000			
	Lack of Fit	0.00	5	0.001	3.890	0.081	
	Pure Error	0.00	5	0.000			
	Cor Total	0.04	16				
Anthocyanin content	Model	220.43	6	36.740	47.890	< 0.0001	significant
	⁽¹⁾ Linear Mixture	188.76	2	94.380	123.020	< 0.0001	
	AB	5.00	1	5.000	6.520	0.029	
	AC	13.18	1	13.180	17.180	0.002	
	BC	6.46	1	6.460	8.420	0.016	
	ABC	4.38	1	4.380	5.710	0.038	
	Residual	7.67	10	0.767			not significant
	Lack of Fit	0.86	5	0.172	0.126	0.980	
	Pure Error	6.81	5	1.360			
	Cor Total	228.10	16				
Total phenol content	Model	118.20	6	19.700	89.340	< 0.0001	significant
	⁽¹⁾ Linear Mixture	41.40	2	20.700	93.880	< 0.0001	
	AB	17.99	1	17.990	81.590	< 0.0001	
	AC	0.61	1	0.606	2.750	0.129	
	BC	13.62	1	13.620	61.780	< 0.0001	
	ABC	2.18	1	2.180	9.860	0.011	
	Residual	2.21	10	0.221			not significant
	Lack of Fit	0.26	5	0.053	0.136	0.976	
	Pure Error	1.94	5	0.388			
	Cor Total	120.41	16				
Total flavonoid content	Model	2437.98	6	406.330	466.820	< 0.0001	significant
	⁽¹⁾ Linear Mixture	1742.13	2	871.060	1000.730	< 0.0001	
	AB	116.27	1	116.270	133.580	< 0.0001	
	AC	258.95	1	258.950	297.500	< 0.0001	
	BC	114.85	1	114.850	131.950	< 0.0001	
	ABC	31.22	1	31.220	35.870	0.000	
	Residual	8.70	10	0.870			not significant
	Lack of Fit	4.43	5	0.886	1.040	0.485	
	Pure Error	4.28	5	0.855			
	Cor Total	2446.69	16				
Antioxidant activity DPPH	Model	127.13	6	21.190	360.660	< 0.0001	significant
	⁽¹⁾ Linear Mixture	96.20	2	48.100	818.750	< 0.0001	

	AB	21.18	1	21.180	360.510	< 0.0001	not significant
	AC	0.06	1	0.058	0.978	0.346	
	BC	17.46	1	17.460	297.200	< 0.0001	
	ABC	0.78	1	0.777	13.220	0.005	
	Residual	0.59	10	0.059			
	Lack of Fit	0.06	5	0.012	0.115	0.984	
	Pure Error	0.53	5	0.105			
	Cor Total	127.72	16				
Antioxidant activity FRAP	Model	113.72	6	18.950	132.290	< 0.0001	significant
	⁽¹⁾ Linear Mixture	106.36	2	53.180	371.190	< 0.0001	
	AB	5.46	1	5.460	38.130	0.000	
	AC	2.38	1	2.380	16.630	0.002	
	BC	5.01	1	5.010	34.980	0.000	
	ABC	3.68	1	3.680	25.670	0.001	
	Residual	1.43	10	0.143			
	Lack of Fit	1.04	5	0.208	2.630	0.156	not significant
	Pure Error	0.40	5	0.079			
	Cor Total	115.15	16				
Ascorbic acid	Model	537.37	6	89.560	317.190	< 0.0001	significant
	⁽¹⁾ Linear Mixture	420.85	2	210.420	745.240	< 0.0001	
	AB	0.71	1	0.707	2.500	0.145	
	AC	14.84	1	14.840	52.550	< 0.0001	
	BC	4.27	1	4.270	15.110	0.003	
	ABC	8.05	1	8.050	28.520	0.000	
	Residual	2.82	10	0.282			
	Lack of Fit	0.57	5	0.114	0.253	0.921	not significant
	Pure Error	2.25	5	0.451			
	Cor Total	540.19	16				

Table 6.59. Optimization criteria for different process and response variables for microgreen-based RTS beverage

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Pomegranate juice	Maximize	42.5	50	1	1	3
B: Microgreen powder	Maximize	0.5	2	1	1	4
C: Water	In range	42.5	50	1	1	3
Colour & Appearance	In range	6.53	7.78	1	1	3
Flavour & Sweetness	In range	6.96	8.06	1	1	3
Consistency	In range	6.88	8	1	1	3
Mouthfeel	In range	6.48	7.83	1	1	3
Overall Acceptability	Maximize	6.57	8.33	1	1	5
Total soluble solids	In range	10.56	12.69	1	1	5
pH	In range	3.63	3.85	1	1	3
Titratable acidity	In range	1.01	1.16	1	1	3
Anthocyanin content	Maximize	35.74	48.63	1	1	5
Total phenol content	Maximize	47.66	57.94	1	1	5
Total flavonoid content	Maximize	57.48	102.12	1	1	5
Antioxidant activity DPPH	Maximize	78.25	86.89	1	1	5
Antioxidant activity FRAP	Maximize	12.37	19.98	1	1	5
Ascorbic acid	Maximize	193.71	213.39	1	1	5

The ANOVA results (**Table 6.58**) confirmed the significance of interactions among pomegranate juice, microgreen powder, and water ($P < 0.05$).

The anola (10%) and kinnow (5%) based RTS beverage prepared, the physicochemical properties such as TSS (15.01 to 15.07°Brix), pH (3.46 to 3.56), and acidity (0.40 to 0.50%) were observed (Balaji & Prasad, 2014). In another study, the RTS beverage from papaya, ginger, and honey prepared and the pH value (2.17 to 2.76), acidity (0.20 to 1.05%), and TSS (13 to 13.50 °Brix) were observed (Debnath et al., 2022). Similarly, the ready-to-serve kinnow and amla-based beverage was prepared, the pH value (3.01 to 4.36), TSS (14.6 to 16.8 °Brix), and acidity (1.3 to 1.92%) were observed in different formulation (Purewal et al., 2022).

6.6.3. Effect of processing variables on biochemical and antioxidant parameters

6.6.3.1. Anthocyanin content

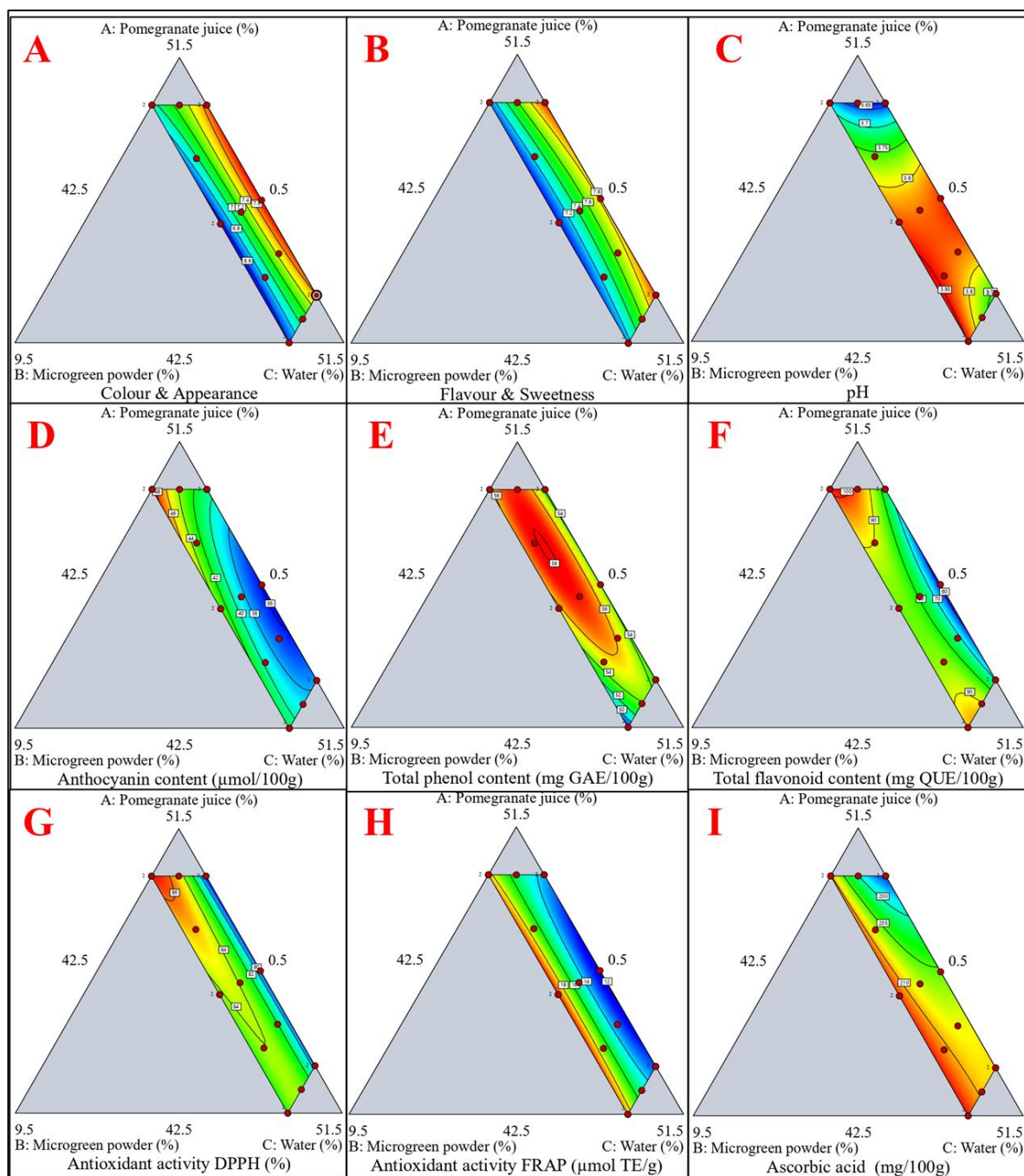


Figure 6.42. Response surface plots showing the effect of pomegranate juice (A), microgreen powder (B), water (C) on various study selected parameters of microgreen-based RTS beverage. Where, A: colour & appearance; B: flavour & sweetness; C: pH; D: anthocyanin content; E: total phenol content; F: Total flavonoid content; G: antioxidant activity DPPH; H: antioxidant activity FRAP; I: ascorbic acid

The anthocyanin content in microgreen-based RTS beverage ranged from 35.74 to 48.63 $\mu\text{mol}/100\text{g}$ was observed in across the formulations (**Table 6.53**). Pomegranate juice was the primary contributor to anthocyanin levels due to its rich natural pigments, which imparted the characteristic red color to the beverage (Alighourchi & Barzegar, 2008). The formulations with higher percentage of pomegranate juice (%) exhibited significantly higher anthocyanin

content, enhancing the beverage visual and nutritional appeal. Microgreen powder also contributed to anthocyanin content, albeit to a lesser extent. The synergistic effect of pomegranate juice and microgreen powder was evident, as confirmed by ANOVA results (**Table 6.58**). However, excessive water content diluted the anthocyanin concentration, reducing its antioxidant efficacy. The response surface plot (**Figure 6.42 (D)**) illustrates the interaction effects among the variables, emphasizing the importance of optimizing their proportions. The regression model equations shown in (**Table 6.57**) for anthocyanin content the variables A, B, C and ABC positive effect and AB, AC, and BC showing the negative effect and satisfactory R^2 values (96.64%); furthermore, R^2 -pred (89.07%), which is typically lower than R^2 measures the model predictive performance on future observations. The soymilk-strawberry RTS beverages prepared from the by adding different levels of soymilk (80 to 95%) and strawberry pulp (5 to 20%) the anthocyanin content was significantly increased with increasing the concentration of strawberry pulp (Verma et al., 2019).

6.6.3.2. Total phenolic and flavonoid content

The total phenolic and flavonoid content in different formulation of microgreen-based RTS beverage ranged from 47.66 to 57.94 mg GAE/100g and 57.48 to 102.12 mg QE/100g across the formulations were observed, respectively (**Table 6.53**). Phenolic and flavonoid compounds, primarily derived from pomegranate juice, play a vital role in the beverage enhancing antioxidant capacity, health benefits and stability (Saparbekova et al., 2022). Formulations of microgreen-based RTS beverage with higher pomegranate juice levels ($\geq 50\%$) exhibited increased phenolic content and flavonoids due to the presence of hydrolyzable tannins and ellagic acid derivatives (Siddiqui et al., 2024). Microgreen powder further enhanced the phenolic content, contributing bioactive compounds such as flavonoids and phenolic acids (quercetin and kaempferol) (Singh et al., 2024). However, addition of more amount of microgreen powder ($\geq 1.5\%$) showing bitterness, which could negatively impact sensory acceptability. The response surface plot (**Figure 6.42 (E and F)**) demonstrates the significant influence of pomegranate juice and microgreen powder on phenolic content. The selected variables shoeing the significant interactions ($P < 0.05$) on total phenol and flavonoid content of microgreen based RTS beverage (**Table 6.58**). The (**Table 6.57**) and (**Table 6.58**) represent the regression coefficients and F-values for the polynomial equations, indicating the suggested model adequacy, significant lack of fit, and satisfactory R^2 values; furthermore, R^2 -pred, which is typically lower than R^2 measures the model predictive performance on future observations. The special cubic model for total phenol and flavonoid content were regarded as suitable for analysis because their R^2 values were all above 98%. Adhikari & Joshi, (2023)

prepared the wheatgrass microgreen juice based RTS beverage, the total phenol (30.18 mg/g) and flavonoids (9.78 mg/g) were observed. The RTS beverage were prepared from kinnow and amla juice and the total phenol content in different formulation 51.29 to 71.35 mg GAE/100 ml observed (Purewal et al., 2022). In another study, sapodilla based RTS beverage prepared and the total phenol (359.12 to 389.00 μg GAE/100 mL) and flavonoid content (111.48 to 492.59 μg CE/g) were observed (Fiaz et al., 2022).

6.6.3.3. Ascorbic acid content

The ascorbic acid content of the microgreen-based RTS beverage ranged from 16.42 to 22.37 mg/100g across the formulations (**Table 6.53**). Pomegranate juice served as the primary source of ascorbic acid, a vital antioxidant known for its role in neutralizing free radicals and enhancing immune function (Asgary et al., 2014). The incorporation of pomegranate juice ($\geq 50\%$) significantly increased the ascorbic acid content. Apart from these, microgreen powder, particularly at moderate levels ($\leq 1\%$), further enhanced the ascorbic acid content due to its rich vitamin C reserves (Singh et al., 2024). Water content played a diluting role, with higher water levels ($\geq 47\%$) reducing the overall concentration of ascorbic acid. The response surface plot (**Figure 6.42 (I)**) highlights the interaction effects between pomegranate juice and microgreen powder and ANOVA results (**Table 6.58**) confirmed significant interactions ($P < 0.05$) among the variables. The regression model equations shown in (**Table 6.57**) for ascorbic acid content the variables A, B, C, AC and BC positive effect and AB, and ABC showing the negative effect and satisfactory R^2 values (98.76%); furthermore, R^2 -pred (98.12%), which is typically lower than R^2 measures the model predictive performance on future observations. Adhikari & Joshi, (2023) prepared the wheatgrass microgreen juice based RTS beverage, the ascorbic acid (14.85 mg/100g) were observed. In another study, fenugreek microgreen based functional juice blends was prepared using kinnow mandarin and aloe vera juice. The optimized beverage the ascorbic acid content 35.62 mg/ 100g were observed (Sharma et al., 2020).

6.6.4. Antioxidant activities

The antioxidant activities of microgreen-based RTS beverage were measured by DPPH and FRAP assays. The antioxidant activity (DPPH and FRAP) ranged from 78.25 to 86.89% and 12.37 to 19.98 μmol TE/g, respectively (**Table 6.53**). Pomegranate juice significantly enhanced antioxidant activity due to its rich composition of anthocyanins, phenolics, and flavonoids (Saparbekova et al., 2022). Microgreen powder further boosted antioxidant activity by providing additional bioactive compounds profile to microgreen-based RTS beverage (Singh et al., 2024). The response surface plots (**Figures 6.42 (G)**) and (**Figure 6.42 (H)**) illustrate the combined effects of the variables on antioxidant activities. Further the ANOVA

results (**Table 6.58**) confirmed significant interactions among pomegranate juice, microgreen powder, and water ($P < 0.05$). The regression model equations shown in (**Table 6.57**) for antioxidant activities of DPPH and FRAP the variables positive effect and satisfactory R^2 values and R^2 -pred which is typically lower than R^2 measures the model predictive performance on future observations. In wheatgrass optimized beverage the DPPH antioxidant scavenging activity (13.33%) were observed. In another study, microgreen optimized blend beverage the reducing power (56.65%), metal chelation activity (65.31%) and free radical scavenging activity (69.09%) were observed (Sharma et al., 2020). The RTS beverage were prepared from kinnow and amla juice and the DPPH (% inhibition) in different formulation 31.89 to 70.13% (Purewal et al., 2022). Similarly, pineapple core powder was utilized for the development of plant-based probiotic beverages, the DPPH range from 64.12 to 72.54% observed (Malini et al., 2023).

6.6.5. Effect of storage conditions on physicochemical properties

The physicochemical properties of the optimized microgreen-based RTS beverage, including pH, total soluble solids, titratable acidity, and overall acceptability, were evaluated at varying temperature conditions (5°C, 25°C, and 37°C). During the selected storage period (**Table 6.60**), the pH of the optimized beverage exhibited minor fluctuations. At storage conditions of 25°C and 37°C, the pH decreased from 3.65 to 3.35, whereas refrigerated storage at 5°C demonstrated superior stability, with pH values ranging from 3.65 to 3.39. Statistical analysis indicated that storage temperature significantly influenced pH stability ($P < 0.05$), with refrigeration (5°C) emerging as the most effective condition for maintaining the pH value, followed by 25°C and 37°C. This reduction in pH can be attributed to microbial fermentation and enzymatic activity, which produce organic acids over time (Kumar et al., 2024). The refrigeration condition (5°C) slowed these processes, thereby preserving the beverage's acidic profile and ensuring microbial safety. The presence of organic acids in pomegranate juice contributed to the pH buffering capacity, maintaining a consistent taste profile (Türkyılmaz, 2013). Additionally, the mineral content in the microgreen powder provided extra buffering effects, mitigating rapid pH shifts (Singh et al., 2024). Our findings align with previous studies on kiwifruit juice stored under different conditions (5, 15, and 25°C) (Kumar et al., 2024). Compared to other similar RTS beverages, the pH levels of our microgreen-based RTS beverage samples fall within the typical range for most RTS fruit beverages (3.03–4.48), ensuring a balanced acidity level likely to be well-received by consumers (Prabhakaran et al., 2024).

Table 6.60. Effect of storage temperature conditions on overall acceptability and physicochemical parameters of optimized microgreen-based RTS beverage

Day	Temperature (°C)	Overall acceptability	pH	Titrateable acidity (%)	Total soluble solid (°Brix)
Day-0	5	7.27±0.21 ^{aA}	3.65±0.01 ^{aA}	1.15±0.00 ^{iC}	12.65±0.01 ^{kC}
	25	7.27±0.21 ^{aA}	3.65±0.01 ^{aB}	1.15±0.00 ^{iB}	12.65±0.01 ^{kB}
	37	7.27±0.21 ^{aA}	3.65±0.01 ^{aB}	1.15±0.00 ^{iA}	12.65±0.01 ^{kA}
Day-5	5	7.32±0.14 ^{abA}	3.63±0.01 ^{aA}	1.16±0.01 ^{ijC}	12.66±0.01 ^{jkC}
	25	7.30±0.14 ^{abA}	3.62±0.01 ^{aB}	1.17±0.01 ^{ijB}	12.66±0.01 ^{jkB}
	37	7.26±0.09 ^{abA}	3.61±0.01 ^{aB}	1.21±0.01 ^{iA}	12.68±0.01 ^{ijkA}
Day-10	5	7.27±0.07 ^{abA}	3.62±0.02 ^{aA}	1.16±0.01 ^{ijC}	12.68±0.01 ^{jkC}
	25	7.22±0.04 ^{abA}	3.58±0.01 ^{abcB}	1.26±0.01 ^{hB}	12.68±0.01 ^{ijkB}
	37	7.17±0.06 ^{abA}	3.57±0.01 ^{abcB}	1.31±0.02 ^{gA}	12.70±0.01 ^{hijA}
Day-15	5	7.07±0.07 ^{abcdeA}	3.60±0.01 ^{abA}	1.20±0.02 ^{ijC}	12.72±0.01 ^{hiC}
	25	7.08±0.06 ^{abcdeA}	3.50±0.01 ^{cdeB}	1.37±0.02 ^{jB}	12.73±0.01 ^{hB}
	37	7.09±0.09 ^{abcdA}	3.49±0.01 ^{cdeB}	1.43±0.01 ^{efA}	12.83±0.01 ^{gA}
Day-20	5	7.06±0.05 ^{abcdeA}	3.52±0.01 ^{bcdA}	1.29±0.01 ^{gC}	12.83±0.01 ^{gC}
	25	7.06±0.06 ^{abcdeA}	3.47±0.01 ^{defB}	1.40±0.01 ^{fgB}	12.85±0.01 ^{fgB}
	37	6.97±0.08 ^{abcdeA}	3.44±0.06 ^{defgB}	1.55±0.01 ^{dA}	12.96±0.01 ^{dA}
Day-25	5	6.83±0.08 ^{bcdeA}	3.42±0.01 ^{efgA}	1.47±0.01 ^{eC}	12.89±0.01 ^{efC}
	25	6.82±0.11 ^{bcdeA}	3.41±0.01 ^{efgB}	1.57±0.02 ^{dB}	12.90±0.01 ^{eB}
	37	6.88±0.08 ^{abcdeA}	3.40±0.01 ^{efgB}	1.77±0.02 ^{dA}	13.28±0.02 ^{bA}
Day-30	5	6.74±0.05 ^{cdeA}	3.39±0.01 ^{fgA}	1.69±0.01 ^{cC}	12.96±0.01 ^{dC}
	25	6.70±0.08 ^{deA}	3.35±0.01 ^{gB}	1.76±0.01 ^{bB}	13.13±0.01 ^{cB}
	37	6.62±0.14 ^{eA}	3.39±0.08 ^{fgB}	1.87±0.01 ^{aA}	13.47±0.01 ^{aA}

Note: The results were presented as mean±standard deviations (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments and different uppercase letters within the column indicate significant (P<0.05) differences in temperature condition for the parameters.

The titrateable acidity of the optimized microgreen-based RTS beverage increased slightly during storage, reflecting the production of organic acids due to microbial activity and enzymatic reactions. Initially, the optimized microgreen-based RTS beverage had an acidity of 1.15%. By the end of the 30 days storage period, the acidity increased to 1.69% at 5°C, 1.76% at 25°C, and 1.87% at 37°C (**Table 6.60**). Similar to the pH trends, higher storage temperatures accelerated the increase in acidity. Lower temperature conditions (5°C and 25°C) effectively slowed the acidification process, preserving the beverage's refreshing taste (Kumar et al., 2024). Natural acids present in pomegranate juice, such as citric and malic acids, played a significant role in maintaining a balanced flavor profile (Hasnaoui et al., 2010). Statistical analysis indicated significant differences in acidity between storage conditions (P<0.05), emphasizing the benefits of lower temperatures. The effect of blending kinnow and amla and storage duration on the titrateable acidity of RTS beverages was notable. Data showed that the

titratable acidity percentage significantly increased from 1.3% to 2.03% with increased storage duration (days) among all prepared beverage formulations (Purewal et al., 2022). In another study, a fruit juice-based cocktail made from mango and pineapple juices showed an increase in titratable acidity from 0.21% to 0.69% over a two-month storage duration (Wazed et al., 2023). Similarly, in sugar beet jaggery-added pomegranate RTS beverages, the acidity value increased from 0.30% to 0.40% under 4°C storage conditions during a 120 days storage period (Ma et al., 2018).

The total soluble solids concentration of the optimized microgreen-based RTS beverage sample was initially 12.65°Brix and increased over time under all storage temperature conditions. On day 30, the TSS value at 5°C reached 12.96°Brix, which was lower compared to the values at 25°C and 37°C (ranging from 13.13 to 13.47°Brix), as shown in **(Table 6.60)**. The increase in TSS of the optimized microgreen-based beverage could be attributed to the hydrolysis of polysaccharides (starch and pectin) into simpler sugars like monosaccharides (glucose and fructose) and oligosaccharides (sucrose) and other constituents (Hemalatha et al., 2018). Statistical analysis confirmed that temperature had a minor but significant effect on TSS content ($P < 0.05$), with refrigerated storage (5°C) yielding the best results, followed by 25°C and 37°C. The use of stabilizers (CMC), during beverage preparation minimized phase separation (Akkarachaneeyakorn & Tinrat, 2015). The inherent sugars from pomegranate juice and the added sugar (5%) contributed to the TSS levels, ensuring a consistent sweetness profile (Kulkarni & Aradhya, 2004). Similar changes in TSS during storage were reported in RTS beverages made from cape gooseberry, stored at 27°C (14.17 to 14.63°Brix) and 4°C (14.13 to 15.03°Brix) over a 90-day storage duration. Comparable changes in TSS were also observed in RTS beverages made from Aloe vera, sweet lime, amla, and ginger (Mishra & Sangma, 2017).

The temperature conditions (5°C and 25°C) significantly helped in maintaining the sensory attributes of the microgreen-based RTS beverage **(Table 6.60)**. Panelists preferred the refrigerated samples due to their fresher taste, better aroma retention, and more stable consistency. In contrast, ambient storage led to increased acidity and astringency, negatively impacting consumer perception. Texture changes, such as sedimentation or phase separation, were more noticeable in ambient stored samples, whereas refrigerated storage helped maintain uniform consistency. Based on the obtained results, with overall acceptability scores below 5, our microgreen-based RTS beverage is considered safe for consumption over a 30-day storage duration under selected storage temperatures (Kumar et al., 2024).

6.6.6. Effect of storage conditions on biochemical and antioxidant parameters

6.6.6.1. Ascorbic acid and anthocyanin content

Ascorbic acid and anthocyanin content showed a gradual decline with increasing temperature ($5^{\circ}\text{C} < 25^{\circ}\text{C} < 37^{\circ}\text{C}$) over the storage period (**Table 6.61**). At 5°C , ascorbic acid and anthocyanin content decreased from 210.63 to 202.34 mg/100g and 48.87 to 47.09 $\mu\text{mol}/100\text{g}$ after 30 days, respectively. The beverage stored at 5°C retained higher levels of these compounds compared to storage at 25°C and 37°C . This loss can be attributed to the oxidative degradation of ascorbic acid, which is accelerated by higher temperatures. The lower temperature of 5°C effectively slowed the degradation process by minimizing exposure to heat and light, known catalysts for oxidative reactions (Kumar et al., 2024). The degradation of anthocyanins is primarily due to their sensitivity to temperature and pH fluctuations, leading to structural breakdown. Refrigeration helped preserve anthocyanins by maintaining a stable and cooler environment, reducing the rate of degradation (Enaru et al., 2021). Statistical analysis confirmed significant differences in ascorbic acid and anthocyanin content retention between the storage conditions ($P < 0.05$), highlighting the importance of refrigeration in maintaining the visual and nutritional quality of the beverage.

Our findings align with previous studies on kiwifruit juice stored under different conditions (5, 15, and 25°C). The higher oxygen barrier feature of glass bottles may have inhibited oxygen flow, lowering the conversion of ascorbic acid into dehydroascorbic acid via aerobic oxidation. Thus, the only available pathway was the anaerobic breakdown of ascorbic acid, occurring at a reduced reaction rate. Furthermore, storage temperature significantly affects the deterioration of ascorbic acid. Ascorbic acid high heat sensitivity increases both its aerobic and anaerobic breakdown at elevated temperatures, such as 37°C and 25°C (Kumar et al., 2024). Another study demonstrated that RTS beverages crafted from *Carissa spinarum* fruit and stored at 4°C exhibited outstanding stability, maintaining ascorbic acid levels at 22.5 to 11.65 mg/100 ml and anthocyanin content at 15.3 to 8.33 mg/100 ml (Prabhakaran et al., 2024). Similarly, a carbonated beverage made from pomegranate juice and stored at ambient ($12.2\text{--}33.1^{\circ}\text{C}$) and cool ($5\text{--}8^{\circ}\text{C}$) conditions for three months showed degradation in ascorbic acid and anthocyanin content. After two months at ambient temperature, the ascorbic acid decreased from 1.12 to 1.01 mg/100 ml and anthocyanins from 1.81 to 0.99 mg/100 ml. At cool storage after three months, the ascorbic acid reduced from 1.12 to 0.94 mg/100 ml and anthocyanins from 1.81 to 0.97 mg/100 ml (Sandhan et al., 2009).

Table 6.61. Effect of storage temperature conditions on biochemical parameters of optimized microgreen powder-based RTS beverage

Day	Temperature (°C)	Anthocyanin content (μmol/100g)	Total phenol content (mg GAE/100g)	Total flavonoid content (mg QE/100g)	Ascorbic acid (mg/100g)
Day-0	5	48.87±0.11 ^{aA}	55.93±0.11 ^{aA}	102.09±0.34 ^{aA}	210.63±0.53 ^{aA}
	25	48.87±0.11 ^{aB}	55.93±0.11 ^{aB}	102.09±0.34 ^{aB}	210.63±0.53 ^{aB}
	37	48.87±0.11 ^{aC}	55.93±0.11 ^{aC}	102.09±0.34 ^{aC}	210.63±0.53 ^{aC}
Day-5	5	48.84±0.10 ^{abA}	55.72±0.24 ^{abA}	101.44±0.16 ^{abA}	209.86±0.56 ^{abA}
	25	48.61±0.13 ^{abcB}	55.62±0.04 ^{abcB}	101.20±0.21 ^{abB}	208.48±0.33 ^{cbB}
	37	48.39±0.11 ^{abcC}	54.46±0.28 ^{cdefC}	101.18±0.08 ^{abC}	205.45±0.09 ^{cdC}
Day-10	5	48.44±0.10 ^{abcA}	55.27±0.11 ^{abcdA}	101.14±0.16 ^{abA}	208.98±1.03 ^{abA}
	25	48.40±0.18 ^{abcB}	55.17±0.17 ^{abcdeB}	101.13±0.15 ^{abcB}	205.41±0.18 ^{cdB}
	37	48.24±0.10 ^{abcC}	54.47±0.25 ^{cdefC}	100.38±0.36 ^{bcC}	201.12±0.16 ^{gC}
Day-15	5	47.89±0.25 ^{bcdA}	54.52±0.24 ^{bcdefA}	100.44±0.27 ^{bcA}	206.35±0.25 ^{cA}
	25	47.32±0.13 ^{defB}	54.33±0.23 ^{defB}	98.54±0.13 ^{deB}	202.48±0.35 ^{fgB}
	37	46.48±0.13 ^{ghC}	52.57±0.40 ^{hiC}	98.29±0.20 ^{deC}	198.58±0.18 ^{hC}
Day-20	5	47.71±0.42 ^{cdeA}	54.02±0.24 ^{efgA}	99.26±0.29 ^{cdA}	204.33±0.14 ^{deA}
	25	46.93±0.29 ^{fgB}	53.00±0.18 ^{ghiB}	95.34±0.44 ^{ghB}	201.39±0.48 ^{gB}
	37	44.58±0.27 ^{jC}	51.17±0.39 ^{jkC}	94.21±0.25 ^{hiC}	194.55±0.09 ^{iC}
Day-25	5	47.39±0.25 ^{defA}	53.67±0.59 ^{fghA}	97.33±0.23 ^{efA}	203.59±0.20 ^{fgA}
	25	46.13±0.13 ^{hiB}	52.92±0.47 ^{ghiB}	94.24±0.30 ^{hiB}	201.64±0.43 ^{gB}
	37	44.03±0.06 ^{jC}	50.17±0.11 ^{kC}	93.35±0.45 ^{ijC}	193.41±0.28 ^{iC}
Day-30	5	47.09±0.11 ^{efgA}	52.57±0.45 ^{hiA}	96.38±0.44 ^{fgA}	202.34±0.55 ^{fgA}
	25	45.51±0.25 ^{iB}	51.92±0.38 ^{ijB}	92.39±0.51 ^{jB}	198.43±0.13 ^{hB}
	37	43.83±0.22 ^{jC}	48.52±0.39 ^{jC}	92.36±0.46 ^{jC}	191.75±0.19 ^{jC}

Note: The results were presented as mean±standard deviations (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments and different uppercase letters within the column indicate significant (P<0.05) differences in temperature condition for the parameters.

6.6.6.2. Total phenol and flavonoid content

Another bioactive compounds, including total phenol and flavonoid content, showed a gradual decline with increasing temperature ($5^{\circ}\text{C} < 25^{\circ}\text{C} < 37^{\circ}\text{C}$) over the storage period (**Table 6.61**). At 5°C , the total phenol and flavonoid content decreased from 55.93 to 55.57 mg GAE/100g and from 102.09 to 96.38 mg QE/100g after 30 days, respectively. Beverages stored at 5°C retained higher levels of these bioactive compounds compared to those stored at 25°C and 37°C . The degradation of phenolic compounds can be attributed to oxidative reactions and interactions with other components in the beverage matrix (Kumar et al., 2024). Flavonoids are particularly susceptible to oxidation and degradation, especially at elevated temperatures, which accelerates their breakdown. Refrigerated storage minimized these reactions, ensuring better retention of phenolic and flavonoid content. Statistical analysis indicated significant differences ($P < 0.05$) between the storage conditions, with refrigeration demonstrating superior efficacy in preserving phenolic compounds and flavonoids. In a study on RTS beverages made from cape gooseberry, higher retention of total phenolic compounds was observed at 4°C (21.83 to 15.56 mg GAE/100 mL) and at 27°C (21.83 to 8.03 mg GAE/100 mL) over a 90-day storage period (Hemalatha et al., 2018). Similarly, in a study on sapodilla-based RTS beverages, total phenol content reduced from 359.12 to 343.53 μg GAE/100 mL and flavonoid content from 181.48 to 111.48 μg CE/g under refrigeration conditions over a 90-day storage duration (Fiaz et al., 2022).

6.6.6.3. Antioxidant activities

Antioxidant activities, measured by DPPH and FRAP assays, declined during storage with increasing temperatures. The highest antioxidant activity was observed at 5°C (78.32% for DPPH and 14.30 $\mu\text{mol TE/g}$ for FRAP), followed by 25°C (72.25% for DPPH and 13.25 $\mu\text{mol TE/g}$ for FRAP) and 37°C (70.13% for DPPH and 12.59 $\mu\text{mol TE/g}$ for FRAP) by day 30 (**Table 6.62**). This reduction in antioxidant activities corresponds to the degradation of bioactive compounds such as ascorbic acid, phenolics, and flavonoids. Lower temperature storage effectively slowed this decline by mitigating oxidative stress and preserving the structural integrity of these compounds. Statistical analysis showed significant differences ($P < 0.05$) in antioxidant activities between the three storage conditions, demonstrating the critical role of refrigeration at 5°C in maintaining the functional properties of the microgreen-based RTS beverage. These findings align with previous studies on various fruit-based RTS beverages, including coconut water (Ceballos et al., 2024), *Carissa spinarum* (Prabhakaran et al., 2024), kinnow and amla (Purewal et al., 2022), and sapodilla (Fiaz et al., 2022).

Table 6.62. Effect of storage temperature conditions on antioxidant and microbial parameters of optimized microgreen-based RTS beverage

Day	Temperature (°C)	Antioxidant activity DPPH (%)	Antioxidant activity FRAP (μmol TE/g)	Total plate count (log cfu/ml)	Yeast and mold (log cfu/ml)
Day-0	5	86.92±0.08 ^{aA}	18.56±0.15 ^{aA}	2.18±0.08 ^{kC}	1.32±0.08 ^{iB}
	25	86.92±0.08 ^{aB}	18.56±0.15 ^{aB}	2.18±0.08 ^{kB}	1.32±0.08 ^{iB}
	37	86.92±0.08 ^{aC}	18.56±0.15 ^{aC}	2.18±0.08 ^{kA}	1.32±0.08 ^{iA}
Day-5	5	86.27±0.16 ^{abA}	18.10±0.09 ^{bA}	2.63±0.13 ^{jkC}	1.72±0.22 ^{hiB}
	25	85.97±0.13 ^{abB}	17.17±0.08 ^{bB}	2.71±0.18 ^{jkB}	1.94±0.06 ^{ghB}
	37	85.22±0.23 ^{bcC}	16.40±0.11 ^{cC}	3.56±0.11 ^{efghiA}	1.89±0.15 ^{ghiA}
Day-10	5	85.52±0.20 ^{abcA}	17.29±0.18 ^{bA}	3.06±0.18 ^{ijC}	1.86±0.09 ^{ghiB}
	25	84.18±0.23 ^{cdB}	16.62±0.28 ^{bcB}	3.24±0.13 ^{ghijB}	2.04±0.08 ^{fghB}
	37	82.17±0.16 ^{efgC}	15.18±0.21 ^{dC}	3.71±0.25 ^{defgA}	2.14±0.06 ^{efghA}
Day-15	5	83.58±0.30 ^{deA}	16.33±0.13 ^{cA}	3.14±0.06 ^{hijC}	1.94±0.08 ^{ghB}
	25	81.68±0.93 ^{fghB}	15.28±0.22 ^{dB}	3.54±0.13 ^{efghiB}	2.13±0.08 ^{efghB}
	37	80.37±0.16 ^{hC}	14.28±0.21 ^{eC}	4.07±0.12 ^{bcdeA}	2.24±0.16 ^{efghA}
Day-20	5	82.41±0.21 ^{efA}	15.53±0.28 ^{dA}	3.40±0.12 ^{fghiC}	2.37±0.23 ^{efgB}
	25	80.85±0.25 ^{ghB}	15.21±0.11 ^{dB}	3.84±0.08 ^{cdefgB}	2.46±0.32 ^{defgB}
	37	78.28±0.28 ^{hC}	13.86±0.11 ^{efC}	4.44±0.11 ^{abcA}	2.70±0.07 ^{cdeA}
Day-25	5	80.29±0.32 ^{iA}	15.13±0.13 ^{dA}	3.78±0.16 ^{defgC}	2.63±0.13 ^{cdefB}
	25	78.25±0.27 ^{iB}	14.21±0.18 ^{eB}	4.04±0.11 ^{bcdeB}	2.71±0.18 ^{bcdB}
	37	71.75±1.03 ^{jC}	13.39±0.15 ^{fC}	4.63±0.34 ^{abA}	3.56±0.11 ^{abA}
Day-30	5	78.32±0.28 ^{iA}	14.30±0.17 ^{eA}	3.93±0.05 ^{bcdeC}	3.06±0.18 ^{bcdB}
	25	72.25±0.16 ^{jB}	13.25±0.11 ^{fgB}	4.26±0.19 ^{abcdB}	3.24±0.13 ^{abcB}
	37	70.13±0.49 ^{kC}	12.59±0.29 ^{gC}	4.73±0.23 ^{aA}	3.71±0.25 ^{aA}

Note: The results were presented as mean±standard deviations (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments and different uppercase letters within the column indicate significant (P<0.05) differences in temperature condition for the parameters.

In these studies, a decline in antioxidant activity, as determined by the DPPH and FRAP assays, was similarly observed.

6.6.7. Effect of storage conditions on microbial parameters

The microbial parameters of the microgreen-based RTS beverage, including total plate count (TPC) and yeast and mold count, were evaluated under different storage conditions (5°C, 25°C, and 37°C). Microbial stability is crucial in determining the shelf life and safety of RTS beverages. The results for microbial parameters are presented in **(Table 6.62)**. The TPC of the microgreen-based RTS beverage increased gradually during storage, with a more pronounced rise observed at 37°C. On the initial day, the TPC was 2.18 log cfu/ml; by day 30, it had reached 3.93 log cfu/ml at 5°C, 4.26 log cfu/ml at 25°C, and 4.73 log cfu/ml at 37°C. Yeast and mold counts followed a similar trend, starting at 1.32 log cfu/ml and rising to 3.06 log cfu/ml at 5°C, 3.24 log cfu/ml at 25°C, and 3.71 log cfu/ml at 37°C by day 30. The higher microbial growth at 25°C to 37°C can be attributed to favorable conditions for bacterial proliferation, such as increased temperature and moisture availability (Kumar et al., 2024). The use of stabilizers and the acidic nature of pomegranate juice also contributed to inhibiting fungal growth (Valero-Mendoza et al., 2022). Lower temperatures significantly inhibited microbial growth by slowing metabolic activity and reducing enzyme functionality in bacteria. Statistical analysis confirmed significant differences ($P < 0.05$) in microbial parameters at different storage conditions, with refrigeration (5°C) proving more effective in maintaining microbial safety. Our findings align with previous studies on cold plasma-treated kiwifruit juice stored at different temperature conditions, including 5°C, 15°C, and 25°C (Kumar et al., 2024). With TPC and yeast and mold count below 6 Log CFU/mL, our microgreen-based RTS beverage is considered safe for consumption based on a 30 days storage duration under selected storage temperatures (Patrignani et al., 2009; Ceballos et al., 2024).

6.6.8. Kinetic modeling of different parameters and shelf-life model

The kinetic modeling of different quality indices in microgreen-based Ready-to-Serve (RTS) beverages is crucial for understanding degradation patterns and predicting the shelf-life of the product (Zhang et al., 2020). Quality indices such as overall acceptability, acidity, ascorbic acid content, and microbial growth exhibit distinct degradation behaviors that must be analyzed to ensure optimal storage and consumer satisfaction. Various kinetic models, including zero-order, first-order, and second-order reactions, provide insight into the reaction mechanisms governing these changes. The Arrhenius, Eyring, and Ball models are widely used to assess temperature dependency and predict shelf-life effectively (Cui et al., 2023; Kumar et

al., 2024). The (Table 6.63) and (Table 6.64) present the experimental values based on the reaction kinetics of different quality indices at multiple storage temperatures.

The reaction order estimation for various parameters, including overall acceptability, acidity, ascorbic acid content, and total plate count, is shown in (Table 6.63). The primary aim of this study is to determine which kinetic model best describes the degradation patterns of these quality indices by evaluating the coefficient of determination (R^2). The reaction rate constant (k) and half-life ($t_{1/2}$) are critical parameters in understanding how rapidly these quality indices change over time. The degradation of overall acceptability was found to follow a zero-order reaction model, as indicated by the highest R^2 values across all storage temperatures. The values for the temperature conditions of 5°C ($R^2 = 0.8973$), 25°C ($R^2 = 0.9115$), and 37°C ($R^2 = 0.9285$) confirm that sensory attributes such as flavor, texture, and aroma degrade at a constant rate, independent of their initial concentration. This means that the rate of sensory degradation remains steady, likely due to the uniform breakdown of volatile compounds and flavoring agents over time (Ortega-Rivas et al., 2012). Furthermore, the reaction rate constant (k) values increased with temperature for 5°C (0.02), 25°C (0.0204), and 37°C (0.0208), confirming that acceptability declines more rapidly at higher storage temperatures. Acidity levels were best modeled using a first-order kinetic equation, with R^2 values indicating that acidity variations are influenced by interactions between reactants, potentially due to microbial activity or enzymatic reactions. The reaction rate constant (k) values also showed an increasing trend with temperature, indicating that acidity changes occur more rapidly at higher temperatures, likely due to increased microbial metabolism and enzymatic hydrolysis (Zheng et al., 2014).

The degradation of ascorbic acid followed first-order kinetics, as evidenced by high R^2 values at 5°C (0.9727), 25°C (0.9445), and 37°C (0.9619). First-order kinetics indicate that the degradation rate is directly proportional to the concentration of ascorbic acid remaining in the beverage. The reaction rate constant (k) showed an increasing trend with temperature, suggesting that ascorbic acid degradation is highly temperature-dependent. The loss of ascorbic acid is primarily due to oxidative reactions, which accelerate at elevated temperatures. Given the essential role of ascorbic acid in antioxidant activity and nutritional value, storage at lower temperatures is recommended to slow down its degradation and extend shelf-life (Yin et al., 2022). Microbial growth, represented by total plate count, exhibited first-order kinetics at lower temperatures (5°C with $R^2 = 0.944$, 25°C with $R^2 = 0.9192$), but the R^2 value at 37°C was lower (0.7515).

Table 6.63. Reaction order estimation of quality indices based on the coefficient of determination (R^2) from the zero-, first- and second-order reactions.

Parameters	Temperature (°C)	Zero order			First order			Second order	
		k	R^2	$t_{1/2}$	k	R^2	$t_{1/2}$	k	R^2
Overall acceptability	5	0.02	0.8973	181.75	0.0028	0.8956	247.552564	0.0004	0.8937
	25	0.0204	0.9115	178.186275	0.00299	0.9076	231.8218	0.0004	0.9036
	37	0.0208	0.9285	174.759615	0.003	0.9214	231.04906	0.0004	0.9141
Acidity	5	0.0169	0.7935	34.0236686	0.0124	0.8189	55.8989662	0.0092	0.8414
	25	0.0183	0.8983	31.420765	0.0131	0.931	52.9119985	0.0095	0.9539
	37	0.025	0.9749	23.00	0.017	0.988	40.7733636	0.0118	0.9931
Ascorbic acid	5	0.3003	0.9726	350.699301	0.0015	0.9727	462.09812	0.000007	0.9728
	25	0.3879	0.9526	271.500387	0.0019	0.9445	364.814306	0.000009	0.9459
	37	0.6234	0.9694	168.936477	0.0031	0.9619	223.595865	0.00002	0.9661
Total plate count	5	0.0564	0.9724	19.3262411	0.0186	0.944	37.26597745	0.0063	0.9001
	25	0.0679	0.9616	16.0530191	0.0213	0.9192	32.54212115	0.0069	0.8634
	37	0.0745	0.8333	14.6308725	0.0215	0.7515	32.23940375	0.0065	0.6638

Note: R^2 , coefficient of determination; k, rate of reaction

Table 6.64. Kinetic parameters of quality indices during storage obtained by the Arrhenius, Eyring, and Ball models.

Parameters	Types of Reactions	Arrhenius model		Eyring models			Ball model	
		Ea (kJ/mol)	R^2	ΔH (kJ/mol)	ΔS (J/(mol.k))	R^2	Z (°C)	R^2
Overall acceptability	Zero order	0.871	0.9569	18.2755	-197.75884	1	0.1138	1
	First order	1.7	0.931	20.4904	-198.8352	1	0.1027	1
Acidity	Zero order	15.4904	0.7312	13.1446	-201.1741	0.9482	0.1517	0.9598
	First order	11.9464	0.7044	9.5997	-202.4534	0.9135	0.1968	0.9396
Ascorbic acid	Zero order	11.6894	0.8583	9.3427	-200.8167	0.9857	0.2006	0.9918
	First order	15.3952	0.8385	87.961	-167.4896	0.9387	0.0259	0.9449
Total plate count	Zero order	10.2513	0.9994	7.9044	-202.41	0.7167	0.2297	0.8501
	First order	7.4761	0.9383	5.1296	-204.1205	0.6652	0.3149	0.8042

Note: R^2 , coefficient of determination; ΔH , enthalpy of activation; ΔS , entropy of activation; Z, storage temperature span necessary for a tenfold decrease in the D value

This suggests that at higher temperatures, microbial proliferation deviates from a simple first-order reaction, likely due to an exponential growth phase beyond a threshold concentration. The reaction rate constant (k) values increased from 0.0186 at 5°C to 0.0215 at 37°C, confirming that microbial activity intensifies as temperature rises. This highlights the importance of refrigeration in controlling microbial spoilage and ensuring product safety over extended storage durations.

The kinetic parameters for different quality indices, using the Arrhenius, Eyring, and Ball models, are provided in **(Table 6.64)**. These models help quantify temperature-dependent changes and predict shelf-life under varying conditions. The Arrhenius model describes how temperature affects reaction rate constants through the calculation of activation energy (E_a). Higher E_a values indicate greater sensitivity to temperature fluctuations, emphasizing the need for controlled storage conditions. For the overall acceptability parameter, the activation energy (E_a) for zero-order and first-order reactions were 0.871 kJ/mol and 1.7 kJ/mol, respectively, indicating moderate temperature sensitivity. For acidity, higher E_a values were observed (zero-order is 15.49 kJ/mol, and first-order is 11.94 kJ/mol), suggesting that acidity changes are strongly influenced by temperature fluctuations. For ascorbic acid, moderate E_a values (zero-order for 11.68 kJ/mol, and first-order for 15.39 kJ/mol) confirm its temperature-dependent degradation, reinforcing the need for cold storage. The microbial growth exhibited low E_a values (zero-order is 10.25 kJ/mol, and first-order is 7.47 kJ/mol), indicating that microbial activity is less dependent on temperature than chemical reactions.

The Eyring model further explores kinetic behavior by evaluating the enthalpy (ΔH) and entropy (ΔS) of activation. For overall acceptability, the enthalpy of activation (ΔH) ranged from 18.27 kJ/mol for zero-order reactions to 20.49 kJ/mol for first-order reactions, suggesting minimal energy input required for degradation. In terms of acidity, ΔH values were 13.14 kJ/mol for zero-order reactions and 9.59 kJ/mol for first-order reactions, reinforcing its moderate susceptibility to temperature-induced changes. Regarding ascorbic acid, high ΔH values of 9.34 kJ/mol for zero-order reactions and 87.96 kJ/mol for first-order reactions demonstrate its vulnerability to thermal degradation. Lastly, for total plate count, the lowest ΔH values of 7.90 kJ/mol for zero-order reactions and 5.12 kJ/mol for first-order reactions confirm that microbial growth is relatively independent of temperature variations.

The Ball model estimates the temperature range necessary for a tenfold decrease in quality, referred to as Z-values. For overall acceptability, Z-values of 0.1138°C for zero-order reactions and 0.1027°C for first-order reactions suggest minimal temperature impact. In terms of acidity, higher Z-values of 0.1517°C for zero-order reactions and 0.1968°C for first-order

reactions indicate a greater temperature effect. For ascorbic acid, Z-values of 0.2006°C for zero-order reactions and 0.0259°C for first-order reactions confirm rapid degradation at higher temperatures. Lastly, for total plate count, Z-values of 0.2297°C for zero-order reactions and 0.3149°C for first-order reactions suggest that microbial growth is highly temperature-dependent.

The kinetic modeling of quality indices in microgreen-based RTS beverages provides essential insights into storage stability. Zero-order kinetics govern overall acceptability, while acidity and microbial growth exhibit first-order behaviors. Ascorbic acid follows first-order degradation, emphasizing the importance of controlled storage conditions. The Arrhenius, Eyring, and Ball models further validate temperature sensitivity, with acidity and ascorbic acid being most affected. These findings contribute to optimizing storage conditions and extending the shelf-life of RTS beverages.

6.6.9. Optimized product cost

The cost estimation for the optimized microgreen-based Ready-to-Serve (RTS) beverage incorporates various expenses, including raw material costs, processing, packaging, and marketing. The total cost of producing 1 liter of the beverage is ₹ 126.81. With a profit margin of 20%, this results in a final price of ₹ 152.17 per liter (**Table 6.65**). Additionally, the production cost of the developed RTS beverage product is estimated to be ₹30.43 per 200 mL, making it highly competitive with commercial products available in the market, which are typically priced between ₹20 and ₹40 per 200 mL in glass bottles (**Table 6.65**). Therefore, from an economic perspective, the product is highly feasible.

Table 6.65. The optimized microgreen-based RTS beverage cost estimation

Ingredients	Quantity	Market price (₹)	Amount used/ 1 L	Total cost (₹)
Microgreens (Radish sango)	1 kg	1258.13	20g	28.30
Pomegranate	1 kg	100	450.00g	50.00
CMC	0.075 kg	219	2.5g	8.47
Sugar	1 kg	85	50g	4.25
Water	1 L	10	425mL	4.25
Packaging cost	1	3	5	15.00
Processing cost				110.27
Marketing and distribution expenses @15% of product				16.54
			Total	126.81
Profit margin @ 20% of cost of product				25.36
			Total	152.17
Microgreen-based RTS beverage cost for 200 mL glass bottles				30.43

6.7. Optimization of variables for microgreen juice-based dairy beverage

To determine the values of the variables microgreen juice (A), dahi (B), and water (C) for the study, response surface methodology was recommended using D-optimal mixture design and conducting 17 runs ($n=3$) treatments of tests. The (Tables 6.66, Table 6.67, and Table 6.68) present the results of sensory, physicochemical, biochemical, and antioxidant parameters scores of average mean values ($n=3$) which were influenced by microgreen juice, dahi, and water. According to the results, the optimal composition for various selected parameters for the functional fermented microgreens-based dairy beverage with microgreen juice was determined to be 9.189% microgreen juice, 60% dahi, and 15.311% mixed with 15% sugar, and 0.50% CMC. The optimization criteria for a functional microgreen juice-based dairy beverage using three main process variables including microgreen juice (5 to 9.5%, maximize), dahi (60 to 64.5%, minimize), and water (15 to 19.5%, minimize), each with set goals and importance levels as shown in (Table 6.69). It also specifies response variables of different parameters. These criteria are designed to balance various selected sensory, physicochemical, biochemical, and antioxidant parameters to create an optimal functional fermented microgreens-based dairy (lassi) beverage formulation. The (Figure 6.43) shows that this combination had a desirability score of 0.781, indicating that it was the best combination for the study. In (Table 6.70), both the observed and predicted responses are presented.

6.7.1. Effect of processing variables on sensory parameters

The sensory quality of microgreen juice-based dairy beverage was evaluated through parameters such as color and appearance, flavour and sweetness, consistency, mouthfeel, and overall acceptability. These parameters were significantly ($P<0.05$) affected by the proportions of the key processing variables including microgreen juice, dahi, and water. These factors, individually and in combination, influenced consumer perceptions and preferences. The results for different parameters are shown in (Table 6.66). The (Table 6.71) and (Table 6.72) represent the regression coefficients and F-values for the polynomial equations, indicating the suggested model's adequacy, significant lack of fit, and satisfactory R^2 values; furthermore, R^2 -pred, which is typically lower than R^2 measures the model predictive performance on future observations. All models were regarded as suitable for analysis because their R^2 values were all above 90%.

Table 6.66. The effect of constituents on sensory parameters of microgreen juice-based diary (lassi) beverage

Treatments	Dahi (%)	Microgreen extract (%)	Water (%)	Colour & Appearance	Flavour & Sweetness	Consistency	Mouthfeel	Overall Acceptability
1	60.00	9.50	15.00	7.81	7.35	7.87	7.52	7.05
2	60.75	5.75	18.00	7.97	7.56	7.88	7.68	7.73
3	60.75	8.00	15.75	7.83	7.48	7.82	7.61	7.24
4	60.00	5.00	19.50	8.09	7.52	7.94	7.75	7.96
5	62.25	5.00	17.25	8.11	7.84	7.97	7.76	7.85
6	62.25	7.25	15.00	7.98	7.79	7.75	7.88	7.33
7	60.00	9.50	15.00	7.81	7.29	7.89	7.48	7.05
8	60.00	7.25	17.25	7.82	7.39	7.85	7.94	7.68
9	63.00	5.75	15.75	8.02	7.66	7.91	7.56	7.54
10	60.00	5.00	19.50	8.09	7.56	7.94	7.79	7.96
11	64.50	5.00	15.00	8.12	7.29	8.07	7.49	7.77
12	61.88	5.75	16.88	7.99	7.62	7.89	7.53	7.57
13	64.50	5.00	15.00	8.08	7.29	8.11	7.60	7.77
14	62.25	5.00	17.25	8.12	7.84	7.97	7.76	7.85
15	60.75	6.88	16.88	7.85	7.43	7.83	7.63	7.47
16	62.25	7.25	15.00	7.98	7.79	7.79	7.83	7.39
17	61.50	6.50	16.50	7.91	7.51	7.84	7.48	7.42

Note: All the experimental results values are presented as mean value (n=3) basis based on each sensory parameter.

Table 6.67. The effect of constituents on physicochemical parameters of microgreen juice-based diary (lassi) beverage

Treatments	Dahi (%)	Microgreen extract (%)	Water (%)	pH	Acidity (% lactic acid)	TSS (°Brix)	Water holding capacity (%)	Syneresis (%)
1	60.00	9.50	15.00	3.91	0.58	22.42	43.69	45.66
2	60.75	5.75	18.00	3.84	0.55	22.29	49.66	51.78
3	60.75	8.00	15.75	3.87	0.57	21.51	44.91	46.95
4	60.00	5.00	19.50	3.88	0.55	22.52	45.93	47.95
5	62.25	5.00	17.25	3.82	0.55	21.96	51.86	52.88
6	62.25	7.25	15.00	3.87	0.57	20.74	41.05	42.05
7	60.00	9.50	15.00	3.92	0.58	21.88	44.05	46.05
8	60.00	7.25	17.25	3.89	0.56	23.17	45.82	47.22
9	63.00	5.75	15.75	3.81	0.56	21.48	48.12	50.18
10	60.00	5.00	19.50	3.88	0.55	22.58	45.93	47.95
11	64.50	5.00	15.00	3.81	0.55	22.53	47.22	49.22
12	61.88	5.75	16.88	3.81	0.56	21.63	50.66	52.66
13	64.50	5.00	15.00	3.82	0.54	22.51	47.22	50.22
14	62.25	5.00	17.25	3.82	0.55	21.96	50.22	51.36
15	60.75	6.88	16.88	3.85	0.57	22.19	47.99	49.99
16	62.25	7.25	15.00	3.89	0.58	20.74	41.25	43.25
17	61.50	6.50	16.50	3.82	0.56	21.52	48.82	50.88

Note: All the experimental results values are presented as mean value (n=3) basis based on each physicochemical parameter.

Table 6.68. The effect of constituents on biochemical and antioxidant parameters of microgreen juice-based diary (lassi) beverage

Treatments	Dahi (%)	Microgreen extract (%)	Water (%)	Anthocyanin content (μmol/100g)	Total phenol content (mg GAE/100g)	Total flavonoid content (mg QE/100g)	Antioxidant activity DPPH (%)	Antioxidant activity FRAP (μmol TE/g)	Ascorbic acid (mg/100g)
1	60.00	9.50	15.00	36.55	42.23	23.43	59.48	8.84	48.86
2	60.75	5.75	18.00	25.88	33.97	13.61	54.11	5.94	34.98
3	60.75	8.00	15.75	32.62	41.76	19.10	58.29	7.38	42.65
4	60.00	5.00	19.50	23.79	29.83	11.98	51.91	5.41	32.96
5	62.25	5.00	17.25	24.91	28.88	12.03	51.82	5.78	34.08
6	62.25	7.25	15.00	33.55	39.85	17.04	56.78	6.67	41.18
7	60.00	9.50	15.00	36.68	42.23	23.43	59.48	8.84	48.86
8	60.00	7.25	17.25	38.68	45.87	17.11	56.62	6.51	38.89
9	63.00	5.75	15.75	24.89	32.28	13.41	54.04	6.06	36.20
10	60.00	5.00	19.50	23.73	29.83	11.98	51.91	5.41	32.96
11	64.50	5.00	15.00	24.42	30.78	11.44	51.66	5.61	34.32
12	61.88	5.75	16.88	22.55	31.53	13.58	54.55	6.15	35.76
13	64.50	5.00	15.00	24.44	30.79	12.54	51.33	5.60	34.35
14	62.25	5.00	17.25	24.25	29.35	11.95	51.88	5.78	34.02
15	60.75	6.88	16.88	28.69	39.23	16.53	55.77	6.59	38.36
16	62.25	7.25	15.00	33.18	37.59	16.57	56.66	6.22	42.28
17	61.50	6.50	16.50	24.18	35.13	15.97	55.55	6.51	37.68

Note: All the experimental results values are presented as mean value (n=3) basis based on each biochemical and antioxidant parameter. Where, GAE: Gallic acid equivalent; QE: Quercetin equivalent

Table 6.69. Optimization criteria for different process and response variables for microgreen-based microgreen juice-based diary (lassi) beverage

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Microgreen juice	Maximize	5	9.5	1	1	5
B: Dahi	Minimize	60	64.5	1	1	5
C: Water	Minimize	15	19.5	1	1	5
Colour & Appearance	Minimize	7.81	8.12	1	1	5
Flavour & Sweetness	Minimize	7.29	7.84	1	1	5
Consistency	In range	7.75	8.11	1	1	3
Mouthfeel	Maximize	7.48	7.94	1	1	5
Overall Acceptability	In range	7.05	7.96	1	1	5
pH	Maximize	3.81	3.92	1	1	3
Acidity	Maximize	0.54	0.5837	1	1	5
Total soluble solids	Maximize	20.74	23.17	1	1	5
Water holding capacity	Minimize	41.05	51.86	1	1	5
Syneresis	Minimize	42.05	52.88	1	1	5
Anthocyanin content	Maximize	22.55	38.68	1	1	5
Total phenol content	Maximize	28.88	45.87	1	1	5
Total flavonoid content	Maximize	11.44	23.43	1	1	5
Antioxidant activity DPPH	Maximize	51.33	59.48	1	1	5
Antioxidant activity FRAP	Maximize	5.41	8.84	1	1	5
Ascorbic acid	Maximize	32.96	48.86	1	1	5

The visual appeal of the dairy beverage, primarily assessed through its color and appearance, was markedly influenced by the microgreen juice (%) concentration. As observed in (Table 6.66), the color and appearance scores ranged from 7.81 to 8.12, with formulations containing moderate levels of microgreen juice (%) scoring the highest (Figure 6.44 (A)). The addition of the excessive amount of microgreen juice (%) resulted in darker hues, which some panel members found less appealing. Lower microgreen juice concentrations (5%) resulted in higher scores for color and appearance (8.08 to 8.12). These formulations achieved a more subtle green tint, which harmonized with the creamy white base of the lassi, making it visually more acceptable to panelists.

Table 6.70. Predicted and observed optimum responses for microgreen juice-based diary (lassi) beverage

Responses	Predicted values	Observed values
Microgreen juice (%)	9.189	
Dahi (%)	60	
Water (%)	15.311	
Colour & Appearance	7.796	7.85±0.45
Flavour & Sweetness	7.324	7.38±0.26
Consistency	7.871	7.93±0.35
Mouthfeel	7.598	7.78±0.18
Overall Acceptability	7.156	8.29±0.10
pH	3.911	4.35±0.01
Acidity (% lactic acid)	0.58	0.62±0.01
TSS (°Brix)	22.362	22.42±0.08
Water holding capacity (%)	44.203	44.32±0.17
Syneresis (%)	46.047	46.30±0.36
Anthocyanin content (µmol/100g)	37.828	37.91±0.11
Total phenol content (mg GAE/100g)	43.941	44.14±0.28
Total flavonoid content (mg QE/100g)	22.489	22.62±0.18
Antioxidant activity DPPH (%)	59.177	59.32±0.20
Antioxidant activity FRAP (µmol TE/g)	8.447	8.30±0.21
Ascorbic acid (mg/100g)	47.228	59.32±0.20

The obtained results from experiments are represented in mean±standard deviation values with displayed significant differences ($P<0.05$).

The regression model in (Table 6.71) confirms that the linear and interaction effects of microgreen juice and water significantly ($P<0.05$) impacted the beverage color and appearance parameter. Excessive water diluted the green intensity, while insufficient water increased the colors harshness. The pudina extract (2.5 to 7.5%) was incorporated into the lassi, sore obtained for color and appearance in the range of 6.88 to 7.50, this study showed that a lower concentration of pudina extract (2.5%) rated highest score (Dhumal et al., 2018). Similarly, the score for color and appearance was increased by 3.0% addition of ginger juice, thereafter score declined simultaneously. The highest score (7.77) was obtained by the treatment lassi incorporated with 3.0% ginger juice, respectively (Raje-Nimbalkar et al., 2023). These findings are slightly lower than our results, this may be due to radish sango microgreens deep purple leaves, and lavender-pink stems, which create a striking visual contrast (Mlinarić et al., 2023).

The proportions of microgreen juice and water significant ($P<0.05$) effect showed on the sensory parameter of flavor and sweetness of microgreen juice-based dairy beverage. The incorporation of radish sango microgreen juice imparted a unique flavor profile characterized by subtle vegetal and slightly pungent flavor (Coronado et al., 2023). However, at higher concentrations of microgreen juice (9.5%), the intensity of these flavors overshadowed the

inherent sweetness of the beverage, leading to lower flavor scores (7.29). These demonstrated that an excess of bioactive compounds such as phenolic compounds, while beneficial for nutritional value, can result in sensory challenges due to the inherent bitterness and pungency of radish microgreens. As shown in (Table 6.71), the quadratic effects of microgreen juice and significant interactions between juice and water were critical in determining flavor. Water played a crucial role in diluting the intensity of microgreens pungent flavor while maintaining a pleasant sweetness, as evidenced by higher flavor scores at water levels of 18% and 19.5%. The mango powder and beetroot powder showed significant ($P < 0.05$) effect on flavor scores of lassi, the highest score (8.25 to 8.50) was obtained for the mango powder (7.5%) and beetroot powder (3%), respectively (Chawla & Sivakumar, 2017). Similar trends have been reported in functional dairy (lassi) beverages such as ginger lassi, carrot lassi, turmeric lassi (Maji et al., 2020), tomato pomace lassi (Kumar et al., 2023), Aloe-probiotic lassi (Kumar et al., 2017), and *Aloe barbadensis* Miller lassi (Hussain et al., 2015). The plant-based additives enhanced flavor and functional appeal but required optimization to maintain palatability.

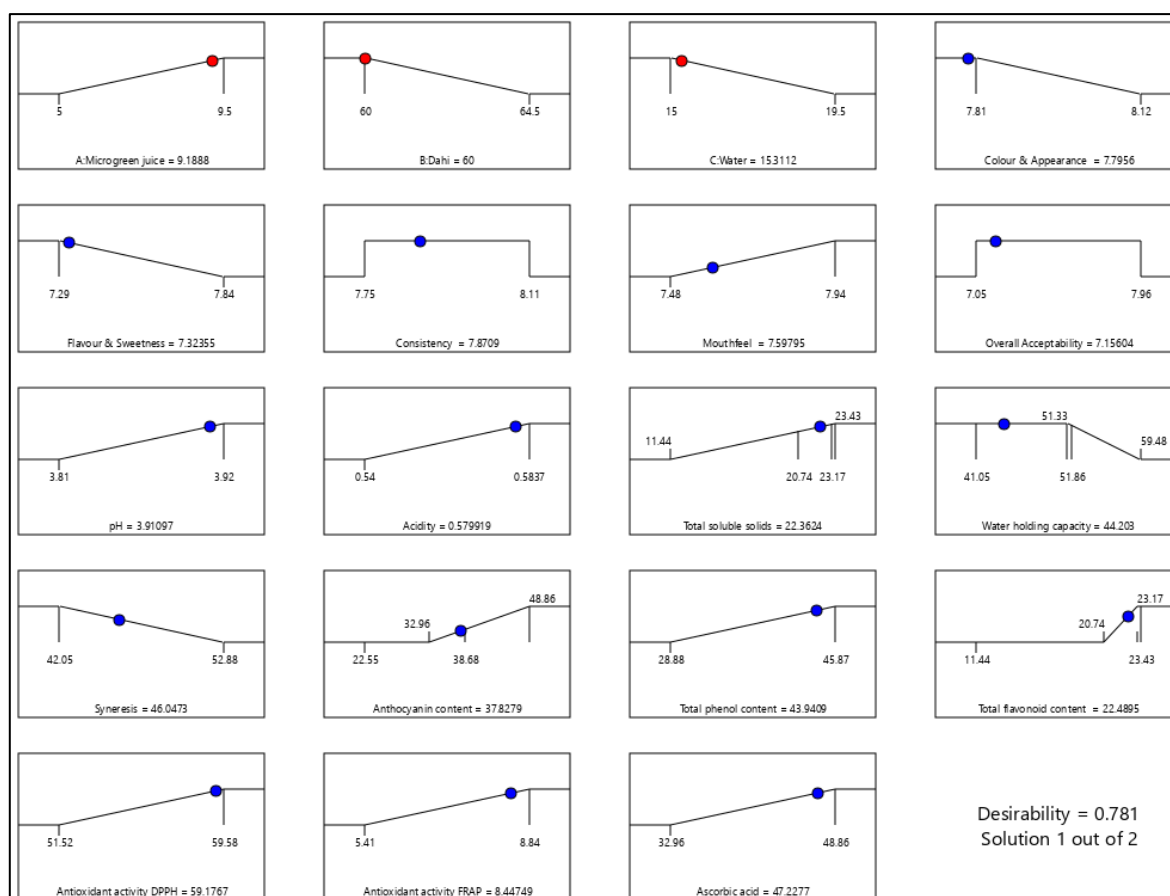


Figure 6.43. Desirability ramp for optimization of microgreen juice-based dairy (lassi) beverage

Consistency and mouthfeel were strongly influenced by the dahi and water concentrations. The dairy beverage represents a smooth, creamy texture and its thickness depends on the ratio of dahi to water (Withers et al., 2013). High dahi concentrations enhanced viscosity (Gallardo-Escamilla et al., 2006), resulting in high consistency scores from 8.11 to 8.07. However, excessively thick formulations can lead to a heavy and less drinkable mouthfeel, which some panelists found undesirable, as reflected in lower mouthfeel scores from 7.49 to 7.60 (**Table 6.66**). The (**Table 6.71**) indicates that the linear effects of dahi and water were significant, with interactions between these variables affecting the final texture of the developed dairy (lassi) beverage. The sensory response surfaces demonstrate in (**Figure 6.44 (C)**) how the interplay of these variables influences consistency and mouthfeel. These results were in agreement with those obtained by Maji et al. (2018) who reported that turmeric extract in different concentrations had a significant effect on the sensory parameters of consistency and mouthfeel of herbal lassi.

Overall acceptability reflects the cumulative impact of sensory attributes and serves as a holistic measure of consumer preference. As seen in (**Table 6.66**), the overall acceptability scores ranged from 7.05 to 7.96 on the 9-point hedonic scale. The response surface graph (**Figure 6.44 (D)**) illustrates the relationship between independent variables and the overall preference of the panelists. Based on the graph, moderate concentrations of microgreen juice tend to result in a higher overall preference for the microgreen juice-based dairy (lassi) beverage. The regression equation (R^2) accounted for 99.82% of the total variation in the overall acceptability of the lassi beverage. The quadratic models for sensory parameters (**Table 6.71**) exhibited significant effects of both dahi and microgreen juice on overall acceptability, emphasizing the importance of balanced proportions. Similarly, as the concentration of fenugreek and spinach microgreen juice increases in the functional beverage, the overall acceptability of the juice blend decreases because of its dark green color. The brown coloration results from the reaction between anthocyanins and tannins, which form brown-colored complexes, giving the juice a dark color and a slightly sour taste (Sharma et al., 2020; Sharma et al., 2021). The moringa-fortified lassi was prepared from moringa leaf powder, this study showed that the increase in the concentration of moringa leaf powder (1.63 to 2.26%) concentration significantly ($P < 0.05$) decreased the overall acceptability score of prepared products from 8.50 to 6.25, respectively (Mistry et al., 2018).

Table 6.71. The predicted models to experimental data in D-optimal design for the effect of microgreen-based microgreen juice-based diary (lassi) beverage

Variables	Regression equation	Mean±SD	C.V. %	R ²	Adjusted R ²	Predicted R ²	Adeq precision
Colour & Appearance	$7.81 \times A + 8.10 \times B + 8.09 \times C + 0.0982 \times AB - 0.5233 \times AC + 0.0804 \times BC - 1.52 \times ABC$	7.98±0.09	0.12	0.9957	0.9932	0.9848	49.12
Flavour & Sweetness	$7.32 \times A + 7.30 \times B + 7.54 \times C + 1.95 \times AB - 0.1775 \times AC + 1.70 \times BC - 6.69 \times ABC$	7.54±0.02	0.29	0.9917	0.9867	0.9749	38.56
Consistency	$7.88 \times A + 8.09 \times B + 7.94 \times C - 0.8554 \times AB - 0.2395 \times AC - 0.1847 \times BC + 0.2665 \times ABC$	7.90±0.01	0.18	0.9844	0.9750	0.9480	33.50
Mouthfeel	$7.50 \times A + 7.54 \times B + 7.77 \times C + 1.32 \times AB + 1.25 \times AC + 0.4053 \times BC - 12.13 \times ABC$	7.66±0.03	0.41	0.9710	0.9537	0.8936	22.64
Overall Acceptability	$7.05 \times A + 7.77 \times B + 7.96 \times C - 0.2073 \times AB + 0.6976 \times AC - 0.0605 \times BC - 6.29 \times ABC$	7.57±0.11	0.20	0.9982	0.9971	0.9943	90.29
pH	$3.91 \times A + 3.81 \times B + 3.88 \times C + 0.0576 \times AB - 0.0229 \times AC - 0.1117 \times BC - 1.10 \times ABC$	3.85±0.06	0.15	0.9833	0.9733	0.9399	26.70
Acidity	$0.5835 \times A + 0.5447 \times B + 0.5476 \times C + 0.0361 \times AB - 0.0165 \times AC + 0.0155 \times BC + 0.0307 \times ABC$	0.56±0.04	0.82	0.9256	0.8810	0.7516	12.99
Total soluble solids	$22.12 \times A + 22.53 \times B + 22.56 \times C - 6.43 \times AB + 3.26 \times AC - 2.28 \times BC - 7.75 \times ABC$	21.98±0.14	0.63	0.9713	0.9541	0.9069	27.05
Water holding capacity	$43.82 \times A + 47.23 \times B + 46.00 \times C - 17.65 \times AB + 3.56 \times AC + 18.12 \times BC + 76.62 \times ABC$	46.73±0.42	0.91	0.9883	0.*812	0.9638	36.66
Syneresis	$43.81 \times A + 49.73 \times B + 48.05 \times C - 20.61 \times AB + 1.27 \times AC + 13.49 \times BC + 107.83 \times ABC$	48.60±0.57	1.49	0.9789	0.9662	0.9347	26.70
Anthocyanin content	$36.55 \times A + 24.52 \times B + 23.77 \times C + 11.45 \times AB + 33.51 \times AC + 2.17 \times BC - 247.71 \times ABC$	28.41±0.30	1.06	0.9981	0.9970	0.9940	82.86
Total phenol content	$42.26 \times A + 30.81 \times B + 29.79 \times C + 8.97 \times AB + 39.45 \times AC - 4.91 \times BC - 107.29 \times ABC$	35.36±0.53	1.50	0.9943	0.9908	0.9800	49.33
Total flavonoid content	$23.43 \times A + 11.95 \times B + 11.97 \times C - 3.74 \times AB - 2.32 \times AC - 0.2499 \times BC + 13.41 \times ABC$	15.38±0.30	1.99	0.9960	0.9935	0.9866	58.67
Antioxidant activity DPPH	$59.49 \times A + 51.51 \times B + 51.92 \times C + 5.01 \times AB + 3.08 \times AC + 1.01 \times BC + 12.02 \times ABC$	54.81±0.27	0.50	0.9939	0.9902	0.9806	44.82
Antioxidant activity FRAP	$8.85 \times A + 5.61 \times B + 5.40 \times C - 3.07 \times AB - 2.48 \times AC + 1.09 \times BC + 10.51 \times ABC$	6.43±0.10	1.61	0.9939	0.9902	0.9785	51.95
Ascorbic acid	$48.84 \times A + 34.32 \times B + 32.97 \times C + 0.4387 \times AB - 8.04 \times AC + 1.66 \times BC - 9.77 \times ABC$	38.14±0.25	0.38	0.9984	0.9974	0.9944	95.35

Table 6.72. ANOVA special cubic model for the different responses

Responses	Source	Sum of Squares	df	Mean Square	F-value	P-value	
Colour & Appearance	Model	0.2185	6	0.0364	389.24	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	0.1801	2	0.09	962.36	< 0.0001	
	AB	0.0008	1	0.0008	8.69	0.0146	
	AC	0.0151	1	0.0151	161.39	< 0.0001	
	BC	0.0006	1	0.0006	5.95	0.0348	
	ABC	0.0034	1	0.0034	36.87	0.0001	
	Residual	0.0009	10	0.0001			
	Lack of Fit	0.0001	5	0	0.1006	0.9876	>0.05
	Pure Error	0.0008	5	0.0002			
	Cor Total	0.2194	16				
Flavour & Sweetness	Model	0.5848	6	0.0975	199.09	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	0.062	2	0.031	63.36	< 0.0001	
	AB	0.3213	1	0.3213	656.43	< 0.0001	
	AC	0.0017	1	0.0017	3.55	0.089	
	BC	0.2504	1	0.2504	511.43	< 0.0001	
	ABC	0.0671	1	0.0671	137.07	< 0.0001	
	Residual	0.0049	10	0.0005			
	Lack of Fit	0.0023	5	0.0005	0.8827	0.5528	>0.05
	Pure Error	0.0026	5	0.0005			
	Cor Total	0.5896	16				
Consistency	Model	0.1374	6	0.0229	105.07	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	0.0621	2	0.031	142.44	< 0.0001	
	AB	0.0617	1	0.0617	282.9	< 0.0001	
	AC	0.0032	1	0.0032	14.52	0.0034	
	BC	0.0029	1	0.0029	13.48	0.0043	
	ABC	0.0001	1	0.0001	0.4889	0.5004	
	Residual	0.0022	10	0.0002			
	Lack of Fit	0.0004	5	0.0001	0.2108	0.9437	>0.05
	Pure Error	0.0018	5	0.0004			
	Cor Total	0.1396	16				
Mouthfeel	Model	0.3379	6	0.0563	55.87	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	0.049	2	0.0245	24.28	0.0001	
	AB	0.1469	1	0.1469	145.71	< 0.0001	
	AC	0.0858	1	0.0858	85.14	< 0.0001	
	BC	0.0142	1	0.0142	14.04	0.0038	
	ABC	0.2208	1	0.2208	219.01	< 0.0001	
	Residual	0.0101	10	0.001			
	Lack of Fit	0.0012	5	0.0002	0.1327	0.9776	>0.05
	Pure Error	0.0089	5	0.0018			
	Cor Total	0.348	16				

Overall Acceptability	Model	1.35	6	0.2249	904.5	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	1.25	2	0.6262	2519	< 0.0001	
	AB	0.0036	1	0.0036	14.56	0.0034	
	AC	0.0268	1	0.0268	107.94	< 0.0001	
	BC	0.0003	1	0.0003	1.27	0.2861	
	ABC	0.0593	1	0.0593	238.68	< 0.0001	
	Residual	0.0025	10	0.0002			
	Lack of Fit	0.0007	5	0.0001	0.3811	0.8434	>0.05
	Pure Error	0.0018	5	0.0004			
	Cor Total	1.35	16				
pH	Model	0.0222	6	0.0037	98.28	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	0.0159	2	0.0079	210.84	< 0.0001	
	AB	0.0003	1	0.0003	7.42	0.0214	
	AC	0	1	0	0.7698	0.4009	
	BC	0.0011	1	0.0011	28.51	0.0003	
	ABC	0.0018	1	0.0018	48.22	< 0.0001	
	Residual	0.0004	10	0			
	Lack of Fit	0.0001	5	0	0.2556	0.9197	>0.05
	Pure Error	0.0003	5	0.0001			
	Cor Total	0.0226	16				
Acidity	Model	0.0027	6	0.0004	20.74	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	0.0025	2	0.0013	58.34	< 0.0001	
	AB	0.0001	1	0.0001	5.08	0.0479	
	AC	0	1	0	0.696	0.4236	
	BC	0	1	0	0.9607	0.3501	
	ABC	0.000001412	1	1.412E-06	0.0654	0.8033	
	Residual	0.0002	10	0			
	Lack of Fit	0.0001	5	0	0.5872	0.7134	>0.05
	Pure Error	0.0001	5	0			
	Cor Total	0.0029	16				
Total soluble solids	Model	6.68	6	1.11	56.47	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	1.34	2	0.6683	33.9	< 0.0001	
	AB	3.48	1	3.48	176.63	< 0.0001	
	AC	0.5874	1	0.5874	29.8	0.0003	
	BC	0.4474	1	0.4474	22.7	0.0008	
	ABC	0.09	1	0.09	4.57	0.0583	
	Residual	0.1971	10	0.0197			
	Lack of Fit	0.0493	5	0.0099	0.3336	0.8732	>0.05
	Pure Error	0.1478	5	0.0296			
	Cor Total	6.88	16				
	Model	153.21	6	25.54	140.49	< 0.0001	< 0.05

Water holding capacity	⁽¹⁾ Linear Mixture	60.53	2	30.27	166.52	< 0.0001	
	AB	26.25	1	26.25	144.42	< 0.0001	
	AC	0.7002	1	0.7002	3.85	0.0781	
	BC	28.27	1	28.27	155.55	< 0.0001	
	ABC	8.81	1	8.81	48.45	< 0.0001	
	Residual	1.82	10	0.1818			
	Lack of Fit	0.388	5	0.0776	0.2714	0.9107	>0.05
	Pure Error	1.43	5	0.2859			
	Cor Total	155.03	16				
Syneresis	Model	153.99	6	25.66	77.19	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	60.48	2	30.24	90.95	< 0.0001	
	AB	35.81	1	35.81	107.69	< 0.0001	
	AC	0.0885	1	0.0885	0.2661	0.6171	
	BC	15.69	1	15.69	47.17	< 0.0001	
	ABC	17.44	1	17.44	52.45	< 0.0001	
	Residual	3.33	10	0.3325			
	Lack of Fit	0.8738	5	0.1748	0.3565	0.859	>0.05
	Pure Error	2.45	5	0.4903			
	Cor Total	157.31	16				
Anthocyanin content	Model	481.7	6	80.28	887.16	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	368.72	2	184.36	2037.2	< 0.0001	
	AB	11.05	1	11.05	122.06	< 0.0001	
	AC	61.92	1	61.92	684.28	< 0.0001	
	BC	0.4039	1	0.4039	4.46	0.0608	
	ABC	92.04	1	92.04	1017.1	< 0.0001	
	Residual	0.9049	10	0.0905			
	Lack of Fit	0.6082	5	0.1216	2.05	0.2248	>0.05
	Pure Error	0.2967	5	0.0593			
	Cor Total	482.61	16				
Total phenol content	Model	490.37	6	81.73	289.54	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	391.66	2	195.83	693.76	< 0.0001	
	AB	6.79	1	6.79	24.04	0.0006	
	AC	85.8	1	85.8	303.97	< 0.0001	
	BC	2.08	1	2.08	7.36	0.0218	
	ABC	17.27	1	17.27	61.17	< 0.0001	
	Residual	2.82	10	0.2823			
	Lack of Fit	0.1584	5	0.0317	0.0595	0.9962	>0.05
	Pure Error	2.66	5	0.5329			
	Cor Total	493.2	16				
Total flavonoid content	Model	231.56	6	38.59	411.03	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	230.19	2	115.09	1225.8	< 0.0001	
	AB	1.18	1	1.18	12.58	0.0053	

	AC	0.2969	1	0.2969	3.16	0.1057	
	BC	0.0054	1	0.0054	0.0573	0.8157	
	ABC	0.2697	1	0.2697	2.87	0.121	
	Residual	0.9389	10	0.0939			
	Lack of Fit	0.2229	5	0.0446	0.3113	0.887	>0.05
	Pure Error	0.716	5	0.1432			
	Cor Total	232.5	16				
Antioxidant activity DPPH	Model	124.54	6	20.76	269.78	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	119.52	2	59.76	776.74	< 0.0001	
	AB	2.11	1	2.11	27.47	0.0004	
	AC	0.5216	1	0.5216	6.78	0.0263	
	BC	0.0871	1	0.0871	1.13	0.3124	
	ABC	0.2167	1	0.2167	2.82	0.1242	
	Residual	0.7694	10	0.0769			
	Lack of Fit	0.7059	5	0.1412	11.13	0.0097	>0.05
	Pure Error	0.0635	5	0.0127			
	Cor Total	125.31	16				
Antioxidant activity FRAP	Model	17.31	6	2.89	270.73	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	15.99	2	7.99	750.18	< 0.0001	
	AB	0.7962	1	0.7962	74.71	< 0.0001	
	AC	0.3396	1	0.3396	31.87	0.0002	
	BC	0.1022	1	0.1022	9.59	0.0113	
	ABC	0.1657	1	0.1657	15.55	0.0028	
	Residual	0.1066	10	0.0107			
	Lack of Fit	0.0053	5	0.0011	0.0525	0.9971	>0.05
	Pure Error	0.1013	5	0.0203			
	Cor Total	17.42	16				
Ascorbic acid	Model	412.2	6	68.7	1021.3	< 0.0001	< 0.05
	⁽¹⁾ Linear Mixture	405.67	2	202.83	3015.3	< 0.0001	
	AB	0.0162	1	0.0162	0.2411	0.634	
	AC	3.56	1	3.56	52.93	< 0.0001	
	BC	0.2363	1	0.2363	3.51	0.0904	
	ABC	0.1432	1	0.1432	2.13	0.1753	
	Residual	0.6727	10	0.0673			
	Lack of Fit	0.0654	5	0.0131	0.1078	0.9856	>0.05
	Pure Error	0.6073	5	0.1215			
	Cor Total	412.87	16				

6.7.2. Effect of processing variables on physicochemical parameters

The physicochemical properties of the microgreen juice-based dairy (lassi) beverage, including pH, titratable acidity (lactic acid %), total soluble solids, WHC, and syneresis, were significantly ($P < 0.05$) influenced by processing variables including, microgreens juice (5–

9.5%), dahi (60–64.5%), and water (15–19.5%). The above-mentioned parameters such as pH, are important parameters in dairy products that influence microbial growth, flavor development, and the coagulation of milk proteins, which is essential for the production of fermented dairy products (cheese, dahi, and yogurt) (Alam et al., 2024). The titratable acidity, expressed as a lactic acid percentage, is critical for assessing fermentation quality and product safety, while total soluble solids contribute to the texture and sweetness of dairy products (Akhi et al., 2023). Furthermore, parameters like WHC and syneresis are vital for determining the texture and stability of products, affecting consumer acceptance and product shelf life (Arab et al., 2022).

The pH value of the microgreen juice-based dairy (lassi) beverage ranged from 3.81 to 3.92 across different formulations were observed, respectively (**Table 6.67**). The obtained results indicated that both microgreen juice (%) and dahi (%) content significantly ($P < 0.05$) influenced the pH value of the beverage, while water (%) addition had a diluting effect. The high level of microgreen juice concentrations (%) reduced the pH due to the presence of organic acids such as ascorbic acid, phenolic compounds, and glucosinolates in radish sango microgreens (Kaur et al., 2018; Mlinarić et al., 2023). Among the different formulated dairy (lassi) beverage the microgreens juice containing 9.5% juice showed a pH of 3.81, among the lowest in the study. Moreover, increased dahi (%) content during the development of dairy (lassi) had a similar acidifying effect observed, as dahi is rich in lactic acid produced during fermentation (Akhi et al., 2023; Alam et al., 2024). The addition of water during lassi preparation, and the high water percentage (%) mitigated the acidifying effects of juice and dahi, leading to a relatively higher pH. The reduction in pH value of dairy (lassi) beverage with higher juice and dahi percentages is attributed to their acidic nature. A low pH enhances the microbial stability of the beverage but must be balanced to avoid over-acidification, which can impact flavor. The addition of microgreens juice (%), dahi (%), and water (%) at the linear level (**Figure 6.44 (C)**) and the quadratic level (**Table 6.71**) significantly ($P < 0.05$) influenced the pH value of the beverage, as indicated in (**Table 6.72**). RSM analysis (**Figure 6.44 (C)**) also supports this finding, and demonstrates the interplay between the variables the concentration of microgreen juice (A), dahi (B), and water (C), with increasing concentration causing a slight decrease in the pH value of formulated dairy (lassi) beverage. The observed pH values align with previous studies on functional dairy (lassi) beverages enriched with plant-based powder/extract additives (Hussain et al., 2015; Kumar et al., 2017; Kumar et al., 2023).

The titratable acidity, in this study, was expressed as a percentage of lactic acid, ranging from 0.54% to 0.58% (**Table 6.66**). The microgreen juice (%) at higher levels increased

titratable acidity, as observed in our finding results. This is consistent with the presence of organic acids in the microgreen juice (Sharma et al., 2020; 2021; Kaur et al., 2018; Mlinarić et al., 2023). Our results are in agreement with Kumar and Mishra (2004), who observed higher acid development (0.729%) in mango soymilk-fortified yogurt. Similarly, in another study on the development of tomato pomace in lassi, the acidity (0.65 to 0.78%) (Kumar et al., 2023) and moringa pod powder added to lassi the acidity (0.24 to 0.65%) (Mistry et al., 2018), where tomato pomace powder and moringa pod powder were increased acid production. Another variable, dahi significantly contributed to acidity due to its lactic acid content, with the increasing concentration of dahi showing an increasing trend in titratable acidity in the study. Apart from these, increased water levels diluted the acidity, resulting in lower titratable acidity values. Titratable acidity increases with higher juice and dahi levels due to their acid contributions (Kumar et al., 2023). The acid content also supports microbial inhibition, extending shelf life. Based on the statistical analysis of ANOVA for titratable acidity response (**Table 6.72**), suggests a special cubic model with an F-value of 20.74 and a P-value ($P < 0.05$) indicating a significant model with a coefficient of determination ($R^2 = 0.9256$) (**Table 6.71**). However, no significance was found for lack of fit (0.7134). These results demonstrate that the percentage of microgreen juice, dahi, and water has an influence on the titratable acidity of the microgreen juice-based dairy (lassi) beverage. The final equation that represents the titratable acidity of the microgreens juice-based dairy (lassi) beverage is shown in (**Table 6.71**).

The total soluble solids content measured the dissolved solids content in microgreen juice-based dairy (beverage), the observed value ranged from 20.74 to 23.17°Brix (**Table 6.67**). Total soluble solids show the presence of sugars, minerals, and organic acids in the beverage (Andrés et al., 2014). The presence of a high percentage of microgreen juice in microgreen juice dairy (lassi) beverages contributes to increased total soluble solid content due to the presence of natural sugars and bioactive compounds (**Figure 6.44 (F)**), with 9.5% microgreen juice added dairy (lassi) recorded the highest total soluble solid content (23.17°Brix). On the other side, dahi significantly ($P < 0.05$) influenced total soluble solid content, as it contains lactose sugar. The formulation with higher dahi content (64.5%), showed slightly elevated total soluble content values (22.89°Brix). The addition of water during the preparation of dairy (lassi) beverage reduced total soluble solid content with 19.5% water recorded as the lowest (21.12°Brix). The final equation represents the total soluble solids content of the microgreen-based microgreen juice-based dairy (lassi) as shown in (**Table 6.71**). The statistical analysis using ANOVA (**Table 6.72**) revealed that the special cubic model showed an F-value of 56.47, with a P-value of less than ($P < 0.05$), suggesting a significant model fit. Additionally, the

coefficient of determination ($R^2 = 0.9713$) indicates a strong correlation (**Table 6.71**), while the lack of fit is found to be insignificant (0.8732). These findings demonstrate that the composition of the microgreen juice-based dairy (lassi) beverage ingredient has a determining effect on the total soluble solids content. The increase in total soluble solids with higher juice and dahi percentage affects the sweetness and mouthfeel sensory parameters (Kumar et al., 2023). However, excessive amounts of total soluble solid content, affect the viscosity of beverage (Kaur et al., 2018). The pudina extract (2.5 to 7.5%) was incorporated into the lassi, and the total soluble solids content ranged from 11.16 to 11.55, this study showed that at lower concentrations of pudina extract (2.5%) observed lower total soluble solids content value (Dhumal et al., 2018). Similar results were reported for the fermented dairy (lassi) product prepared with varying levels of mango powder (2.5 to 7.5%) and beetroot powder (1 to 4%). The TSS values for mango powder lassi (9.80 to 17%) and beetroot powder lassi (9.80 to 11.30%) were observed (Chawla & Sivakumar, 2017).

WHC, a measure of the beverages ability to retain water and resist syneresis and syneresis, the separation of liquid from the solid matrix, is an undesirable attribute that impacts the beverages stability and visual appeal (Akhi et al., 2023). The WHC and syneresis in developed microgreen juice-based dairy (lassi) beverage ranged from 41.05% to 51.86% and 42.05% to 52.88%, respectively (**Table 6.66**). Both parameters help in maintaining the texture and stability of dairy-based beverages. The increased percentage of microgreen juice improved WHC slightly and reduced syneresis, likely due to the binding properties of bioactive compounds (Sanyukta et al., 2023). Increased dahi (%) levels significantly enhanced WHC and minimized syneresis by forming a cohesive protein network that retains water. On the opposite side, an increase in a concentration of water (%) during the preparation of microgreen juice dairy (lassi) reduced WHC and increased syneresis by weakening the structural integrity of the beverage. The improvement in WHC with dahi and microgreen juice concentrations is attributed to the protein-bioactive interactions that stabilize the beverage matrix. A high level of WHC ensures a creamy texture and prevents phase separation, enhancing the appearance of the product (Kumar et al., 2021).

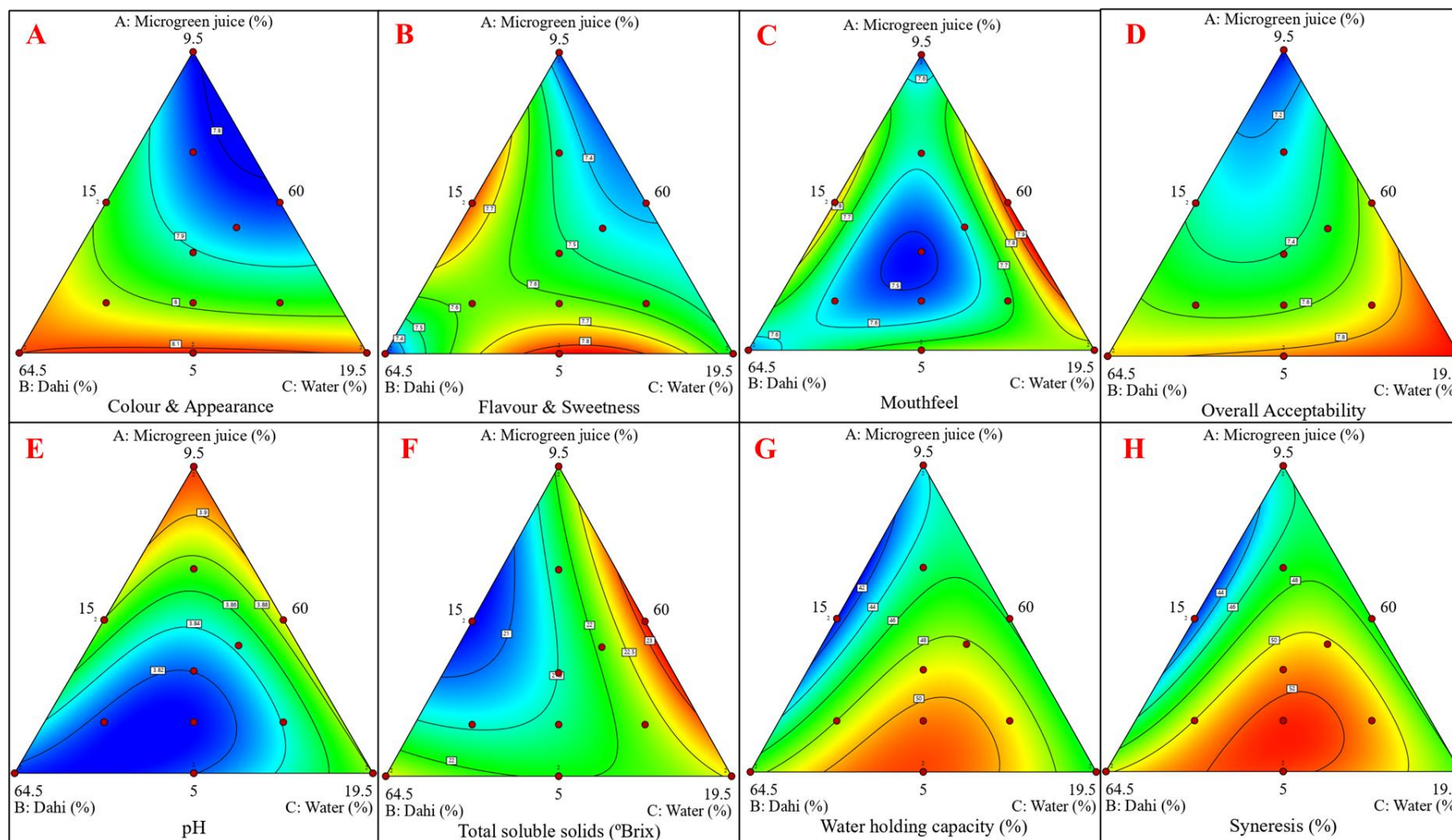


Figure 6.44. Response surface plots showing the effect of microgreen juice (A), dahi (B), and water (C) on microgreen juice-based dairy (lassi) beverage selected study parameters. Where, A: Colour & Appearance; B: Flavour & Sweetness; C: Mouthfeel; D: Overall acceptability; E: pH; F: Total soluble solids; G: Water holding capacity; H: Syneresis.

On the other side, the reduction in syneresis with higher microgreen juice and dahi percentage highlights their stabilizing role in the beverage matrix. Lower syneresis improves the products shelf life, texture, and consumer acceptability (Kumar et al., 2023). The **(Figure 6.44 (G and H))** depicts the relationship between these parameters and the processing variables, showing that higher dahi and juice levels optimize this parameter. The **(Table 6.71)** shows positive significant ($P<0.05$) linear and quadratic effects of microgreens juice and dahi on WHC and syneresis. The regression model high R^2 value (0.9883 and 0.9789) indicates strong predictive reliability. The statistical analysis using ANOVA **(Table 6.72)** revealed that the special cubic model showed an F-value of 140.49 and 77.19, with a P-value of less than ($P<0.05$), suggesting a significant model fit. The observed syneresis trends align with studies on lassi enriched with tomato pomace powder, where excessive fiber and bioactive composition high content ingredients weakened gel structures (Kumar et al., 2023) and similarly, for the WHC, lassi supplemented with Aloe *barbadensis* Miller juice (Hussain et al., 2014). The processing variables (microgreens juice, dahi, and water) had significant impacts on the physicochemical properties of the microgreen juice-based lassi beverage. Increasing microgreens juice and dahi concentrations improved acidity, total soluble solids, WHC, and reduced syneresis, enhancing the beverages stability and sensory characteristics. Conversely, higher water percentage significantly reduced these effects, highlighting the need for careful optimization.

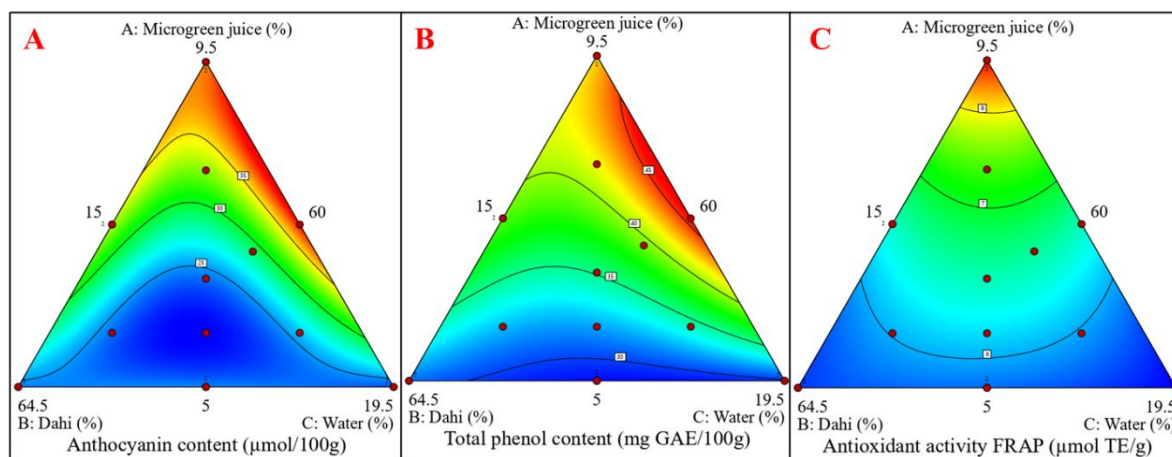


Figure 6.45. Response surface plots showing the effect of microgreen juice (A), dahi (B), and water (C) on microgreen juice-based dairy (lassi) beverage selected study parameters. Where, A: Anthocyanin content; B: Total phenol content; C: Antioxidant activity FRAP.

6.7.3. Effect of processing variables on biochemical and antioxidant parameters

The biochemical and antioxidant parameters of the microgreen juice-based lassi beverage are important indicators of its nutritional and functional quality. These parameters, including ascorbic acid content, anthocyanin content, TPC, total flavonoid content, and

antioxidant activities (DPPH and FRAP), were evaluated under different concentration percentages (%) of microgreens juice, dahi, and water. The obtained results for biochemical and antioxidant parameters are presented in (Table 6.68).

6.7.3.1. Ascorbic acid and anthocyanin content

Ascorbic acid (vitamin C), a potent antioxidant, is another critical bioactive compound in the beverage and it contributes to immune defense (Sobczak et al., 2022). Its content in microgreen juice containing dairy (lassi) beverage ranged from 32.96 to 48.86 mg/100g, respectively observed (Table 6.68). The high percentage of microgreen juice significantly increased the ascorbic acid content of prepared dairy (lassi) beverage. The regression model (Table 6.71) confirmed significant ($P < 0.05$) linear effects of microgreen juice, with minimal influence from dahi and water. The orange peel powder was added at varying levels (1.5 to 5%) to the lassi, and the ascorbic acid content in the prepared lassi significantly increased up to 136 mg/ 100g by adding 2.5% orange peel powder (Ananthakumar & Narayanan, 2021). In another study, *Mangifera indica* (15 to 20%) and *Myrciaria dubia* (5 to 10%) were incorporated into the yogurt, the ascorbic acid content in the range of 57.3 to 115.1 mg/100g observed, respectively (Renteria et al., 2022).

Anthocyanins, known for their antioxidant properties, are abundant in radish sango microgreens (Zhang et al., 2018). The anthocyanin content in the dairy (lassi) beverage ranged from 22.55 to 38.68 $\mu\text{mol}/100\text{ g}$ (Table 6.68). The highest anthocyanin levels were observed in formulations with higher microgreen juice content, such as 9.5%, demonstrating that microgreen juice is the primary source of anthocyanins. The regression analysis in (Table 6.71) revealed significant ($P < 0.05$) linear and quadratic effects of microgreen juice on anthocyanin content. Interactions with dahi and water were also influential. The excess water (19.5%) reduced the anthocyanin concentration. Conversely, formulations with lower water and higher juice concentrations achieved enhanced anthocyanin retention. The response surface plot for anthocyanin content (Figure 6.45 (A)) revealed a direct correlation between microgreen juice concentration and this bioactive compound. The increase in anthocyanin levels reflects the pigmentation properties of radish sango microgreens, which are rich in these natural antioxidants. The different concentrations of mulberry pomace powder (1 to 3%) were incorporated into the preparation of fortified stirred yogurt, from this study the anthocyanin content of 23.29 to 79.09 g cyanidin-3glucoside/g was observed (Du et al., 2021).

6.7.3.2. Total phenol and flavonoid content

Phenolic and flavonoid compounds are vital for antioxidant activity and contribute to the dairy (lassi) beverage functional properties. The TPC and flavonoid content of the

microgreen juice-based dairy (lassi) beverage ranged from 28.88 to 45.87 mg GAE/100g and 11.44 to 23.43 mg QE/100g were observed in developed dairy (lassi) beverage samples, respectively (**Table 6.68**). Formulations with higher microgreen juice (%) concentrations exhibited elevated total phenol and flavonoid content. This increase is attributed to the abundant phenolic compounds in radish sango microgreens, which are concentrated in the juice. The (**Figure 6.44 (B)**) depicts the relationship between TPC and the processing variables, showing that higher dahi and juice significantly ($P<0.05$) increased the phenolic content. The (**Table 6.71**) shows positive significant ($P<0.05$) linear and quadratic effects of microgreen juice and dahi on phenol and flavonoid content. The regression model high R^2 value (0.9943 and 0.9960) indicates strong predictive reliability. The statistical analysis using ANOVA (**Table 6.72**) revealed that the special cubic model showed an F-value of 289.56 and 411.03, with a P-value of less than ($P<0.05$), suggesting a significant model fit. Our results are consistent with findings from Varnaite et al. (2022) and Alqahtani et al. (2020), who observed a similar trend in TPC in yogurt with cranberry and tomato pomace. In another study, antioxidant-enriched herbal lassi incorporated with lemongrass, the TPC observed in formulated lassi is 50.28 mg GAE/100g, respectively (Shendurse et al., 2024). Additionally, Maji et al. (2020) reported a TPC range of 0.124 to 0.226 mg GAE/gm for various types of herbal lassi (ginger, carrot, turmeric), which was lower than our results.

6.7.3.4. Antioxidant activity

The antioxidant activities of the lassi were analyzed using the DPPH radical scavenging activity and FRAP. Both the antioxidant properties showed significant ($P<0.05$) variation based on the processing variables of microgreen juice, dahi, and water. The DPPH and FRAP antioxidant activity ranged from 51.33% to 59.48% and 5.41 to 8.84 $\mu\text{mol TE/g}$, were observed respectively (**Table 6.68**). Microgreens juice-based dairy (lassi) beverage formulations with higher microgreens juice (%) exhibited greater antioxidant properties. This trend aligns with the higher levels of anthocyanins, phenolics, and flavonoids in these formulations. Radish sango microgreens are rich in phenolics, flavonoids, anthocyanins, and ascorbic acid (Singh et al., 2024). Higher microgreen juice concentrations directly enhance the bioactive compound content and antioxidant activities (Sharma et al., 2020). However, excessive juice levels may lead to sensory challenges (Kaur et al., 2018). The regression model (**Table 6.69**) confirmed significant linear and quadratic effects of juice concentration on the antioxidant activities of (DPPH and FRAP). The addition of water significantly reduced the activity by diluting the antioxidant compounds. The dahi provides a protective environment for bioactive compounds, reducing oxidative degradation of ascorbic acid and phenolic compounds. Moderate dahi (%)

enhances the solubility and stability of phenolics and flavonoids. The regression analysis (**Table 6.71**) revealed significant interactions between microgreen juice and water, with optimal combinations enhancing the radical scavenging activity and ferric-reducing power of the microgreen juice-based dairy (lassi) beverage. In (**Figure 6.45 (C)**) further illustrates the positive correlation between microgreen juice and the antioxidant activity of FRAP. The regression model for antioxidant activity of DPPH and FRAP high R^2 value (0.9902 and 0.9902) indicates strong predictive reliability. The statistical analysis using ANOVA (**Table 6.72**) revealed that the special cubic model showed an F-value of 269.78 and 270.73, with a P-value of less than ($P < 0.05$), suggesting both significant models fit. The DPPH scavenging activity of symbiotic lassi with honey was recorded at 28.43% (Shah et al., 2016). Additionally, lassi enhanced with tomato pomace powder exhibited DPPH antioxidant activity ranging from 13.11% to 28.12% (Kumar et al., 2023). These values are somewhat lower compared to our findings. In another study, antioxidant-enriched herbal lassi incorporated with Lemongrass, antioxidant capacity such as DPPH and FRAP were observed in formulated lassi is 33.53 and 24.62 $\mu\text{M TE}/100\text{g}$, respectively (Shendurse et al., 2024).

6.7.4. Effect of storage conditions on physicochemical properties

The physicochemical properties of the optimized microgreen juice-based dairy (lassi) beverage were evaluated during storage at three different temperatures (5°C , 15°C , and 25°C) over 15 days. During this storage time, various physicochemical parameters were analyzed including pH, acidity, total soluble solids, WHC, and syneresis. These parameters are critical in determining the quality, stability, and shelf life of the dairy (lassi) beverage.

The pH of the dairy-based lassi consistently decreased during storage across all temperature conditions, indicating increased acidity due to microbial fermentation and chemical changes. On day 0th day, the pH value was observed to be 4.35 for all storage conditions. By the end of the 15th day storage period, the pH decreased to 4.05 at 5°C , 3.99 at 15°C , and 3.91 at 25°C (**Table 6.73**). Temperature significantly affected ($P < 0.05$) the pH value of the optimized microgreen juice-based dairy (lassi) beverage, with higher storage temperatures accelerating the decline in pH (Ziaro et al., 2023). At 25°C , microbial activity was more pronounced, leading to the rapid production of lactic acid and a steeper drop in pH. In contrast, the lower temperature condition (5°C) slowed microbial activity, maintaining a higher pH for a longer period (Łopusiewicz et al., 2019).

Table 6.73. Effect of storage temperature conditions on physicochemical parameters of microgreen juice-based diary (lassi) beverage.

Days	Temperature (°C)	Overall acceptability	pH	Acidity (% lactic acid)	Total soluble solid (°Brix)	Water holding capacity (%)	Syneresis (%)
Day-0	5	8.29±0.10 ^{aA}	4.35±0.01 ^{aA}	0.62±0.01 ^{cC}	22.42±0.08 ^{aA}	44.32±0.17 ^{fB}	46.30±0.36 ^{dB}
	15	8.29±0.10 ^{aA}	4.35±0.01 ^{abAB}	0.62±0.01 ^{bB}	22.42±0.08 ^{aB}	44.32±0.17 ^{fB}	46.30±0.36 ^{dA}
	25	8.29±0.10 ^{aA}	4.35±0.01 ^{bB}	0.62±0.01 ^{aA}	22.42±0.08 ^{aC}	44.32±0.17 ^{fB}	46.30±0.36 ^{dA}
Day-3	5	8.17±0.06 ^{abA}	4.31±0.01 ^{aA}	0.63±0.01 ^{cC}	22.34±0.01 ^{abA}	44.42±0.04 ^{efB}	46.57±0.03 ^{dB}
	15	8.12±0.02 ^{abB}	4.30±0.01 ^{abbAB}	0.63±0.01 ^{bB}	22.29±0.01 ^{bB}	44.50±0.01 ^{efB}	46.57±0.03 ^{dA}
	25	8.01±0.04 ^{abcC}	4.29±0.01 ^{bB}	0.65±0.01 ^{ahA}	22.18±0.01 ^{cC}	44.65±0.14 ^{efA}	46.59±0.01 ^{dA}
Day-6	5	8.13±0.04 ^{abA}	4.29±0.01 ^{abA}	0.69±0.01 ^{gC}	22.30±0.01 ^{abA}	44.71±0.10 ^{efB}	46.83±0.08 ^{dB}
	15	7.84±0.16 ^{bcdB}	4.27±0.01 ^{abAB}	0.71±0.02 ^{gB}	22.21±0.02 ^{bcB}	44.79±0.08 ^{defB}	46.88±0.11 ^{cdA}
	25	7.84±0.08 ^{cdC}	4.28±0.02 ^{bB}	0.75±0.01 ^{fA}	22.11±0.13 ^{cC}	44.82±0.18 ^{defA}	47.12±0.19 ^{cdA}
Day-9	5	7.93±0.07 ^{abcA}	4.17±0.01 ^{acA}	0.75±0.04 ^{fC}	22.26±0.01 ^{abcA}	44.92±0.04 ^{deB}	46.92±0.04 ^{bcdB}
	15	7.68±0.10 ^{bdeB}	4.12±0.01 ^{abcdAB}	0.80±0.01 ^{eB}	21.89±0.01 ^{fB}	44.89±0.08 ^{deB}	47.32±0.19 ^{cdA}
	25	7.34±0.21 ^{cfgC}	4.10±0.01 ^{bdB}	0.82±0.01 ^{aA}	21.76±0.01 ^{cC}	45.82±0.18 ^{bA}	46.82±0.62 ^{bcdA}
Day-12	5	7.51±0.10 ^{aefA}	4.06±0.01 ^{adA}	0.83±0.01 ^{cC}	21.98±0.01 ^{eA}	45.27±0.11 ^{cdB}	47.07±0.11 ^{cdB}
	15	7.38±0.09 ^{bfgB}	3.98±0.05 ^{abAB}	0.90±0.02 ^{bB}	21.77±0.01 ^{bB}	45.49±0.23 ^{bcB}	48.07±0.26 ^{bcdA}
	25	7.10±0.12 ^{cC}	4.01±0.12 ^{bB}	0.98±0.01 ^{aA}	21.35±0.01 ^{cC}	45.92±0.04 ^{abA}	47.87±0.16 ^{abA}
Day-15	5	7.27±0.12 ^{agA}	4.05±0.07 ^{deA}	0.93±0.01 ^{cC}	21.55±0.01 ^{aA}	45.59±0.13 ^{bcB}	46.90±0.64 ^{cdB}
	15	7.06±0.06 ^{bB}	3.99±0.01 ^{abAB}	0.99±0.01 ^{bB}	21.14±0.01 ^{bB}	45.84±0.13 ^{bB}	48.55±0.14 ^{aA}
	25	6.25±0.10 ^{cC}	3.91±0.01 ^{bB}	1.13±0.01 ^{aA}	21.10±0.01 ^{cC}	46.42±0.18 ^{aA}	48.85±0.13 ^{aA}

Note: The results were presented as mean±standard deviations (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments and different uppercase letters within the column indicate significant (P<0.05) differences in temperature condition for the parameters.

This decreasing trend in pH value for the optimized microgreen juice-based dairy (lassi) beverage aligns with increased microbial activity, as evidenced by total plate counts (**Table 6.75**). Fermented dairy products like dahi contain naturally occurring lactic acid bacteria, which likely contributed to the acidification by fermenting residual sugars. The decrease in pH during storage is primarily attributed to the metabolic activity of lactic acid bacteria, which convert lactose into lactic acid (Pandhi & Poonia, 2021). The rate of acid production increases with temperature, consistent with the Arrhenius behavior of biochemical reactions. Additionally, interactions between microgreen juice and dairy proteins may contribute to the beverage buffering capacity, slowing the rate of pH decline at lower temperatures. Similarly, Krishna et al. (2019) observed a similar increase in the pH of probiotic lassi over a 12th day storage period. In another study, Aloe vera-supplemented synbiotic lassi exhibited a pH increase to 4.30-4.35 over a 16-day storage period (Kumar et al., 2021). Previous studies on fermented dairy products have also documented a reduction in pH with increasing storage temperatures (>10°C), which aligns with our current observations (George et al., 2010; Shah et al., 2016; Kumar et al., 2017).

The acidity (% lactic acid) of the beverage increased over time, consistent with the observed pH trends. Initially, the optimized microgreen juice-based dairy (lassi) beverage had an acidity of 0.62%. By the end of the 15th day storage period, the acidity increased to 0.93% at 5°C, 0.99% at 15°C, and 1.13% at 25°C. Similar to the pH trends, higher storage temperatures accelerated the increase in acidity. At 25°C, the growth rate of lactic acid bacteria and enzymatic activity was higher, resulting in rapid acid production in the dairy (lassi) beverage (Rawat et al., 2022). The increase in acidity negatively impacted sensory attributes, particularly flavor, and overall acceptability. Conversely, at 5°C, the lower temperature effectively slowed the rate of acidification, preserving sensory quality for a longer period (Rani et al., 2023). The increase in acidity is primarily driven by the fermentation of lactose into lactic acid by lactic acid bacteria (Shah et al., 2016). A similar increasing trend was observed for titratable acidity in herbal honey lassi, which rose from 0.77% to 1.38% over a 28th day storage period (Kumar et al., 2020). The presence of bioactive compounds from radish sango microgreens, which may act as prebiotics, could also stimulate lactic acid bacteria growth, further influencing acidity (Lee et al., 2023).

Total soluble solids (°Brix), which indicate the presence of dissolved solids such as sugars and minerals, remained relatively stable in the optimized dairy (lassi) beverage during storage under different conditions (**Table 6.73**). On the initial day, the total soluble solids content was 22.42°Brix. By the 15th day of storage, the total soluble solids content had decreased to a range of 21.10 to 21.55°Brix, with the decline being more pronounced at the

higher temperature of 25°C. This reduction could be attributed to the utilization of sugars by microbes during fermentation, which reduces the concentration of soluble solids in the dairy (lassi) beverage (Silva et al., 2017; Birwal et al., 2024). The stability of total soluble solids indicates that the beverage formulation maintained its structural integrity, with minimal breakdown of macronutrients such as proteins and polysaccharides during storage (Furlán et al., 2011). Similar trends were observed for the whey-based papaya beverage (Saravanakumar, & Manimegalai, 2001), whey-based fermented beverage prepared by blending using pineapple and strawberry juice (Kaur et al., 2018).

The effect of storage temperature on the WHC and syneresis of the optimized microgreen juice-based dairy (lassi) beverage is presented in (**Table 6.73**). On the 0th day, the WHC and syneresis were observed to be 44.32% and 46.30%, respectively, across all storage conditions. By the end of the 15th day storage period, these values increased to 45.92% and 46.90% at 5°C, 45.84% and 48.55% at 15°C, and 46.42% and 48.85% at 25°C, respectively. Temperature had a significant ($P<0.05$) effect on the WHC and syneresis of the optimized microgreen juice-based dairy (lassi) beverage, with higher storage temperatures (25°C) accelerating these trends. Higher temperature conditions led to a more rapid decline in WHC, indicating destabilization of the dairy (lassi) beverage (Hussain et al., 2015). This destabilization is likely due to increased protein (casein) denaturation and whey separation at elevated temperatures. During this study, the optimized dairy (lassi) beverage stored at 5°C retained better WHC, maintaining the creamy texture of the beverage (Asaduzzaman et al., 2021). The presence of stabilizers like CMC in the formulation likely mitigated these effects, particularly at lower temperatures (Kumar et al., 2021). Similarly, the increase in syneresis was more pronounced at higher temperatures, consistent with greater destabilization of the protein matrix and higher rates of microbial activity at elevated temperatures (Kumar et al., 2021). The increasing syneresis negatively impacted the texture and visual appeal of the beverage, making it less desirable to consumers.

The overall acceptability of the microgreen juice-based dairy (lassi) beverage, evaluated on a 9-point hedonic scale, varied significantly with storage temperature (5°C, 15°C, and 25°C) over 15 days (**Table 6.73**). On the initial day, the optimized formulation scored the highest acceptability (8.15), attributed to its balanced flavor, creamy texture, and fresh appearance. By the end of the 15th day storage period, these values decline to 7.27 at 5°C, 7.06 at 15°C, and 6.25 at 25°C, respectively. Minimal changes in sensory attributes were noted, as refrigeration conditions (5°C) effectively slowed biochemical and microbial activities. These results emphasize that refrigeration at 5°C effectively preserves the microgreen juice-based

dairy (lassi) beverage sensory appeal for up to 15 days, while higher temperatures (15°C and 25°C) lead to accelerated degradation, reducing its consumer acceptability.

6.7.5. Effect of storage conditions on biochemical parameters

6.7.5.1. Ascorbic acid and anthocyanin content

The ascorbic acid and anthocyanin content, key antioxidants in the microgreen juice-based dairy (lassi) beverage, exhibited a significant decline over the storage period, as illustrated in **(Table 6.74)**. Throughout the storage duration, both ascorbic acid and anthocyanin levels decreased steadily, with the rate of decline significantly ($P<0.05$) influenced by temperature conditions. By day 15, the ascorbic acid and anthocyanin content measured at 5°C were 56.75 mg/100g and 35.39 µmol/100g, at 15°C were 52.59 mg/100g and 35.19 µmol/100g, and at 25°C were 47.65 mg/100g and 34.96 µmol/100g, respectively. These results are consistent with prior studies on fermented dairy beverages that reported significant temperature-dependent losses of ascorbic acid during storage (Znamirowska et al., 2021; Ahmed et al., 2023). The reduction was more pronounced at 25°C compared to 5°C and 15°C storage conditions (Martinsen et al., 2020).

Both antioxidants are highly susceptible to oxidative degradation: ascorbic acid converts to dehydroascorbic acid upon exposure to oxygen and light, while anthocyanins degrade due to increased oxidative and enzymatic activity. Anthocyanins are particularly sensitive to temperature, pH, and oxidative conditions, undergoing hydrolysis of glycosidic bonds which leads to loss of color and antioxidant activity (Peñaloza et al., 2022). The degradation of anthocyanins not only diminishes the antioxidant activities of the dairy (lassi) beverage but also its visual appeal, as color intensity fades over time (Mefleh et al., 2022). Refrigeration significantly reduced the degradation rate, preserving both the functional and sensory attributes of the optimized product. This aligns with findings from studies on anthocyanin-enriched dairy beverages, where low-temperature storage was shown to enhance pigment stability (Ścibisz et al., 2019; Hapuarachchi et al., 2023). Under refrigeration (5°C), the degradation of ascorbic acid and anthocyanins was slower, preserving more of these bioactive compounds compared to higher temperatures.

6.7.5.2. Total phenol and flavonoid content

On the initial day, the optimized microgreen juice-based dairy (lassi) beverage contained 44.14 mg GAE/100 mg of total phenols and 22.62 mg QE/100 mg of total flavonoids. These contents significantly ($P<0.05$) decreased over the storage period as storage time and temperature increased. The highest retention of TPC and flavonoid content was observed at lower temperatures (5°C>15°C>25°C) over the 15 day storage period, as shown in **(Table**

6.74). By the end of day 15, the TPC and flavonoid content at 5°C were 42.15 mg GAE/100 mg and 20.42 mg QE/100 mg, at 15°C were 40.96 mg GAE/100 mg and 20.11 mg QE/100 mg, and at 25°C were 40.19 mg GAE/100 mg and 19.89 mg QE/100 mg, respectively.

Phenolic compounds were more stable at lower temperatures. These findings are consistent with studies on functional dairy products enriched with herbal lassi fortified with turmeric extract (Maji et al., 2018), pea milk-fortified dahi with jamun seed extract (Pandhi & Poonia, 2021), and yogurt fortified with goji berries (Taneva & Zlatev, 2020), where phenolic and flavonoid compound stability was found to be temperature-dependent. At 25°C, phenolic degradation was more pronounced due to oxidative reactions and enzymatic breakdown (Salazar-Orbea et al., 2023). Refrigeration helped retain phenolic compounds, preserving the beverage's antioxidant potential for a longer duration. The degradation of phenolics is attributed to enzymatic oxidation (polyphenol oxidase activity) and polymerization reactions, which are exacerbated by higher temperatures (Moon et al., 2020).

Similarly, flavonoid degradation was significantly higher at elevated temperatures, with the most pronounced loss observed at 25°C. Flavonoid content degradation occurs through oxidation and interactions with proteins and other matrix components (Chung et al., 2015). The rate of these reactions increases at 25°C, while 5°C conditions slow them, preserving total flavonoids for longer periods.

6.7.5.3. Free fatty acid and thiobarbituric acid content

The free fatty acid content, an indicator of lipid hydrolysis, increased during the storage period in the optimized microgreen juice-based dairy (lassi) beverage (Jiang et al., 2021). In the fresh beverage sample, the free fatty acid content was 0.42 µg/g, rising to 0.77 µg/g at 5°C, 0.84 µg/g at 15°C, and 0.97 µg/g at 25°C by day 15, as shown in (**Table 6.74**). A similar trend was observed in synbiotic lassi samples, where free fatty acid content increased from 0.56 to 0.85 µg/g during 28 days of storage (Sharma et al., 2015). The increase in free fatty acid was more pronounced at 25°C due to enhanced enzymatic activity and oxidation processes (Macedo & Xavier, 2015). Elevated free fatty acid levels can contribute to off-flavors and reduced sensory acceptability, especially at higher temperatures. Lipid hydrolysis is catalyzed by lipase enzymes, releasing free fatty acids from triglycerides (Yadav et al., 2007; Singh et al., 2024). This process is temperature-sensitive, with higher temperatures enhancing enzyme activity. Bioactive compounds in microgreen juice may influence lipid stability by providing antioxidants that delay oxidation.

Table 6.74. Effect of storage temperature conditions on biochemical parameters of optimized microgreen juice-based dairy (lassi) beverage

Days	Temperature (°C)	Anthocyanin content (μmol/100g)	Total phenol content (mg GAE/100g)	Total flavonoid content (mg QE/100g)	Ascorbic acid (mg/100g)	Free fatty acid content (μg/g)	Thiobarbituric acid content (mg MDA/kg)
Day-0	5	37.91±0.11 ^{aA}	44.14±0.28 ^{aA}	22.62±0.18 ^{aA}	59.32±0.20 ^{aA}	0.42±0.01 ^{iC}	0.022±0.00 ^{iC}
	15	37.91±0.11 ^{aA}	44.14±0.28 ^{aB}	22.62±0.18 ^{aB}	59.32±0.20 ^{aB}	0.42±0.01 ^{iB}	0.022±0.00 ^{iB}
	25	37.91±0.11 ^{aB}	44.14±0.28 ^{aC}	22.62±0.18 ^{aB}	59.32±0.20 ^{aC}	0.46±0.01 ^{iA}	0.022±0.00 ^{iA}
Day-3	5	37.36±0.09 ^{abA}	44.27±0.65 ^{abA}	22.20±0.08 ^{abA}	59.17±0.13 ^{abA}	0.57±0.01 ^{hC}	0.029±0.00 ^{ijC}
	15	37.31±0.16 ^{abcA}	43.17±0.08 ^{bcdB}	22.10±0.13 ^{abB}	58.62±0.21 ^{abB}	0.59±0.01 ^{ghB}	0.034±0.00 ^{hiB}
	25	36.99±0.18 ^{bcB}	43.10±0.13 ^{cdeC}	22.25±0.08 ^{abB}	56.66±0.30 ^{efC}	0.63±0.01 ^{defghA}	0.036±0.00 ^{ghiA}
Day-6	5	37.07±0.07 ^{bcA}	43.76±0.32 ^{abcA}	21.93±0.11 ^{abcA}	58.56±0.30 ^{abcA}	0.60±0.01 ^{fghC}	0.037±0.00 ^{ghiC}
	15	37.09±0.11 ^{bcA}	42.43±0.14 ^{defB}	21.74±0.22 ^{bcdB}	58.07±0.06 ^{cdB}	0.67±0.02 ^{defB}	0.037±0.00 ^{ghiB}
	25	36.14±0.17 ^{defB}	42.42±0.04 ^{defC}	21.57±0.23 ^{bcdB}	55.43±0.28 ^{gC}	0.71±0.02 ^{cdA}	0.041±0.00 ^{efghA}
Day-9	5	36.67±0.21 ^{cdeA}	43.15±0.02 ^{cdA}	21.52±0.37 ^{bcdA}	57.56±0.12 ^{cdA}	0.63±0.01 ^{efghC}	0.040±0.00 ^{fghC}
	15	36.79±0.17 ^{bcdA}	41.92±0.13 ^{fghB}	21.22±0.05 ^{cdeB}	56.22±0.28 ^{fgB}	0.70±0.01 ^{cdeB}	0.043±0.00 ^{defgB}
	25	35.96±0.28 ^{fgB}	42.09±0.09 ^{fgC}	21.02±0.18 ^{defgB}	51.33±0.14 ^{iC}	0.77±0.03 ^{bcA}	0.051±0.00 ^{bcdA}
Day-12	5	36.25±0.18 ^{defA}	42.55±0.30 ^{defA}	21.11±0.05 ^{defA}	57.10±0.08 ^{deA}	0.66±0.01 ^{defgC}	0.043±0.00 ^{defgC}
	15	36.09±0.11 ^{efA}	41.33±0.27 ^{ghB}	20.78±0.16 ^{efghB}	55.48±0.33 ^{gB}	0.78±0.02 ^{bcB}	0.048±0.00 ^{cdeB}
	25	35.31±0.21 ^{eB}	40.34±0.15 ^{iC}	20.32±0.18 ^{ghiB}	49.20±0.24 ^{jC}	0.84±0.02 ^{bA}	0.054±0.00 ^{bcA}
Day-15	5	35.39±0.26 ^{ghA}	42.15±0.16 ^{efgA}	20.42±0.18 ^{fghiA}	56.75±0.13 ^{defA}	0.77±0.02 ^{bcC}	0.047±0.00 ^{cdefC}
	15	35.19±0.11 ^{hA}	40.96±0.14 ^{hiB}	20.11±0.06 ^{hiB}	52.59±0.21 ^{hB}	0.84±0.04 ^{bB}	0.057±0.00 ^{abB}
	25	34.96±0.14 ^{hB}	40.19±0.08 ^{iC}	19.89±0.36 ^{iB}	47.65±0.17 ^{kC}	0.97±0.02 ^{aA}	0.065±0.00 ^{aA}

Note: The results were presented as mean±standard deviations (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments and different uppercase letters within the column indicate significant (P<0.05) differences in temperature condition for the parameters.

Thiobarbituric acid content, an indicator of lipid oxidation, also increased during storage. In the fresh beverage sample, thiobarbituric acid content was 0.022 mg MDA/kg, rising to 0.047 mg MDA/kg at 5°C, 0.057 mg MDA/kg at 15°C, and 0.065 mg MDA/kg at 25°C by day 15, as shown in (Table 6.74). These values align with findings for long-life kheer, which ranged from 0.073 to 0.081 stored at 37°C (Jha et al., 2011). Higher storage temperatures accelerated lipid oxidation, resulting in increased thiobarbituric acid content. At 25°C, oxidative degradation of lipids occurred more rapidly, increasing malondialdehyde formation (Jain et al., 2014; Shiby et al., 2017). Lipid oxidation involves the formation of free radicals and reactive oxygen species, leading to malondialdehyde as a byproduct. Lower temperature storage effectively minimized this process, preserving the sensory and oxidative stability of the optimized beverage product (Jain et al., 2014). Antioxidants from microgreen juice likely mitigated oxidation to some extent, but the rate of oxidation increased with temperature.

6.7.5.4. Effect of storage conditions on antioxidant activities

Antioxidant activities, measured through DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP), are vital indicators of the functional properties of the microgreen juice-based dairy (lassi) beverage. These activities are influenced by bioactive compounds such as phenolics, flavonoids, anthocyanins, and ascorbic acid present in the beverage. Storage conditions, including temperature (5°C, 15°C, and 25°C) and duration (15 days), significantly impacted these parameters, as shown in (Table 6.75).

The DPPH radical scavenging activity of the optimized microgreen juice-based dairy (lassi) beverage decreased over the storage period, reflecting the gradual degradation of antioxidant compounds. The highest antioxidant activity was observed at 5°C (56.75%), followed by 15°C (52.59%) and 25°C (47.65%) by day 15. Ferric reducing antioxidant power, another measure of antioxidant potential, showed a similar declining trend over storage. On the initial day, FRAP activity was 8.30 µmol TE/g, which decreased to 6.27 µmol TE/g at 5°C, 6.81 µmol TE/g at 15°C, and 5.97 µmol TE/g at 25°C by day 15. Similar observations were made in dahi prepared from pea milk and jamun seed extract, where DPPH activity declined from 80.1% to 54.3% under refrigeration (4 to 7°C) over 21 days (Pandhi & Poonia, 2021). In fruit-fortified probiotic buttermilk (lassi), DPPH radical scavenging activity decreased from 64.22% to 38.93%, and FRAP assay optical density decreased from 0.657±0.02 to 0.423±0.01 during storage for 15 days (Kumar & Kumar, 2016).

At 5°C, the decline in DPPH activity was slower, indicating better preservation of antioxidant compounds, while at 15°C and 25°C, the degradation was more pronounced. The decline in DPPH activity is directly correlated with the degradation of phenolics, flavonoids,

and ascorbic acid. These bioactive compounds are primary contributors to the radical scavenging potential of the microgreen juice-based dairy (lassi) beverage. FRAP activity is largely influenced by phenolic and flavonoid content, as these compounds possess reducing capabilities (Firuzi et al., 2004). Their degradation over time, especially at elevated temperatures, contributes to the decline in FRAP activity. Additionally, enzymatic activity and exposure to oxygen at higher temperatures accelerate these reactions, leading to a loss of FRAP activity (Henríquez et al., 2011).

6.7.6. Effect of storage conditions on microbial parameters

Microbial quality is a critical aspect of the stability and safety of any food product, including dairy beverages like the microgreen juice-based lassi beverage. The growth of microorganisms during storage can reduce the shelf life, sensory attributes, and safety of the beverage (Fusco et al., 2020). In this study, the microbial parameters of the optimized microgreen juice-based dairy (lassi) beverage were assessed under three different storage conditions (5°C, 15°C, and 25°C) over a period of 15 days. The primary microbial parameters analyzed were total plate count, and yeast and mold count. These parameters provide insights into the effectiveness of storage conditions in preventing microbial contamination and proliferation.

On the initial day, the total plate count was 3.07 log cfu/ml, which increased to 5.75 log cfu/ml at 5°C, 6.18 log cfu/ml at 15°C, and 6.54 log cfu/ml at 25°C by day 15 (**Table 6.75**). The growth of yeasts and molds can lead to off-flavors, discoloration, and a decrease in product quality, making it essential to monitor their growth during storage (Sørhaug, 2011). The addition of different levels of orange juice significantly ($P < 0.05$) influenced the standard plate count (SPC) in the lassi samples during all the days of the storage period; the SPC count ranged from 5.99 to 7.34×10^7 cfu/g over a 7 days storage period (Jadhav et al., 2014). In another study, the SPC count of turmeric lassi increased from 4.78 to 8.60 log cfu/ml over 9 days of storage (Maji et al., 2018). The observed differences in total plate count between storage conditions highlight the importance of temperature control in extending shelf life. At 5°C, the microbial load remained within acceptable limits for dairy beverages, preserving both safety and sensory quality (Kumar et al., 2024). The total count values in the lassi beverage were lower than the 10^6 cells/mL established by microbiological quality guidelines for ready-to-eat food (Fikry et al., 2020), suggesting that the optimized lassi is microbiologically stable during its shelf-life period. This stability might be due to the high content of antioxidants in the lassi, which can act as preservative agents.

Table 6.75. Effect of storage temperature conditions on antioxidant and microbial parameters of optimized microgreen juice-based dairy (lassi) beverage

Days	Temperature (°C)	Antioxidant activity DPPH (%)	Antioxidant activity FRAP (μmol TE/g)	Total plate count (log cfu/ml)	Yeast and mold (log cfu/ml)
Day-0	5	59.32±0.20 ^{aA}	8.30±0.21 ^{aA}	3.07±0.23 ^{gB}	2.21±0.08 ^{gB}
	15	59.32±0.20 ^{aB}	8.30±0.21 ^{aA}	3.07±0.23 ^{gA}	2.21±0.08 ^{gB}
	25	59.32±0.20 ^{aC}	8.30±0.21 ^{aB}	3.07±0.23 ^{gA}	2.21±0.08 ^{gA}
Day-3	5	59.17±0.13 ^{abA}	8.04±0.04 ^{aA}	3.94±0.08 ^{fgB}	2.26±0.09 ^{fgB}
	15	58.62±0.21 ^{abB}	8.00±0.06 ^{abA}	4.05±0.14 ^{efA}	2.24±0.06 ^{gB}
	25	56.66±0.30 ^{efC}	7.92±0.06 ^{abB}	4.12±0.21 ^{efA}	2.54±0.06 ^{efgA}
Day-6	5	58.56±0.30 ^{abcA}	7.84±0.10 ^{bcA}	4.10±0.24 ^{efB}	2.32±0.21 ^{fgB}
	15	58.07±0.06 ^{cdB}	7.46±0.14 ^{bcA}	4.98±0.06 ^{bcdA}	2.33±0.08 ^{fgB}
	25	55.43±0.28 ^{gC}	7.21±0.09 ^{cdB}	4.90±0.13 ^{cdeA}	2.83±0.08 ^{defgA}
Day-9	5	57.56±0.12 ^{cdA}	7.39±0.03 ^{cdA}	4.54±0.50 ^{defB}	3.11±0.18 ^{deB}
	15	56.22±0.28 ^{fgB}	7.23±0.12 ^{cdA}	5.67±0.26 ^{bcdA}	2.90±0.07 ^{defB}
	25	51.33±0.14 ^{iC}	6.75±0.20 ^{defB}	5.89±0.15 ^{abA}	3.30±0.21 ^{cdA}
Day-12	5	57.10±0.08 ^{deA}	7.21±0.06 ^{cdA}	5.09±0.28 ^{bcdB}	3.86±0.11 ^{cB}
	15	55.48±0.33 ^{gB}	6.96±0.07 ^{cdeA}	5.88±0.08 ^{abA}	3.90±0.07 ^{cB}
	25	49.20±0.24 ^{iC}	6.44±0.16 ^{efgB}	6.14±0.08 ^{aA}	4.66±0.29 ^{bA}
Day-15	5	56.75±0.13 ^{defA}	6.27±0.17 ^{fgA}	5.75±0.24 ^{abcB}	4.69±0.42 ^{bB}
	15	52.59±0.21 ^{hB}	6.81±0.09 ^{deA}	6.18±0.21 ^{aA}	4.91±0.08 ^{bB}
	25	47.65±0.17 ^{kC}	5.97±0.11 ^{gB}	6.54±0.35 ^{aA}	5.89±0.15 ^{aA}

Note: The results were presented as mean±standard deviations (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments and different uppercase letters within the column indicate significant (P<0.05) differences in temperature condition for the parameters.

Similarly, the yeast and mold count on the initial day was 2.21 log cfu/ml, which increased to 4.69 log cfu/ml at 5°C, 4.91 log cfu/ml at 15°C, and 5.89 log cfu/ml at 25°C by day 15 (**Table 6.75**). The lower counts observed under refrigerated conditions indicate the efficacy of low-temperature storage in mitigating fungal contamination, thereby preserving the product's sensory and microbiological quality. Similar results were reported for different types of lassi beverages, including lassi prepared from orange juice (Jadhav et al., 2014), sweetened lassi (George et al., 2010), and symbiotic lassi containing honey (Sharma et al., 2015). The increase in total plate count and yeast and mold count with storage temperature can be attributed to the activation of microbial enzymes and metabolic processes that occur more rapidly at higher temperatures (Melaku et al., 2024). Refrigeration slows down microbial growth significantly by reducing the metabolic rates of bacteria.

6.7.7. Kinetic modeling of different parameters and shelf-life model

The reaction order for various quality indices, including overall acceptability, acidity, free fatty acid content (FFA), thiobarbituric acid (TBA), total plate count, and yeast and mold count, was determined by fitting the experimental data to zero, first, and second-order reaction models. The best-fit model was identified based on the coefficient of determination (R^2) values, as illustrated in (**Table 6.76**). A well-fitted model is characterized by the highest R^2 value. Based on the obtained data, the zero-order model exhibited the best fit, with R^2 values ranging from 0.901 to 0.998. This was followed by the first-order model (R^2 : 0.862 to 0.993), while the second-order model had the lowest R^2 values (0.808 to 0.991). Consequently, it was concluded that the zero-order model most accurately represented the changes in quality indices, consistent with previous research on food deterioration kinetics (Singh et al., 2024; Kumar et al., 2024).

The reaction rate constants (k) for different quality indices demonstrated a significant effect of temperature on degradation kinetics. The absolute k values increased with temperature, underscoring the importance of low-temperature storage for maintaining product quality. The values for overall acceptability, acidity, free fatty acid, thiobarbituric acid, total plate count, and yeast and mold count increased with storage temperature, indicating a positive k for the selected parameters in the zero-order reaction. The temperature's significant impact on the reaction rate highlights the necessity of storing the optimized microgreen-based dairy (lassi) beverage at low temperatures. This aligns with earlier research findings that showed enhanced absolute k values with temperature for acidity and sensory evaluation (Zhi et al., 2017), free fatty acid, and thiobarbituric acid (Kumar & Mishra, 2004). Moreover, it was observed that quality degradation occurred more rapidly at 25°C compared to 5°C, with degradation rates being 3 to 6 times higher at the higher temperature.

Table 6.76. Reaction order estimation of quality indices based on the coefficient of determination (R^2) from the zero-, first- and second-order reactions.

Parameters	Temperature (°C)	Zero order			First order		Second order	
		k	R^2	$t_{1/2}$	k	R^2	k	R^2
Overall acceptability	5	0.0694	0.915	59.73	0.0089	0.909	0.0011	0.902
	10	0.0815	0.988	50.86	0.0106	0.983	0.0014	0.977
	25	0.1279	0.941	32.41	0.0175	0.920	0.0024	0.897
Acidity	5	0.0212	0.959	14.50	0.0283	0.973	0.0383	0.982
	10	0.0262	0.968	11.74	0.0339	0.976	0.0445	0.978
	25	0.0344	0.953	8.94	0.0413	0.979	0.0510	0.991
Free fatty acid	5	0.0191	0.898	10.99	0.0329	0.862	0.0583	0.808
	10	0.0255	0.945	8.24	0.0412	0.889	0.0690	0.819
	25	0.0305	0.970	6.89	0.0442	0.932	0.0664	0.868
Thiobarbituric acid	5	0.0016	0.960	6.72	0.0489	0.911	1.5344	0.845
	10	0.0021	0.970	5.12	0.0573	0.916	1.6530	0.822
	25	0.0027	0.972	3.98	0.0659	0.907	1.7848	0.798
Total plate count	5	0.1646	0.974	9.33	0.0382	0.961	0.0091	0.925
	10	0.2066	0.932	7.43	0.0451	0.888	0.0102	0.833
	25	0.2322	0.956	6.61	0.0491	0.915	0.0108	0.857
Yeast and mould	5	0.1715	0.888	6.43	0.0541	0.912	0.0178	0.922
	10	0.1816	0.854	6.07	0.0561	0.894	0.0181	0.920
	25	0.2403	0.901	4.59	0.6560	0.958	0.0191	0.989

Note: R^2 , coefficient of determination; k, rate of reaction

Table 6.77. Kinetic parameters of quality indices during storage obtained by the Arrhenius, Eyring, Ball models.

Parameters	Arrhenius model		Eyring models			Ball model		
	Ea (kJ/mol)	R ²	ΔH (kJ/mol)	ΔS (J/(mol.k))	R ²	Z (°C)	D _{ref} (day)	R ²
Overall acceptability	20.622	1.000	18.275	-197.759	1.000	0.114	12.270	1.000
Acidity	15.490	0.963	13.145	-201.174	0.948	0.152	38.168	0.960
Free fatty acid	14.143	0.847	11.797	-201.837	0.793	0.166	39.216	0.842
Thiobarbituric acid	16.318	0.918	13.972	-203.369	0.891	0.144	476.190	0.915
Total plate count	10.251	0.811	7.905	-201.417	0.717	0.230	4.840	0.805
Yeast and mould	11.689	0.991	9.343	-200.817	0.986	0.201	5.507	0.992

Note: R², coefficient of determination; ΔH, enthalpy of activation; ΔS, entropy of activation; Z, storage temperature span necessary for a tenfold decrease in the D value; D_{ref}, D value at the reference temperature.

To further comprehend temperature effects, the reaction rate constants were modeled using the Arrhenius, Eyring, and Ball models and the results were presented in (Table 6.77), showed that the activation energy (Ea) values obtained using the Arrhenius model ranged from 10.251 to 20.622 kJ/mol. The highest Ea was recorded for overall acceptability (20.622 kJ/mol), indicating its high sensitivity to temperature fluctuations. Even a slight increase in temperature significantly reduced the sensory properties of the optimized microgreen-based dairy beverage (Table 6.77). The Eyring model provided further thermodynamic insights by calculating entropy (ΔS) and activation enthalpy (ΔH). The enthalpy values (ΔH) closely matched those of Ea, while the entropy values (ΔS) were negative across all indices. This indicated a structured transition state during degradation, suggesting that the quality deterioration process was non-spontaneous and primarily driven by temperature increases (Table 6.77).

The Ball model estimated D_{ref} and Z values to determine the temperature sensitivity of each parameter. Among the quality indices, overall acceptability had the lowest Z-value (0.114°C) and the highest Ea and ΔH values, reinforcing its susceptibility to thermal degradation. The Z-value measures the temperature range required for a tenfold decrease in quality; a low Z-value for overall acceptability means that minor temperature variations substantially impact sensory degradation. Comparatively, microbial growth indices such as total plate count and yeast and mold count had higher Z-values (0.201 and 0.230), indicating

that microbial spoilage was less sensitive to temperature fluctuations than sensory and biochemical parameters. However, their significant activation energies emphasized the necessity of maintaining low temperatures to restrict microbial proliferation.

Using the Arrhenius equation (**Table 6.66**), the shelf-life prediction of microgreen-based dairy (lassi) beverage at 5°C was 59.73, 14.50, 10.99, 6.72, 9.33, and 6.43 days based on overall acceptability, acidity, FFA, TBA, total plate count, and yeast and mold count, respectively. The kinetic modeling of quality indices, including overall acceptability, acidity, FFA content, TBA values, and microbial parameters, highlighted the pivotal role of storage temperature in determining the rate of changes. Refrigeration at 5°C effectively minimized degradation processes and microbial proliferation, thereby extending the shelf life of the microgreen-based dairy beverage. These findings emphasize the critical importance of temperature control in preserving the functional, sensory, and microbial quality of innovative dairy products.

6.7.8. Optimized product cost

The raw material costs involved in preparing microgreen-based dairy (lassi) are detailed in (**Table 6.78**). The manufacturing cost for producing the microgreen-based dairy (lassi) amounts to ₹124.75. The production cost of the developed product is estimated to be ₹40.54 per 200 mL, making it highly competitive with commercial lassi products available in the market (**Table 6.78**). Therefore, from an economic perspective, the product is highly feasible.

Table 6.78. The optimized microgreen-based dairy (lassi) beverage cost estimation

Ingredients	Amount/ 1 kg	Cost (₹)
Microgreens (Radish sango)	92.00 g	42.00
Dahi	600.00 g	45.00
CMC	2.50 g	8.47
Sugar	150.00 g	12.75
Water	153.11 ml	1.53
Packaging cost		15.00
Processing cost		124.75
Marketing and distribution expenses at 25% of product		124.75+31.19=155.94
Profit margin at 30% of cost of product		155.94+46.78=202.72
Total		202.72
Production cost for 200 mL		40.54

6.8. Optimization of variables for microgreen juice-based RTS beverage

The optimization of microgreen juice-based RTS beverage formulation was performed using the D-optimal design in response surface methodology (RSM). The independent variables included (A) microgreen juice (10–20%), (B) pomegranate juice (40–54.5%), and (C) water (30–40%), while the responses evaluated were sensory parameters, physicochemical

properties, and antioxidant properties and obtained results were shown in (Table 6.79, Table 6.80, and Table 6.81). The optimization criteria for a microgreen juice-based RTS beverage using three main process independent variables including (A) microgreen juice (10–20%, maximize), (B) pomegranate juice (40–54.5%, in range), and (C) water (30–40%, water), each with set goals and importance levels as shown in (Table 6.82). The model predicted an optimal formulation with 20% microgreen juice, 44.20% pomegranate juice, and 30.30% water and this combination provided the highest This combination provided the highest desirability ramp (Figure 6.47) indicating an optimization score of 0.812, affirming the model reliability. The predicted values for key responses closely matched the observed values, confirming model reliability (Table 6.83).

6.8.1. Effect of processing variables on sensory parameters

The sensory characteristics, color and appearance play a crucial role in the visual appeal and consumer preference for beverages. The color intensity in the optimized formulation was significantly influenced by the concentration of microgreen and pomegranate juices. The colour and appearance scores for microgreen juice-based RTS beverage, ranging from 6.81 to 7.99 were observed in different formulations (Table 6.79). The mechanism behind these variations lies in the pigment composition of the ingredients. Pomegranate juice is rich in anthocyanins, which impart a deep red hue (Mena et al., 2012), while radish microgreens contain anthocyanins and chlorophyll, leading to a greenish tint when in higher concentrations (Rizvi et al., 2024). The combination of both led to a blended hue, with increased microgreen juice content shifting the beverage towards a darker tone, potentially affecting consumer preference. The statistical model validation (Table 6.84) for color and appearance showed a high R^2 value of 0.9992, confirming strong predictability. The ANOVA results (Table 6.85) indicated a significant influence ($P < 0.05$) of microgreen and pomegranate juice concentrations on color and appearance. The regression model equations shown in (Table 6.84) for flavour and sweetness the variables A, B, C, AB, AC and BC positive effect and ABC showing the negative effect. The response surface plot (Figure 6.46 (A)) visually depicts the interaction effects, showing that increased pomegranate juice enhanced redness, while excess microgreen juice resulted in a darker shade, reducing acceptability.

Flavour perception in microgreen juice-based RTS beverages is a function of the balance between sweetness, acidity, and bitterness. The flavour and sweetness scores ranged from 6.48 to 7.64 were observed in prepared microgreen juice-based RTS beverage, as indicated in (Table 6.79). Pomegranate juice provides natural sugars and organic acids, mainly citric and malic acids, contributing to sweetness and tanginess (Tezcan et al., 2009).

Table 6.79. The effect of constituents on sensory parameters of microgreen juice-based RTS beverage

Treatments	Microgreen juice (%)	Pomegranate juice (%)	Water (%)	Colour & Appearance	Flavour & Sweetness	Consistency	Mouthfeel	Overall Acceptability
1	10.00	44.50	40.00	7.55	6.88	7.83	6.99	7.03
2	10.00	49.50	35.00	7.99	7.64	7.91	7.28	7.65
3	15.00	49.50	30.00	7.46	7.13	7.92	6.96	7.12
4	10.00	44.50	40.00	7.59	6.66	7.83	6.66	7.66
5	13.33	47.83	33.33	7.67	6.98	8.03	6.83	7.35
6	20.00	40.00	34.50	6.54	6.92	7.63	6.73	6.55
7	17.25	40.00	37.25	6.81	6.88	7.79	6.93	6.59
8	20.00	44.50	30.00	6.73	6.82	7.68	6.57	6.59
9	10.00	54.50	30.00	7.64	7.09	7.87	6.85	7.58
10	15.00	49.50	30.00	7.46	7.13	7.84	6.96	7.19
11	16.67	46.33	31.50	7.28	6.85	7.89	6.71	6.96
12	10.00	49.50	35.00	7.99	7.64	7.91	7.28	7.65
13	11.50	46.33	36.67	7.74	7.06	8.12	6.96	7.39
14	10.00	54.50	30.00	7.64	7.09	7.87	6.85	7.58
15	14.90	44.70	34.90	7.41	6.77	8.04	6.67	7.04
16	14.50	40.00	40.00	6.86	6.48	7.82	6.86	6.44
17	20.00	44.50	30.00	6.79	6.75	7.57	6.67	6.59

Note: All the experimental results values are presented as mean value (n=3) basis based on each sensory parameter.

Microgreen juice, on the other hand, introduces glucosinolates and phenolic compounds, which can impart a slightly bitter or pungent taste at higher concentrations. Additionally, the presence of polyphenols in microgreens contributes to astringency, which was noticeable in formulations with higher concentrations (Bafumo et al., 2024). The statistical validation (**Table 6.84**) revealed a R^2 value of 0.9786, indicating a strong correlation between the formulation variables and flavor perception and regression model equations for this parameter the variables A, B, C, AB, AC and BC positive effect and ABC showing the negative effect. The ANOVA results (**Table 6.85**) confirmed that microgreen and pomegranate juice concentrations had a significant effect ($P < 0.05$) on flavor. The response surface plot (**Figure 6.46 (B)**) highlighted that higher pomegranate juice improved flavor acceptability, whereas excess microgreen juice led to increased bitterness.

Consistency is critical in determining the mouthfeel and viscosity of the beverage. The results (**Table 6.79**) showed that the formulations containing 13.33–15% microgreen juice had the highest consistency scores (8.03–7.92). In contrast, beverages with 20% microgreen juice had slightly lower consistency scores (7.57–7.68). The higher consistency in these formulations can be attributed to the pectin content in pomegranate juice (Ismail et al., 2014), along with CMC (0.5%), which acts as a stabilizer, preventing phase separation. Microgreen juice contains soluble fibers, which contribute to viscosity; however, at higher concentrations, the suspension of microgreen particles led to sedimentation, slightly affecting uniformity (Wu et al., 2024). The statistical analysis showed an R^2 value of 0.9914 (**Table 6.84**), confirming model adequacy. ANOVA results (**Table 6.85**) indicated a significant impact ($P < 0.05$) of microgreen juice levels on beverage consistency. The response surface plot (**Figure 6.46 (C)**) demonstrated that microgreen juice concentration had a direct effect on viscosity, with excess amounts leading to inconsistency.

Mouthfeel refers to the texture, body, and overall sensory perception of the beverage in the mouth. The microgreen juice-based RTS beverage scores ranged from 6.59 to 7.28, with the highest values observed in formulations containing 10–15% microgreen juice. A slight decline in mouthfeel acceptability was noticed at 20% microgreen juice. The factors affecting mouthfeel include fiber concentration, presence of stabilizers, and phenolic compounds (George et al., 2024). The pungent compounds from microgreens, such as glucosinolates and isothiocyanates, can also affect the sensory perception of mouthfeel (Bafumo et al., 2024). Statistical validation (**Table 6.84**) confirmed a strong model fit ($R^2 = 0.9722$), with ANOVA results (**Table 6.85**) showing a significant influence ($P < 0.05$) of microgreen juice content on mouthfeel perception. The regression model equations for this parameter the variables A, B, C,

AB, AC and BC positive effect and ABC showing the negative effect. The response surface plot (**Figure 6.46 (D)**) indicates that moderate levels of microgreen juice and pomegranate juice resulted in optimal mouthfeel acceptability. Overall acceptability integrates all sensory attributes and is the key determinant of consumer preference. As seen in (**Table 6.79**), the overall acceptability scores ranged from 6.44 to 7.58 on the 9-point hedonic scale were observed in microgreen juice-based RTS beverage. The regression model (**Table 6.84**) demonstrated a high R^2 value of 0.9983, confirming that formulation variables significantly affected overall acceptability. The ANOVA results (**Table 6.85**) indicated strong statistical significance ($P < 0.05$).

The RTS beverage prepared from spinach microgreens juice, pomegranate juice, and pineapple juice was evaluated for color and appearance (scores ranging from 7.6 to 9.0), consistency (7.1 to 8.2), mouthfeel (6.1 to 7.9), and overall acceptability (6.3 to 7.4) as reported by Sharma et al. (2021). Another study by Kashudhan et al. (2016) assessed a wheatgrass-pomegranate-based therapeutic beverage, with sensory scores ranging from 5.0 to 6.1. Similarly, a functional beverage prepared from fenugreek microgreens, kinnow mandarin, and aloe vera, mixed in different ratios and sweetened with sorbitol and stevia, was evaluated for various sensory parameters. Scores for color and appearance ranged from 3.0 to 9.0, mouthfeel from 3.1 to 8.5, consistency from 4.1 to 8.2, and overall acceptability from 4.1 to 9.42 (Sharma et al., 2020). Different formulations of a novel drink powder produced with varying concentrations of pumpkin pulp and seed powder had color scores ranging from 7.5 to 8.7 and overall acceptability scores between 7.0 and 8.3 (Halim et al., 2024). Additionally, whey-fruit-based beverages prepared from red beetroot peel (1 to 6%) showed a similar trend in color and appearance scores (Stoica et al., 2024). The consistency score of a Ready-to-Drink (RTD) beverage made from whey and beetroot juice ranged from 7.8 to 8.3, aligning with the findings of Theba et al. (2024).

6.8.2. Effect of processing variables on physicochemical parameters

The physicochemical properties of microgreen juice-based RTS beverages were evaluated based on total soluble solids, pH, and titratable acidity parameters and the results are summarized in (**Table 6.80**). The total soluble solids, represents the concentration of dissolved solids, primarily sugars, in the beverage. The total soluble solids values ranged from 11.47 to 12.25°Brix, with the highest values observed in formulations containing higher pomegranate juice concentrations (49.5–54.5%). This can be attributed to the natural sugar content in pomegranate juice, which contributes to the sweetness and mouthfeel of the beverage (Tezcan et al., 2009). Conversely, higher microgreen juice and water levels slightly reduced TSS due to

the dilution effect. The statistical model validation (**Table 6.84**) confirmed a strong correlation between formulation variables and total soluble solids, with an R^2 value of 0.9699. The ANOVA results (**Table 6.85**) indicated a significant effect ($P < 0.05$) of pomegranate juice concentration on total soluble solids. The response surface plot (**Figure 6.46 (E)**) showed that increasing pomegranate juice levels enhanced total soluble solids content.

Table 6.80. The effect of constituents on physicochemical parameters of microgreen juice-based RTS beverage

Treatments	Microgreen juice (%)	Pomegranate juice (%)	Water (%)	TSS (°Brix)	pH	Titrate ble acidity (%)
1	10.00	44.50	40.00	11.89	4.05	0.25
2	10.00	49.50	35.00	12.25	4	0.24
3	15.00	49.50	30.00	11.73	4.04	0.22
4	10.00	44.50	40.00	11.95	4.05	0.25
5	13.33	47.83	33.33	11.88	4.02	0.24
6	20.00	40.00	34.50	11.62	4.13	0.26
7	17.25	40.00	37.25	11.47	4.1	0.24
8	20.00	44.50	30.00	11.68	4.11	0.25
9	10.00	54.50	30.00	12.07	4.06	0.24
10	15.00	49.50	30.00	11.73	4.04	0.22
11	16.67	46.33	31.50	11.64	4.05	0.24
12	10.00	49.50	35.00	12.25	4	0.24
13	11.50	46.33	36.67	11.91	4.02	0.24
14	10.00	54.50	30.00	12.06	4.06	0.23
15	14.90	44.70	34.90	11.56	4.04	0.24
16	14.50	40.00	40.00	11.51	4.12	0.24
17	20.00	44.50	30.00	11.56	4.15	0.25

Note: All the experimental results values are presented as mean value (n=3) basis based on each physicochemical parameter.

The pH values ranged from 4.00 to 4.15, with formulations containing higher pomegranate juice concentrations exhibiting lower pH values due to the presence of organic acids. The ANOVA results (**Table 6.85**) confirmed that pomegranate juice had a statistically significant effect ($P < 0.05$) on pH. The regression model showed an R^2 value of 0.9953, indicating strong predictability. The response surface plot (**Figure 6.46 (F)**) demonstrated that higher pomegranate juice concentrations decreased pH, while microgreen juice had a stabilizing effect. Titratable acidity is a crucial parameter influencing flavor sharpness and microbial stability. The titratable acidity values ranged from 0.22 to 0.26%, with higher values recorded in formulations containing higher microgreen and pomegranate juice concentrations. The presence of ascorbic acid, citric acid, and phenolic acids contributed to the increased

acidity levels. The statistical analysis (**Table 6.84**) indicated an R^2 value of 0.9943, confirming the model reliability. The ANOVA results (**Table 6.85**) showed a significant ($P < 0.05$) effect of both microgreen and pomegranate juice concentrations on titratable acidity. The response surface plot (**Figure 6.46 (G)**) illustrated that higher pomegranate juice led to an increase in acidity, while increased water content diluted acidity. The optimized formulation (20% microgreen juice, 44.20% pomegranate juice, 30.30% water) achieved balanced total soluble solids (11.72°Brix), pH (4.13), and titratable acidity (0.27%), ensuring ideal taste, stability, and consumer acceptability (**Table 6.83**).

The RTS beverage prepared from amla (10%) and kinnow (5%) exhibited physicochemical properties such as total soluble solids (15.01 to 15.07°Brix), pH (3.46 to 3.56), and acidity (0.40 to 0.50%) as observed by Balaji & Prasad (2014). In another study, an RTS beverage made from papaya, ginger, and honey was evaluated, showing pH values of 2.17 to 2.76, acidity ranging from 0.20 to 1.05%, and total soluble solids between 13 and 13.50 °Brix (Debnath et al., 2022). Similarly, a ready-to-serve beverage based on kinnow and amla was prepared, with observed pH values ranging from 3.01 to 4.36, total soluble solids from 14.6 to 16.8 °Brix, and acidity between 1.3 and 1.92% in different formulations (Purewal et al., 2022). The RTS beverage made from spinach microgreens juice, pomegranate juice, and pineapple juice had total soluble solids ranging from 8.02 to 14.07°Brix and acidity between 0.26 and 0.38%, as reported by Sharma et al. (2021). Another study by Kashudhan et al. (2016) assessed a wheatgrass-pomegranate-based therapeutic beverage, showing pH values of 3.8 to 4.44 and total soluble solids ranging from 13 to 20°Brix. Additionally, a functional beverage prepared from fenugreek microgreens, kinnow mandarin, and aloe vera, mixed in different ratios and sweetened with sorbitol and stevia, was evaluated for total soluble solids and titratable acidity. The total soluble solids ranged from 6 to 22.5°Brix, while titratable acidity was observed to be between 0.31 and 2.99 (Sharma et al., 2020).

6.8.3. Effect of processing variables on biochemical and antioxidant parameters

6.8.3.1. Biochemical parameters

The biochemical and antioxidant properties of microgreen juice-based RTS beverages were analyzed to evaluate the nutritional and functional attributes of the formulations. Anthocyanins are natural pigments with potent antioxidant properties, contributing to the color and health benefits of the beverage (Khoo et al., 2017). The anthocyanin content in microgreen juice-based RTS different formulations ranged from 46.29 to 59.86 $\mu\text{mol}/100\text{g}$, with the highest values observed in formulations containing higher pomegranate juice content (above 49.50%). Pomegranate juice is a rich source of anthocyanins, particularly delphinidin, cyanidin, and

pelargonidin derivatives, which enhance color stability and antioxidant capacity (Noda et al., 2001). The statistical validation (**Table 6.84**) revealed a strong correlation ($R^2 = 0.9894$) between processing variables and anthocyanin content and the regression model equations for this parameter the variables A, B, C, AB, and ABC positive effect and AC and BC showing the negative effect. The ANOVA results (**Table 6.85**) indicated that pomegranate juice concentration had a significant effect ($P < 0.05$). The response surface plot (**Figure 6.46 (A)**) demonstrated that increasing pomegranate juice levels enhanced anthocyanin retention, while excessive microgreen juice slightly reduced anthocyanin stability due to polyphenol interactions and enzymatic oxidation.

Total phenolic content and total flavonoid content are key indicators of antioxidant potential. The total phenolic content ranged from 36.27 to 41.59 mg GAE/100g, while total flavonoid content varied from 71.49 to 98.66 mg QE/100g were observed in the different microgreen juice-based RTS beverage formulations (**Table 6.81**). The highest values were recorded in formulations with higher microgreen juice levels ($\geq 15\%$), as microgreens are rich in phenolic acids, flavonoids, and glucosinolates, which contribute to radical scavenging activity (Rizvi et al., 2024; Bafumo et al., 2024). The statistical model (**Table 6.84**) confirmed a significant impact of microgreen juice on total phenolic content ($R^2 = 0.9925$) and total flavonoid content ($R^2 = 0.9976$). The ANOVA results (**Table 6.85**) indicated that both microgreen and pomegranate juice levels had a significant effect ($P < 0.05$) on polyphenol content. The response surface plots (**Figure 6.46 (C)**) and (**Figure 6.46 (D)**) showed that moderate pomegranate juice levels (44–49%) combined with higher microgreen juice (15–20%) resulted in optimal phenolic and flavonoid content, likely due to synergistic interactions between polyphenols, anthocyanins, and ascorbic acid.

Ascorbic acid is a crucial antioxidant that enhances immune function, collagen synthesis, and free radical neutralization. The ascorbic acid content in different formulations ranged from 197.54 to 226.92 mg/100g (**Table 6.81**), with the highest values observed in formulations containing higher pomegranate juice concentrations ($\geq 49.5\%$). Pomegranate juice is naturally rich in ascorbic acid, which contributes to the beverage overall antioxidant potential (Saleem et al., 2025). The statistical analysis (**Table 6.84**) indicated a strong correlation ($R^2 = 0.9916$) between processing variables and ascorbic acid content. The ANOVA results (**Table 6.85**) confirmed that pomegranate juice concentration had a significant effect ($P < 0.05$). The response surface plot (**Figure 6.46 (B)**) demonstrated that increasing pomegranate juice levels significantly improved ascorbic acid retention.

Table 6.81. The effect of constituents on biochemical and antioxidant parameters of microgreen juice-based RTS beverage

Treatments	Microgreen juice (%)	Pomegranate juice (%)	Water (%)	Anthocyanin content ($\mu\text{mol}/100\text{g}$)	Total phenol content (mg GAE/100g)	Total flavonoid content (mg QE/100g)	Antioxidant activity DPPH (%)	Antioxidant activity FRAP ($\mu\text{mol TE/g}$)	Ascorbic acid (mg/100g)
1	10.00	44.50	40.00	46.29	41.59	71.49	86.82	12.95	199.96
2	10.00	49.50	35.00	47.11	40.31	77.99	89.19	12.94	208.03
3	15.00	49.50	30.00	56.26	36.27	93.78	89.58	12.66	228.64
4	10.00	44.50	40.00	46.99	41.59	72.01	86.01	12.95	198.26
5	13.33	47.83	33.33	55.21	36.82	87.48	88.04	13.51	207.75
6	20.00	40.00	34.50	53.27	40.04	96.48	89.33	12.52	202.67
7	17.25	40.00	37.25	52.86	38.14	93.23	89.26	12.2	212.88
8	20.00	44.50	30.00	56.21	40.62	98.66	88.53	12.96	211.04
9	10.00	54.50	30.00	52.02	37.25	73.58	88.66	13	206.56
10	15.00	49.50	30.00	56.26	36.74	94.02	89.58	12.66	227.64
11	16.67	46.33	31.50	57.51	37.21	95.32	88.36	13.27	212.68
12	10.00	49.50	35.00	47.11	40.01	76.23	89.19	12.94	208.03
13	11.50	46.33	36.67	50.88	38.95	80.78	87.66	13.38	202.25
14	10.00	54.50	30.00	52.02	37.29	73.58	87.95	13	206.21
15	14.90	44.70	34.90	56.39	36.81	89.63	86.26	13.63	204.96
16	14.50	40.00	40.00	52.53	38.41	82.68	86.97	12.66	203.42
17	20.00	44.50	30.00	59.86	40.62	98.66	87.94	12.44	205.05

Note: All the experimental results values are presented as mean value (n=3) basis based on each biochemical and antioxidant parameter. Where, GAE: Gallic acid equivalent; QE: Quercetin equivalent

Table 6.82. Optimization criteria for different process and response variables microgreen juice-based RTS beverage

Name	Goal	Lower Limit	Upper Limit	Importance
A: Microgreen juice	Maximize	10.00	20.00	5
B: Pomegranate juice	In range	40.00	54.50	5
C: Water	In range	30.00	40.00	5
Colour & Appearance	In range	6.54	7.99	1
Flavour & Sweetness	In range	6.48	7.64	1
Consistency	In range	7.57	8.06	1
Mouthfeel	In range	6.59	7.28	5
Overall acceptability	Maximize	6.51	7.32	1
Total soluble solids	In range	11.47	12.25	5
pH	maximize	4.00	4.14	5
Titrateable acidity	Maximize	0.22	0.26	5
Anthocyanin content	Maximize	46.90	59.86	5
Total phenol content	Maximize	36.27	41.59	3
Total flavonoid content	Maximize	71.49	98.66	5
Antioxidant activity DPPH	Maximize	86.26	89.57	1
Antioxidant activity FRAP	Maximize	12.37	13.63	1
Ascorbic acid	Maximize	197.54	226.92	1

Table 6.83. Predicted and observed optimum responses for microgreen juice-based RTS beverage

Responses	Predicted values	Observed values
Microgreen juice	20	
Pomegranate juice	44.201	
Water	30.299	
Colour & Appearance	6.752	6.88±0.07
Flavour & Sweetness	6.739	6.83±0.15
Consistency	7.622	7.91±0.06
Mouthfeel	6.59	6.74±0.12
Overall acceptability	6.935	7.72±0.19
Total soluble solids (°Brix)	11.605	11.72±0.01
pH	4.126	4.13±0.00
Titrateable acidity (%)	0.253	0.27±0.01
Anthocyanin content (µmol/100g)	58.626	58.79±0.08
Total phenol content (mg GAE/100g)	40.404	41.06±0.24
Total flavonoid content (mg QE/100g)	98.467	98.49±0.24
Antioxidant activity DPPH (%)	87.938	88.16±0.26
Antioxidant activity FRAP (µmol TE/g)	12.695	12.81±0.11
Ascorbic acid (mg/100g)	204.929	205.12±0.16

The obtained results from experiments are represented in mean±standard deviation values with displayed significant differences (P<0.05).

Table 6.84. The predicted models to experimental data in D-optimal design for the effect of microgreen juice-based RTS beverage

Variables	Regression equation	Mean±SD	C.V. %	R ²	Adjusted R ²	Predicted R ²	Adeq precision
Colour & Appearance	$5.68 \times A + 7.64 \times B + 6.53 \times C + 2.19 \times AB + 2.79 \times AC + 3.24 \times BC - 3.04 \times ABC$	7.36±0.01	0.22	0.9992	0.9987	0.9976	136.02
Flavour & Sweetness	$6.11 \times A + 7.09 \times B + 4.85 \times C + 1.67 \times AB + 5.66 \times AC + 5.66 \times BC - 19.73 \times ABC$	6.99±0.05	0.80	0.9786	0.9657	0.9375	32.63
Consistency	$7.17 \times A + 7.88 \times B + 7.68 \times C + 0.9728 \times AB + 1.43 \times AC + 0.54 \times BC + 4.12 \times ABC$	7.86±0.04	0.20	0.9914	0.9862	0.9746	43.20
Mouthfeel	$5.88 \times A + 6.85 \times B + 6.00 \times C + 2.08 \times AB + 3.10 \times AC + 3.10 \times BC - 15.99 \times ABC$	6.89±0.03	0.56	0.9722	0.9555	0.9214	25.43
Overall Acceptability	$6.89 \times A + 7.14 \times B + 7.10 \times C - 0.0430 \times AB - 1.98 \times AC + 0.8413 \times BC + 0.0930 \times ABC$	7.02±0.12	0.19	0.9983	0.9973	0.9948	93.81
pH	$4.29 \times A + 4.06 \times B + 4.21 \times C - 0.4361 \times AB - 0.5844 \times AC - 0.4715 \times BC + 0.5649 \times ABC$	4.06±0.003	0.09	0.9953	0.9925	0.9867	56.83
Titrateable acidity	$0.3142 \times A + 0.2339 \times B + 0.2616 \times C - 0.1808 \times AB - 0.1839 \times AC - 0.03250 \times BC + 0.4929 \times ABC$	0.23±0.01	0.39	0.9943	0.9909	0.9840	62.95
Total soluble solids	$11.67 \times A + 12.07 \times B + 11.26 \times C - 0.8343 \times AB + 0.26 \times AC + 1.97 \times BC - 5.24 \times ABC$	11.81±0.05	0.46	0.9699	0.9518	0.9045	22.71
Anthocyanin content	$57.96 \times A + 52.03 \times B + 52.45 \times C + 11.31 \times AB - 9.54 \times AC - 24.12 \times BC + 126.20 \times ABC$	53.03±0.55	1.04	0.9894	0.9831	0.9675	33.29
Total phenol content	$47.88 \times A + 37.27 \times B + 42.32 \times C - 19.08 \times AB - 27.61 \times AC + 4.57 \times BC - 26.50 \times ABC$	38.75±0.20	0.51	0.9925	0.9880	0.9736	39.66
Total flavonoid content	$90.77 \times A + 73.53 \times B + 57.35 \times C + 62.75 \times AB + 74.19 \times AC + 42.36 \times BC - 112.62 \times ABC$	85.62±0.62	0.72	0.9976	0.9961	0.9919	68.40
Antioxidant activity DPPH	$84.63 \times A + 88.11 \times B + 79.50 \times C + 11.68 \times AB + 27.99 \times AC + 19.33 \times BC - 107.42 \times ABC$	88.12±0.23	0.26	0.9737	0.9579	0.9220	21.48
Antioxidant activity FRAP	$12.73 \times A + 13.01 \times B + 13.28 \times C - 1.07 \times AB - 2.28 \times AC - 0.9284 \times BC + 30.41 \times ABC$	12.91±0.06	0.52	0.9789	0.9662	0.9361	28.51
Ascorbic acid	$155.84 \times A + 206.24 \times B + 172.96 \times C + 167.62 \times AB + 186.44 \times AC + 65.44 \times BC - 583.36 \times ABC$	208.38±0.95	0.45	0.9916	0.9865	0.9751	48.11

Table 6.85. ANOVA special cubic model for the different responses

Responses	Source	Sum of Squares	df	Mean Square	F-value	P-value	
Colour & appearance	Model	3.250	6	0.542	1974.7	< 0.05	significant
	⁽¹⁾ Linear Mixture	2.740	2	1.370	4999.11	< 0.05	
	AB	0.119	1	0.119	431.54	< 0.05	
	AC	0.100	1	0.100	364.62	< 0.05	
	BC	0.260	1	0.260	945.68	< 0.05	
	ABC	0.010	1	0.010	34.82	0.0002	
	Residual	0.003	10	0.000			
	Lack of Fit	0.000	5	0.000	0.06	0.9967	not significant
	Pure Error	0.003	5	0.001			
	Cor Total	3.260	16				
Flavour & sweetness	Model	1.430	6	0.238	76.08	< 0.05	significant
	⁽¹⁾ Linear Mixture	0.555	2	0.277	88.52	< 0.05	
	AB	0.069	1	0.069	22.04	0.0008	
	AC	0.413	1	0.413	131.65	< 0.05	
	BC	0.828	1	0.828	264.12	< 0.05	
	ABC	0.404	1	0.404	128.77	< 0.05	
	Residual	0.031	10	0.003			
	Lack of Fit	0.005	5	0.001	0.18	0.9604	not significant
	Pure Error	0.027	5	0.005			
	Cor Total	1.460	16				
Consistency	Model	0.308	6	0.051	191.46	< 0.05	significant
	⁽¹⁾ Linear Mixture	0.141	2	0.071	263.75	< 0.05	
	AB	0.023	1	0.023	87.16	< 0.05	
	AC	0.026	1	0.026	97.50	< 0.05	
	BC	0.007	1	0.007	27.70	0.0004	
	ABC	0.018	1	0.018	65.63	< 0.05	
	Residual	0.003	10	0.000			
	Lack of Fit	0.001	5	0.000	0.28	0.9077	not significant
	Pure Error	0.002	5	0.000			
	Cor Total	0.311	16				
Mouthfeel	Model	0.525	6	0.088	58.28	< 0.05	significant
	⁽¹⁾ Linear Mixture	0.212	2	0.106	70.55	< 0.05	
	AB	0.107	1	0.107	71.06	< 0.05	
	AC	0.217	1	0.217	144.34	< 0.05	
	BC	0.238	1	0.238	158.16	< 0.05	

	ABC	0.265	1	0.265	176.56	< 0.05	
	Residual	0.015	10	0.002			
	Lack of Fit	0.003	5	0.001	0.25	0.925	not significant
	Pure Error	0.012	5	0.002			
	Cor Total	0.540	16				
Overall acceptability	Model	1.080	6	0.179	979.80	< 0.05	significant
	⁽¹⁾ Linear Mixture	0.691	2	0.346	1888.23	< 0.05	
	AB	0.000	1	0.000	0.25	0.6281	
	AC	0.050	1	0.050	274.32	< 0.05	
	BC	0.018	1	0.018	95.49	< 0.05	
	ABC	0.000	1	0.000	0.05	0.8293	
	Residual	0.002	10	0.000			
	Lack of Fit	0.001	5	0.000	0.46	0.7904	not significant
	Pure Error	0.001	5	0.000			
	Cor Total	1.080	16				
Total soluble solids	Model	0.954	6	0.159	53.68	< 0.05	significant
	⁽¹⁾ Linear Mixture	0.736	2	0.368	124.27	< 0.05	
	AB	0.017	1	0.017	5.80	0.0368	
	AC	0.001	1	0.001	0.29	0.5998	
	BC	0.096	1	0.096	32.47	0.0002	
	ABC	0.028	1	0.028	9.60	0.0113	
	Residual	0.030	10	0.003			
	Lack of Fit	0.021	5	0.004	2.27	0.1943	not significant
	Pure Error	0.009	5	0.002			
	Cor Total	0.984	16				
pH	Model	0.029	6	0.005	355.42	< 0.05	significant
	⁽¹⁾ Linear Mixture	0.017	2	0.009	632.72	< 0.05	
	AB	0.005	1	0.005	342.68	< 0.05	
	AC	0.004	1	0.004	320.55	< 0.05	
	BC	0.006	1	0.006	400.55	< 0.05	
	ABC	0.000	1	0.000	24.15	0.0006	
	Residual	0.000	10	0.000			
	Lack of Fit	0.000	5	0.000	0.37	0.8504	not significant
	Pure Error	0.000	5	0.000			
	Cor Total	0.029	16				
Titrateable acidity	Model	0.002	6	0.000	290.33	< 0.05	significant
	⁽¹⁾ Linear Mixture	0.001	2	0.000	358.20	< 0.05	

	AB	0.001	1	0.001	920.88	< 0.05	
	AC	0.000	1	0.000	496.23	< 0.05	
	BC	0.000	1	0.000	34.56	0.0002	
	ABC	0.000	1	0.000	287.31	< 0.0001	
	Residual	0.000	10	0.000			
	Lack of Fit	0.000	5	0.000	1.58	0.3143	not significant
	Pure Error	0.000	5	0.000			
	Cor Total	0.002	16				
Anthocyanin content	Model	285.970	6	47.660	155.84	< 0.05	significant
	⁽¹⁾ Linear Mixture	235.140	2	117.570	384.40	< 0.05	
	AB	3.160	1	3.160	10.33	0.0093	
	AC	1.170	1	1.170	3.83	0.0789	
	BC	14.370	1	14.370	46.97	< 0.05	
	ABC	16.510	1	16.510	53.98	< 0.05	
	Residual	3.060	10	0.306			
	Lack of Fit	0.726	5	0.145	0.31	0.8871	not significant
	Pure Error	2.330	5	0.467			
	Cor Total	289.030	16				
Total phenol content	Model	53.370	6	8.890	220.50	< 0.05	significant
	⁽¹⁾ Linear Mixture	12.680	2	6.340	157.16	< 0.05	
	AB	8.990	1	8.990	222.82	< 0.05	
	AC	9.800	1	9.800	242.98	< 0.05	
	BC	0.515	1	0.515	12.78	0.0051	
	ABC	0.728	1	0.728	18.05	0.0017	
	Residual	0.403	10	0.040			
	Lack of Fit	0.247	5	0.049	1.58	0.3136	not significant
	Pure Error	0.156	5	0.031			
	Cor Total	53.770	16				
Total flavonoid content	Model	1616.080	6	269.350	690.81	< 0.05	significant
	⁽¹⁾ Linear Mixture	1479.980	2	739.99	1897.89	< 0.05	
	AB	97.220	1	97.220	249.35	< 0.05	
	AC	70.790	1	70.790	181.55	< 0.05	
	BC	44.290	1	44.290	113.59	< 0.05	
	ABC	13.150	1	13.150	33.73	0.0002	
	Residual	3.900	10	0.390			
	Lack of Fit	2.190	5	0.437	1.28	0.3977	not significant

	Pure Error	1.710	5	0.343			
	Cor Total	1619.980	16				
Antioxidant activity DPPH	Model	20.490	6	3.420	61.70	< 0.05	significant
	⁽¹⁾ Linear Mixture	5.840	2	2.920	52.78	< 0.05	
	AB	3.370	1	3.370	60.89	< 0.05	
	AC	10.080	1	10.080	182.06	< 0.05	
	BC	9.220	1	9.220	166.66	< 0.05	
	ABC	11.960	1	11.960	216.14	< 0.05	
	Residual	0.554	10	0.055			
	Lack of Fit	0.408	5	0.082	2.81	0.1403	not significant
	Pure Error	0.145	5	0.029			
	Cor Total	21.040	16				
Antioxidant activity FRAP	Model	2.130	6	0.354	77.18	< 0.05	significant
	⁽¹⁾ Linear Mixture	0.496	2	0.248	54.02	< 0.05	
	AB	0.028	1	0.028	6.19	0.0321	
	AC	0.067	1	0.067	14.57	0.0034	
	BC	0.021	1	0.021	4.64	0.0568	
	ABC	0.959	1	0.959	208.84	< 0.0001	
	Residual	0.046	10	0.005			
	Lack of Fit	0.009	5	0.002	0.26	0.9185	not significant
	Pure Error	0.037	5	0.007			
	Cor Total	2.170	16				
Ascorbic acid	Model	1070.430	6	178.40	196.22	< 0.05	significant
	⁽¹⁾ Linear Mixture	346.720	2	173.36	190.67	< 0.05	
	AB	693.650	1	693.65	762.91	< 0.05	
	AC	447.000	1	447.00	491.63	< 0.05	
	BC	105.740	1	105.740	116.30	< 0.05	
	ABC	352.810	1	352.81	388.04	< 0.05	
	Residual	9.090	10	0.909			
	Lack of Fit	1.290	5	0.258	0.17	0.965	not significant
	Pure Error	7.800	5	1.560			
	Cor Total	1079.520	16				

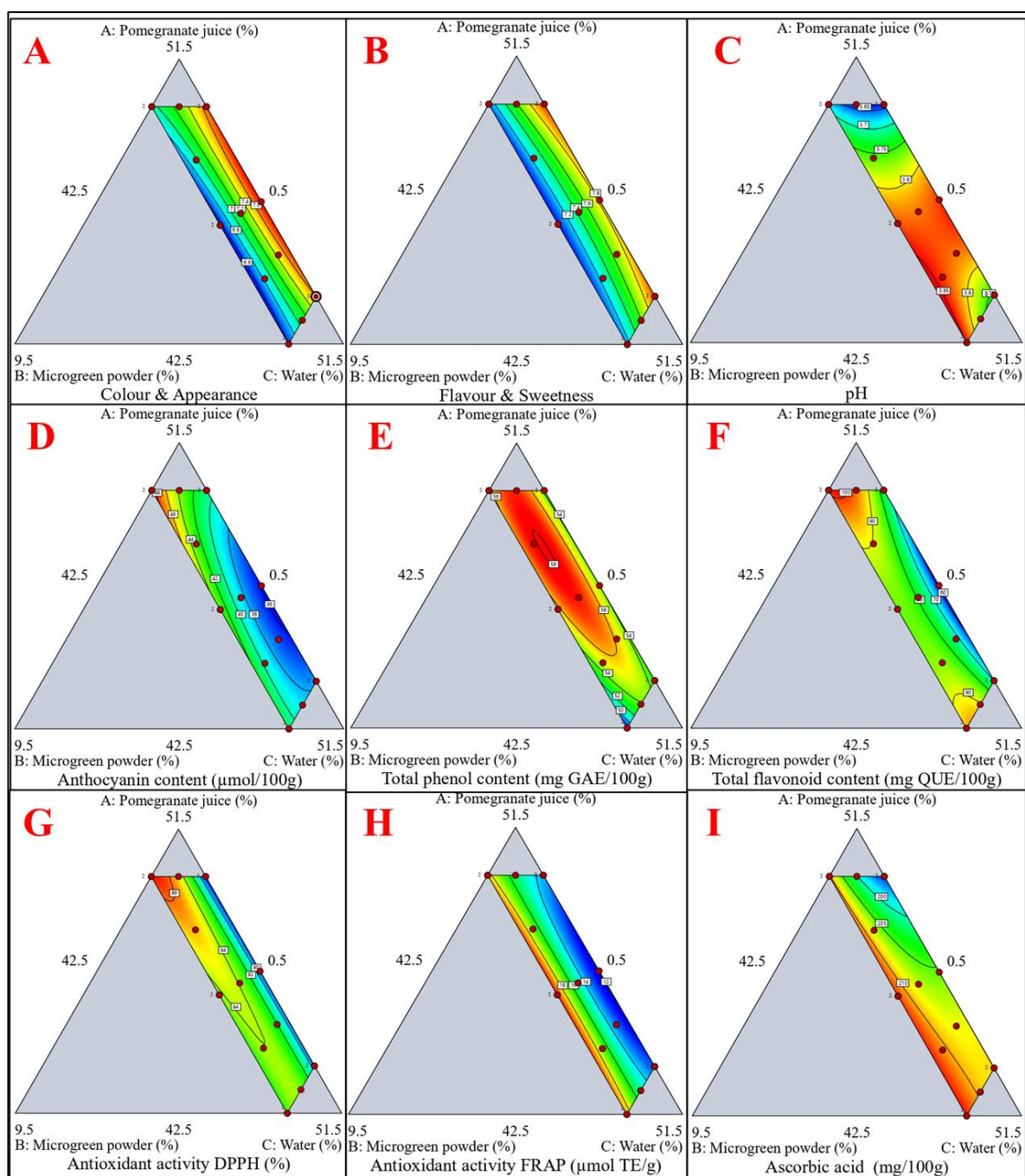


Figure 6.46. Response surface plots showing the effect of pomegranate juice (A), microgreen powder (B), water (C) on various study selected parameters of microgreen-based RTS beverage. Where, A: colour & appearance; B: flavour & sweetness; C: pH; D: anthocyanin content; E: total phenol content; F: Total flavonoid content; G: antioxidant activity DPPH; H: antioxidant activity FRAP; I: ascorbic acid

Our results on the selected biochemical parameters of the microgreen juice-based RTS beverage align with previous studies. Verma et al. (2019) reported that the anthocyanin content in soymilk-strawberry RTS beverages increased significantly with the addition of different levels of soymilk (80 to 95%) and strawberry pulp (5 to 20%). Purewal et al. (2022) reported that the total phenol content in kinnow and amla juice-based RTS beverages ranged from 51.29

to 71.35 mg GAE/100 mL. In another study, a sapodilla-based RTS beverage was prepared with total phenol content ranging from 359.12 to 389.00 μg GAE/100 mL and flavonoid content between 111.48 to 492.59 μg CE/g (Fiaz et al., 2022). Sharma et al. (2020) prepared a fenugreek microgreen-based functional juice blend using kinnow mandarin and aloe vera juice, with an optimized ascorbic acid content of 35.62 mg/100g. Kashudhan et al. (2016) assessed a wheatgrass-pomegranate-based therapeutic beverage, reporting ascorbic acid content ranging from 0.122 to 0.62 mg/mL.

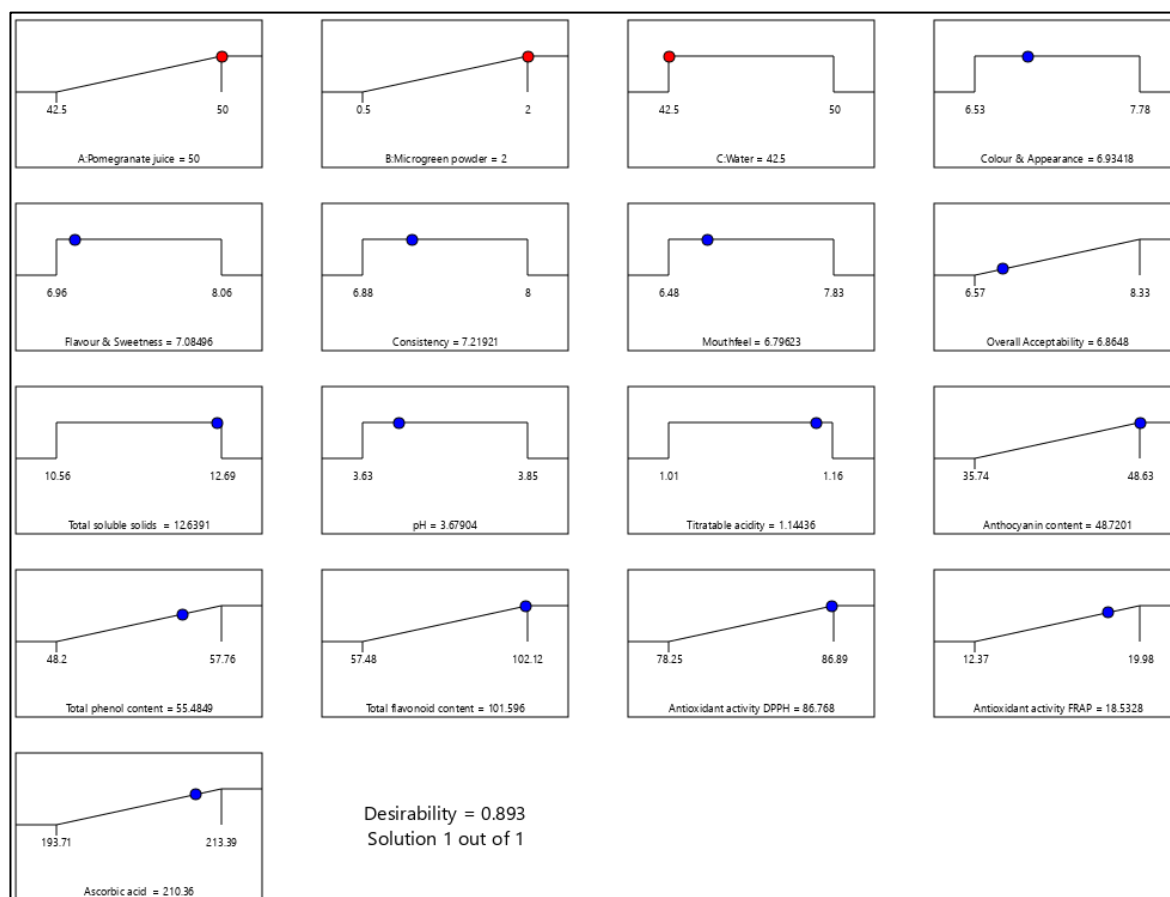


Figure 6.47. Desirability ramp for optimization of microgreen-based RTS beverage

6.8.3.2. Antioxidant activities

The antioxidant properties of the microgreen juice-based RTS beverage were evaluated using DPPH radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP). The DPPH inhibition ranged from 86.26 to 89.57%, while FRAP values varied from 12.37 to 13.63 μmol TE/g were observed among the formulation (**Table 6.81**). The highest antioxidant activities were observed in formulations containing higher microgreen juice levels ($\geq 15\%$) and moderate pomegranate juice levels (44–49%). The statistical model validation (**Table 6.84**) confirmed strong predictability for DPPH ($R^2 = 0.9737$) and FRAP ($R^2 = 0.9789$). The regression model equations shown in (**Table 6.84**) for antioxidant activity (DPPH) the variables

A, B, C, AB, AC and BC positive effect and ABC showing the negative effect, antioxidant activity (FRAP) the variables A, B, C, and ABC positive effect and AB, AC and BC showing the negative effect. The ANOVA results (**Table 6.85**) demonstrated that both microgreen and pomegranate juice concentrations had significant effects ($P < 0.05$) on antioxidant activities. The response surface plots (**Figure 6.46 (E)** and (**Figure 6.46 (F)**) showed that an optimal combination of microgreen juice and pomegranate juice resulted in maximum antioxidant potential, due to the synergistic action of anthocyanins, phenolics, and vitamin C. The biochemical and antioxidant analysis confirmed that processing variables significantly influenced the nutritional properties of the microgreen juice-based RTS beverage. The optimized microgreen juice-based RTS beverage formulation (20% microgreen juice, 44.20% pomegranate juice, 30.30% water) exhibited maximum anthocyanin content (59.86 $\mu\text{mol}/100\text{g}$), total phenolic content (41.06 mg GAE/100g), total flavonoid content (98.49 mg QE/100g), ascorbic acid content (205.12 mg/100g), and antioxidant activities (DPPH: 88.16% and FRAP: 12.81 $\mu\text{mol TE/g}$) as shown in (**Table 6.83**). These results highlight the functional potential of microgreen and pomegranate juice blends in enhancing beverage stability, antioxidant capacity. In the optimized wheatgrass beverage, a DPPH antioxidant scavenging activity of 13.33% was observed. Another study on an optimized microgreen blend beverage reported reducing power of 56.65%, metal chelation activity of 65.31%, and free radical scavenging activity of 69.09% (Sharma et al., 2020). The RTS beverage prepared from kinnow and amla juice exhibited DPPH (% inhibition) values ranging from 31.89 to 70.13% in different formulations (Purewal et al., 2022). Similarly, pineapple core powder was utilized for the development of plant-based probiotic beverages, with DPPH values ranging from 64.12 to 72.54% (Malini et al., 2023).

6.8.4. Effect of storage conditions on physicochemical properties and overall acceptability

The physicochemical properties and overall acceptability of the optimized microgreen juice-based RTS beverage were significantly influenced by storage conditions, particularly temperature and storage duration. Throughout the storage period, a consistent decline in pH was observed, with an initial value of 4.13 decreasing more rapidly at higher temperatures. By day 30, the pH had dropped to 3.78 at 5°C, 3.67 at 25°C, and 3.57 at 37°C (**Table 6.86**). This decline is primarily attributed to the accumulation of organic acids, oxidation of phenolic compounds, and microbial metabolic activity leading to the production of acidic metabolites (Türkyılmaz, 2013; Kumar et al., 2024). The most pronounced reduction occurred at 37°C, confirming that elevated temperatures accelerate biochemical degradation and microbial proliferation, thereby destabilizing the beverage matrix. Simultaneously, titratable acidity

increased from an initial 0.27% to 0.66% at 5°C, 0.71% at 25°C, and 0.82% at 37°C. This rise in acidity is indicative of enzymatic and microbial metabolic reactions, which convert carbohydrates into organic acids, primarily citric and ascorbic acids, contributing to the beverage's tartness and further reducing pH (Kumar et al., 2024). These findings align with previous studies on kiwifruit juice stored at various temperatures (5, 15, and 25°C) (Kumar et al., 2024). Compared to other RTS beverages, the pH levels of the microgreen-based RTS beverage fall within the standard range for most fruit-based RTS beverages (3.03–4.48), suggesting an appropriate balance of acidity that enhances consumer acceptance (Prabhakaran et al., 2024). Similarly, a study on chestnut rose beverage examined the impact of packaging materials (polyethylene terephthalate and polyethylene naphthalate) and storage temperatures (4, 25, 37, and 55°C) on shelf life. The results demonstrated that higher temperatures accelerated the degradation of macromolecular compounds, promoted physical and chemical reactions, and ultimately compromised beverage stability (Wang et al., 2021). The effect of blending kinnow and amla and storage duration on the titratable acidity of RTS beverages was notable. Data showed that the titratable acidity percentage significantly increased from 1.3% to 2.03% with increased storage duration (days) among all prepared beverage formulations (Purewal et al., 2022). In another study, a fruit juice-based cocktail made from mango and pineapple juices showed an increase in titratable acidity from 0.21% to 0.69% over a two-month storage duration (Wazed et al., 2023). Similarly, in sugar beet jaggery-added pomegranate RTS beverages, the acidity value increased from 0.30% to 0.40% under 4°C storage conditions during a 120 days storage period (Ma et al., 2018).

The total soluble solids content in the optimized microgreen juice-based RTS beverage exhibited an increasing trend during storage (**Table 6.86**), rising from an initial 11.72°Brix to 12.66°Brix at 5°C, 13.15°Brix at 25°C, and 13.56°Brix at 37°C by day 30. This increase can be attributed to the hydrolysis of polysaccharides into simpler sugars, which enhances their solubility within the liquid matrix. Elevated temperatures accelerated this process, likely due to enhanced enzymatic degradation of complex carbohydrates, resulting in a perceptibly sweeter taste at the early stages of storage (Hemalatha et al., 2018). Statistical analysis indicated that temperature had a minor but significant impact on total soluble solids content ($P < 0.05$), with refrigerated storage at 5°C maintaining the most stable composition, followed by 25°C and 37°C. However, prolonged storage at 37°C was associated with increased microbial proliferation, potentially leading to fermentation, which contributed to notable acidity changes and flavor degradation. Similar trends in total soluble solids variation during storage have been reported in RTS beverages made from cape gooseberry, where total soluble

solids increased from 14.17 to 14.63°Brix at 27°C and from 14.13 to 15.03°Brix at 4°C over a 90 days storage period. Comparable patterns were also observed in RTS formulations containing Aloe vera, sweet lime, amla, and ginger (Mishra & Sangma, 2017). Additionally, Wazed et al. (2023) reported an increase in total soluble solids from 11.01 to 14.83°Brix in a mango-pineapple juice cocktail stored at 4°C, further supporting the trend of sugar concentration enhancement over time.

Table 6.86. Effect of storage temperature conditions on physicochemical parameters of optimized microgreen juice-based RTS beverage

Days	T (°C)	Overall acceptability	pH	Titrateable acidity (%)	Total soluble solid (°Brix)
Day-0	5	7.72±0.19 ^{aA}	4.13±0.00 ^{aA}	0.27±0.01 ^{iC}	11.72±0.01 ^{mC}
	25	7.72±0.19 ^{aB}	4.13±0.00 ^{aB}	0.27±0.01 ^{iB}	11.72±0.01 ^{mB}
	37	7.72±0.19 ^{aB}	4.13±0.00 ^{aC}	0.27±0.01 ^{iA}	11.72±0.01 ^{mA}
Day-5	5	7.58±0.10 ^{abA}	4.13±0.01 ^{aA}	0.28±0.01 ^{hiC}	11.78±0.01 ^{lC}
	25	7.32±0.16 ^{abcdB}	4.12±0.00 ^{aB}	0.29±0.01 ^{hiB}	11.87±0.01 ^{kB}
	37	7.34±0.06 ^{abcB}	4.09±0.01 ^{abC}	0.39±0.01 ^{gA}	11.95±0.01 ^{jA}
Day-10	5	7.40±0.11 ^{abcA}	4.10±0.01 ^{abA}	0.32±0.01 ^{hC}	11.81±0.01 ^{lC}
	25	7.20±0.12 ^{bcdeB}	4.07±0.02 ^{bcB}	0.37±0.01 ^{gB}	11.95±0.01 ^{jB}
	37	7.20±0.05 ^{bcdeB}	3.95±0.0 ^{ldC}	0.54±0.02 ^{eA}	12.17±0.01 ^{hA}
Day-15	5	7.12±0.14 ^{cdefA}	4.03±0.01 ^{cA}	0.39±0.01 ^{gC}	11.97±0.01 ^{jC}
	25	6.97±0.08 ^{cdefgB}	3.97±0.01 ^{dB}	0.49±0.01 ^{fB}	12.13±0.01 ^{hiB}
	37	6.97±0.08 ^{cdefgB}	3.80±0.01 ^{efC}	0.64±0.01 ^{dA}	12.62±0.01 ^{eA}
Day-20	5	7.11±0.12 ^{cdefA}	3.95±0.01 ^{dA}	0.49±0.01 ^{fC}	12.11±0.01 ^{iC}
	25	6.85±0.08 ^{efgB}	3.82±0.01 ^{efB}	0.55±0.01 ^{eB}	12.55±0.01 ^{fB}
	37	6.81±0.14 ^{efgB}	3.75±0.01 ^{gC}	0.71±0.01 ^{bcA}	12.84±0.01 ^{dA}
Day-25	5	6.80±0.02 ^{efgA}	3.83±0.0 ^{leA}	0.57±0.01 ^{eC}	12.24±0.01 ^{gC}
	25	6.68±0.09 ^{fgB}	3.73±0.01 ^{gB}	0.67±0.01 ^{cdB}	12.74±0.01 ^{cB}
	37	6.87±0.06 ^{defgB}	3.65±0.01 ^{hC}	0.73±0.01 ^{bA}	13.13±0.01 ^{bA}
Day-30	5	6.69±0.03 ^{fgA}	3.78±0.01 ^{fgA}	0.66±0.01 ^{dC}	12.66±0.01 ^{eC}
	25	6.68±0.06 ^{fgB}	3.67±0.01 ^{hB}	0.71±0.01 ^{bcB}	13.15±0.01 ^{bB}
	37	6.57±0.06 ^{gB}	3.57±0.02 ^{iC}	0.82±0.01 ^{aA}	13.56±0.01 ^{aA}

Note: The results were presented as mean±standard deviations (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments and different uppercase letters within the column indicate significant (P<0.05) differences in temperature condition for the parameters.

The overall acceptability scores followed a declining in optimized microgreen juice-based RTS beverage at selected temperature condition (5°C, 25°C, and 37°C) with initial values overall acceptability score of 7.72 on the 9-point hedonic scale dropping to 6.69 at 5°C, 6.68 at 25°C, and 6.57 at 37°C by the end of the study (**Table 6.86**). Sensory deterioration was more evident in samples stored at higher temperatures, where increasing acidity, loss of freshness, and perceptible off-flavors led to lower acceptability scores. The combination of increasing

total soluble solids and acidity at higher temperatures altered the beverage organoleptic properties, making it less appealing compared to samples stored at 5°C, which retained a more balanced taste and stability over time. These findings align with previous research on functional beverages, where temperature-dependent on physicochemical properties has been reported (Wazed et al., 2023; Kumar et al., 2024).

6.8.5. Effect of storage conditions on biochemical and antioxidant properties

6.8.5.1. Ascorbic acid and anthocyanin content

Ascorbic acid and anthocyanin content exhibited a progressive decline with increasing storage temperature over the 30-day period in the optimized microgreen juice-based RTS beverage (**Table 6.87**). At 5°C, ascorbic acid content decreased from 205.12 mg/100g to 188.56 mg/100g, while at 25°C and 37°C, the reduction was more pronounced, reaching 186.23 mg/100g and 184.63 mg/100g, respectively. A similar trend was observed for anthocyanins, with levels dropping from 58.79 µmol/100g to 55.47 µmol/100g at 5°C, and further declining to 54.36 µmol/100g at 25°C and 51.43 µmol/100g at 37°C.

The decline in ascorbic acid content is primarily attributed to oxidative degradation, which is exacerbated at higher temperatures. Storage at 5°C effectively slowed this process by minimizing exposure to heat and light, both of which accelerate oxidation (Kumar et al., 2024). Similarly, the degradation of anthocyanins is largely influenced by their sensitivity to temperature and pH variations, leading to structural breakdown. Refrigerated storage provided a more stable environment, thereby reducing the rate of anthocyanin degradation and preserving color stability (Enaru et al., 2021). Our findings are consistent with previous studies on kiwifruit juice stored at varying temperatures (5, 15, and 25°C), where glass bottles with high oxygen barrier properties minimized oxygen diffusion, thereby reducing aerobic oxidation of ascorbic acid into dehydroascorbic acid. Consequently, anaerobic degradation remained the primary pathway, occurring at a slower rate. Temperature played a crucial role in ascorbic acid degradation, with higher temperatures (25°C and 37°C) accelerating both aerobic and anaerobic breakdown due to its heat sensitivity (Kumar et al., 2024). Similarly, RTS beverages from *Carissa spinarum* stored at 4°C exhibited remarkable stability, maintaining ascorbic acid levels between 22.5 and 11.65 mg/100 ml and anthocyanin content between 15.3 and 8.33 mg/100 ml (Prabhakaran et al., 2024). A carbonated pomegranate juice beverage stored at ambient (12.2–33.1°C) and cool (5–8°C) conditions for three months also showed progressive losses in ascorbic acid and anthocyanins, emphasizing the role of lower temperatures in preserving bioactive compounds (Sandhan et al., 2009).

6.8.5.2. Total phenol and flavonoid content

The total phenol and flavonoid content exhibited a temperature-dependent decline in optimized microgreen juice-based RTS beverages (**Table 6.87**). Initially, total phenol content was 41.06 mg GAE/100g, decreasing to 39.13 mg GAE/100g at 5°C, 38.46 mg GAE/100g at 25°C, and 37.15 mg GAE/100g at 37°C. Similarly, flavonoid content dropped from 98.49 mg QE/100g to 94.65 mg QE/100g at 5°C, with greater losses at 25°C (92.73 mg QE/100g) and 37°C (91.37 mg QE/100g). This degradation is primarily attributed to oxidative reactions and polymerization, which accelerate at higher temperatures. Statistical analysis confirmed significant differences ($P<0.05$) among storage conditions, highlighting refrigeration condition (5°C) protective effect.

Table 6.87. Effect of storage temperature conditions on biochemical parameters of optimized microgreen juice-based RTS beverage

Days	T (°C)	Anthocyanin content (μmol/100g)	Total phenol content (mg GAE/100g)	Total flavonoid content (mg QE/100g)	Ascorbic acid (mg/100g)
Day-0	5	58.79±0.08 ^{aA}	41.06±0.24 ^{aA}	98.49±0.24 ^{aA}	205.12±0.16 ^{aA}
	25	58.79±0.08 ^{aB}	41.06±0.24 ^{aB}	98.49±0.24 ^{aB}	205.12±0.16 ^{aB}
	37	58.79±0.08 ^{aC}	41.06±0.24 ^{aC}	98.49±0.24 ^{aC}	205.12±0.16 ^{aC}
Day-5	5	58.35±0.16 ^{abA}	40.96±0.10 ^{abA}	98.24±0.11 ^{aA}	204.62±0.30 ^{aA}
	25	58.28±0.21 ^{abcB}	40.26±0.19 ^{bcdB}	98.00±0.17 ^{abB}	204.25±0.16 ^{aB}
	37	57.60±0.25 ^{bcdeC}	40.43±0.13 ^{abcC}	97.63±0.13 ^{abcC}	201.58±0.20 ^{bcC}
Day-10	5	57.99±0.20 ^{abcdA}	40.80±0.13 ^{abcA}	97.80±0.09 ^{abcA}	202.60±0.16 ^{bA}
	25	57.49±0.10 ^{cdeB}	40.51±0.16 ^{abcB}	97.33±0.29 ^{bcB}	201.70±0.17 ^{bcB}
	37	56.30±0.19 ^{fgC}	40.16±0.21 ^{cdC}	95.22±0.14 ^{fcC}	200.26±0.32 ^{cC}
Day-15	5	57.40±0.22 ^{deA}	40.65±0.08 ^{abcA}	97.06±0.23 ^{cdA}	201.50±0.23 ^{cdA}
	25	57.09±0.11 ^{efB}	40.23±0.17 ^{cdB}	95.42±0.42 ^{efB}	200.55±0.31 ^{deB}
	37	55.29±0.25 ^{iC}	39.31±0.25 ^{eC}	94.26±0.19 ^{ghC}	198.38±0.35 ^{fcC}
Day-20	5	57.08±0.13 ^{efA}	40.26±0.10 ^{bcdA}	96.18±0.12 ^{deA}	198.33±0.29 ^{fA}
	25	56.22±0.20 ^{ghB}	39.61±0.11 ^{deB}	94.61±0.16 ^{fgB}	198.33±0.15 ^{fB}
	37	53.46±0.28 ^{kC}	38.36±0.28 ^{ghC}	93.12±0.11 ^{iC}	190.21±0.23 ^{hC}
Day-25	5	56.21±0.12 ^{ghA}	39.65±0.17 ^{deA}	95.29±0.38 ^{efA}	192.43±0.35 ^{gA}
	25	55.62±0.23 ^{ghiB}	39.19±0.08 ^{eB}	93.43±0.33 ^{hiB}	190.33±0.31 ^{hB}
	37	52.30±0.25 ^{lC}	37.74±0.11 ^{hiC}	92.22±0.11 ^{jkC}	185.34±0.14 ^{jkC}
Day-30	5	55.47±0.32 ^{hiA}	39.13±0.14 ^{efA}	94.65±0.13 ^{fgA}	188.56±0.39 ^{iA}
	25	54.36±0.18 ^{ijB}	38.46±0.18 ^{fgB}	92.73±0.19 ^{ijB}	186.23±0.30 ^{jB}
	37	51.43±0.28 ^{mC}	37.15±0.16 ^{iC}	91.37±0.22 ^{kC}	184.63±0.20 ^{kC}

Note: The results were presented as mean±standard deviations (n=3). The different lowercase letters within the column indicate significant ($P<0.05$) differences among all treatments and different uppercase letters within the column indicate significant ($P<0.05$) differences in temperature condition for the parameters.

Comparable trends were observed in RTS beverages from cape gooseberry, where phenolic retention was higher at 4°C (21.83 to 15.56 mg GAE/100 mL) than at 27°C (21.83 to 8.03 mg GAE/100 mL) over 90 days (Hemalatha et al., 2018). Similarly, sapodilla-based RTS beverages and fermented apple juice showed phenolic degradation due to enzymatic oxidation and chemical reactions during storage (Fiaz et al., 2022; Bai et al., 2022).

6.8.5.3. Antioxidant activities

The antioxidant activity of the optimized microgreen juice-based RTS beverage, assessed through DPPH and FRAP assays, showed a gradual decline over the storage period (**Table 6.88**). Initially, DPPH scavenging activity was 88.16%, decreasing to 78.46% at 5°C, 72.33% at 25°C, and 70.23% at 37°C. Likewise, FRAP values dropped from 12.81 µmol TE/g to 10.22 µmol TE/g at 5°C, with more pronounced reductions at 25°C (9.99 µmol TE/g) and 37°C (9.27 µmol TE/g). This decrease in antioxidant potential can be attributed to the degradation of bioactive compounds such as phenolics, flavonoids, and ascorbic acid, which play a key role in neutralizing free radicals (Soobrattee et al., 2005). Refrigeration at 5°C proved most effective in preserving antioxidant stability, as evidenced by significant differences ($P < 0.05$) among storage temperatures. These results are consistent with previous studies on fruit-based RTS beverages, including coconut water (Ceballos et al., 2024), *Carissa spinarum* (Prabhakaran et al., 2024), kinnow and amla (Purewal et al., 2022), and sapodilla (Fiaz et al., 2022), where similar declines in antioxidant activity were reported.

6.8.6. Effect of storage conditions on microbial parameters

The microbiological stability of the microgreen juice-based RTS beverage was significantly affected by storage conditions, with microbial counts increasing over time, particularly at higher temperatures (25°C and 37°C). The total plate count and yeast and mold counts were measured over a 30 days storage period at 5°C, 25°C, and 37°C to assess microbial growth and potential spoilage. Microbial spoilage is a major concern for ready-to-serve beverages as it can lead to undesirable changes in taste, texture, and safety, ultimately reducing shelf life and consumer acceptability (Chern et al., 2018). The initial total plate count was 1.96 log cfu/mL, which increased progressively over time across all storage conditions. At 5°C, TPC reached 3.90 log cfu/mL, while at 25°C and 37°C, the counts rose significantly, reaching 4.31 log cfu/mL and 4.74 log cfu/mL, respectively (**Table 6.88**). The differences between storage conditions indicate that microbial proliferation is heavily influenced by temperature, with higher temperatures promoting bacterial growth and metabolic activity. At refrigeration temperature (5°C), bacterial growth was slower due to reduced metabolic activity and enzyme function, leading to delayed spoilage. On the other hand, at 25°C and 37°C, microbial growth

accelerated due to optimal conditions for bacterial replication, resulting in a substantial increase in total plate count over time (Cabello-Olmo et al., 2020).

Table 6.88. Effect of storage temperature conditions on antioxidant and microbial parameters of optimized microgreen juice-based RTS beverage

Days	T(°C)	Antioxidant activity DPPH (%)	Antioxidant activity FRAP (μmol TE/g)	Total plate count (log cfu/ml)	Yeast and mold (log cfu/ml)
Day-0	5	88.16±0.26 ^{aA}	12.81±0.11 ^{aA}	1.96±0.10 ^{iC}	1.23±0.08 ^{iC}
	25	88.16±0.26 ^{aB}	12.81±0.11 ^{aA}	1.96±0.10 ^{iB}	1.23±0.08 ^{iB}
	37	88.16±0.26 ^{aC}	12.81±0.11 ^{aB}	1.96±0.10 ^{iA}	1.23±0.08 ^{iA}
Day-5	5	86.41±0.04 ^{bA}	12.57±0.08 ^{abA}	2.57±0.20 ^{hiC}	1.70±0.26 ^{hiC}
	25	86.02±0.20 ^{bB}	12.52±0.12 ^{abcA}	2.74±0.21 ^{ghB}	1.91±0.03 ^{fghB}
	37	85.26±0.17 ^{bcC}	11.95±0.16 ^{dB}	3.59±0.14 ^{cdefA}	1.88±0.16 ^{fghiA}
Day-10	5	85.52±0.20 ^{bcA}	12.09±0.11 ^{bcdA}	3.01±0.25 ^{fghC}	1.82±0.15 ^{ghiC}
	25	84.23±0.16 ^{cdB}	12.00±0.08 ^{cdA}	3.29±0.15 ^{cdefB}	2.04±0.08 ^{efghB}
	37	82.22±0.23 ^{fcC}	11.57±0.13 ^{defB}	3.73±0.28 ^{cdefA}	2.19±0.13 ^{defghA}
Day-15	5	83.58±0.30 ^{deA}	11.89±0.13 ^{dA}	3.25±0.09 ^{efghC}	1.80±0.13 ^{ghiC}
	25	81.83±0.72 ^{fgB}	11.82±0.08 ^{deA}	3.61±0.17 ^{cdefB}	2.18±0.16 ^{defghB}
	37	80.37±0.16 ^{hC}	10.51±0.22 ^{hiB}	4.09±0.15 ^{abcdA}	2.36±0.18 ^{defgA}
Day-20	5	82.46±0.28 ^{efA}	11.26±0.11 ^{efA}	3.43±0.08 ^{defgC}	2.40±0.22 ^{defgC}
	25	80.85±0.25 ^{ghB}	11.16±0.11 ^{fgA}	3.84±0.08 ^{bcdeB}	2.51±0.25 ^{cdefB}
	37	78.28±0.28 ^{iC}	10.21±0.21 ^{hijB}	4.47±0.11 ^{abA}	2.76±0.01 ^{bcdA}
Day-25	5	80.37±0.21 ^{hA}	10.67±0.16 ^{ghA}	3.64±0.07 ^{cdefC}	2.60±0.03 ^{cdeC}
	25	78.29±0.22 ^{iB}	10.55±0.13 ^{hiA}	4.08±0.14 ^{abcdB}	2.78±0.13 ^{bcdB}
	37	71.93±0.77 ^{jC}	9.86±0.15 ^{jB}	4.70±0.27 ^{aA}	3.75±0.13 ^{aA}
Day-30	5	78.46±0.37 ^{iA}	10.22±0.19 ^{hijA}	3.90±0.13 ^{bcdeC}	3.15±0.33 ^{abcC}
	25	72.33±0.19 ^{jB}	9.99±0.13 ^{ijA}	4.31±0.26 ^{abcB}	3.29±0.21 ^{abB}
	37	70.23±0.49 ^{kC}	9.27±0.16 ^{kB}	4.74±0.35 ^{aA}	3.80±0.15 ^{aA}

Note: The results were presented as mean±standard deviations (n=3). The different lowercase letters within the column indicate significant (P<0.05) differences among all treatments and different uppercase letters within the column indicate significant (P<0.05) differences in temperature condition for the parameters.

Yeasts and molds are common spoilage microorganisms in RTS beverages, particularly in those containing fruit and vegetable extracts, which provide an ideal substrate for fungal growth. The initial yeasts and molds count was 1.23 log cfu/mL, which increased over time under all storage conditions. By the end of the storage period, yeasts and molds count had risen to 3.15 log cfu/mL at 5°C, 3.29 log cfu/mL at 25°C, and 3.80 log cfu/mL at 37°C (**Table 6.88**). Yeast and mold proliferation was significantly higher at 37°C, which can be attributed to the favorable conditions for fungal growth, including elevated temperature and increased moisture content. Fungal contamination in RTS beverages can lead to off-flavors, gas production, and visible spoilage, negatively impacting sensory quality and consumer acceptance (De Carvalho

et al., 2024). Refrigeration at 5°C was the most effective in controlling yeast and mold growth, as lower temperatures slow down spore germination and mycelial development. Our findings align with previous studies on cold plasma-treated kiwifruit juice stored at different temperature conditions, including 5°C, 15°C, and 25°C (Kumar et al., 2024). With total plate count and yeast and mold count below 6 log CFU/mL, our microgreen juice-based RTS beverage is considered safe for consumption based on a 30 days storage duration under selected storage temperatures (Patrignani et al., 2009; Ceballos et al., 2024).

6.8.7. Kinetic modeling of different parameters and shelf-life model

The shelf-life and quality deterioration kinetics of microgreen juice-based Ready-to-Serve (RTS) beverage were investigated using various kinetic models to predict changes in key quality parameters under different storage conditions. The reaction order for quality indices such as overall acceptability, acidity, ascorbic acid, and total plate count was analyzed using zero-order, first-order, and second-order kinetic models based on the coefficient of determination (R^2). Additionally, the Arrhenius and Eyring models were employed to determine the kinetic parameters of these quality indices during storage, providing insights into the temperature dependency of degradation rates and the thermodynamic stability of the beverage.

To assess degradation kinetics, reaction order estimation was performed at different storage temperatures (5°C, 25°C, and 37°C). The (Table 6.89) and (Table 6.90) present the experimental study values based on the reaction kinetics of different quality indices at multiple storage temperatures. The reaction order estimation for various parameters, including overall acceptability, acidity, ascorbic acid content, and total plate count, is shown in (Table 6.89). The primary objective of this to determine which kinetic model best describes the degradation patterns of these quality indices by evaluating the coefficient of determination (R^2). The reaction rate constant (k) and half-life ($t_{1/2}$) are crucial parameters for understanding how rapidly these quality indices change over time.

The degradation of overall acceptability was found to follow a zero-order reaction model, as indicated by the highest R^2 values across all storage temperatures. The values for the temperature conditions of 5°C ($R^2 = 0.9800$), 25°C ($R^2 = 0.9329$), and 37°C ($R^2 = 0.9274$) confirm that sensory attributes such as flavor, texture, and aroma degrade at a constant rate, independent of their initial concentration. This steady rate of sensory degradation is likely due to the uniform breakdown of volatile compounds and flavoring agents over time. Additionally, the reaction rate constant (k) values increased with temperature, confirming that acceptability declines more rapidly at higher storage temperatures.

Table 6.89. Reaction order estimation of quality indices based on the coefficient of determination (R^2) from the zero-, first- and second-order reactions.

Parameters	T (°C)	Zero order			First order		Second order	
		k	R^2	$t_{1/2}$	k	R^2	k	R^2
Overall acceptability	5	0.035	0.9800	109.348	0.0049	0.9792	0.0007	0.9777
	25	0.034	0.9329	113.864	0.0048	0.9417	0.0007	0.9495
	37	0.034	0.9274	113.196	0.0048	0.9338	0.0007	0.9389
Acidity	5	0.014	0.9556	9.854	0.0324	0.9781	0.0806	0.9795
	25	0.016	0.9791	8.385	0.0355	0.9742	0.0834	0.9446
	37	0.018	0.9532	7.542	0.0806	0.8856	0.0735	0.791
Ascorbic acid	5	0.560	0.9098	183.307	0.0028	0.9047	0.00001	0.8994
	25	0.628	0.8973	163.390	0.0032	0.891	0.00002	0.8846
	37	0.743	0.9455	138.054	0.0038	0.9428	0.00002	0.94
Total plate count	5	0.060	0.9460	16.361	0.0206	0.8923	0.0074	0.8244
	25	0.073	0.9321	13.351	0.0237	0.8657	0.008	0.7843
	37	0.081	0.8151	12.144	0.0241	0.7149	0.0077	0.616

Note: R^2 , coefficient of determination; k, rate of reaction

Table 6.90. Kinetic parameters of quality indices during storage obtained by the Arrhenius and Eyring models.

Parameters	Arrhenius model		Eyring models		
	Ea (kJ/mol)	R^2	ΔH (kJ/mol)	ΔS (J/(mol.k))	R^2
Overall acceptability	0.8968	0.8158	3.2023	-207.9426	0.891
Acidity	6.0867	0.9909	5.7707	-204.8667	0.7518
Ascorbic acid	6.1396	0.899	6.8159	-200.7182	0.955
Total plate count	6.9149	0.9999	6.4703	-228.7176	0.6702

Note: Ea: activation energy; R^2 , coefficient of determination; ΔH , enthalpy of activation; ΔS , entropy of activation

Acidity levels were best modeled using a zero-order kinetic equation, with R^2 values indicating that acidity variations are influenced by interactions between reactants, potentially due to microbial activity or enzymatic reactions. The reaction rate constant (k) values also showed an increasing trend with temperature, indicating that acidity changes occur more rapidly at higher temperatures, likely due to increased microbial metabolism and enzymatic hydrolysis.

The degradation of ascorbic acid followed zero-order kinetics, as evidenced by high R^2 values at 5°C (0.9098), 25°C (0.8973), and 37°C (0.9455). Zero-order kinetics indicate that the degradation rate is directly proportional to the concentration of ascorbic acid remaining in the beverage. The reaction rate constant (k) showed an increasing trend (0.560 to 0.743) with temperature, suggesting that ascorbic acid degradation is highly temperature-dependent. The

loss of ascorbic acid is primarily due to oxidative reactions, which accelerate at elevated temperatures. Given the essential role of ascorbic acid in antioxidant activity and nutritional value, storage at lower temperatures is recommended to slow down its degradation and extend shelf-life.

Microbial growth, represented by total plate count, exhibited first-order kinetics at lower temperatures (5°C with $R^2 = 0.9460$, 25°C with $R^2 = 0.9321$), but the R^2 value at 37°C was lower (0.8151). This suggests that at higher temperatures, microbial proliferation deviates from a simple first-order reaction, likely due to an exponential growth phase beyond a threshold concentration. The reaction rate constant (k) values increased from 0.060 at 5°C to 0.081 at 37°C, confirming that microbial activity intensifies as temperature rises. This highlights the importance of refrigeration in controlling microbial spoilage and ensuring product safety over extended storage durations.

The temperature dependence of degradation reactions and kinetic parameters of quality indices were determined using the Arrhenius and Eyring models (**Table 6.90**). The activation energy (E_a) values calculated using the Arrhenius model revealed that total plate count had the highest E_a (6.9149 kJ/mol) for zero-order, indicating that temperature significantly impacts the acidification process. Conversely, overall acceptability had the lowest E_a values (0.8968 kJ/mol), suggesting that sensory deterioration is less influenced by temperature fluctuations. The Eyring model provided additional insights into the thermodynamic properties of degradation reactions. The enthalpy of activation (ΔH) values confirmed that ascorbic acid content required the highest energy input for degradation (6.8159 kJ/mol), aligning with its high activation energy in the Arrhenius model. Entropy of activation (ΔS) values were negative for all parameters, indicating a decrease in molecular randomness during degradation, characteristic of enzymatic and non-enzymatic oxidation reactions.

Based on the kinetic modeling results, shelf-life estimation was performed using degradation rate constants from the best-fitting kinetic model. The estimated half-life ($t_{1/2}$) for overall acceptability at 5°C was 109.348 days, decreasing to 113.196 days at 37°C. For acidity, $t_{1/2}$ values ranged from 9.854 days (5°C) to 7.542 days (37°C), suggesting that acidity increased at a faster rate under higher temperatures. The shelf-life of ascorbic acid, based on zero-order kinetics, was estimated at 183.307 days at 5°C but declined to 138.05 days at 37°C, demonstrating significant losses in antioxidant content over time. Microbial stability, as indicated by total plate count, showed rapid deterioration at higher temperatures, with $t_{1/2}$ values decreasing from 16.361 to 12.144 days. This indicates that microbial spoilage is a primary

factor affecting the overall shelf-life of the RTS beverage, necessitating stringent storage conditions.

The kinetic modeling results highlight the importance of refrigerated storage (5°C) in preserving the sensory, biochemical, and microbiological quality of microgreen juice-based RTS beverages. Higher temperatures accelerate oxidative and microbial degradation, reducing shelf-life and consumer acceptability. The activation energy and enthalpy values further support that acidity and microbial growth are the most temperature-sensitive parameters, necessitating controlled storage conditions. Future research should explore additional preservation techniques, such as natural antioxidants, active packaging, and controlled atmosphere storage, to enhance the stability and shelf-life of RTS beverages.

6.8.8. Optimized product production cost

The cost estimation for the optimized microgreen juice-based RTS beverage includes various expenses such as raw materials, processing, packaging, and marketing. The total cost of producing 1 liter of the beverage is ₹192.11. With a profit margin of 20%, the final price is ₹230.53 per liter (**Table 6.91**). Furthermore, the production cost of the developed RTS beverage product is calculated at ₹46.11 per 200 mL, making it highly competitive with existing commercial products in the market.

Table 6.91. The optimized microgreen juice-based RTS beverage cost estimation

Ingredients	Quantity	Market price (₹)	Amount used/ 1 Liter	Total Cost (₹)
Microgreens (Radish sango)	1 kg	1258.13	200 g	91.30
Pomegranate	1 kg	100	450.00 g	45.00
CMC	0.075 kg	219	2.5 g	8.47
Sugar	1 kg	85	50 g	4.25
Water	1 L	10	303 mL	3.03
Packaging cost	1	3	5	15.00
Processing cost			Total	167.05
Marketing and distribution expenses @15% of product				25.06
			Total	192.11
Profit margin @ 20% of cost of product				38.42
			Total	230.53
Optimized microgreen juice-based RTS beverage cost for 200 mL				46.11

Summary and Conclusion

This study presents a comprehensive investigation into the cultivation, optimization, and application of microgreens, encompassing growing media, light treatments, nutrient management, postharvest handling, drying behavior, powder characterization, and functional food development. The evaluation of alternative growing media (soil, cocopeat, and hydroponics) revealed that cocopeat was the most effective substrate, significantly enhancing shoot height, shoot width, and the accumulation of bioactive compounds. Among the selected microgreens, flax crops and radish sango exhibited superior morphological traits compared to broccoli, cabbage, pak choi, beetroot, and red amaranthus. Nutritional profiling showed that microgreens generally possess high moisture content and relatively low fat and protein levels. Flax crops had the highest total chlorophyll, beetroot showed elevated carotenoids, and cabbage excelled in ascorbic acid content. Radish sango microgreens demonstrated the highest levels of anthocyanins, total phenolics, flavonoids, and antioxidant activity, as measured by DPPH and FRAP assays.

The study also explored the influence of LED light spectra on microgreen growth and biochemical composition. Red and blue LED lights significantly improved fresh weight, shoot height, and antioxidant properties. Blue light enhanced chlorophyll and carotenoid synthesis, while red light improved photosynthetic efficiency. These findings suggest that targeted light wavelengths can be strategically used to enhance specific nutritional attributes in microgreens. In addition, foliar spray applications of calcium chloride (CaCl_2) and potassium nitrate (KNO_3) were optimized using response surface methodology (RSM). The identified optimal concentrations 18 mM CaCl_2 and 8 mM KNO_3 significantly improved growth parameters, pigment levels, and antioxidant capacity in radish sango microgreens, highlighting the role of nutrient management in enhancing microgreen quality.

Postharvest storage and processing techniques were also evaluated to determine their impact on shelf-life and quality retention. Storage at 5°C effectively reduced microbial growth and preserved antioxidant activity compared to 25°C. Metalized polyester packaging minimized oxygen exposure and retained bioactive compounds, while washing treatments with citric acid and sodium hypochlorite improved microbial safety and antioxidant retention. Drying experiments revealed that tray drying at 60°C with optimized loading density reduced drying time and improved energy efficiency. The Midilli et al. model provided the best fit for drying kinetics, and moisture diffusivity ranged from 5.35×10^{-7} to $1.273 \times 10^{-6} \text{ m}^2/\text{s}$. The lowest specific electric energy consumption was observed at 60°C, indicating that higher drying temperatures can enhance efficiency without compromising quality.

Further, the study examined the effects of particle size distribution on the functional, antioxidant, and thermal properties of radish sango microgreen powder. Finer particle sizes (75 μm) showed higher antioxidant activity and phenolic content due to increased surface area and extractability, although they also exhibited greater oxidative susceptibility. Thermal analysis revealed decomposition patterns that influence powder stability, emphasizing the importance of selecting particle sizes based on intended applications in nutraceutical, food, or pharmaceutical formulations. Finally, two microgreen-based functional beverages a dairy lassi and a ready-to-serve (RTS) beverage were developed and optimized. The lassi formulation (70% dahi and 2.5% microgreen powder) and the RTS beverage (with pomegranate juice) demonstrated high bioactive content and shelf stability for up to 30 days at 5°C. Kinetic modeling confirmed the retention of antioxidant compounds, supporting the commercial potential of microgreen-based functional beverages.

In conclusion, this study underscores the importance of optimizing cultivation and processing parameters to enhance the yield, nutritional quality, and antioxidant properties of microgreens. The findings provide valuable insights for researchers, growers, and food technologists aiming to integrate microgreens into sustainable agriculture and functional food systems. By refining growing media, light treatments, nutrient applications, postharvest handling, and product development, microgreens can be positioned as high-value crops with significant health and commercial benefits.

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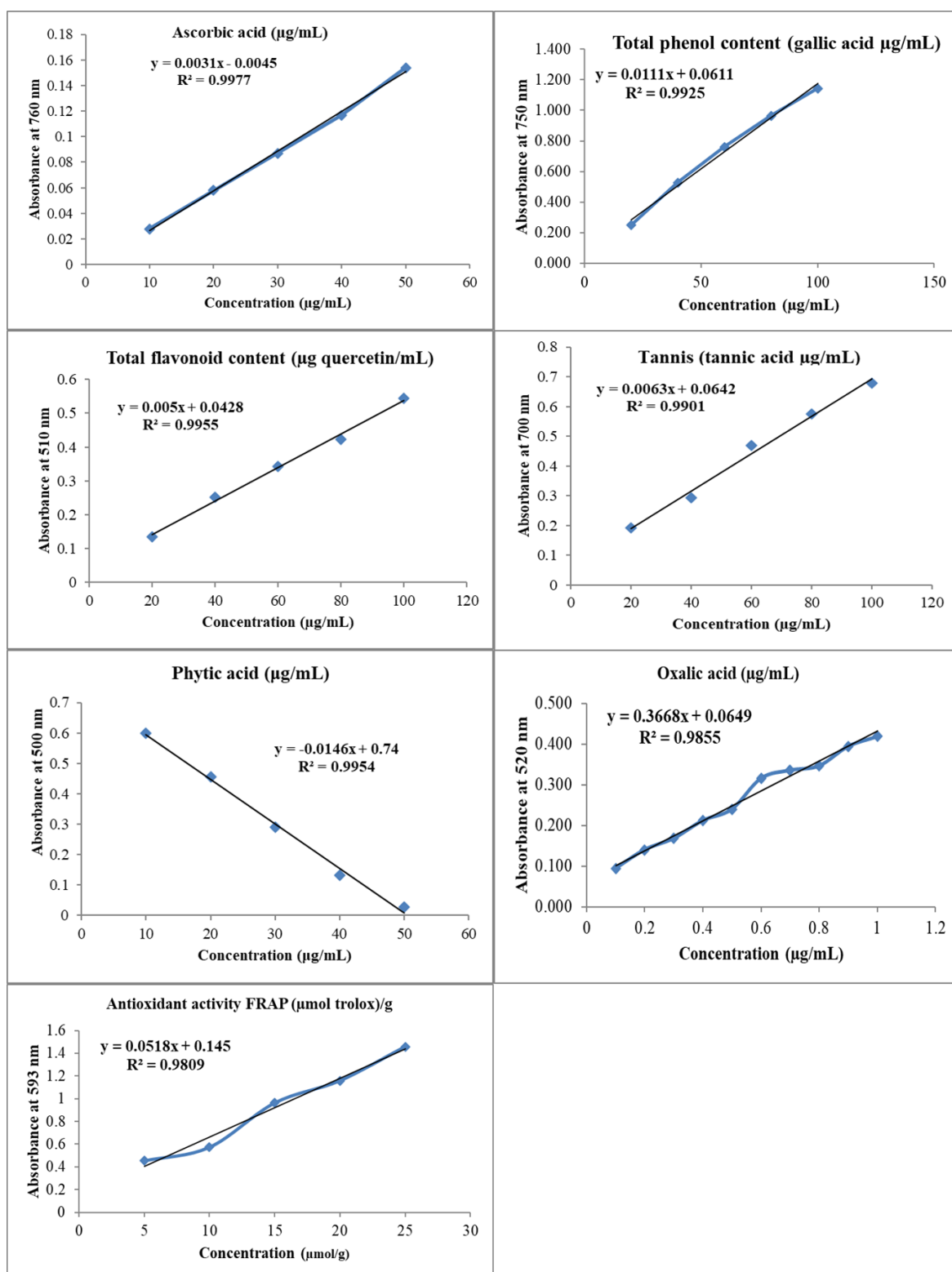
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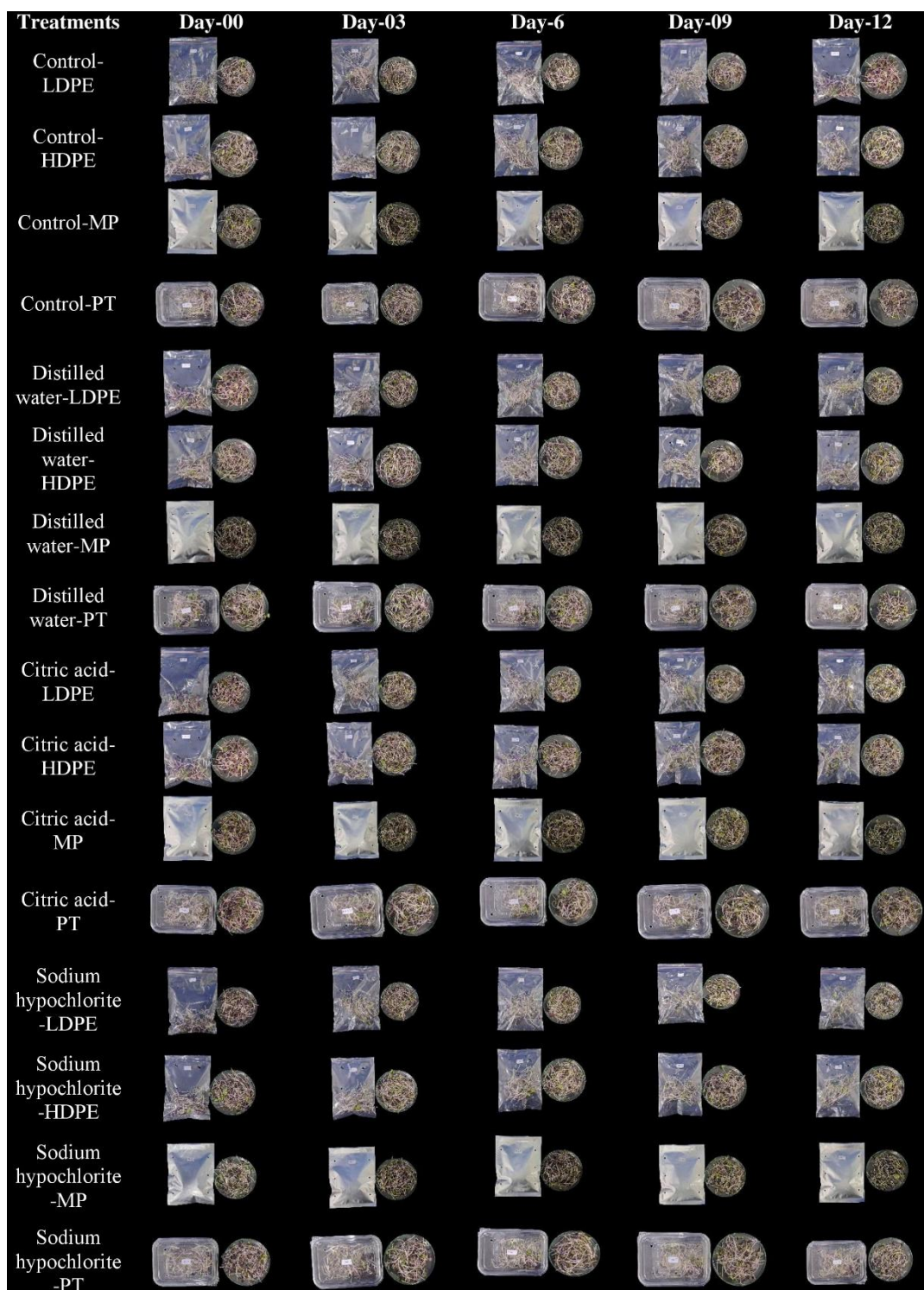
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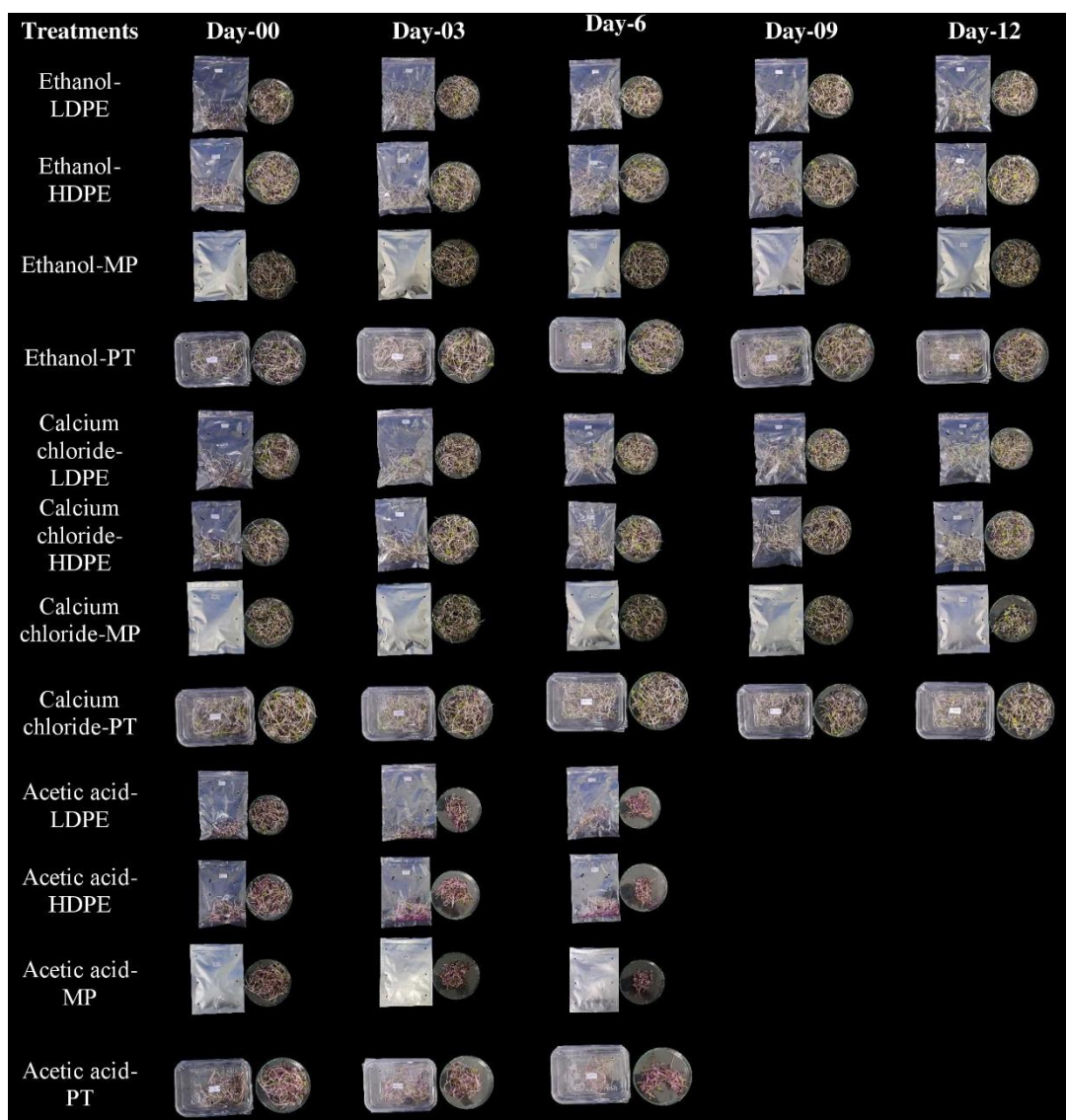
APPENDICES



Appendices Figure 1. Standard curves for different biochemical compounds



Appendices Figure 2 (A). Effect of different washing treatments on radish sango microgreens stored in various packaging materials at 5°C storage condition.



Appendices Figure 2 (B). Effect of different washing treatments on radish sango microgreens stored in various packaging materials at 5°C storage condition.

Appendices Table 1. Effect of different washing treatments on radish sango microgreens stored in various packaging materials at 5°C on weight loss, electrolyte leakage, overall quality, and off-odor.

Washing treatments	Packaging materials	Days	Weight loss (%)	Electrolyte leakage (%)	Overall quality	Off-odor
Acetic acid	HDPE	Day-0	0.00±0.00aC	1.87±0.46aB	7.30±0.14cB	0.95±0.07aB
		Day-3	2.96±0.06aC	3.20±0.28aB	6.43±0.05cB	2.20±0.64aB
		Day-6	7.42±0.03aC	25.80±0.57aB	6.00±0.15cB	4.33±0.24aB
	LDPE	Day-0	0.00±0.00aB	1.87±0.46aB	7.30±0.14cC	0.95±0.07aA
		Day-3	2.88±0.06aB	3.00±0.28aB	6.90±0.05cC	1.91±0.21aA
		Day-6	7.28±0.06aB	25.70±0.14aB	5.59±0.13cC	4.74±0.19aA
	MP	Day-0	0.00±0.00aD	1.87±0.46aC	7.30±0.14cA	0.95±0.07aC
		Day-3	2.94±0.03aD	3.70±0.14aC	6.56±0.19cA	1.95±0.30aC
		Day-6	7.42±0.14aD	23.70±0.14aC	6.07±0.08cA	3.96±0.02aC
	PT	Day-0	0.00±0.00aA	1.87±0.46aA	7.30±0.14cD	0.95±0.07aA
		Day-3	2.86±0.03aA	3.40±0.14aA	6.86±0.11cD	2.66±0.11aA
		Day-6	7.26±0.03aA	23.53±0.04aA	5.64±0.37cD	4.36±0.33aA
Calcium chloride	HDPE	Day-0	0.00±0.00dC	1.54±0.31eB	8.20±0.00abB	0.55±0.07bcB
		Day-3	1.36±0.06dC	1.93±0.02eB	8.33±0.09abB	0.69±0.05bcB
		Day-6	2.56±0.06dC	1.93±0.02eB	7.76±0.08abB	1.74±0.08bcB
		Day-9	2.66±0.08dC	2.49±0.07eB	6.41±0.04abB	2.75±0.04bcB
		Day-12	3.04±0.11dC	4.39±0.07eB	5.77±0.03abB	3.72±0.01bcB
	LDPE	Day-0	0.00±0.00dB	1.54±0.31eB	8.20±0.00abC	0.55±0.07bcA
		Day-3	1.76±0.06dB	1.90±0.01eB	8.39±0.65abC	0.76±0.01bcA
		Day-6	2.56±0.06dB	1.90±0.01eB	7.52±0.11abC	1.95±0.02bcA
		Day-9	2.82±0.03dB	2.39±0.28eB	6.34±0.01abC	2.87±0.12bcA
		Day-12	3.06±0.03dB	4.34±0.21eB	5.49±0.22abC	3.72±0.03bcA
	MP	Day-0	0.00±0.00dD	1.54±0.31eC	8.20±0.00abA	0.55±0.07bcC

		Day-3	1.30±0.03dD	1.97±0.01eC	8.51±0.12abA	0.70±0.06bcC
		Day-6	2.50±0.03dD	1.97±0.01eC	7.55±0.47abA	1.73±0.10bcC
		Day-9	2.56±0.06dD	2.27±0.01eC	6.48±0.06abA	2.81±0.02bcC
		Day-12	2.88±0.06dD	4.22±0.08eC	5.74±0.06abA	3.40±0.05bcC
	PT	Day-0	0.00±0.00dA	1.54±0.31eA	8.20±0.00abD	0.55±0.07bcA
		Day-3	1.86±0.03dA	1.94±0.01eA	7.91±0.04abD	0.76±0.03bcA
		Day-6	2.64±0.06dA	1.94±0.01eA	7.44±0.11abD	1.79±0.19bcA
		Day-9	2.74±0.03dA	2.89±0.06eA	6.14±0.02abD	3.44±0.63bcA
		Day-12	3.16±0.06dA	4.87±0.09eA	5.44±0.02abD	3.82±0.04bcA
	Citric acid	HDPE	Day-0	0.00±0.00eC	2.08±0.15cB	8.45±0.07aB
Day-3			1.50±0.03eC	1.94±0.01cB	8.23±0.11aB	0.62±0.06bcB
Day-6			2.30±0.03eC	2.39±0.08cB	7.69±0.01aB	1.74±0.05bcB
Day-9			2.34±0.08eC	3.07±0.06cB	6.61±0.12aB	2.35±0.39bcB
Day-12			3.44±0.06eC	4.09±0.04cB	5.79±0.01aB	3.88±0.06bcB
LDPE		Day-0	0.00±0.00eB	2.08±0.15cB	8.45±0.07aC	0.65±0.21bcA
		Day-3	1.68±0.06eB	1.92±0.04cB	8.18±0.39aC	0.73±0.04bcA
		Day-6	2.08±0.06eB	2.77±0.17cB	7.42±0.04aC	1.93±0.01bcA
		Day-9	2.28±0.06eB	3.12±0.10cB	6.46±0.04aC	2.83±0.03bcA
		Day-12	3.48±0.06eB	4.32±0.38cB	5.43±0.07aC	3.93±0.01bcA
MP		Day-0	0.00±0.00eD	2.08±0.15cC	8.45±0.07aA	0.65±0.21bcC
		Day-3	1.30±0.03eD	1.99±0.02cC	8.27±0.06aA	0.52±0.04bcC
		Day-6	2.06±0.03eD	2.75±0.32cC	7.85±0.04aA	1.67±0.01bcC
		Day-9	2.18±0.03eD	3.35±0.25cC	6.68±0.06aA	2.65±0.01bcC
		Day-12	3.34±0.03eD	4.40±0.18cC	5.78±0.02aA	3.82±0.02bcC
PT		Day-0	0.00±0.00eA	2.08±0.16cA	8.45±0.07aD	0.50±0.00bcA
		Day-3	1.86±0.03eA	2.08±0.11cA	7.85±0.07aD	0.84±0.07bcA
		Day-6	2.26±0.03eA	2.45±0.30cA	7.29±0.10aD	1.86±0.08bcA

Distilled water		Day-9	2.40±0.06eA	3.50±0.23cA	6.23±0.04aD	2.95±0.06bcA
		Day-12	3.54±0.03eA	4.80±0.19cA	5.77±0.02aD	3.87±0.04bcA
	HDPE	Day-0	0.00±0.00dC	1.86±0.16bB	8.40±0.14abB	0.25±0.07cB
		Day-3	1.54±0.03dC	1.86±0.05bB	8.23±0.11abB	0.62±0.06cB
		Day-6	2.34±0.03dC	2.86±0.19bB	7.67±0.02abB	1.72±0.03cB
		Day-9	2.42±0.03dC	2.86±0.19bB	6.59±0.13abB	2.71±0.01cB
		Day-12	3.34±0.14dC	5.81±0.12bB	5.51±0.04abB	3.73±0.04cB
	LDPE	Day-0	0.00±0.00dB	1.86±0.16bB	8.40±0.14abC	0.25±0.07cA
		Day-3	1.70±0.03dB	1.88±0.06bB	8.18±0.39abC	0.73±0.04cA
		Day-6	2.50±0.03dB	2.89±0.06bB	7.39±0.08abC	1.91±0.01cA
		Day-9	2.70±0.03dB	3.29±0.64bB	6.44±0.07abC	2.86±0.05cA
		Day-12	3.54±0.08dB	5.87±0.04bB	5.25±0.03abC	3.87±0.04cA
	MP	Day-0	0.00±0.00dD	1.86±0.16bC	8.40±0.14abA	0.25±0.07cC
		Day-3	1.34±0.03dD	1.74±0.06bC	8.27±0.06abA	0.52±0.04cC
		Day-6	2.12±0.06dD	2.83±0.06bC	7.79±0.01abA	1.64±0.03cC
		Day-9	2.28±0.06dD	2.83±0.06bC	6.67±0.10abA	2.68±0.02cC
		Day-12	3.46±0.08dD	4.93±0.08bC	5.60±0.03abA	3.57±0.04cC
	PT	Day-0	0.00±0.00dA	1.86±0.16bA	8.40±0.14abD	0.25±0.07cA
		Day-3	1.82±0.03dA	1.93±0.04bA	7.85±0.07abD	0.84±0.07cA
		Day-6	2.18±0.03dA	2.97±0.01bA	7.14±0.02abD	1.91±0.01cA
		Day-9	2.34±0.08dA	2.99±0.01bA	6.27±0.02abD	2.96±0.05cA
		Day-12	3.42±0.03dA	5.94±0.06bA	5.37±0.03abD	3.92±0.04cA
Ethanol	HDPE	Day-0	0.00±0.00cC	1.76±0.30dB	8.10±0.14abB	0.55±0.07bB
		Day-3	1.38±0.03cC	1.97±0.01dB	8.25±0.06abB	0.62±0.10bB
		Day-6	2.54±0.08cC	1.97±0.01dB	7.70±0.01abB	1.78±0.01bB
		Day-9	2.84±0.06cC	2.87±0.13dB	6.41±0.04abB	2.75±0.04bB
		Day-12	3.78±0.14cC	4.62±0.08dB	5.43±0.07abB	3.93±0.01bB

	Sodium hypochlorite	LDPE	Day-0	0.00±0.00cB	1.76±0.30dB	8.10±0.14abC	0.55±0.07bA
			Day-3	1.74±0.03cB	1.93±0.04dB	8.23±0.44abC	0.77±0.02bA
			Day-6	2.48±0.06cB	1.93±0.04dB	7.52±0.11abC	1.95±0.02bA
			Day-9	2.74±0.08cB	2.93±0.05dB	6.34±0.01abC	2.87±0.12bA
			Day-12	3.56±0.11cB	4.88±0.12dB	5.37±0.03abC	3.92±0.04bA
		MP	Day-0	0.00±0.00cD	1.76±0.30dC	8.10±0.14abA	0.55±0.07bC
			Day-3	1.24±0.06cD	1.99±0.01dC	8.27±0.03abA	0.50±0.06bC
			Day-6	2.50±0.03cD	1.99±0.01dC	7.75±0.10abA	1.69±0.01bC
			Day-9	2.84±0.06cD	2.94±0.08dC	6.48±0.06abA	2.81±0.02bC
			Day-12	3.44±0.06cD	4.67±0.12dC	5.79±0.01abA	3.85±0.04bC
		PT	Day-0	0.00±0.00cA	1.76±0.30dA	8.10±0.14abD	0.55±0.07bA
			Day-3	1.88±0.06cA	2.03±0.01dA	7.85±0.05abD	0.76±0.03bA
			Day-6	2.68±0.06cA	2.03±0.01dA	7.85±0.04abD	1.67±0.01bA
			Day-9	2.94±0.14cA	2.93±0.16dA	6.14±0.02abD	2.94±0.08bA
			Day-12	3.72±0.11cA	5.08±0.06dA	5.68±0.02abD	3.85±0.02bA
		HDPE	Day-0	0.00±0.00eC	1.86±0.16dB	8.35±0.21aB	0.55±0.07bcB
			Day-3	1.48±0.06eC	1.93±0.01dB	8.20±0.01aB	0.62±0.10bcB
			Day-6	2.30±0.03eC	1.93±0.01dB	7.69±0.01aB	1.75±0.06bcB
			Day-9	2.38±0.08eC	2.73±0.28dB	6.56±0.11aB	2.65±0.04bcB
			Day-12	3.22±0.03eC	4.72±0.26dB	5.78±0.03aB	3.85±0.04bcB
		LDPE	Day-0	0.00±0.00eB	1.86±0.16dB	8.35±0.21aC	0.55±0.07bcA
			Day-3	1.66±0.08eB	1.90±0.01dB	8.23±0.46aC	0.77±0.02bcA
			Day-6	2.14±0.03eB	1.90±0.01dB	7.52±0.11aC	1.95±0.02bcA
			Day-9	2.30±0.03eB	3.39±0.14dB	6.42±0.02aC	2.87±0.02bcA
			Day-12	3.50±0.03eB	4.34±0.07dB	5.37±0.10aC	3.92±0.07bcA
		MP	Day-0	0.00±0.00eD	2.08±0.16dC	8.35±0.21aA	0.55±0.07bcC
			Day-3	1.26±0.03eD	1.94±0.01dC	8.34±0.07aA	0.50±0.06bcC

Control	PT	Day-6	2.04±0.06eD	1.94±0.01dC	7.87±0.01aA	1.63±0.01bcC
		Day-9	2.32±0.11eD	2.40±0.06dC	6.68±0.06aA	2.66±0.05bcC
		Day-12	3.16±0.06eD	4.40±0.06dC	5.68±0.02aA	3.85±0.02bcC
		Day-0	0.00±0.00eA	2.08±0.16dA	8.35±0.21aD	0.55±0.07bcA
		Day-3	1.82±0.03eA	2.09±0.10dA	7.90±0.02aD	0.77±0.04bcA
		Day-6	2.24±0.11eA	2.09±0.10dA	7.32±0.06aD	1.86±0.06bcA
		Day-9	2.52±0.06eA	3.14±0.16dA	6.29±0.05aD	2.88±0.04bcA
		Day-12	3.32±0.06eA	4.94±0.02dA	5.70±0.08aD	3.87±0.04bcA
	HDPE	Day-0	0.00±0.00bC	1.43±0.16bB	8.25±0.07bB	0.35±0.07bcB
		Day-3	1.56±0.06bC	1.89±0.01bB	8.05±0.07bB	0.55±0.07bcB
		Day-6	2.64±0.11bC	2.79±0.13bB	7.61±0.08bB	1.74±0.06bcB
		Day-9	2.90±0.08bC	3.84±0.06bB	6.62±0.09bB	2.73±0.04bcB
		Day-12	3.66±0.14bC	5.80±0.01bB	5.47±0.02bB	3.74±0.03bcB
	LDPE	Day-0	0.00±0.00bB	1.43±0.16bB	8.25±0.07bC	0.35±0.07bcA
		Day-3	1.72±0.06bB	1.94±0.07bB	7.95±0.07bC	0.65±0.07bcA
		Day-6	2.52±0.06bB	2.38±0.07bB	7.27±0.04bC	1.93±0.04bcA
		Day-9	2.88±0.11bB	3.63±0.28bB	6.39±0.08bC	2.91±0.02bcA
		Day-12	3.82±0.08bB	5.58±0.35bB	5.19±0.08bC	3.92±0.04bcA
	MP	Day-0	0.00±0.00bD	1.43±0.16bC	8.25±0.07bA	0.35±0.07bcC
		Day-3	1.40±0.06bD	1.77±0.05bC	8.11±0.01bA	0.40±0.00bcC
		Day-6	2.60±0.06bD	2.36±0.04bC	7.77±0.02bA	1.67±0.02bcC
		Day-9	2.88±0.06bD	2.91±0.60bC	6.74±0.06bA	2.67±0.01bcC
		Day-12	3.68±0.06bD	5.46±0.04bC	5.59±0.01bA	3.65±0.01bcC
	PT	Day-0	0.00±0.00bA	1.43±0.16bA	8.25±0.07bD	0.35±0.07bcA
		Day-3	1.78±0.03bA	1.97±0.06bA	7.85±0.07bD	0.80±0.14bcA
		Day-6	2.58±0.03bA	2.96±0.04bA	7.03±0.01bD	1.94±0.03bcA
		Day-9	3.08±0.17bA	3.91±0.04bA	6.19±0.05bD	2.95±0.06bcA

	Day-12	3.84±0.11bA	5.91±0.11bA	5.34±0.02bD	3.97±0.04bcA

Results are expressed as mean ± standard deviation of three replicates. The different lowercase letters within the column indicate significant (p<0.05) differences based on washing treatments and different uppercase letters within the column indicate significant (p<0.05) differences among the packaging materials.

Appendices Table 2. Effect of different washing treatments on radish sango microgreens stored in various packaging materials at 5°C on total chlorophyll content, total carotenoid content, anthocyanin content, and total phenol content.

Washing treatments	Packaging materials	Days	Total chlorophyll content (µg/g)	Total carotenoid content (µg/g)	Anthocyanin content (µmol/100 g)	Total phenol content (mg GAE/100g)
Acetic acid	HDPE	Day-0	36.35±0.39eC	8.45±0.17cA	66.70±0.04aA	334.14±1.27aB
		Day-3	33.89±0.53eC	7.73±0.13cA	65.39±0.50aA	311.80±1.53aB
		Day-6	22.27±0.42eC	7.02±0.32cA	61.86±0.08aA	306.40±0.25aB
	LDPE	Day-0	36.35±0.39eCB	8.45±0.17cB	66.70±0.04aB	334.14±1.27aD
		Day-3	34.03±0.32eCB	7.96±0.19cB	66.38±0.27aB	313.87±0.64aD
		Day-6	24.81±1.06eCB	7.75±0.14cB	61.70±0.15aB	307.57±0.64aD
	MP	Day-0	36.35±0.39eA	8.45±0.17cA	66.70±0.04aA	334.14±1.27aA
		Day-3	33.89±0.53eA	7.96±0.10cA	65.31±0.08aA	312.70±1.02aA
		Day-6	23.60±0.35eA	6.37±0.06cA	61.61±0.27aA	308.33±0.83aA
	PT	Day-0	36.35±0.39eB	8.45±0.17cC	66.70±0.04aC	334.14±1.27aC
		Day-3	33.86±0.62eB	7.79±0.15cC	64.82±0.31aC	308.11±0.13aC
		Day-6	23.52±0.46eB	6.22±0.08cC	61.61±0.20aC	306.76±0.51aC
Calcium chloride	HDPE	Day-0	36.52±0.08cC	8.42±0.05aA	66.77±0.13bcA	335.50±0.64aB
		Day-3	34.50±0.26cC	8.11±0.14aA	65.95±0.10bcA	329.91±0.38aB
		Day-6	33.64±0.12cC	7.88±0.08aA	64.06±0.01bcA	315.18±0.57aB
		Day-9	33.06±0.09cC	7.87±0.13aA	61.02±0.36bcA	308.20±0.51aB
		Day-12	29.04±0.10cC	7.74±0.20aA	56.30±0.31bcA	299.01±0.76aB
	LDPE	Day-0	36.52±0.08cCB	8.42±0.05aB	66.77±0.13bcB	335.50±0.64aD

		Day-3	34.28±0.05cCB	8.16±0.00aB	66.03±0.25bcB	323.78±0.64aD
		Day-6	33.47±0.46cCB	7.89±0.25aB	63.57±0.25bcB	316.85±0.51aD
		Day-9	33.18±0.34cCB	7.58±0.07aB	60.86±0.36bcB	306.85±0.38aD
		Day-12	30.27±0.11cCB	7.28±0.26aB	56.02±0.23bcB	298.83±0.00aD
	MP	Day-0	36.52±0.08cA	8.42±0.05aA	66.77±0.13bcA	335.50±0.64aA
		Day-3	33.94±0.02cA	7.92±0.11aA	65.95±0.36bcA	330.99±0.64aA
		Day-6	33.58±0.53cA	7.80±0.24aA	63.57±0.22bcA	317.39±0.76aA
		Day-9	32.49±0.50cA	8.08±0.05aA	61.19±0.10bcA	309.68±0.06aA
		Day-12	31.62±0.38cA	7.86±0.04aA	56.10±0.34bcA	299.41±0.06aA
	PT	Day-0	36.52±0.08cB	8.42±0.05aC	66.77±0.13bcC	335.50±0.64aC
		Day-3	34.28±0.05cB	8.11±0.01aC	66.03±0.01bcC	332.79±0.64aC
		Day-6	33.36±0.17cB	7.90±0.05aC	63.24±0.25bcC	315.68±0.64aC
		Day-9	33.12±0.43cB	7.54±0.13aC	60.12±0.25bcC	306.35±0.32aC
		Day-12	30.27±0.11cB	7.22±0.18aC	54.18±0.29bcC	296.53±0.57aC
Citric acid	HDPE	Day-0	36.55±0.01aC	8.46±0.04aA	66.84±0.24bA	335.05±1.27bB
		Day-3	36.55±0.01aC	8.46±0.04aA	66.34±0.24bA	329.37±1.40bB
		Day-6	32.84±0.09aC	7.92±0.10aA	64.45±0.13bA	315.95±0.76bB
		Day-9	32.64±0.52aC	7.82±0.16aA	61.01±0.13bA	303.96±0.64bB
		Day-12	32.64±0.52aC	7.81±0.21aA	56.16±0.47bA	297.30±0.64bB
	LDPE	Day-0	36.55±0.01aCB	8.46±0.04aB	66.84±0.24bB	335.05±1.27bD
		Day-3	36.55±0.01aCB	8.46±0.04aB	66.34±0.24bB	323.87±0.76bD
		Day-6	33.30±0.13aCB	7.93±0.04aB	63.55±0.24bB	315.68±0.64bD
		Day-9	32.94±0.49aCB	7.90±0.13aB	60.19±0.13bB	304.41±1.27bD
		Day-12	32.40±1.25aCB	7.87±0.30aB	55.67±0.24bB	297.57±0.25bD
	MP	Day-0	36.55±0.01aA	8.46±0.04aA	66.84±0.24bA	335.05±1.27bA
		Day-3	36.55±0.01aA	8.46±0.04aA	66.42±0.13bA	330.54±1.27bA
		Day-6	33.58±0.53aA	7.80±0.24aA	63.63±0.13bA	314.82±0.32bA

Distilled water	PT	Day-9	33.50±0.42aA	7.21±0.18aA	61.33±0.13bA	305.81±1.97bA
		Day-12	33.50±0.42aA	7.00±0.18aA	56.16±0.22bA	297.97±0.06bA
		Day-0	36.36±0.27aB	8.46±0.05aC	66.82±0.26bC	335.05±1.27bC
		Day-3	36.36±0.27aB	8.46±0.05aC	66.50±0.26bC	332.34±1.27bC
		Day-6	33.50±0.42aB	7.84±0.18aC	63.46±0.09bC	313.47±0.45bC
		Day-9	33.47±0.37aB	7.20±0.19aC	60.17±0.09bC	305.45±2.74bC
		Day-12	33.47±0.37aB	7.01±0.22aC	54.02±0.21bC	295.90±0.83bC
	HDPE	Day-0	37.43±0.27bcC	8.29±0.15bA	67.07±0.12bcA	337.75±1.27bB
		Day-3	37.43±0.27bcC	8.29±0.15bA	66.75±0.35bcA	329.64±1.27bB
		Day-6	34.77±0.11bcC	7.75±0.01bA	64.28±0.12bcA	314.30±0.42bB
		Day-9	29.15±0.12bcC	7.51±0.16bA	60.58±0.22bcA	307.16±0.70bB
		Day-12	28.50±0.37bcC	7.31±0.32bA	56.21±0.10bcA	296.71±1.08bB
	LDPE	Day-0	37.43±0.27bcCB	8.29±0.15bB	67.07±0.12bcB	337.75±1.27bD
		Day-3	37.43±0.27bcCB	8.29±0.15bB	66.83±0.23bcB	322.88±0.64bD
		Day-6	34.37±0.68bcCB	7.69±0.13bB	63.46±0.35bcB	314.73±0.57bD
		Day-9	29.81±0.05bcCB	7.22±0.19bB	59.94±0.24bcB	305.90±0.45bD
		Day-12	26.79±0.14bcCB	6.78±0.18bB	55.34±0.24bcB	297.03±0.76bD
	MP	Day-0	37.43±0.27bcA	8.29±0.15bA	67.07±0.12bcA	337.75±1.27bA
		Day-3	37.43±0.27bcA	8.29±0.15bA	66.66±0.00bcA	330.54±1.27bA
		Day-6	33.77±0.22bcA	8.16±0.15bA	63.54±0.23bcA	316.03±0.75bA
		Day-9	33.09±0.75bcA	7.55±0.36bA	60.75±0.46bcA	307.66±0.25bA
		Day-12	31.01±0.91bcA	7.91±0.10bA	55.91±0.35bcA	297.75±0.25bA
	PT	Day-0	37.43±0.27bcB	8.29±0.15bC	67.07±0.12bcC	337.75±1.27bC
		Day-3	37.43±0.27bcB	8.29±0.15bC	66.42±0.12bcC	331.44±1.27bC
		Day-6	33.34±0.16bcB	8.04±0.27bC	63.13±0.12bcC	312.97±0.64bC
		Day-9	30.11±1.00bcB	7.31±0.25bC	59.85±0.12bcC	305.32±0.51bC
		Day-12	30.38±0.61bcB	6.92±0.16bC	54.19±0.23bcC	295.05±0.76bC

Ethanol	HDPE	Day-0	36.47±0.01bC	8.36±0.02aA	66.84±0.23cA	334.14±1.27bB
		Day-3	36.47±0.01bC	8.36±0.02aA	66.35±0.24cA	330.81±0.89bB
		Day-6	33.71±0.23bC	7.76±0.09aA	63.72±0.23cA	316.17±0.83bB
		Day-9	33.06±0.09bC	7.84±0.15aA	61.01±0.12cA	307.30±0.76bB
		Day-12	29.08±0.16bC	7.63±0.13aA	55.36±0.45cA	296.40±0.38bB
	LDPE	Day-0	36.47±0.01bCB	8.36±0.02aB	66.84±0.23cB	334.14±1.27bD
		Day-3	36.47±0.01bCB	8.36±0.02aB	66.10±0.35cB	322.57±0.32bD
		Day-6	33.52±0.39bCB	7.87±0.23aB	63.30±0.10cB	315.45±0.32bD
		Day-9	33.18±0.34bCB	7.55±0.09aB	60.67±0.10cB	306.40±0.25bD
		Day-12	30.19±0.22bCB	7.37±0.14aB	55.25±0.10cB	297.30±0.13bD
	MP	Day-0	36.47±0.01bA	8.36±0.02aA	66.84±0.23cA	334.14±1.27bA
		Day-3	36.47±0.01bA	8.36±0.02aA	66.02±0.24cA	330.09±0.64bA
		Day-6	33.58±0.53bA	7.92±0.10aA	63.55±0.24cA	314.91±0.06bA
		Day-9	32.49±0.50bA	8.01±0.07aA	61.09±0.23cA	307.88±0.06bA
		Day-12	31.62±0.38bA	7.86±0.04aA	56.33±0.01cA	297.93±0.13bA
	PT	Day-0	36.47±0.01bB	8.36±0.02aC	66.84±0.23cC	334.14±1.27bC
		Day-3	36.47±0.01bB	8.36±0.02aC	65.69±0.46cC	332.88±0.51bC
		Day-6	33.40±0.23bB	7.91±0.09aC	63.23±0.23cC	313.65±0.45bC
		Day-9	33.12±0.43bB	7.43±0.01aC	59.45±0.23cC	305.90±0.96bC
		Day-12	30.27±0.11bB	7.27±0.19aC	54.28±0.11cC	295.86±0.51bC
Sodium hypochlorite	HDPE	Day-0	36.57±0.02bC	8.54±0.02aA	67.00±0.01bA	334.59±0.64bB
		Day-3	36.57±0.02bC	8.54±0.02aA	66.50±0.22bA	329.37±1.40bB
		Day-6	33.71±0.23bC	7.89±0.05aA	64.29±0.10bA	315.86±0.89bB
		Day-9	33.14±0.20bC	7.81±0.28aA	60.76±0.25bA	305.05±0.13bB
		Day-12	29.01±0.05bC	7.72±0.12aA	56.23±0.63bA	298.20±0.64bB
	LDPE	Day-0	36.57±0.02bCB	8.54±0.02aB	67.00±0.01bB	334.59±0.64bD
		Day-3	36.57±0.02bCB	8.54±0.02aB	66.75±0.13bB	322.16±0.89bD

		Day-6	33.52±0.39bCB	7.97±0.10aB	63.63±0.13bB	316.62±0.57bD
		Day-9	33.15±0.29bCB	7.41±0.04aB	60.35±0.13bB	305.90±0.83bD
		Day-12	30.21±0.02bCB	7.29±0.24aB	55.65±0.26bB	298.29±0.51bD
	MP	Day-0	36.52±0.05bA	8.48±0.10aA	67.07±0.09bA	333.69±0.64bA
		Day-3	36.52±0.05bA	8.48±0.10aA	66.58±0.09bA	331.76±0.83bA
		Day-6	33.58±0.53bA	7.93±0.15aA	63.95±0.09bA	316.94±0.13bA
		Day-9	32.49±0.50bA	8.08±0.05aA	61.08±0.02bA	305.95±0.89bA
		Day-12	31.54±0.49bA	7.77±0.08aA	56.15±0.28bA	298.78±0.19bA
	PT	Day-0	36.52±0.05bB	8.48±0.10aC	67.07±0.09bC	333.69±0.64bC
		Day-3	36.52±0.05bB	8.48±0.10aC	65.92±0.60bC	332.03±0.83bC
		Day-6	33.40±0.23bB	7.90±0.11aC	63.38±0.21bC	313.87±1.02bC
		Day-9	33.12±0.43bB	7.54±0.13aC	59.93±0.21bC	305.32±1.27bC
		Day-12	30.27±0.11bB	7.25±0.19aC	54.09±0.33bC	295.95±1.02bC
Control	HDPE	Day-0	37.68±0.09dC	8.32±0.21bA	67.32±0.23bcA	340.00±0.64bB
		Day-3	35.24±0.32dC	8.07±0.05bA	67.23±0.35bcA	329.19±1.91bB
		Day-6	34.77±0.11dC	7.79±0.03bA	64.20±0.23bcA	310.54±0.76bB
		Day-9	29.15±0.12dC	7.54±0.11bA	59.27±0.23bcA	306.98±0.70bB
		Day-12	28.50±0.37dC	7.38±0.19bA	56.07±0.12bcA	296.22±0.38bB
	LDPE	Day-0	37.68±0.09dCB	8.32±0.21bB	67.32±0.23bcB	340.00±0.64bD
		Day-3	35.71±0.05dCB	8.18±0.16bB	67.15±0.23bcB	322.88±0.64bD
		Day-6	34.37±0.68dCB	7.70±0.08bB	63.54±0.23bcB	313.87±0.64bD
		Day-9	30.04±0.05dCB	7.14±0.13bB	59.11±0.46bcB	305.77±0.64bD
		Day-12	26.79±0.14dCB	6.82±0.24bB	55.25±0.35bcB	296.76±0.64bD
	MP	Day-0	37.68±0.09dA	8.32±0.21bA	67.32±0.23bcA	340.00±0.64bA
		Day-3	34.31±0.17dA	8.19±0.11bA	67.15±0.46bcA	331.44±1.27bA
		Day-6	33.77±0.22dA	8.19±0.12bA	63.38±0.46bcA	308.83±1.15bA
		Day-9	33.09±0.75dA	7.63±0.31bA	59.11±0.46bcA	307.52±0.19bA

	PT	Day-12	31.01±0.91dA	7.96±0.02bA	55.82±0.46bcA	297.57±0.25bA
		Day-0	37.68±0.09dB	8.32±0.21bC	67.32±0.23bcC	340.00±0.64bC
		Day-3	33.41±0.12dB	8.20±0.20bC	67.07±0.12bcC	330.99±0.64bC
		Day-6	33.34±0.16dB	8.16±0.18bC	62.96±0.12bcC	307.48±0.51bC
		Day-9	30.11±1.00dB	7.31±0.25bC	59.43±0.23bcC	304.86±0.64bC
		Day-12	30.38±0.61dB	6.95±0.11bC	54.02±0.46bcC	295.59±0.25bC

Results are expressed as mean ± standard deviation of three replicates. The different lowercase letters within the column indicate significant (p<0.05) differences based on washing treatments and different uppercase letters within the column indicate significant (p<0.05) differences among the packaging materials.

Appendices Table 3. Effect of different washing treatments on radish sango microgreens stored in various packaging materials at 5°C on total flavonoid content, ascorbic acid, total glucosinolate content, and antioxidant activities (DPPH and FRAP).

Washing treatments	Packaging materials	Days	Total flavonoid content (mg QE/100 g)	Ascorbic acid (mg/100g)	Total glucosinolate (μmol/g)	Antioxidant activity DPPH (%)	Antioxidant activity FRAP (μmol TE/g)
Acetic acid	HDPE	Day-0	31.00±0.71aA	365.81±1.37aB	39.81±0.17aB	73.03±0.24aB	33.38±0.41aB
		Day-3	28.25±0.21aA	353.10±0.18aB	36.74±0.26aB	64.74±0.16aB	27.91±0.41aB
		Day-6	24.75±0.21aA	346.97±0.27aB	29.70±0.17aB	55.90±0.39aB	16.12±0.81aB
	LDPE	Day-0	31.00±0.71aB	365.81±1.37aC	39.81±0.17aC	73.03±0.24aC	33.38±0.41aC
		Day-3	26.40±0.14aB	352.65±0.46aC	36.50±0.09aC	65.18±0.16aC	26.91±1.02aC
		Day-6	24.65±0.07aB	348.77±4.11aC	29.64±0.43aC	56.06±0.16aC	15.54±0.41aC
	MP	Day-0	31.00±0.71aA	365.81±1.37aA	39.81±0.17aA	73.03±0.24aA	33.38±0.41aA
		Day-3	28.40±0.14aA	354.35±0.23aA	37.10±0.09aA	65.91±0.24aA	28.20±0.41aA
		Day-6	24.95±0.35aA	348.42±0.23aA	29.34±0.51aA	56.45±0.24aA	14.82±0.20aA
	PT	Day-0	31.00±0.71aB	365.81±1.37aD	39.81±0.17aD	73.03±0.24aD	33.38±0.41aC
		Day-3	25.60±0.14aB	352.90±0.46aD	36.26±0.09aD	63.68±0.39aD	26.19±0.41aC
		Day-6	25.10±0.42aB	346.94±0.23aD	29.28±0.43aD	55.67±0.08aD	14.10±0.41aC
Calcium chloride	HDPE	Day-0	33.50±1.41bA	368.06±1.82bB	40.11±0.26bB	72.69±0.24bB	33.96±0.41bcB

		Day-3	30.94±0.79bA	366.71±0.09bB	39.11±0.07bB	71.36±0.24bB	30.36±0.20bcB
		Day-6	29.25±0.21bA	361.06±0.50bB	33.31±0.17bB	64.07±0.16bB	25.61±0.41bcB
		Day-9	23.90±0.28bA	317.42±0.46bB	28.86±0.34bB	53.45±0.08bB	21.44±0.20bcB
		Day-12	18.10±0.28bA	297.61±0.09bB	27.24±0.43bB	48.61±0.31bB	14.53±0.61bcB
	LDPE	Day-0	33.50±1.41bB	368.06±1.82bC	40.11±0.26bC	72.69±0.24bC	33.96±0.41bcC
		Day-3	30.75±0.92bB	366.29±0.23bC	38.54±0.08bC	71.58±0.24bC	29.50±0.20bcC
		Day-6	28.70±0.57bB	359.26±0.50bC	32.77±0.26bC	63.79±0.39bC	24.75±0.81bcC
		Day-9	23.45±0.21bB	316.68±0.68bC	28.86±0.17bC	53.00±0.39bC	19.42±0.61bcC
		Day-12	17.50±0.14bB	295.90±0.23bC	26.58±0.17bC	47.66±0.24bC	12.81±0.20bcC
	MP	Day-0	33.50±1.41bA	368.06±1.82bA	40.11±0.26bA	72.69±0.24bA	33.96±0.41bcA
		Day-3	30.95±0.64bA	367.32±0.14bA	39.81±1.70bA	71.36±0.24bA	30.50±1.22bcA
		Day-6	28.75±0.78bA	360.48±0.60bA	33.91±0.34bA	64.85±0.16bA	26.76±0.81bcA
		Day-9	23.40±0.71bA	317.45±0.23bA	28.98±0.17bA	54.67±0.08bA	22.01±0.61bcA
		Day-12	18.40±0.42bA	296.45±0.27bA	27.90±0.17bA	49.22±0.55bA	14.96±0.41bcA
	PT	Day-0	33.50±1.41bB	368.06±1.82bD	40.11±0.26bD	72.69±0.24bD	33.96±0.41bcC
		Day-3	30.35±0.35bB	366.32±0.18bD	37.76±0.33bD	70.47±0.24bD	29.50±0.20bcC
		Day-6	27.85±0.64bB	359.77±0.59bD	32.47±0.17bD	63.24±0.24bD	22.45±0.41bcC
		Day-9	23.40±0.42bB	314.68±0.23bD	28.14±0.34bD	54.00±0.24bD	20.43±0.41bcC
		Day-12	16.75±0.35bB	282.42±0.32bD	26.22±0.17bD	47.55±0.24bD	13.96±0.20bcC
Citric acid	HDPE	Day-0	32.95±0.64cdA	368.06±0.91bcB	40.05±0.17cB	73.03±0.24cdB	34.68±0.61abB
		Day-3	30.43±0.09cdA	366.71±0.09bcB	38.34±0.14cB	70.19±0.31cdB	31.80±0.61abB
		Day-6	28.85±0.49cdA	360.81±0.14bcB	32.65±0.09cB	63.52±0.16cdB	24.75±0.81abB
		Day-9	23.35±0.07cdA	315.71±0.14bcB	28.44±0.26cB	51.95±0.47cdB	19.71±0.61abB
		Day-12	17.00±0.14cdA	296.61±0.59bcB	26.34±0.17cB	47.89±0.24cdB	14.39±0.41abB
	LDPE	Day-0	32.95±0.64cdB	368.06±0.91bcC	40.05±0.17cC	73.03±0.24cdC	34.68±0.61abC
		Day-3	30.45±0.78cdB	365.94±0.27bcC	38.06±0.08cC	70.02±0.08cdC	30.22±0.41abC
		Day-6	28.35±0.07cdB	359.26±0.50bcC	32.41±0.09cC	63.13±0.24cdC	24.17±0.41abC

		Day-9	23.05±0.21cdB	315.00±0.14bcC	28.33±0.10cC	52.50±0.31cdC	18.70±0.41abC
		Day-12	16.75±0.07cdB	295.16±0.36bcC	25.86±0.17cC	47.16±0.47cdC	12.95±0.41abC
	MP	Day-0	32.95±0.64cdA	368.06±0.91bcA	40.05±0.17cA	73.03±0.24cdA	34.68±0.61abA
		Day-3	30.33±0.08cdA	367.29±0.09bcA	38.67±0.09cA	70.13±0.08cdA	32.95±0.20abA
		Day-6	28.40±1.27cdA	362.16±0.05bcA	33.61±0.26cA	64.29±0.16cdA	26.33±0.20abA
		Day-9	23.30±0.14cdA	316.48±0.23bcA	28.56±0.26cA	50.50±0.16cdA	20.58±0.20abA
		Day-12	17.90±0.28cdA	296.84±0.27bcA	26.22±0.17cA	49.61±0.47cdA	14.82±0.20abA
	PT	Day-0	33.10±0.85cdB	367.74±0.46bcD	40.17±0.00cD	72.91±0.08cdD	34.53±0.41abC
		Day-3	30.15±0.35cdB	366.39±0.27bcD	37.82±0.26cD	69.63±0.16cdD	31.65±0.41abC
		Day-6	27.15±0.35cdB	360.58±0.36bcD	31.87±0.17cD	62.85±0.31cdD	23.02±0.81abC
		Day-9	23.00±0.14cdB	314.06±0.18bcD	27.90±0.17cD	50.78±0.08cdD	19.57±0.41abC
		Day-12	17.90±0.14cdB	282.19±0.64bcD	25.19±0.09cD	47.05±0.31cdD	13.96±0.20abC
Distilled water	HDPE	Day-0	33.95±0.64cdA	368.71±0.00bcB	40.11±0.09cB	73.03±0.08dB	34.39±0.20cB
		Day-3	30.41±0.10cdA	366.61±0.14bcB	38.22±0.14cB	69.52±0.31dB	30.79±0.41cB
		Day-6	29.20±0.34cdA	360.39±0.46bcB	32.41±0.09cB	63.18±0.16dB	25.04±0.41cB
		Day-9	22.35±0.07cdA	315.06±0.14bcB	28.08±0.26cB	52.61±0.16dB	19.57±0.41cB
		Day-12	17.10±0.00cdA	296.29±0.14bcB	26.10±0.17cB	47.27±0.16dB	13.96±0.20cB
	LDPE	Day-0	33.95±0.64cdB	368.71±0.00bcC	40.11±0.09cC	73.03±0.08dC	34.39±0.20cC
		Day-3	30.10±0.71cdB	365.74±0.46bcC	37.98±0.14cC	69.69±0.24dC	29.50±0.20cC
		Day-6	28.04±0.20cdB	358.61±0.50bcC	32.17±0.26cC	62.79±0.24dC	23.88±0.41cC
		Day-9	22.15±0.07cdB	314.58±0.46bcC	27.96±0.09cC	52.89±0.08dC	18.42±0.81cC
		Day-12	16.75±0.07cdB	294.19±0.82bcC	25.68±0.09cC	46.72±0.16dC	12.66±0.41cC
	MP	Day-0	33.95±0.64cdA	368.71±0.00bcA	40.11±0.09cA	73.03±0.08dA	34.39±0.20cA
		Day-3	30.32±0.08cdA	367.39±0.87bcA	38.43±0.08cA	69.24±0.24dA	31.65±0.41cA
		Day-6	28.78±0.73cdA	361.84±0.41bcA	33.31±0.51cA	64.24±0.24dA	25.90±0.41cA
		Day-9	23.15±0.07cdA	315.84±0.68bcA	28.20±0.26cA	52.89±0.08dA	20.29±0.20cA
		Day-12	17.80±0.42cdA	296.87±0.32bcA	26.28±0.09cA	49.00±0.24dA	14.53±0.20cA

	PT	Day-0	33.95±0.64cdB	368.71±0.00bcD	40.11±0.09cD	73.03±0.08dD	34.39±0.20cC
		Day-3	30.00±0.42cdB	366.39±0.36bcD	37.34±0.09cD	68.80±0.24dD	30.22±0.41cC
		Day-6	26.90±0.57cdB	360.74±0.14bcD	31.57±0.26cD	62.01±0.08dD	23.02±0.41cC
		Day-9	22.00±0.14cdB	314.35±0.23bcD	27.48±0.09cD	51.56±0.39dD	19.42±0.20cC
		Day-12	17.85±0.07cdB	282.81±0.59bcD	25.31±0.26cD	46.50±0.16dD	13.67±0.20cC
Ethanol	HDPE	Day-0	32.10±0.57dA	367.42±0.91dB	39.81±0.17cB	72.80±0.08cB	32.81±1.22dB
		Day-3	29.95±0.35dA	365.16±0.46dB	38.31±0.10cB	71.47±0.08cB	30.79±0.41dB
		Day-6	29.00±0.14dA	356.33±0.41dB	33.07±0.17cB	63.13±0.24cB	23.60±1.22dB
		Day-9	23.40±0.28dA	315.29±0.18dB	27.96±0.26cB	52.67±0.39cB	19.42±0.20dB
		Day-12	17.25±0.49dA	295.97±0.32dB	26.58±0.17cB	46.55±0.39cB	13.38±0.61dB
	LDPE	Day-0	32.10±0.57dB	367.42±0.91dC	39.81±0.17cC	72.80±0.08cC	32.81±1.22dC
		Day-3	30.35±0.78dB	365.16±0.46dC	38.30±0.13cC	70.19±0.16cC	29.78±1.02dC
		Day-6	28.20±0.14dB	357.71±0.78dC	32.47±0.17cC	62.07±0.16cC	25.90±0.41dC
		Day-9	23.05±0.21dB	315.39±0.23dC	27.42±0.51cC	52.56±0.39cC	18.56±0.61dC
		Day-12	16.65±0.07dB	294.84±0.09dC	25.98±0.17cC	46.38±0.31cC	11.65±0.20dC
	MP	Day-0	32.10±0.57dA	367.42±0.91dA	39.81±0.17cA	72.80±0.08cA	32.81±1.22dA
		Day-3	29.88±0.13dA	366.26±0.18dA	38.39±0.14cA	71.30±0.31cA	31.08±0.41dA
		Day-6	28.50±0.85dA	355.58±0.14dA	33.91±0.34cA	63.79±0.39cA	24.17±0.41dA
		Day-9	23.15±0.64dA	315.68±0.46dA	28.20±0.26cA	53.56±0.08cA	20.58±0.20dA
		Day-12	17.90±0.28dA	296.52±0.18dA	26.88±0.09cA	47.05±0.31cA	14.96±0.41dA
	PT	Day-0	32.10±0.57dB	367.42±0.91dD	39.81±0.17cD	72.80±0.08cD	32.81±1.22dC
		Day-3	30.00±0.14dB	364.97±0.18dD	37.46±0.26cD	69.91±0.08cD	30.07±0.20dC
		Day-6	27.25±0.07dB	354.61±0.32dD	32.35±0.17cD	62.12±0.08cD	24.75±0.81dC
		Day-9	22.75±0.21dB	313.13±0.59dD	27.30±0.17cD	54.67±0.39cD	18.85±0.61dC
		Day-12	16.62±0.02dB	281.65±0.14dD	25.56±0.43cD	46.11±0.08cD	12.37±0.41dC
Sodium hypochlorite	HDPE	Day-0	32.50±0.00cdA	368.39±0.46bcB	40.05±0.00bB	72.86±0.00cdB	33.67±0.81abB
		Day-3	30.44±0.08cdA	366.45±0.46bcB	38.93±0.19bB	70.52±0.31cdB	32.09±0.61abB

		Day-6	28.80±0.14cdA	360.52±0.27bcB	32.83±0.17bB	63.79±0.24cdB	25.47±0.20abB
		Day-9	23.45±0.07cdA	316.26±0.64bcB	28.74±0.17bB	51.22±0.08cdB	21.01±0.41abB
		Day-12	17.55±0.07cdA	297.00±0.05bcB	27.06±0.51bB	48.16±0.16cdB	14.24±0.20abB
	LDPE	Day-0	32.50±0.00cdB	368.39±0.46bcC	40.05±0.00bC	72.86±0.00cdC	33.67±0.81abC
		Day-3	30.55±0.92cdB	365.81±0.46bcC	38.42±0.25bC	70.41±0.16cdC	30.79±0.41abC
		Day-6	27.95±0.07cdB	359.90±0.50bcC	32.59±0.17bC	63.40±0.16cdC	24.46±0.41abC
		Day-9	23.35±0.07cdB	315.32±0.32bcC	28.51±0.15bC	52.34±0.24cdC	19.28±0.41abC
		Day-12	17.65±0.35cdB	295.58±0.23bcC	26.28±0.09bC	47.89±0.24cdC	12.66±0.41abC
	MP	Day-0	33.00±0.71cdA	368.06±0.91bcA	40.17±0.17bA	72.75±0.16cdA	33.96±1.22abA
		Day-3	30.85±0.64cdA	366.77±0.00bcA	39.87±0.09bA	70.63±0.16cdA	32.23±0.81abA
		Day-6	28.40±1.13cdA	361.90±0.41bcA	33.79±0.34bA	64.40±0.16cdA	26.47±0.41abA
		Day-9	23.45±0.07cdA	316.87±0.32bcA	28.80±0.26bA	50.22±0.08cdA	21.73±0.20abA
		Day-12	18.10±0.57cdA	296.13±0.18bcA	27.72±0.09bA	48.33±0.24cdA	14.53±0.20abA
	PT	Day-0	33.00±0.71cdB	368.06±0.91bcD	40.17±0.17bD	72.75±0.16cdD	33.96±1.22abC
		Day-3	30.35±0.21cdB	366.16±0.05bcD	38.06±0.43bD	70.52±0.16cdD	29.50±0.61abC
		Day-6	27.15±0.21cdB	359.97±0.05bcD	32.17±0.26bD	63.13±0.24cdD	24.17±1.22abC
		Day-9	23.35±0.07cdB	314.39±0.64bcD	28.14±0.17bD	50.67±0.24cdD	20.72±0.41abC
		Day-12	16.60±0.14cdB	282.10±0.14bcD	26.10±0.34bD	47.05±0.16cdD	13.53±0.41abC
Control	HDPE	Day-0	35.00±0.71bcA	368.39±0.46cB	40.11±0.09dB	72.91±0.08eB	34.10±0.20dB
		Day-3	30.35±0.07bcA	366.48±0.05cB	38.00±0.17dB	68.30±0.16eB	30.22±0.41dB
		Day-6	28.67±0.10bcA	359.87±0.27cB	32.05±0.26dB	62.74±0.16eB	24.75±0.81dB
		Day-9	22.15±0.07bcA	314.45±0.09cB	27.60±0.26dB	51.50±0.16eB	18.85±0.61dB
		Day-12	17.00±0.14bcA	295.68±0.18cB	25.56±0.26dB	46.44±0.24eB	13.09±0.61dB
	LDPE	Day-0	35.00±0.71bcB	368.39±0.46cC	40.11±0.09dC	72.91±0.08eC	34.10±0.20dC
		Day-3	30.00±0.71bcB	365.55±0.46cC	37.70±0.09dC	68.52±0.16eC	29.21±0.20dC
		Day-6	27.62±0.17bcB	358.45±0.46cC	30.85±0.43dC	61.74±0.31eC	23.45±0.20dC
		Day-9	21.70±0.14bcB	313.94±0.46cC	27.12±0.60dC	51.72±0.31eC	17.84±0.41dC

	MP	Day-12	16.65±0.07bcB	293.94±0.46cC	25.07±0.09dC	46.05±0.16eC	11.94±0.20dC
		Day-0	35.00±0.71bcA	368.39±0.46cA	40.11±0.09dA	72.91±0.08eA	34.10±0.20dA
		Day-3	30.35±0.07bcA	367.52±0.59cA	38.33±0.12dA	68.13±0.24eA	31.37±0.41dA
		Day-6	29.13±1.65bcA	361.32±0.23cA	33.01±0.60dA	63.13±0.24eA	25.76±0.61dA
		Day-9	22.70±0.28bcA	315.42±0.36cA	28.08±0.43dA	52.00±0.24eA	20.00±0.61dA
		Day-12	17.60±0.14bcA	296.48±0.68cA	26.10±0.17dA	47.83±0.16eA	14.39±0.41dA
	PT	Day-0	35.00±0.71bcB	368.39±0.46cD	40.11±0.09dD	72.91±0.08eD	34.10±0.20dC
		Day-3	30.15±0.35bcB	366.29±0.23cD	37.28±0.17dD	68.02±0.24eD	29.50±0.20dC
		Day-6	26.85±0.21bcB	359.84±0.23cD	30.37±0.26dD	61.23±0.08eD	22.30±0.20dC
		Day-9	21.65±0.21bcB	313.39±0.23cD	26.88±0.43dD	49.22±0.24eD	19.14±0.20dC
		Day-12	17.65±0.21bcB	282.42±0.23cD	24.95±0.26dD	45.88±0.24eD	13.38±0.20dC

Results are expressed as mean ± standard deviation of three replicates. The different lowercase letters within the column indicate significant ($p<0.05$) differences based on washing treatments and different uppercase letters within the column indicate significant ($p<0.05$) differences among the packaging materials.

Appendices Table 4. Effect of different washing treatments on radish sango microgreens stored in various packaging materials at 5°C on microbial parameters.

Washing treatments	Packaging materials	Days	Total plate count (log CFU/g)	Yeast and mould count (log CFU/g)
Acetic acid	HDPE	Day-0	2.50±0.14bB	1.65±0.21dCB
		Day-3	3.46±0.65bB	2.50±0.14dCB
		Day-6	6.83±0.13bB	6.83±0.13dCB
	LDPE	Day-0	2.50±0.14bA	1.65±0.21dAB
		Day-3	3.88±0.06bA	2.50±0.14dAB
		Day-6	6.88±0.06bA	6.88±0.06dAB
	MP	Day-0	2.50±0.14bB	1.65±0.21dC
		Day-3	3.94±0.03bB	2.50±0.14dC
		Day-6	6.94±0.03bB	6.94±0.03dC
	PT	Day-0	2.50±0.14bA	1.65±0.21dA
		Day-3	3.86±0.03bA	2.50±0.14dA
		Day-6	6.91±0.04bA	6.91±0.04dA
Calcium chloride	HDPE	Day-0	2.60±0.28cB	1.65±0.21cCB
		Day-3	2.36±0.06cB	2.60±0.28cCB
		Day-6	3.31±0.13cB	4.31±0.13cCB
		Day-9	4.31±0.13cB	6.68±0.57cCB
		Day-12	6.68±0.57cB	7.18±0.14cCB
	LDPE	Day-0	2.60±0.28cA	1.65±0.21cAB
		Day-3	2.85±0.18cA	2.60±0.28cAB
		Day-6	3.85±0.18cA	5.05±0.10cAB
		Day-9	5.05±0.10cA	6.76±0.14cAB
		Day-12	6.76±0.14cA	7.76±0.14cAB
	MP	Day-0	2.60±0.28cB	1.65±0.21cC
		Day-3	2.34±0.08cB	2.60±0.28cC
		Day-6	3.24±0.06cB	4.24±0.06cC
		Day-9	4.24±0.06cB	6.64±0.35cC
		Day-12	6.64±0.35cB	7.64±0.35cC
	PT	Day-0	2.60±0.28cA	1.65±0.21cA
		Day-3	2.76±0.17cA	2.60±0.28cA
		Day-6	3.76±0.17cA	4.36±0.40cA
		Day-9	4.36±0.40cA	6.82±0.32cA
		Day-12	6.82±0.32cA	7.32±0.39cA
Citric acid	HDPE	Day-0	2.45±0.07cB	1.65±0.07cCB
		Day-3	2.54±0.17cB	2.45±0.07cCB
		Day-6	3.59±0.24cB	3.98±0.76cCB
		Day-9	3.98±0.76cB	6.98±0.65cCB
		Day-12	6.98±0.65cB	7.48±0.06cCB
	LDPE	Day-0	2.45±0.07cA	1.65±0.07cAB

		Day-3	2.68±0.20cA	2.45±0.07cAB
		Day-6	3.83±0.13cA	4.66±0.09cAB
		Day-9	4.66±0.09cA	6.86±0.37cAB
		Day-12	6.86±0.37cA	7.36±0.33cAB
	MP	Day-0	2.45±0.07cB	1.65±0.07cC
		Day-3	2.30±0.03cB	2.45±0.07cC
		Day-6	3.30±0.03cB	4.36±0.11cC
		Day-9	4.36±0.11cB	6.36±0.11cC
		Day-12	6.36±0.11cB	7.36±0.11cC
	PT	Day-0	2.45±0.07cA	1.65±0.07cA
		Day-3	2.91±0.04cA	2.45±0.07cA
		Day-6	3.91±0.04cA	4.87±0.10cA
		Day-9	4.87±0.10cA	8.37±0.81cA
		Day-12	8.37±0.81cA	7.87±0.10cA
Distilled water	HDPE	Day-0	2.60±0.28bB	1.85±0.07bCB
		Day-3	3.77±0.18bB	2.60±0.28bCB
		Day-6	4.77±0.18bB	4.59±0.24bCB
		Day-9	4.59±0.24bB	6.59±1.17bCB
		Day-12	6.59±1.17bB	8.59±0.24bCB
	LDPE	Day-0	2.60±0.28bA	1.85±0.07bAB
		Day-3	3.61±0.30bA	2.60±0.28bAB
		Day-6	4.61±0.30bA	4.78±0.20bAB
		Day-9	4.78±0.20bA	7.28±0.51bAB
		Day-12	7.28±0.51bA	8.78±0.20bAB
	MP	Day-0	2.60±0.28bB	1.85±0.07bC
		Day-3	3.29±0.16bB	2.60±0.28bC
		Day-6	4.59±0.27bB	4.34±0.08bC
		Day-9	4.34±0.08bB	6.84±0.63bC
		Day-12	6.84±0.63bB	8.34±0.08bC
	PT	Day-0	2.60±0.28bA	1.85±0.07bA
		Day-3	3.71±0.04bA	2.60±0.28bA
		Day-6	4.76±0.11bA	4.91±0.04bA
		Day-9	4.91±0.04bA	7.41±0.75bA
		Day-12	7.41±0.75bA	8.41±0.66bA
Ethanol	HDPE	Day-0	2.55±0.21cB	1.40±0.14cCB
		Day-3	2.38±0.03cB	2.55±0.21cCB
		Day-6	3.46±0.14cB	4.28±0.09cCB
		Day-9	4.28±0.09cB	6.28±0.09cCB
		Day-12	6.28±0.09cB	7.28±0.09cCB
	LDPE	Day-0	2.55±0.21cA	1.40±0.14cAB
		Day-3	3.74±0.03cA	2.55±0.21cAB
		Day-6	4.59±0.10cA	4.85±0.18cAB

		Day-9	4.85±0.18cA	6.85±0.18cAB
		Day-12	6.85±0.18cA	7.85±0.18cAB
	MP	Day-0	2.55±0.21cB	1.40±0.14cC
		Day-3	2.24±0.06cB	2.55±0.21cC
		Day-6	3.35±0.10cB	4.24±0.06cC
		Day-9	4.24±0.06cB	6.19±0.13cC
		Day-12	6.19±0.13cB	7.19±0.13cC
	PT	Day-0	2.55±0.21cA	1.40±0.14cA
		Day-3	2.88±0.06cA	2.55±0.21cA
		Day-6	3.86±0.08cA	4.91±0.04cA
		Day-9	4.91±0.04cA	6.91±0.04cA
		Day-12	6.91±0.04cA	7.91±0.04cA
Sodium hypochlorite	HDPE	Day-0	2.30±0.14cB	1.50±0.00cCB
		Day-3	2.48±0.06cB	2.30±0.14cCB
		Day-6	3.48±0.06cB	4.51±0.21cCB
		Day-9	4.51±0.21cB	6.76±0.14cCB
		Day-12	6.76±0.14cB	7.76±0.14cCB
	LDPE	Day-0	2.30±0.14cA	1.50±0.00cAB
		Day-3	2.66±0.08cA	2.30±0.14cAB
		Day-6	3.66±0.08cA	5.24±0.11cAB
		Day-9	5.24±0.11cA	6.24±0.11cAB
		Day-12	6.24±0.11cA	7.24±0.11cAB
	MP	Day-0	2.30±0.14cB	1.55±0.07cC
		Day-3	2.26±0.03cB	2.30±0.14cC
		Day-6	3.36±0.11cB	4.35±0.10cC
		Day-9	4.35±0.10cB	6.50±0.31cC
		Day-12	6.50±0.31cB	7.50±0.31cC
	PT	Day-0	2.30±0.14cA	1.55±0.07cA
		Day-3	2.82±0.03cA	2.30±0.14cA
		Day-6	3.87±0.10cA	4.80±0.17cA
		Day-9	4.80±0.17cA	6.80±0.17cA
		Day-12	6.80±0.17cA	7.80±0.17cA
Control	HDPE	Day-0	3.35±0.07aB	2.15±0.07aCB
		Day-3	4.56±0.06aB	3.77±0.18aCB
		Day-6	5.41±0.12aB	5.77±0.18aCB
		Day-9	5.77±0.18aB	7.77±0.18aCB
		Day-12	7.77±0.18aB	8.77±0.18aCB
	LDPE	Day-0	3.35±0.07aA	2.15±0.07aAB
		Day-3	4.72±0.06aA	3.61±0.30aAB
		Day-6	5.77±0.13aA	5.61±0.30aAB
		Day-9	5.61±0.30aA	7.61±0.30aAB

	MP	Day-12	7.61±0.30aA	8.61±0.30aAB
		Day-0	3.35±0.07aB	2.15±0.07aC
		Day-3	4.40±0.06aB	3.29±0.16aC
		Day-6	5.40±0.06aB	5.59±0.27aC
		Day-9	5.59±0.27aB	6.59±0.27aC
		Day-12	6.59±0.27aB	8.59±0.27aC
	PT	Day-0	3.35±0.07aA	2.15±0.07aA
		Day-3	4.78±0.03aA	3.71±0.04aA
		Day-6	5.88±0.11aA	5.74±0.08aA
		Day-9	5.74±0.08aA	7.24±0.63aA
		Day-12	7.24±0.63aA	8.74±0.08aA

Results are expressed as mean ± standard deviation of three replicates. The different lowercase letters within the column indicate significant ($p<0.05$) differences based on washing treatments and different uppercase letters within the column indicate significant ($p<0.05$) differences among the packaging materials.

LIST OF PUBLICATIONS

Sr. No.	Title of paper with author names	Name of journal / conference	Published date	Issn no/vol no, issue no	Indexing in Scopus/ Web of Science/UGC -CARE list (please mention)
1	Comparative analysis of morphological, nutritional, and bioactive properties of selected microgreens in alternative growing medium Mahendra Gunjal, Jyoti Singh, Jaspreet Kaur, Sawinder Kaur, Vikas Nanda, Chandra Mohan Mehta, Vishesh Bhadariya, Prasad Rasane*	South African Journal of Botany	2024/2/1	Volume 165	Scopus
2	Microgreens: Cultivation practices, bioactive potential, health benefits, and opportunities for its utilization as value-added food Mahendra Gunjal, Jyoti Singh, Jaspreet Kaur, Sawinder Kaur, Vikas Nanda, Ankita Sharma, Prasad Rasane*	Food Bioscience	2024/9/18	Volume 62	Scopus
3	Assessment of bioactive compounds, antioxidant properties and morphological parameters in selected microgreens cultivated in soilless media Mahendra Gunjal, Jyoti Singh, Sawinder Kaur, Vikas Nanda, Riaz Ullah, Zafar Iqbal, Sezai Ercisli, Prasad Rasane*	Scientific Reports	2024/10/9	Volume 14 Issue 1	Scopus
4	Process optimization for microgreens-based dairy (lassi) beverage: Consumer acceptability,	Journal of Stored Products Research	2025/5/1	Volume 112	Scopus

	bioactive composition, and storage life using kinetic modelling Mahendra Gunjal, Atul Khalangre, Jyoti Singh, Sawinder Kaur, Sezai Ercisli, Emine Macit, Prasad Rasane*				
5	Effect of convective drying temperature and tray load density on bioactive compounds, antioxidant properties, and functional quality of radish Sango microgreens Mahendra Gunjal, Atul Khalangre, Mansuri M Tosif, Jyoti Singh, Sawinder Kaur, Riaz Ullah, Prasad Rasane*	Food Chemistry	2025/5/17	Volume 488	Scopus
6	Novel approach for development and optimization of microgreens-based functional dairy beverage: Antioxidant enhancement, consumer acceptability, and kinetic shelf-life modeling Mahendra Gunjal, Atul Khalangre, Jyoti Singh, Sezai Ercisli, Prasad Rasane*	Food Chemistry: X	2025/5/22	Volume 28	Scopus
7	Postharvest Technologies Enhance Quality, Antioxidant Activity, Microbial Control, and Shelf-Life of Radish Sango Microgreens Mahendra Gunjal, Jyoti Singh, Sawinder Kaur, Prasad Rasane*	Journal of Food Process Engineering	2025/08/03	Volume 48	Scopus



Comparative analysis of morphological, nutritional, and bioactive properties of selected microgreens in alternative growing medium

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ABSTRACT

Microgreens from different botanical species seven grown using in two selective medium (Soil and Cocopeat) were examined for morphological, proximate, chemical composition, and antioxidant activities of selected microgreen species. The selected microgreens grown in two different growing substrates (soil and cocopeat) showed a significant effect on their quality parameters. Flaxseed and radish sango microgreens show good results in morphological parameters (shoot height and length, and yield) compared with broccoli, cabbage, pak choi, beetroot, and red amaranthus microgreens. Microgreens contain high levels of moisture content (91.92–95.33 g/100 g, FW) and relatively low amounts of fat (2.23–7.58 g/100 g, DW) and protein (2.04–6.11 g BSA/100 g, DW) content were reported. Comparative analysis of microgreens concluded flaxseed and cabbage microgreens have the greatest total chlorophyll content (26.28–29.16 $\mu\text{g/g}$, FW), meanwhile the microgreens obtained from beetroot had the greatest levels of carotenes (7.42–8.09 $\mu\text{g/g}$, FW) and cabbage microgreens shown high level of ascorbic acid content (329.20–438.71 mg/100 g, FW). Whereas radish sango microgreens contains high level of anthocyanins (28.06–45.87 $\mu\text{mol}/100\text{ g}$, FW), total phenol content (241.98–667.48 mg GAE/100g, FW), total flavonoid content (27.64–39.44 mg QUE/100 g, FW) and antioxidant activity (DPPH and FRAP) (61.71–89.24 %, FW and 14.39–17.46 $\mu\text{mol TE/g}$, FW) compared to other microgreens. Further, all results were compared and verified by principle component analysis. Throughout the research, it was noticed and concluded that growing media is crucial for the growth and development of microgreens, and any modifications to the substrate will have an important effect on both the nutritional value and morphological traits of microgreens. Overall, cocopeat cocopeat-based growing medium was the most effective growing medium for the cultivation of microgreens to increase microgreens plant growth, yield, nutritional, biochemical composition, and antioxidant activity of microgreens.

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1. Introduction

Microgreens are immature greens obtained from seeds of various cereals, legumes, pulses, vegetables, herbs, and spices and it has two completely developed cotyledons (true leaves or without true leaves). In recent years, they have gained substantial popularity as a fresh culinary novelty. Despite their diminutive size, microgreens offer surprisingly potent flavors, vivid colors, and a crunchy texture. Microgreens are used as a fresh ingredient or for salad garnishing purposes (Frąszczak and Kula-Maximenko, 2022). The high level nutritional and antioxidant activities of microgreens make them an emerging novel functional food (Kyriacou et al., 2016). Many research

studies reported that microgreens possess higher nutrient value than their mature counterparts (Xiao et al., 2012; Yadav et al., 2018; El-Nakhel et al., 2020; Singh et al., 2022). Their elevated phytonutrients and antioxidant compounds show good anticancer and anti-inflammatory properties (Kopsell et al., 2014; Moreira-Rodríguez et al., 2017; Ghooora et al., 2020).

The microgreens growth phase can last anywhere between 7 and 28 days from emergence, depending on the growing method, growing condition (temperature, humidity, and photoperiods), and genotype. They are harvested when the cotyledons are completely developed and still firm, which occurs after the appearance of tiny pairs of first true leaves. The length of microgreens varies by species between 10 and 20 cm (Di Gioia et al., 2016; Treadwell et al., 2020; Xiao et al., 2012). Moreover, there is growing interest in their commercial production due to the growth of the urban agricultural sector.

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Microgreens: Cultivation practices, bioactive potential, health benefits, and opportunities for its utilization as value-added food

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ABSTRACT

In recent years, changes in diet patterns and preferences for fresh food commodities, ready-to-eat food products, functional foods, and nutraceuticals have increased. Microgreens (young vegetable greens) are a relatively new form of product that has gained popularity and is also referred to as 'vegetable confetti'. It is used to improve the sensorial characteristics, viz. appearance and flavor of foods such as salads and main dishes. Compared with seeds and their mature counterparts, microgreens contain greater levels of functional nutrients (minerals, vitamins, antioxidants, and phenolic compounds). This comprehensive review briefly describes the different families of microgreens used for the cultivation of microscale products and highlights their health-promoting bioactive compounds, such as antioxidants, phenolics, pigments, minerals, and vitamins, which are critically associated with the sustainable developmental goals of good health and welfare. In addition to these important factors affecting the cultivation of microgreens, such as their species, type, growth medium, use of nutrients, bio-fortification, and use of advanced illumination systems, preharvest and postharvest factors affecting microgreens are also addressed.

1. Introduction

Over the last few decades, microgreens have gradually become popular as new forms of specialized vegetables and functional foods. They are very sensitive and immature leafy greens harvested from the first two pairs of leaves and marketed as a whole, including the leaves, stems, and cotyledons. Their cultivation started in the late 1980s, and their interest has widely increased due to their nutritional value (Treadwell et al., 2010). They are a relatively new form of product that has gained increasing popularity and are frequently referred to as the 'vegetable confetti'. In addition to their potential health benefits, microgreen cultivation has different advantages, including ease of cultivation, a short cultivation duration, flexibility for indoor farming, and a high level of income for cultivars with an approximate price of \$30–50 per pound. It is more sustainable than mature crops (herbs and vegetables) because it requires a lower footprint of fertilizers, water, and space for cultivation (Kyriacou et al., 2020). Microgreens are young immature vegetables harvested 7–21 days after germination and are typically 1–3 inches tall. They consist of a stem, cotyledonary leaves,

and true leaves. Unlike sprouts, which include seeds and roots, microgreens are consumed without roots. They present delicate textures and distinct flavors and are richer in minerals and micronutrients than fully-grown plants. The primary distinctions between microgreens, baby greens, sprouts, and vegetables are shown in Fig. 1.

For the cultivation of microgreens, different species of seeds are used, such as vegetables, herbs, grains, cereals, legumes, and beans (Ebert, 2022). A wide range of growing methods are employed, including indoor, outdoor, and regulated environments such as greenhouses and creating systems (growing systems) that include soil and soilless (Di Gioia & Santamaria, 2015). Currently, illumination systems are used for the cultivation of microgreens, but these systems have not been systematically studied to enhance nutrients and bioactive compounds in detail (Meas et al., 2020; Xiao, Lester, et al., 2014). The selection of appropriate species, growth methods, types of substrates, seed quality and purity, sowing methods and germination processes, irrigation practices, fertilizers used, harvesting methods, phytosanitary qualities, and postharvest storage conditions are the most important factors for the commercialization and home-growth cultivation of

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OPEN Assessment of bioactive compounds, antioxidant properties and morphological parameters in selected microgreens cultivated in soilless media

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The study investigated the effect of soilless media (burlap), on the morphological traits and antioxidant activities of microgreens from Brassicaceae, Amaranthaceae, and Linaceae families. The results revealed significant variations were observed in the selected morphological, biochemical composition, and antioxidant capacity of the microgreens. The radish sango and microgreens showed superior morphological characteristics compared to other microgreens. The elemental composition analysis revealed consistent moisture, ash, fat, fiber, and protein content across all families. The results revealed significant variations in the biochemical composition and antioxidant capacity of the microgreens, depending on the growing medium and between microgreens. Notably, microgreens differed in photosynthetic pigment profiles, with flaxseed and cabbage showing the highest chlorophyll content of 26.59 to 27.18 µg/g, FW and carotenoid content in a range of 3.74 to 6.39 µg/g, FW was observed in microgreens. The radish sango and beetroot microgreens exhibited elevated anthocyanin levels of 27.94–28.25 µmol/100 g, FW. Biochemical analysis indicated varying levels of ascorbic acid (177.58 to 256.46 mg/100 g, FW), total glucosinolate content (4.09 to 47.38 µmol/g, FW), phenolic content (131.44 to 298.56 mg GAE/100 g, FW), and flavonoid content (10.94 to 18.14 mg QUE/100 g, FW) were observed in selected microgreens families. Radish sango microgreens demonstrated the highest DPPH (76.82%, FW) and ABTS (88.49%, FW) radical scavenging activities, indicating superior antioxidant potential. The study showed that Brassicaceae microgreens are particularly rich in bioactive and antioxidant properties. Additionally, studies could assess the economic feasibility and scalability of soilless cultivation methods for microgreens to support their inclusion in sustainable agricultural practices and health-promoting diets.

The practice of cultivating various types of vegetable crops without soilless cultivation (comprising hydroponic and substrate-based) growing method, has become a practical alternative to conventional agriculture, especially in areas where soil quality is poor or there's limited arable land¹. This approach offers more benefits over soil based cultivation, including its help to enhance yields, optimize nutrient utilization, extend harvesting periods, and decrease vulnerability to soil-borne pathogens. Furthermore, the increasing impacts of climate change on agriculture farming practices and the diminishing availability of freshwater reserves emphasize the critical need to adopt effective farming techniques like hydroponic systems. These systems have the potential to mitigate the difficulties arising from water scarcity in agriculture, as evidenced by several recent studies^{1–3}.

Microgreens, which are immature versions of leafy vegetables, are gaining attention in the culinary world due to their distinctive flavors and rich nutrient profiles⁴. They are identified by their early developmental stage, usually showcasing two developed cotyledon leaves and the beginnings of the first set of true leaves^{5,6}. Commercial

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Process optimization for microgreens-based dairy (lassi) beverage: Consumer acceptability, bioactive composition, and storage life using kinetic modelling

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ABSTRACT

This study focuses on the development and optimization of a microgreens-based dairy beverage (lassi) using response surface methodology, aiming to enhance its bioactive composition, sensory acceptability, and storage stability. The optimal formulation, determined as 70% dahi and 2.5% microgreens powder with a desirability score of 0.818, significantly enriched the lassi with phenolics (114.00 mg GAE/100g), flavonoids (67.77 mg QUE/100g), anthocyanins (48.23 $\mu\text{mol}/100\text{g}$), and ascorbic acid (152.77 mg/100g), achieving an overall acceptability score of 8.60. Kinetic modeling evaluated the stability of bioactive compounds under storage conditions (5 °C, 15 °C, and 25 °C). At 5 °C, the highest retention of bioactive compounds, lower total plate count (5.69 log cfu/mL), and yeast and mold count (4.69 log cfu/mL) were observed after 15 days. The Arrhenius and Eyring models were applied to analyze temperature-dependent reaction rates, and the zero-order reaction model was combined with the Arrhenius model to predict shelf life. Shelf-life predictions at 5 °C were 21.875 days for overall acceptability, 12.696 days for acidity, 21.875 days for free fatty acids, 10.576 days for thiobarbituric acid, and 11.403 days for total plate count. The optimized lassi was found economically viable, with a production cost of ₹36.10 per 200 mL, aligning with market prices. These findings underscore the potential of radish sango microgreens for creating nutritionally superior, antioxidant-rich dairy beverages with broad commercial and health appeal.

1. Introduction

The growing interest in functional foods and beverages is driven by increasing consumer awareness of the link between diet and health. Dairy products, especially fermented beverages like Mishti Doi, Shrikhand, Buttermilk, and Lassi, are widely recognized for their probiotic benefits and adaptability for fortification with bioactive compounds (Hussain et al., 2015). Functional ingredients are commonly incorporated into traditional dairy products for their nutritional, organoleptic, and functional properties (Shiby et al., 2017). Fermentation enhances the nutritional value and bioavailability of nutrients, with lactic acid bacteria effectively eliminating toxic and anti-nutritional compounds in various food products (Kumar et al., 2023).

Microgreens are known for their high concentration of bioactive components, surpassing their mature counterparts in nutrients. They are young, nutrient-dense seedlings of vegetables and herbs and are an exceptional source of bioactive compounds, including phenolics, flavonoids, vitamins, and antioxidants. Despite their well-established nutritional advantages, the potential of microgreens in dairy-based functional beverages remains largely unexplored. Incorporating microgreens into dairy products offers a novel approach to creating functional beverages with enhanced nutritional and sensory attributes (Singh et al., 2024a). Recently, microgreens in forms such as juice and powder have been utilized in innovative food products like beverages (Sharma et al., 2020, 2021), seasonings (Devi et al., 2023; Abraham and Vijayan, 2022), probiotic-infused products (Mishra et al., 2024), yogurt (Gunjal

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Effect of convective drying temperature and tray load density on bioactive compounds, antioxidant properties, and functional quality of radish Sango microgreens

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ABSTRACT

This study examined the effects of convective drying parameters, including temperature (40, 50, and 60 °C) and tray loading density (0.057 and 0.113 g/cm²), on the bioactive compounds, antioxidant properties, and structural characteristics of radish sango microgreens powder. Drying at 50 °C with 0.057 g/cm² preserved the highest anthocyanins (43.23 μmol/100 g), phenolics (189.45 mg GAE/100 g), and antioxidant activities (DPPH 43.13 %, and FRAP 10.80 μmol TE/g), while drying at 50 °C with 0.113 g/cm² preserved the highest flavonoids (5.86 mg QUE/100 g) and ascorbic acid (239.18 mg/100 g) of radish sango microgreens. Degradation kinetics followed a first-order model, with thermal sensitivity varying among compounds. FTIR analysis revealed functional groups linked to bioactive compounds, while SEM showed flake-like particles enhancing solvent interaction. These findings highlight the significance of controlled drying in retaining functional and structural qualities, supporting the potential of radish sango microgreens in functional food and pharmaceuticals applications.

1. Introduction

Microgreens are young edible plants harvested at the cotyledonary stage and have garnered significant attention in recent years due to their rich nutritional and functional properties. Among all the microgreens, radish sango microgreens (*Raphanus sativus* L. var. sango) are particularly valued for their vibrant purple pigmentation and high concentrations of various types of bioactive compounds including phenolics, anthocyanins, ascorbic acid, and glucosinolates (Mlinarić et al., 2023; Gunjal, Singh, Kaur, Nanda, et al., 2024). These compounds contribute to the antioxidant, anti-inflammatory, and anticancer properties of radish sango microgreens, making them a potential candidate for functional food applications (Singh et al., 2024). Their high nutrient levels and bioactive components make them ideal for incorporation into various food formulations. However, the processing and storage of these delicate greens pose considerable challenges due to their perishable nature and susceptibility to nutrient degradation (Kumar & Singh, 2024; Sanyukta et al., 2023).

To address these challenges, drying is a widely adopted preservation method used to extend the shelf life of microgreens while concentrating their bioactive components for diverse applications, including powders, extracts, and nutraceuticals (Sanyukta et al., 2023; Mansouri et al., 2024; Gunjal, Kaur, Rasane, Singh, et al., 2024). Among the various drying techniques, convective drying is widely used due to its simplicity, cost-effectiveness, and scalability (Motegaonkar et al., 2024). This method involves the use of heated air to remove moisture, thereby reducing microbial activity and enzymatic degradation. However, the application of high temperatures during convective drying can lead to the degradation of thermolabile bioactive compounds, structural alterations, and changes in functional quality characteristics (Akbar et al., 2023; Khalangre, Mirza, Chavan, et al., 2024 & Khalangre, Mirza, Sharma, et al., 2024). These changes are influenced by critical factors such as drying temperature, air velocity, and tray loading density, which must be optimized to retain the nutritional and functional quality of the final product (Akbar et al., 2023; Chakraborty et al., 2023).

The degradation kinetics of bioactive compounds during drying have

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Novel approach for development and optimization of microgreens-based functional dairy beverage: Antioxidant enhancement, consumer acceptability, and kinetic shelf-life modeling

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ABSTRACT

This study optimizes a microgreens-based dairy (lassi) beverage using response surface methodology to enhance antioxidant composition, sensory attributes, and shelf stability. The ideal formulation (9.189 % microgreen juice, 60 % dahi, and 15.311 % water) achieved a desirability score of 0.781, significantly improving bioactive compounds. The optimized lassi contained phenolics (44.14 mg GAE/100 g), flavonoids (22.62 mg QUE/100 g), anthocyanins (37.91 $\mu\text{mol}/100\text{ g}$), and ascorbic acid (59.32 mg/100 g), with an acceptability rating of 8.29. Kinetic modeling assessed bioactive stability under varying storage conditions, with the highest retention at 5 °C after 15-days. Temperature-dependent degradation rates were analyzed using the Arrhenius, Eyring, and Ball models, while shelf-life prediction was based on a zero-order reaction model combined with the Arrhenius equation. The estimated shelf life at 5 °C based on microbial load was 9.33 days ($t_{1/2}$), with production cost, aligning with market trends. These findings highlight commercial potential of radish sango microgreens in developing a nutritious, antioxidant-rich lassi beverage.

1. Introduction

The rising interest in functional foods and beverages is driven by increasing consumer awareness of the relationship between diet and health. Dairy products, especially fermented beverages such as Mishti Doi, Shrikhand, Buttermilk, and Lassi, are highly valued for their probiotic benefits and potential for fortification with bioactive compounds (Hussain, Patil, Yadav, & Singh, 2014). Traditional dairy products are often enhanced with functional ingredients to improve nutritional, sensory, and functional properties (Rasane, Kailey, & Singh, 2017). Fermentation significantly enhances nutrient bioavailability by promoting the activity of lactic acid bacteria, which can remove toxic and anti-nutritional compounds (Kumar, Meena, Rai, & Duary, 2023).

Microgreens, recognized for their high bioactive content, provide greater nutritional benefits than their mature counterparts. These young vegetable and herb seedlings are rich in phenolics, flavonoids, vitamins, and antioxidants, making them ideal for incorporation into functional dairy beverages (Singh et al., 2024). Recent studies have explored microgreens in various food applications, including beverages (Sharma

et al., 2020; Sharma et al., 2021), seasonings, probiotic-infused products, yogurt, and bakery items (Gunjal et al., 2024b). Integrating microgreens into lassi enhances its nutritional profile while catering to the increasing demand for plant-based functional foods (Raje-Nimbal-kar, Patil, Raskar, Kadav, & Joshi, 2023). However, optimizing microgreens-based dairy (lassi) requires balancing sensory attributes, nutritional quality, and storage stability.

Fermentation plays a key role in improving the functional properties of dairy products (Zhi et al., 2017). The metabolic activity of lactic acid bacteria, including *Lactobacillus*, *Streptococcus*, *Enterococcus*, and *Lactococcus*, along with probiotic strains such as *Bifidobacterium* and yeast species like *Saccharomyces* spp., enhances nutrient bioavailability and stabilizes bioactive compounds (Shah, Mokashe, & Mishra, 2016). Additionally, fermentation contributes to desirable sensory characteristics, such as texture and flavor, which are crucial for consumer acceptance (Ścibisz, Ziarno, & Mitek, 2019). The incorporation of radish sango microgreens into the fermentation process presents challenges, including maintaining bioactive compound stability and minimizing potential off-flavors (Singh et al., 2024).

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ORIGINAL ARTICLE

Postharvest Technologies Enhance Quality, Antioxidant Activity, Microbial Control, and Shelf-Life of Radish Sango Microgreens

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ABSTRACT

This study examines the effects of postharvest techniques, including washing treatments, packaging materials, and storage temperatures, on the quality, antioxidant properties, microbial load, and shelf life of radish sango microgreens stored at 5°C and 25°C. The washing treatments comprised citric acid, sodium hypochlorite, ethanol, calcium chloride, acetic acid, and distilled water, with unwashed samples serving as a control. Various packaging materials, including low-density polyethylene (LDPE), high-density polyethylene (HDPE), metalized polyester (MP), and polystyrene trays (PT), were assessed. Key quality parameters such as antioxidant activity (DPPH and FRAP), ascorbic acid, glucosinolate, anthocyanin, total phenols, flavonoids, pigments, and microbial load were analyzed. Microgreens stored at 5°C in MP packaging combined with calcium chloride, citric acid, or sodium hypochlorite exhibited reduced microbial proliferation and retained higher antioxidant levels. Conversely, storage at 25°C accelerated quality deterioration and increased microbial contamination, especially in PT packaging. Principal component analysis (PCA) indicated strong associations between antioxidant activity, bioactive compounds, and 5°C storage. The findings suggest optimal postharvest approaches to enhance the shelf life and commercial viability of microgreens.

1 | Introduction

Microgreens, the young seedlings of edible vegetables and herbs, have gained considerable attention in recent years due to their exceptional nutrient density, distinct flavors, and visually appealing appearance. These nutrient-dense young greens are recognized for their higher concentrations of vitamins, minerals, antioxidants, and phytochemicals compared to their mature counterparts (Singh, Kaur, et al. 2024; Singh, Singh, et al. 2024). Among various microgreens, radish sango (*Raphanus sativus* var. *longipinnatus*), a member of the Brassicaceae family, stands out due to its rich antioxidant profile and potential health-promoting effects (Gunjal, Singh, Kaur, Kaur, Nanda, Sharma, et al. 2024). These microgreens contain a unique combination of

nutrients and bioactive compounds, such as glucosinolates, anthocyanins, and vitamins, making them an appealing option for both consumers and researchers. Their anti-inflammatory, anticancer, and cardioprotective properties further increase their value as a functional food source (Patil et al. 2023).

Fresh produce, including microgreens, is highly sensitive to various environmental factors that can affect their quality, nutrient content, and shelf life (Sharma et al. 2023). Factors such as preharvest conditions, postharvest treatments, storage environments, and packaging play a crucial role in determining the final quality of microgreens (Dalal et al. 2020). Proper handling and storage strategies are essential for extending shelf life and maximizing the retention of health-promoting compounds

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LIST OF CONFERENCES

Sr. No.	Name of the conference	Organized by	Title of oral/poster presentation	Date of conference
1	Global Indian Young Scientists Research and Innovation Conference	Global Indian Scientists & Technocrats Forum (GIS	Nutritional and biochemical characterization of selected microgreens are grown in two different growing mediums	31/05/2023
2	Recent trends in Smart and Sustainable Agriculture for Food Security (SSAFS-2022)	School of Agriculture, Lovely Professional University, Punjab.	Evaluating the Physical Properties of Selected Varieties of Microgreens	21/01/2022



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This is to certify that **Dr./Mr./Ms. Mahendra Gunjal** of **Lovely Professional University, Jalandhar** has participated in Oral presentation on the topic entitled **Evaluating the Physical Properties of Selected Varieties of Microgreens** in the International Conference on **"Recent trends in Smart and Sustainable Agriculture for Food Security"** (SSAFS-2022) held from 21-22 January 2022, organized by School of Agriculture, Lovely Professional University, Punjab.

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