CHARACTERIZATION, FUNCTIONALITY AND UTILIZATION OF RADISH LEAVES FOR ITS VALUE ADDITION

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By

Jasleen Kaur

Registration Number: 41800519

Supervised by.
Dr. Prerna Gupta (19657)
Department of Food Technology and Nutrition (Assistant Professor)
Lovely Professional University

Co-Supervised by
Dr. Poorva Sharma (19600)
Department of Food Technology and Nutrition (Assistant Professor)
Lovely Professional University

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Signature:

Research Supervisor : Dr. Prerna Sharma

Affiliation : Department of Food Technology and Nutrition, Lovely Professional University, Phagwara, Punjab.

Date: 4.7.22

Signature:

Research Co – Supervisor : Dr. Poorva Sharma

Affiliation : Department of Food Technologyand Nutrition, Lovely Professional University, Phagwara, Punjab.

Date:4.7.22

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(Jasleen Kaur)

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ABSTRACT

Research on waste valorization and utilization of food by-products are increasing day-by-day. This trend not only protect the environment from pollution but also waste and by-products released from food industries. In this study, optimization of harvesting stage and storage conditions of radish leaves has been done by using response surface methodology. Different packaging materials such as paper, cling film and LDPE (low density polyethylene) were tried for storage. Tray drying of radish leaves was investigated at temperatures of 45°C, 55°C and 65°C.Twelve different models namely Newton, Page, Modified page model, Henderson and Pabis, Modified Henderson and Pabis, Logarithmic, Singh model, Midillii model, Two term, Two term exponential and Wang and Singh models were fitted to experimental drying data. The obtained dried leaves, 55°C were crushed manually prior to grinding to powder using a mixer grinder. The particle size distribution was further analysed by sieving through the sieve shaker which contains sieves of different mesh sizes (24 BSS, 36 BSS, 56 BSS, 72 BSS, 100 BSS, 200 BSS, 300 BSS). The dried radish leaves were mixed with the spice mix in different treatment combinations of chutney and soup mix and then stored into different packaging materials (aluminium pouches, PET containers and LDPE pouches) over the period of six months under ambient conditions. It was further analysed for its physico-chemical and functional properties.Maximum quality retention in terms of moisture content (94.44 %), ascorbic acid (35.37 mg/100g), DPPH scavenging activity (45.32 %), total phenols (684.025 mgGAE/100gm), total flavonoids (1041.066 mg quercetin/100gm) and chlorophyll (48.795 mg/100gm) was observed with 8.55 cm length of leaves and 4°Cstorage temperature on 24 hours. The applied model was found suitable for current study with 94% of anticipated values. The highest R^2 with the lowest Chi-square and root mean square error (RMSE) were selected as statistical criteria to evaluate how well the tested models fit the drying data. Moisture diffusivity values were varied from 3.74×10-11 to 6.28×10-11 m2/s. The temperature dependent activation energy (E_a) was determined as 23.043 kJ/mol . The fine powder fraction obtained from 300BSS was observed best in terms of higher water-binding characteristic which enhances the quality of powders. The optimized powder were formulated into soup and chutney mix , the formulation scored best based on nine point hedonic scaling were then packed into aluminiumpouch, LDPE pouch and PET containers. The aluminium pouch packed product gave best results as analysed on the basis their functional properties wherein the minimum decrease in pH and increase in acidity was observed from (3.63±0.02to 2.81±0.04) and (1.02±0.02% to 2.68±0.03 %) respectively, minimum increase in TSS was reported from (52.31±0.02 to 52.89±0.01) .Storage had a significant effect on viscosity of chutney mix where the values varied between (811 ±3.535 to 737 ± 7.071 poise).Bulk density of chutney mix in aluminium pouches ranged (0.455±0.005 to 0.541±0.007 g/cm^3), minimum increase in true density ranging from (1.102±0.002 to1.239±0.004), maximum decrease in porosity (0.591 ± 0.001 to 0.558±0.009) as compared to LDPE pouches and PET containers. Similarly, for the soup mix aluminum pouches reported minimum increase in TSS (5.05± 0.01 to 5.28 ± 0.06^o Brix), minimum decrease in pH (5.92 ± 0.02 to 5.73 ± 0.07) , minimum increase in acidity (2.81 ± 0.04 % to 3.12 ± 0.05 %). However, decline in viscosity is reported in all packages (8.55 ± 0.03 to 7.89 ± 0.05 poise). The bulk density reported in soup mix stored in aluminium pouches ranges (0.755 ± 0.003 to 0.809 ± 0.004) , minimum increase in true density and maximum decrease in porosity was reported for soup mix stored in aluminium pouches which was in the range of (1.425 ± 0.004 to 1.517 ± 0.001) and (0.475±0.003to 0.461±0.008) respectively.

Keywords: DPPH scavenging activity ;LDPE (low density polyethylene); Midillii model;response surface methodology;root mean square error (RMSE)

CHAPTER 1 : INTRODUCTION

The impulsive boom in the growing population is underlining the fame of foods like fruits and vegetables and this trend is probably going to go on later on Wadhwa et al., (2016). This consequently is determining the expanded off shoots and waste materials to different uses. Green parts of numerous produces are palatable as well as scrumptious. Radish, beets, turnips, and carrots are much of the time accessible in the grocery stores without any leaf which is a humiliation.

Radish (*Raphanus sativus*) is a popular eatable root product that is grown and enjoyed all over the world due to its potential nutritional value and potential health benefits.It’s for sure stacked with supplements as well asbeneficial biochemical compounds. It’s a flexible produce indigenously used for improving different diseases, for example, cough, anticancer, gastrointestinal inconvenience, liver issues, constipation, dyspepsia, gallbladder issues, joint inflammation, gall and kidney stones, etc. (Singh et al.,2013). The harvest's root section is typically consumed raw, cooked, or cured, as well as in beverage form, but consumption of the green portion in raw or cooked form is less common (Banihani et al., 2017). Radish leaves postulates good amount of nutrients making it a power pack thereby palliates ample of diseases and hence improves various bodily functions. According to the findings by Goyeneche et al. 2018), radish leaves have moderate levels of carbohydrates, between 4040 and 5835 mg/100 g fresh weight (FW), and crude fibre, between 610 and 2578 mg/100 g FW.The leaves accounted for more roughage which ease out in digestion and reduces the problems of constipation and bloating. Radish leaves are likewise a decent protein source, around 22 types of proteins have recognized and about 0.002% of essential oils were found (Afsharypuor et al.,2005). 3810 mg/100 g FW of protein was discovered to be present in the leaves.The greens of the plant are laden with abundance of vitamins like thiamine, niacin, riboflavin, pantothenic acid, folates, Vitamin B6, C, K and E which is good for the essential working of the human body (Prasad et al.,2016). With a value of 752.64 mg/100 g FW, calcium was determined to be the mineral with the highest concentration.Other minerals reported in the leaves were Cu, Fe, Mn, Zn, Mg, Na, K with a concentration of 0.11 ± 0.00, 3.74 ± 0.03, 0.50 ± 0.03, 0.39 ± 0.01, 57.04 ± 0.46, 298.58 ± 2.56, 495.31 ± 0.49 g/100 g respectively. All things considered, these green portions are delicate, have a very limited shelf life (1-2 days) at room temperature, and are deemed to be extremely perishable goods .

Storage temperature, length of leaves and capacity term are a few significant variables which influence the nature of end product. Ideal temperature of storage conditions changes relying on fruit or vegetables. For a few refrigerated delicate foods grown from the ground, the utilization of low temperature stockpiling unfavorably influences quality credits and causes the deterioration all the more quickly (Xiao et al., 2014). Subsequently, the choice of ideal temperature for storing is critical. Supplement content of baby radish green leaf get adjusted with leaf length; thus, approximate length determination is of most extreme significance. To keep up with the extended shelf life, Modified Atmospheric Packaging (MAP) is a successful innovation. This strategy is effectively used in new and minimally processed vegetables, like spinach (*Spinacia oleracea L.*), lettuce (*Lactuca sativa L.*), broccoli (*Brassica oleracea L.* cv. Acadi), and mushrooms (*Agaricus bisporus* cv. U3 Sylvan 381) (Sandhya et al., 2010). Sharangi et al., (2015) have studied the effect of different packaging materials(brown papers, news paper, laminated papers, A4 poly pack , A5 poly pack , box with leaf cushioning , control) on freshly harvested coriander leaves under both room and refrigerated conditions. They reported the stability of 10 days for leaves packed in A5 poly pack and laminated paper under both roomand refrigerated conditions.The temperature and time of storage are examined in this evaluation in order to maintain the quality of young radish greens.Additionally, impact of various packaging materials is contemplated to keep up with quality and enhancing the shelf-life of baby radish greens.Changing mindsets from the basic food habits and shifting towards nutraceuticals as an alternative to medicines and treatments for combating diseases is the modern approach of the people nowadays. This paradigm shift has arisen from the frustrations of lack of medical aids and expensive treatments associated with chronic diseases; Patwardhan et al., (2015). Radish leaves revealed bioactivities with variety of health benefits which presently supports its use for protection against the degenerative diseases Chihoub et al., (2019). So, there is a need to utilize the full potential of these unconventional bio-resources and converting them into valorised products which is an important current research area nowadays. The major limitations in using these bio-resources is their high moisture content which makes its perishable and hence promotes the spoilage. Therefore, different processes or combination of those processes had been tried. Drying is one of the best and suitable process to enhance the shelf life of the bio-waste. The principle objective of drying is to minimize the water activity to control the growth of microbes, permitting retention of quality of the products during long periods of storage and facilitate the usage of leaves, which can contribute to the supply, availability, and demand in the market because of ease in transport and storage (Coradi et al., 2014).There are various methods available for drying such as freeze drying, vacuum drying and tray drying.The high processing cost of freeze drying and vacuum drying limits its application. Therefore in this study,tray drying has been used for the drying of radish leaves. Though, loss of color, flavor, nutritional components and taste is the major challenge associated with this method. Therefore, drying kinetics has been applied to optimize the drying conditions for the maximum retention of nutrients.In a tray dryer, drying was conducted at temperatures of 45 °C, 55 °C, and 65 °C with constant air velocity of 0.5 m/s; the drying times for the leaves were 12 h, 10 h, and 8 h, respectively. Twelve distinct drying numerical models, including the Henderson and Pabis, Page, Logarithmic and Newton (Lewis), Modified Page, Singh, and Middlli models, were used to fit the moisture information. Measurable analysis predicted that the page model would be the best representation of the drying characteristics of radish leaves.Most suitable model was chosen by acquiring the greatest worth of relapse coefficient (R^2) and least worth of Root Mean Square Error (RSME) and chi square (χ^2).

Dehydrated green tops are potential source of bioactive compounds and micronutrients and, thus, can be used to enhance traditional recipes as well as commercial convenience foods (Singh et al., 2014). Foods like instant soups and chutney mix are bracketed in the category of leading dried foods. Instant soup mixes are a composite of several substances like starch, salt, spices, flavors, and flavor enhancers whereas chutney mix is a blend of mint leaves, coriander leaves and spices. Instant soup and chutney mixes gained popularity among all dried and dehydrated products as they are easily accessible in preparation and are considered as universally comfort foods. Soups are consumed for their nutritional advantages, and by patients whose solid intake is decreased because of different physiological circumstances. Soup formulations can be done as nutritionally balanced by integrating whole grains, vegetables, and legumes. Instant soup blends can be utilized as an alternative nourishment product for breakfast as they can satisfy the needs of energy and nutrients expected by the body (Sunyoto and Futiawati, 2012). Being a liquid food, a decent soup powder ought to be cooked in minimum time, holding nutrients and tastefulness practically like newly cooked items (Abeysinghe and Illeperuma, 2006). Dried soup blends enjoy a benefit of insurance from oxidative and enzymatic deterioration and flavor stability for extended periods as long as one year at room temperature (Wakeel et al., 2007). They don't require refrigeration; apply lightweight for transportation and accessible overall around the year (Rekha et al.,2010). Functional ingredients can be integrated into soup powder to give health advantages.

Radish greens being a hub of vitamins, micronutrients, and other phytochemicals such as glucosinolate, catalase, caffeic acid, pelargonidin, and others offers them with a distinctive nutraceutical status with therapeutic benefits. The leafy greens exhibits potential as fortified food with enhanced nutritional quality and therefore can be substituted into existing products in the formulation of health foods. The leaves can be utilized into soup mix as per the study conducted by (Sudarshan et al., 2017) where formulation of instant soup mix was done using sprouted horse gram(25g) and radish leaves (6-12.5g) which was later analysed for its sensory and microbial properties. Similarly study conducted by (Joshi et al., 2020) stated that incorporation of radish leaf powder for instant soup mix preparation enhanced the antioxidant activity by 5 % which further helped in stabilizing the soup for upto the period of two months.Synthesizing these insights helps to encourage the consumption ofunconventional greens in the preparation of novel food products. The unconventional greens can be converted to powders which can be further used to design soup mixes and chutney powders with sufficient health- promoting properties.

Objectives of the study:

The present study has been planned with the following objectives:

- To optimize the harvesting stage and storage conditions for fresh radish leaves.
- To standardize drying conditions for radish leaves.
- To prepare and evaluate radish leaves based health food.
- To study the effect of storage conditions on storage stability of developed products

CHAPTER 2: REVIEW OF LITERATURE

Horticultural produce primarily, fruits and vegetables are majorly grown crops that offer critical nutrients which are of high value to human health. (Sagar et al., 2018). The constant growth in the world's population emphasises the growing demand for produce output, and this trend is anticipated to continue in the next years; Wadhwa et al.,(2016) .Thus, the increasing levels of waste and losses are derived in response. Major causes resulting in waste generation are : a) fruits and vegetables contain indigestible parts that must be removed (peels, seeds, roots, skins, kernels, shells, leaves etc.) raw fruit and vegetables are liable to spoilage before consumption; and iii) because FVWs are economical / affordable than meat and fish, buyers are negligent about spoilage (Sagar et al., 2018). The green tops (regarded as waste) of several veggies are generally not just tasty but also edible. It furnishes discomfiture that radishes, beets, turnips, and carrots are frequently sold in markets without any leaves. Despite the origin, these underspent residual biomasses are potential bioresource of health giving components, comprising phenolic substances, carotenoid, dietetic fiber and polyunsaturated fatty acids, etc. This accession compromises innovative approach for efficient utilization of worldwide, local raw materials/byproducts. The obtained by-products are opulent in carotenoids, apposite for producing functional foods and thus helping reduce food loss and provide novice solutions to food industries and primary producers, who find market niches to sell their by products.

According to phylogenetic studies of the Brassicaceae family, radish (*Raphanus sativus L.*) is an annual or biennial plant related to the genus Raphanus and included in the Rapa/Oleacera lineage (Nishio, 2017). It is a major eatable root crop that is widely grown and consumed because to its favourable nutritional profile and advantageous effects on human health. *Raphanus* was initially originated in Europe and Asia. It is 30-90 cm in height and its root varies in size as well (Gutiérrez et al., 2004). Though it can withstand extreme temperatures, the ideal temperature for the legitimate growth and development of roots in radish is 10-15.5^{o}C (Kaneko and Matsuzawa 1993). In the northern plains of India, it is recommended for late sowing particularly from September to March, whereas in the mountaneous region it is cultivated from March to July. The flora is ready for harvesting in 30-50 days after cultivating and withdrawn of the soil when it reaches its acceptable esculent size

(Singh and Singh 2013). The crop's root is typically consumed raw, cooked, pickled, or in juice form, whereas the leaf is primarily consumed cooked or raw as a vegetable. (Banihani, 2017). The crop is found in various skin colors such as red, purple, black, yellow, and white but the flesh is always white. The naturally occurring pigments anthocyanin are responsible for the red colour of the roots, whilst volatile isothiocyanates, which are common in nations like Japan, the Philippines, and Hawai'i, are responsible for the distinctive bitter flavour and taste (Gupta et al., 2003 ; Nishio, 2017).Generally, the enlarged roots aremainly consumed, but leaves and buds could also be contemplated as a part of a healthy diet.

Among the common vegetables, radish is one of the richest sources of iron, calcium, and metallic element, sodium. Radish leaves have additional calcium, phosphorus, ascorbic acid, and protein than the roots. *Raphanus sativus* is a hub of several phytochemicals such as glucosinolate, catalase, caffeic acid, pelargonidin, and others. Glucosinolate are the most abundant phytochemicals present in radish which acts as the shield to fungal diseases and pest infestation, metabolism of sulfur and nitrogen, regulation of growth in the plant (Ludwig-Müller et al., 2000). It is also a well-known vegetable that is frequently used in homes to cure a wide range of conditions, including cough, cancer, gastrointestinal pain, liver problems, constipation, dyspepsia, problems with the gallbladder, arthritis, gallstones, kidney stones, etc (Singh and Singh 2013). Black radish juice has laxativeand tonic action on viscous and indirectly enhances the flow of digestive fluid (Manivannan et al., 2019). Hence, the leafy part of the radish is an fantastic resource of biologically active compounds with free radical eradicating potential for treating various ailmentssuch as hypertension, cardiometabolic disorders which is very benficial for investigators and the pharmacological companies (Lim et al., 2012 ; Manivannan et al., 2019). Keeping in mind, the present review has been aimed to explore the *Raphanus sativus* in terms of phytochemistry, traditional uses, and health benefits.

2.1 Nutritional composition of radish

Radish is one of the potent sources of essential nutrients and unique bioactive compounds which are profoundly beneficial to human health and fight against various diseases (Banihani, 2017). Quantitative and qualitative information on the proximate composition of radish was disclosed by numerous investigations. Radish roots and leaves both include a variety of water-soluble vitamins, including complex vitamin B (B1, B2, B3, B6, and B9), vitamin C, as well as minerals like potassium, phosphorus, magnesium, calcium, and iron that serve as a fundamental dietary supplement for the body's maintenance.

2.2 Radish leaves

Radish leaves possess moderate levels of carbohydrates ranging between 4040 - 5835 mg/100g F.W. and the crude fiber ranging between 610 -2578 mg/100g F.W. (Goyeneche et al., (2015) and Ankita and Prasad et al., (2015)). The leaves accounted for more roughage which ease out in digestion and reduces the problems of constipation and bloating. Leaves of radish are also anample protein source, with approximately 22 different types of proteins identified and approximately 0.002 percent essential oils found (Afsharypuor and Balam, 2005). Higher protein concentrations presented by the leaves than roots. i.e. 3810 mg/100g F.W. (Goyeneche et al., 2015). The greens of the plant are laden with abundance of vitamins like thiamine, niacin, riboflavin, pantothenic acid, folates, Vitamin B, 6, C,K and E which aids in bodies proper functioning (Prasad , 2016). Themineral that present in ample amount was calcium with a value of 752.64 mg/100g F.W. The other minerals reported in the leaves were Cu, Fe, Mn, Zn, Mg, Na, K was found to be 0.11 ± 0.00, 3.74 ± 0.03, 0.50 ± 0.03, 0.39 ± 0.01, 57.04 ± 0.46, 298.58 ± 2.56, 495.31 ± 0.49 g/100g respectively. These nutrients making radish leaves a power pack thereby palliates ample of diseases and hence improves various bodily functions.

2.3 Radish roots

The reports indicated the carbohydrates and protein content in roots to be about 3030mg/100gm F.W. and 570 mg/100gm F.W. (Goyeneche et al., 2015). The radish roots have 0.57 ± 0.09, 0.07 ± 0.01, 0.77 ± 0.07, 0.32 ± 0.07 and 3.03 g per 100g of crude protein, lipid content, ash, crude fiber, and carbohydrates respectively. Lu et al., (2008) studied 42 radish cultivators and showed that total soluble sugar (TTS) present in radish were ranging from 2.233 to 15.457g kg-1, whereas the fiber which is crude was between 4.507 to 18.546 g kg^{-1}. The amount of various vitamins varied from 0.1416 to 0.3341 g kg^{-1}. Radish roots are the extravagant source of vitamins which are soluble in water like ascorbic acid, vitamin B complex especially vitamin B6, and riboflavin. Additionally, it also conations iron, potassium, calcium, phosphorous, and magnesium (Khattak, 2011; Sabishruthi et al., (2018). Copper, Iron, Manganese, Zinc, Calcium, Magnesium, Sodium, Potassium mineral content in radish roots was determined to be 0.05 0.00, 0.15 0.01, 0.07 0.01, 0.24 0.00, 147.87 1.31, 14.98 0.02, 104.95 0.18, and 380.11 0.87 g/100g, respectively (Goyeneche et al., 2015). They also contains some trace elements like fluorine, iodine, aluminum, manganese, barium, titanium, lithium, and silicon (Singh and Singh, 2013).

Table 2.1: Comparative analysis of nutritive composition of radish leaves and roots.

Composition		**Amount**	
		Leaves	**Root**
Macronutrients	Energy	-	-
	Moisture	90.3%	94 %
	Carbohydrates	4.04 g	3.03 g
	Fat	2.7 %	0.1 %
	Proteins	0.1 %	0.7 %
	Diatery fibers	0.9 %	0.8 %
Micronutrients	**Vitamin**		
	Vitamin A	Vitamin A	Vitamin A
	Vitamin C	Vitamin C	Vitamin C
	Vitamin B_6	Vitamin B_6	Vitamin B_6
	Vitamin K	Vitamin K	Vitamin K
	Thiamin	Thiamin	Thiamin
	Riboflavin	Riboflavin	Riboflavin
	Nicotinic acid	Nicotinic acid	Nicotinic acid
	Minerals	**Minerals**	**Minerals**
	Iron	Iron	Iron
	Phosphorous	Phosphorous	Phosphorous
	Sodium	-	10.7 mg
	Calcium	310 mg	50 mg
	Copper	0.11 mg	-
	Magnesium	-	10.2 mg
Bioactive compounds	Flavonoid	-	43.5 mg
	Total protein	-	6.5%
	Glucosinolates	-	59.69–163.91 mg
	Total sugar	-	19.6 g
	Total phenolic compound	695.07 mg	240 mg
	Carotin	3.955 mg	-

Source: Shyamala and Singh, 1987; Kaymak *et al*., 2010; Anon, 2011; Jing *et al*., 2012;Srivastava *et al*., 2013; Azam *et al*., 2013; Singh and Singh, 2013; Tsouvaltzis and Brecht, 2014; Karaca and Velioglu, 2014; Goyeneche*et al*., 2015

2.4 Bioactive compounds present in radish and their therapeutic property

Radish is a root vegetable which is grown as well as consumed all around the world, they are rich in various bioactive compounds, and by fusing of radish roots and leaves in our diet can provide daily nutrition and bioactive compounds present in them which will help to minimize the risk of lethal chronic diseases cancer, neurogenerative disease, cardiovascular disease (Rhodes, 1994; Manivannan et al., 2019). The most important bioactive compounds found in radish are flavonoids, polyphenols, glucosinolates like glucoraphanin, glucoraphanin, glucoerucin, glucoraphanin, glucobrassicin, 4-methioxyglucobrassicin, 4-hydroxyglucobrassicin, and neoglucobrassicin as well as isothiocyanates which includes sulforaphene, sulforaphane, and indole-3-carbinol, which makes it one of the important source of an antioxidant compound and therefore used in the treatment of various liver diseases like cancer, diabetic and other disease caused due to oxidative damage (Lugasi et al., 1998; Malik et al., 2010; Ishida et al., 2015; Baenas et al., 2016). Some compounds like vitamin C, flavonoids, polyphenol compounds (caffeoylmalic acid, p-coumaroylmalic acid, feruloylmalic acid) act as strong antioxidant agent as it helps in preclude the excessive generation of free radicals in the body and prevents cell damage as well as an alteration in the body (Brandi et al., 1984; Khattak, 2011; Luo et al., 2018) Among cruciferous plants radishes are distinct because they comprises glucosinolates, which acts as precursor compounds to isothiocyanates.

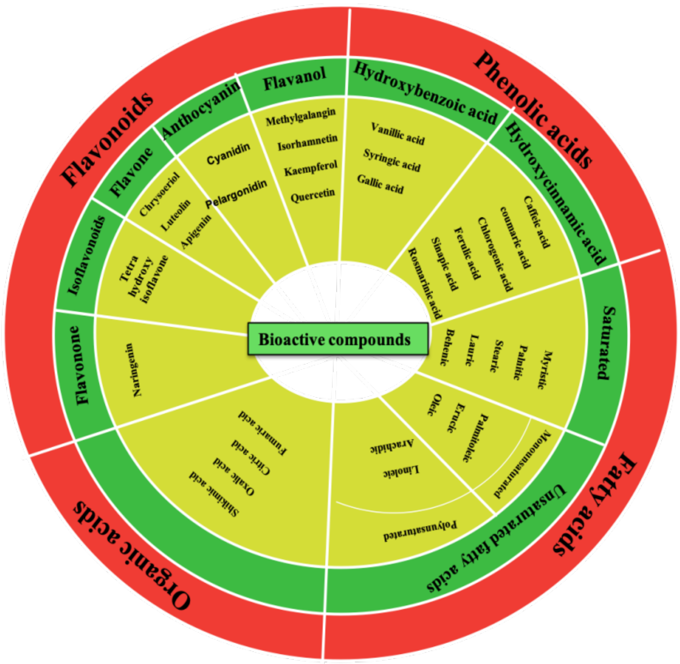

Fig:2.1 Bioactive compounds in radish leaves and roots

2.5 Radish leaves

The bioactive compounds discovered from alcoholic extract of radish leaves includes tannins, glycosides, resins, flavonoids, alkaloids, and saponin whereas coumarins and terpenes are the only two chemicals detected from watery extract. 6.41mg/g of myricetin, 4.88mg/g of catechin and 0.79 mg/g of quercetin is detected in the leaves of radish (Beevi et al.,2010). In radish leaves 6.3mg/100g of β- carotene and 2.4mg/100g of 3-hydroxy-β-ionone are identified under terpenes (Xiao et al.,2012). The flavonols kaemferol-3-O-rhamnoside and quercetin-3-O-rhamnosyl-galactoside make up roughly 35% and 34% of all flavonols in the leaf lamina, respectively (Khan et al., 2017 ; Jing et al.,2012).The most abundant anthocyanins in leaf lamina and leaf petiole is cyanindin accounts for about 54% and 100% respectively. Apigenin-7-O-neohesperidoside,Apigenin-7-O-rutinoside,Chrysoeriol-7-O-apiosyl-glucoside, Luteolin-7-O-glucoside are the flavones were also perceived in of radishleaves, whereas the flavanols discovered in radish leaves include Isorhamnetin-3-O-p-coumaroyl-caffeoylsophorotrioside-7-O-glucoside, Kaempferol-3-O-glucoside, Kaempferol-3-O-glucosyl-rhamnosyl-glucoside, Kaempferol-3-O-rutinoside, Kaempferol-3-O-xy (Singh et al.,2017).

Total phenolic component of radish leaves and roots high amount of total phenolic compound (TPC), but TPC of radish leaves were almost double than roots, whereas the flavonoid content which is the special class of phenolic compound was about four times higher in leaves than root (Goyeneche et al., 2015). Radish leaves have a total phenolic concentration of 125.3 mg GAE/g, according to Noman et al.,(2021) whereas their total flavonoid content is 44.5 mg QE/g. Other studies revealed that the total phenolic content of radish leaves was 29 mg/100g (Shesha et al., 2014) and 695 mgGAE/100g (Goyeneche et al., 2015).Various epidemiological evidence has shown that leaves being rich in glucosinolates is used in the treatment of different types of cancer like anticancer, antioxidant, and cardioprotective activity (Delahaut and Newenhouse, 1998; Lim and Kim, 2015). The IC50 value for the radish leaves in the DPPH assay was 216.8 g/mL, indicating that they had more anti-free radical action. Investigations were done by Chiad et al., (2018) evaluated that radish leaves extract has a stronger anti-cancerous activity as they showed an IC_{50} value of 217 ± 2.1, 287 ± 2.3, 224 ± 2.2 and 453.2 ± 1.9 in A549, HepG2, MCF-7and MDA-MB-231cell lines respectively. The spicy flavor of radish leaves and roots is predominantly owing to the occurrence of different compounds like myrosinase, glucosinolates, and isothiocyanates (Nakamura et al., 2008).

2.6 Radish roots

Radish roots were also found to be a significant source of polyphenolic compounds but lesser that its leaves. A good amount of sinapic acid (4.83 mg/g) and catechin (10.54 mg/g) was found in the of radish rootsextract of water (Khokhar and Magnusdottir, 2002.). major flavonoids present in radish roots are 6-prenyl-naringenin, a flavonoid is discovered in roots of radish While the flavonols found in roots are isorhamnetin-3-O-p-coumaryl-caffeoylsophorotrioside-7-O-malonyl-glucoside,kaempferol-3-O-caffeoyl-sophoroside-7-O glucoside, and kaempferol-3-O-feruloyl-sophoroside-7-Oglucoside (Khan.et al.,2017). The anthocyanins generated from roots include delphinidin-3-O-rutinoside, cyanidin-3-O-caffeoyl-p-coumaroyl-sophoroside-5-O-glucoside, and cyanidin-3-O-di-p-coumaroyl-sophoroside-5-Omalonylglucoside (Singh et al., 2017).According to quantitative analysis, when the values are expressed as mg/100 g F.W. squalene in radish marushka roots comes out on top (40.9 mg/100 g F.W.), with two carotenoids coming in at positions two and three, respectively (-carotene and lutein/zeaxanthin in the sprouts of the opal cultivar with 6.3 mg/100 g F.W. and 5.5 mg. Glucosinolates are present in good amount in red radish roots with total content of 163.1 mg/100g D.W. Main glucosinolates present in roots of radish are glucoraphenin, gluconapin, glucoerucin, glucoraphasatin, 4-Hydroxyglucobrassicin and 4-Methoxyglucobrassicin (S. Lim et al.,2016). When the data was labelled in percentage form for the concentration’s analysis; 4-(methylthio)butyl isothiocyanate (erucin) provided the highest concentration with 25.7 percent in white radish roots. The only compound that was studied, measured in mg/100 g D.W., was 4-(methyl-sulfinyl)butyl isothiocyanate (sulforaphane) (Im et al., 2010). It was found to have the highest concentration in roots in white radish (5.26 mg/100 g D.W.), but it was also found to have the highest concentration in leaves in red radish (4.21 mg/100 g)

The total phenolic content of radish roots is 95.8 of GAE/g, whereas the total flavonoid content is 24.4 mg of QE/g (Noman et al., 2021) Higher antioxidant activity was found in radish roots which was determined by ABTS (IC_{50} = 549 µg/mL) and DPPH assay (IC_{50} = 359.7 µg/mL). Total anthocyanin content present in roots is 160.7mg/100g. The anthocyanins generated from roots include delphinidin-3-O-rutinoside, cyanidin-3-O-caffeoyl-p-coumaroyl-sophoroside-5-O-glucoside, and cyanidin-3-O-di-p-coumaroyl-sophoroside-5-Omalonylglucoside (Singh et al.,2017). Red radish root has a total glucosinolate concentration (TGC) of 163.1 mg/100g D.W. (Jing et al., 2012).Other glucosinolates detected in roots are aliphatic, progoitrin, glucoraphenin, gluconapin, glucoerucin, glucoraphasatin, 4-Hydroxyglucobrassicin and 4-Methoxyglucobrassicin (Lim et al.,2016). 90% of cyanidins are found in root periderm whereas 65% in root xylem (Jing et al., 2012). Raphasatin and sulforapheneare the chief isothiocyanates found in radish roots (Kim et al.,2015).

2.7 Functional activities of Radish

Radish has been a part of the human diet for ages due to its rich and rare combination of phytochemicals (Beevi et al., 2009). The leaves are usually been disposed of but were found to have essential nutrients, bioactive compounds, and strong antioxidant property (Figure 3) (Beevi et al., 2010).Because of the presence of these biologically more active compounds like glucosinolates, flavonoids, and isothiocyanates in radish leaves, it gained a greater interest in pharmacological industries for designing anti-inflammation and anti-cancer drugs (Manivannan et al.,2019). In past, the extract of radish has been utilized to treat various diseases like stomach problems, cardiovascular diseases, urinary infection, hepatic inflammation, and an ulcer (Goyeneche et al., 2015).

2.8 Antioxidative and Antityrosinase activity

Numerous investigations, both *in vitro* and *in vivo*, have depicted that radish leaves extract have a effective antioxidant consequence, which is associated to glucosinolates, isothiocyanates, vitamin C, etc., (Lim et al., 2015; Baenas et al., 2016). Radish leaves have a strong ability to scavenge reactive oxygen species (Goyeneche et al., 2015; Goyeneche et al., 2018; Agarwal & Varma, 2014; Beevi et al., 2010) ; (Bischoff, 2016; Lugasi et al., 2005) and this activity is influenced by their high levels of As a result, radish leaves might be particularly effective at preventing stress-related oxidative illnesses. Agarwal and Varma (2014) reported the DPPH and ABTS scavenging assays,in their studies wherein the ethanolic extract of radish leaves displayed dose-dependent radical scavenging activity at 10, 50, 100, 500, and 1,000 g/mL with IC50 values of 216.8 g/mL and 326.7 g/mL, respectively (Noman et al., 2021). In a research, the prior treatment of F2 fraction i.e. the fraction of oven-dried radish leaves, fractioned by diverse solventpolarity and AB-8 macro porous resins column significantly increased the cell viability and internal antioxidant activity and decreased malondialdehyde (MDA) action depending on dose. The pre-treatment of the F2 fraction also increased the level of intracellular reactive oxygen species (ROS) and restored the loss of mitochondrial membrane potential (MMP) caused by hydrogen peroxide in human foetal fibroblast cells. Because it upregulated the anti-apoptosis protein (Bcl-2) and downregulated the pro-apoptosis protein (Bax), this could be the action's potential mechanism (Luo et al., 2018). In order to determine the efficacy of radish root extracts as a skin-whitening and anti-aging ingredient in cosmetic products, Jakmatakul et al., (2009) evaluated the antioxidant and antityrosinase activities in two types of radish root extracts (freeze-dried juice and extract of methanol).The juice which isfreeze-dried depicted more potency of tyrosinase prevention (IC50 = 3.09 mg/ml) as compared to extract of methanol (IC50 = 9.62 mg/ml) due to ample contents of compounds which are phenolic and ascorbic acid injuice which is dried by the freezing method contributing to its colossal antityrosinase and antioxidantpotential.. As a result, *R. Sativus* extract root seemed to be suitable for application as natural skinwhitening and anti-aging agent provided that further purification or a novice extracting techniques is established to attain extracts with greater effectiveness.

The results demonstrated by Eugenio et al., (2017), stated that the radish leaves have the highest concentration of total phenolics (86.16 ± 4.51 mg catechin/g), followed by yam, beet (6.1 mg GAE/g), sweet potato (17.3 mg GAE/g), and carrot leaves (9.3 mg GAE/g). Radish leaves also had the highest antioxidant activity, which has a significant impact on the potential to protect against reactive species damage. As a result, these are probably regarded as a healthy human diet.

The outcomes of this investigation conducted by Rathi et al., (2019) showed that the phenolic compounds in the extracts made from the white radishes' leaves were higher than those in the red radishes' leaves. Further research has shown that, in all solvents other than cold water and 50% ethanol, the TPC (Total Phenolic Content) and FRSA (Free Radical Scavenging Activity) of extracts made from white radishes' leaves were also found to be significantly higher ($P< 0.05-0.001$) than those made from red radish leaves.

In their work, Kumar et al., (2018) showed that radish leaves methanolic extract contains a variety of phytonutrients, including alkaloids, reducing sugar, flavonoids, glycosides, cardiac glycosides, tannins, phenolic compounds, saponin, amino acids, terpenoids, and steroids. In contrast to leaves, roots of radish depicts fewer phytochemical ingredients in all the extract. Therefore, in this investigation, the leaf extract's higher antioxidant capacity could be accredited to the presence of flavonoids and phenolic compounds compared to the root extract, which lacked both of these substances.

According to Silva et al., (2020) among the samples of radish leaf extract examined, the ethyl acetate fraction had a higher antioxidant capacity, possibly because it included more flavonoids. The potential of this reason was further shown by the fact that the ethyl acetate fraction displayed a percentage of free radical sequestration that was equivalent to that of quercetin and Vitamin C.

Goyeneche et al., (2015) showed in their research that the total phenolic content of the leaves was nearly twice as high (695.07 mg GAE/100 g d.m.) as that of the roots, and the total flavonoid content was four times higher (1042.73 mg quercetin/100 g d.m.). As a result, leaves have been shown to be great antioxidants, which shows that leaves may be used as a source of bioactive substances.

2.9 Antimicrobial activity

The ability of the leaves and roots of radish to fight off microbes has been the subject of extensive research. The antibacterial activity of acetone and hexane extracts of radish leaves was carried out by Beevi et al., (2009) against foodborne and resistant pathogens. In their experiment they found that the acetone and hexane leaf extracts at 1mg/mL showed inhibition zone of 13.74±1.58mm and 12.35±0.48mm against acetone and hexane extract respectively in *Bacillus subtilis,* 20.19±3.42mm and 12.58±0.91mm against *Staphylococcus aureus,* 19.21±1.53mm and 10.20±0.30mm against *Staphylococcus epidermidis,* 16.48±2.36 mm and 12.29±0.39mm against *Escherichia coli,* 22.45±0.69 and 11.80±0.90 against *Enterobacter cloacae* respectively. The 'raphanin' found in radish seeds and leaves is known to have antibacterial and antifungal properties. As a result, it has strong antimicrobial effects on major bacterial strains like *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Escherichia coli*. Juice of root has potential against many food borne pathogenic and bacteria causing food spoilage such as *Listeria, Micrococcus, Enterococcus, Lactobacillus and Pedicoccus* species. Radish seeds also contain antibacterial proteins (Shukla et al., 2011).The pungent quality of radish roots is caused by trans-4-methylthio-3-butenyl-isothiocyanate, which is then transformed to 2-Thioxo-3-pyrrolidinecarbaldehyde, which has a MIC of 50–400 g/ml against fungus and bacteria.The sporicidal and bactericidal activities contribute to defence against fungal and bacterial actions of the radish (Gutiérrez and Perez, 2004). Collectively, radish roots and leaves depicts prominent antimicrobial properties, signifying the potentials to become candidates for natural antibacterial agents that could be used to prolong the storage life of foodstuff.

2.10 Antidiabetic effect

One of the main chronic diseases that seriously endanger public health is diabetes mellitus. In recent years, this condition has become more common.The fat-soluble radish extract was found to play a significant function in lipid metabolism, whereas the water-soluble radish extract showed benefits in the hypoglycemic response (Taniguchi et al., 2007). It was proposed that the water-soluble extract contains glucosidase-inhibiting or insulin-like components, such as polyphenolic compounds (Broadhurst et al., 2000 ; Taniguchi et al., 2007). Raphanus sativus root juices antidiabetic effects were investigated by Shukla et al. in 2010. The glycemic properties of *Raphanus sativus L*. (*Brassicacea*e) root juice were examined in the study to identify its glycemic potential. Normal and streptozotocin (STZ)-induced sub- and mild-diabetic rats received oral dosages of the extract in the range of 100, 200, 300, and 400 mg/ kgbody weight (bw). Glibenclamide was utilised as the reference medication. During investigations on fasting blood glucose, the dose of 300 mg kg 1 bw was identified as the most effective dose, lowering the level of blood glucose (BGL) by 33.4 percent at 6 hours (FBG).However, the glucose tolerance test (GTT) revealed that sub- and slightly diabetic rats experienced reductions of 23.8 and 28.3 percent at the same time as normal rats receiving the same dose. Normal rats experienced a maximum reduction in BGL of 15.9 percent after 3 hours.

Normally, it is recognized that the sulfonylureas, comprising glibenclamide, yield hypoglycemia in normal as well as in animals which are diabeticbypancreatic ß-cells stimulation to emanate ample insulin (Amalraj et al., 1998). The considerable drop in blood glucose levels seen in diabetic rats treated with Raphanus sativus juice and glibenclamide may be attributable to residual pancreatic mechanism stimulation, potentially by accumulative peripheral glucose use, as reported by Earth et al., (1996). Lipopolysaccharides (LPS) were isolated by Gutiérrez and Perez (2004) from radish with an ED50 of 0.4–100 ng/ml and an activating macrophage. These compounds are used as antidiabetic medications in the pharmaceutical and veterinary fields. Additionally, it was shown that radish contain coenzyme Q10, also known as ubiquinone, an antioxidant coenzyme that is fat-soluble and a component of the electron transport chain in human mitochondria. This antioxidant coenzyme also delays the onset of type 2 diabetes(Nakamura et al., 2016; Lazourgui et al., 2016). Coenzyme Q10 at 1600–2000 mg kg1 of body weight in mice weighing 25–30 g, which is equivalent to 130.1–162.6 mg/kg of body weight in people, may be an efficient therapeutic approach for lowering oxidative stress in glaucomatous neurodegeneration, according to Lee et al., (2014) in vivo system evaluation.

Kim et al., (2014) reported that the main catalytic enzyme for the conversion of starch to glucose is α -amylase, together with α -glucosidase. Therefore, its manipulation is thought to be a successful method for managing glucose homeostasis in diabetes. Radish leaves suppressed α -glucosidase activity more effectively. Stronger α -glucosidase inhibitory activity was obtained in saengshik by increasing each ingredient's concentration from 0.001 to 10 mg/ml which consist of radish leaves as well.

2.11 Anticarcinogenic activity-

Modern chronic diseases, particularly cancer, are among the most common health problems worldwide, with a high mortality rate Khan et al., (2020). According to the WHO, cancer is the second most common cause of death worldwide, causing close to 10 million fatalities annually (2021). In low- and middle-income nations, cancer claims the lives of roughly 70% of people, or one in every six people worldwide. The use of complementary and alternative medicine in conjunction with standard medical procedures is growing. Many illnesses, including cancer, have been treated with natural remedies and herbal therapy. Khan et al., (2020) has reported that the radish leaves has exhibited the anticancer capabilities by preventing cancer cell multiplication and causing apoptosis, according to multiple studies employing different cancer cell lines. Wang et al., (2017) stated that the ethanolic extract of radish leaves has been accounted as an antitumor agent as it straightly helps in the inhibition of cells of tumor by hindering the growth by inducing apoptosis ; Kim et al., (2011). Several authors have proved that glucosinolates, flavonoids, polyphenols, and their derivatives which are abundantly present in radish leaves, as well as roots, have a strong anticarcinogenic activity (Fenwick et al., 1983). In another experiment, the ethanolic extract of radish leaves showed the anti-proliferative effect in A549, MCF-7, Hep-G2, and MDA-MB-231 cell line with IC_{50} values of 217 ± 2.1µg/mL, 287 ± 2.3µg/mL, 224 ± 2.2µg/mL and 453.2 ± 1.9 µg/mL respectively and this may be due to the compound rutin present in radish leaves (Noman et al., 2021).To test its antiproliferation activities, ethanolic extracts of the aerial parts of *Raphanus sativus L* (ERL) were applied to the human MDA-MB-231 breast cancer cell line at concentrations of 100, 200, or 300 g/mL. The results demonstrated that the ethanolic extract of Raphanus sativus L's aerial part inhibits cellular proliferation in the MDA-MB-231 human breast cancer cell line in a dose-dependent manner, possibly via the ErbB-Akt pathway. The extract decreased the protein expression of both ErbB and Akt in the cells in a dose-dependent manner, but significantly at 200 and 300 g/mL, respectively (Kim et al., 2011). The breast cancer cell lines SKBR-3 and MDA-MB-231 were discovered to be inhibited by glucoraphenin, which is contained in radish seeds and hydrolyzed to sulforaphene. Sulforaphane was discovered to induce G2/M cell cycle arrest, disrupt cytoskeletal architecture, and lessen the ability of cancer cells to proliferate. Sulforaphane caused concentration-dependent apoptosis, inhibited oxidative stress, and elevated the Bax:Bcl2 ratio and ADRP levels (Pawlik et al., 2017).

Evaluation done by Gutiérrez et al., (2004) states that Raphanus sativus roots and leaves have been used as antibacterial and anticarcinogenic agents all over the world. It also has antiviral properties, as demonstrated by the effectiveness of the leaf's aqueous extract against the influenza virus. The antioxidant activity of pigment of red radish (pelargodinin -3-sophoroside-5-glucoside) was nearly similar to that of BHT at the same concentration. On addition of 0.01pigment, the inhibition ratio exceeded to more than93%. The antioxidant activity was also contributed by the caffeic acid present in the radish.

Standard antioxidant trials have indicated that *Raphanus sativus* aerial sections, according to Beevi et al., (2010) . They have significant antioxidant and radical scavenging activity and are often discarded. *R. sativus* was comparable to typically rich sources like green tea and black tea in terms of total polyphenolic levels, which were 86.16 and 78.77 mg/g dry extract, respectively. By acting as chain-breaking antioxidants, *Raphanus sativus* leaves and stem have demonstrated their ability to effectively scavenge free radicals such DPPH, superoxide anion, hydrogen peroxide, and nitric oxide.

2.12 Anti-inflammatory activity

In many disorders, inflammation increases morbidity and mortality. Numerous physiological and cellular processes are regulated by mediators such cytokines, tumour necrosis factor (TNF), interleukins (IL), prostaglandins (PGs), and thromboxanes during the course of inflammation. Some of the most prevalent inflammatory-related disorders worldwide include rheumatoid arthritis, various carcinomas, atherosclerosis, and asthma. Majid et al.,(2018). Due to its anti-inflammatory properties, Raphanus sativus is a viable candidate for study as a natural remedy for treating and preventing inflammatory diseases. (Kim et al., 2021; Saleh-Abbes et al., 2015). The hydroalcoholic extract of Raphanus sativus root and leaves juice considerably reduced the carageenan and formalin-induced paw edoema in rats, as shown by Kamble et al., (2013) in their study. In comparison to radish root juice (28.46 percent (carageenan), 30.43 percent), radish leaf juice showed stronger inhibition (41.90 percent [carageenan], 42.10 percent [formalin]) and an anti-inflammatory effect (formalin). The potent anti-inflammatory impact may be attributed to the presence of flavonoids, phytosterols, and tannins as well as the control of important inflammatory mediators such histamine, serotonin, prostaglandins, bradykinin, angiotensin, tachykinin, platelet activating factor, and substance-p. In the raw 264.7 cell lines, black radish has also been examined for lipopolysaccharide (LPS) and interleukin (IL)-6-mediated inflammatory responses. In response to black radish extract, pro-inflammatory cytokines like IL-1, IL-6, tumour necrosis factor (TNF), and prostaglandin E2 were generated, even though COX-2 and inducible NO synthase (iNOS) levels were reduced. Additionally, studies have indicated that black radish extract may reduce inflammation through specific immunomodulatory actions. These actions may be caused by the activation of the

NRF2/HO-1 signal transduction pathway and repression of the STAT3/JAK2 signalling pathway (Jeon et al., 2020). Similarly anti-inflammatory effect of Korean radish was also evaluated against RAW 264.7 cell lines. Significant reduction of lung neutrophils and myeloperoxidase in broncho alveolar lavage fluid (BALF) was observed in mice treated with Korean radish. The decreased level of IL6 in BALF induced by LPS was observed. Glycyrrhizin was found to be most potent candidate for anti-inflammatory effect as suggested by HPLC-MS (Kim and Nam, 2017). In general, radish extracts could reduce inflammation by upping the antioxidant defence system and reducing the expression of inflammatory cytokines.Numerous phytochemicals and nutrients found in radish root and leaves may enhance microbial metabolism and tight junction barriers, reducing intestinal damage brought on by inflammation.

The result, conducted by Park et al., (2017) indicate the modulating of the signal transduction pathways, and demonstrated that the radish leaf chloroform fraction reduced the generation of pro-inflammatory mediators that Lipopolysaccharide (LPS) produced in RAW 264.7 macrophages. According to these results, the RSL extract is considered to be a potentially useful and affordable alternative in order to cure inflammatory diseases.

Kamble et al .,(2013) used both acute and chronic models of inflammation in their study and showed that radish leaf juice provided a more substantial anti-inflammatory impact than radish root juice.The hydroalcoholic extract of Raphanus sativus showed strong anti-inflammatory activity due to the presence of flavonoids, phytosterols, and tannins as well as the inhibition of important inflammatory mediators like histamine, serotonin, prostaglandins, bradykinin, angiotensin, tachykinin, platelet activating factor, and substance-p. Since it closely resembles real arthritis, it can be utilised as one of the best test procedures to identify anti-arthritic and anti-inflammatory drugs.

2.13 Antibacterial activities

In a study by Chiad et al., (2018), the watery extract of radish plant leaves outperformed alcohol and antibiotic extract in inhibiting Streptococcus mutans, while alcoholic extract outperformed watery extract in inhibiting Staphylococcus aureus. They evaluated the antibacterial effects of the watery and alcoholic extract of radish leaves and the antibiotic (Gentamycin) on the inhibition of bacteria causing gingivitis and tooth decay.

According to the findings of the study conducted by Al-mossawei et al., (2014); the alcoholic radish leaf extract inhibited the growth of Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Candida albicans.With an increase in extract concentration, the level of inhibition grew.

Beevi et al., (2009) stated that the complete range of organisms was susceptible to the acetone extract of R. sativus leaf extract, but the hexane extracts only significantly inhibited Salmonella typhimurium and had a weak inhibitory effect against the other bacteria. Staphylococcus epidermidis and Staphylococcus aureus were significantly inhibited by acetone extract of the leaf.

2.14 Antihypertensive activities

According to Kim et al., (2010) spontaneously hypertensive rats (SHR), adding dry radish leaf powder to the meal decreased systolic blood pressure, however a 5 percent supplementation had no more of an impact than a 2.5 percent supplementation. A notable ACE-inhibitory impact was seen in the pulmonary tissue of rats with hypertension and rats with normal blood pressure after supplementation

2.15 Other bioactive effects

In addition to the health advantages listed above, new research has identified other bioactivities of radish leaves and roots, including hepatoprotective, neuroprotective, antihypertensive, and antiulcer characteristics. In Sprague-Dawley male rats fed a high cholesterol diet, the groups supplemented with 5 and 10 percent of radish leaf powder demonstrated a significant increase in hepatic glutathione peroxidase activity and a significant decrease in hepatic hydrogen peroxide content, hepatic superoxide radical content in mitochondria, hepatic TBARS values, and hepatic carbonyl values (Rhee et al., 2005). The radish leaves have also demonstrated neuroprotective properties, especially in very severe neurodegenerative illnesses like Alzheimer's disease. When mice were given the compound erucamide, which is produced from dried radish leaf extract, at doses of 20 and 40 mg/kg daily, the effects of the TMT on learning and memory, as determined by the Y-maze and passive avoidance tests, were significantly attenuated (Kim et al., 2018).While to check the nephroprotective effects, the rifampicin induced male albino rats were fed with aqueous and ethanolic extract of radish leaves at 2g/kg b.w in for 28 days. They discovered that the radish leaf extracts, both aqueous and ethanolic, had nephroprotective effects against rifampicin-induced toxicity in adult albino rabbits by lowering nephrotoxic indicators such blood urea nitrogen (BUN), serum creatinine (CR), and blood urea (BU) (Khalid et al., 2018). In a study by Kim et al. (2010), it was discovered that giving Wistar rats dried radish leaf powder (DRLP) orally for four weeks resulted in a greater decrease in systolic blood pressure (SBP) at 2.5 percent than at 5 percent. This antihypertensive effect was attributed to DRLP's ACE-inhibitory activity and increased Na excretion (Kim et al., 2010).While oral treatment of radish leaf ethyl acetate extract at a dose of 90 mg/kg body weight dramatically lowered systolic blood pressure (SBP) in male Wistar rats from 214 mmHg to 166 mmHg, which may be because the extract raises NO levels in the blood and enhances antioxidant activity (Chung et al., 2012). Radish leaves have also demonstrated some antiulcer effects. Ghayur and Gilani (2005) have confirmed that radish leaves extract has shown gastrointestinal and uterotonic activity which was due to the presence of saponin and alkaloids. In another experiment, the ethanolic extract, aqueous extract (AQRS), the Coliform fraction (CRS), and Ethyl acetate fraction (EARS) showed a noteworthy lessening in ulcer index as equaled to the control group in acetic acid-induced chronic gastric ulcer model at 200mg/kg while ethanolic extract (ERS), aqueous extract (AQRS), and ethyl acetate fraction (EARS) pylorus ligation-induced chronic gastric ulcer model in male albino Wistar rats demonstrated a substantial reduction in ulcer index, gastric juice volume, free acidity, and total acidity at 200mg/kg (Devaraj et al., 2011).

HYPOTHESIS

Presently as far as unconventional greens are concerned, radish leaves are known to be bountiful source of nutrients that exhibits its potential to be utilized in the food formulations both as functional and nutraceutical ingredient. The basic approach is to use the radish greens for increased consumption in human diet. The present study therefore focusses on the incorporation of radish leaves powder in the preparation of instant soup and chutney mixes to provide the consumer avail the maximum benefits.

CHAPTER 3: MATERIALS AND METHODS

3.1 Preparation of sample

We purchased radish seeds (*R. sativus* var. ivory white) from the local Jalandhar, Punjab, market. Dibbling technique was used to plant seeds in the horticulture ranch of Lovely Professional University. The thirty seven-day stretch in November, which saw temperature variations between 18 and 25 °C, saw the completion of seed planting. The soil was sandy and had a pH of 7.2. Based on the length of the leaves, the leaf collection was completed. On 28, 37, and 52 days after planting, young (4 cm), mature (8 cm), and over-mature (12 cm) leaves were collected. Cutting stem ends with scissors after cleaning them with 70% ethanol marked the end of the gathering process.After reap, radish leaves were investigated before any treatment and plants with abandons were disposed of.

3.2 Choosing Packaging Material and Storage Conditions of radish leaves

Low density polyethylene (LDPE), cling wrap, and paper sacks were used to package young (4 cm), mid-mature (8 cm), and over-mature (12 cm) leaves. All of the samples were stored at ambient temperature (22 °C) and at (4 °C), and they were then dissected for quality traits such moisture content, chlorophyll, ascorbic acid, DPPH scavenging activity, total phenols, and flavonoids for up to 5 days.

3.2.1 Moisture Content

Moisture content is analysed using standard method of AOAC. Hot air oven is preheated before keeping the sample. 5g sample is taken and kept in hot air oven at 105°C for 1 hour. After an hour petridish was taken out from hot air oven and kept it in desiccator for cooling. After taking the values, petridish with sample again keep in hot air oven for 10 minutes and this method is repeated till concordant values are obtained.

$$\text{Moisture content }\% = \frac{\text{Initial weight of sample} - \text{final weight of sample}}{\text{Initial weight of sample}} \times 100$$

3.2.2 Chlorophyll Value

A portable SPAD 502 Chlorophyll Meter was used to non-destructively assess the amount of chlorophyll (Chl) (Minolta Co., Ltd.)

3.2.3 Antioxidant activity

Antioxidant activity (also known as free radical scavenging activity) was evaluated using Brand-Williams et al (1995)'s approach. DPPH (2, 2-diphenyl-1-picrylhydrazyl) was used as a source of free radicals. The absorbance was measured at 515 nm for 30 minutes, or until it reached a steady state, in a cuvette that contained 0.1 mL of sample extract and 3.9 mL of 6x10-5 mol/L DPPH in methanol. We used methanol as a blank. The remaining DPPH concentration was calculated using the equation below:

$$\text{Antioxidant activity \%} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \text{ x } 100$$

3.2.4 Ascorbic acid(Ranganna, 1986)

AOAC (2004) technique was used to measure the ascorbic acid content using the dye 2, 6-dichlorophenol indophenol. A known volume of the substance was extracted in 3 percent m-phosphoric acid and coloured to produce the desired pink colour. The following formula was used to determine the results, which were then expressed as mg per 100 g of sample:

$$\text{Ascorbic acid}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{Titer (ml)} \times \text{Dye factor}\left(\text{ascorbic acid}\,\frac{\text{mg}}{\text{ml}}\right) \times \text{Volumemade up (ml)}}{\text{extraction taken for estimation (ml)} \times \text{weight of sample}}$$

3.2.5 Total phenolic content

Following the procedure described by Niroula et al., the total phenolic content of the leaves was measured using the Folin-Ciocalteu reagent (2019). Briefly, 2.5 ml of 10% Folin-Ciocalteu reagent and 0.5 ml of methanolic extract were combined. 2.5 ml of sodium carbonate (7.5 percent, w/v) was added after 5 minutes, stirred, and then incubated at 45°C for 45 minutes. A spectrophotometer was used to measure the absorbance at 765 nm in comparison to a blank solution.Blank solution was prepared by using 0.5 ml methanol at the place of sample extract. All assays were performed in triplicates. TPC was calculated on the basis of calibration curve of gallic acid and expressed as standard curve of concentration (0.002-0.006 mg/ml)

3.2.6 Total flavonoid content

According to the method described by Zhao et al., (2018) the centralizations of flavonoids were measured with a colorimetric examination in mind. Quercetin was briefly used as the standard. Flavonoids were extracted from proposition tests using 10 mL of 60 percent ethanol watery and a weighted 1.00 g test. Additionally, these specimens underwent a 3000g centrifugation. The supernatant was transferred to a 25 mL volumetric cup and then fixed to the 25 mL mark using 60 percent ethanol. A 25 mL cylinder was filled with 1.5 mL of concentrate, 4.5 mL of purified water, and 1 mL of 5 percent (w v 1) NaNO2 arrangements.1 mL of the 10 percent (w v) Al (NO3)3 arrangements was added to the mixture after the mixture had been hatching for 6 min. Prior to adding 10 ml of 4 percent (w v) NaOH solutions and fixing it to 25 mL with 60 percent ethanol fluid, the mixture was allowed to sit for 6 minutes. Finally, the mixture was reacted for 15 minutes, and the absorbance of the combination arrangement was measured against a clear containing 5 mL of extraction dissolvable using a spectrophotometer at 510 nm. Tests were independently dissected three times, and the mean of the three tests was used to report the absolute flavonoid concentration as mg quercetin equivalent per g dry weight (DW).

3.3 Drying method

Therefore, in research, drying was done using a tray dryer, at 3 different temperatures, to assess the impact of different drying temperatures on the bioactivity, thermal, and structural aspects of leaves (45, 55 and 65 ^{o} C).We carried out the drying processes till the equilibrium moisture content was reached. An electric weighing balance was used to detect moisture loss every 30 minutes (Model PM 2000; Adventurer OHAUS, Meller, Switzerland). Data sets were recorded as moisture ratio (MR) versus drying time for various experimental drying cycles (t).

Moisture ratio and Drying rate

The moisture ratio and drying rate (DR) was calculated by using following equations;

$$MR = \frac{M-Me}{Mo-Me} \dots\dots\dots (1)$$

Where MR denotes the moisture ratio, M denotes the dry-based moisture content at time t, Me denotes the dry-based moisture equilibrium constant, and M0 denotes the dry-based moisture content starting point.

$$DR = \frac{M(t+dt) - Mt}{dt} \text{ .... (2)}$$

The moisture content at time t+dt is denoted by M(t+dt),

the moisture content at time t, is denoted by Mt,

and the time difference is denoted by dt.

3.3.1 Mathematical Modelling

The mathematical models, include Newton's, Page's, Henderson and Pabis', Pabis's modified, logarithmic, Table 1 lists the models that were chosen to suit the experimental data and describe the drying curve equations: the Singh model, Midillii model, two-term and two-term exponential models, and Wang and Singh.

Statistica 10.0 10.'s entire data set was used to estimate the parameters of each model using non-linear regression. The coefficient of determination was a key consideration in selecting the optimal model to describe the drying curve (R2). The largest R2 values, the least chi-square (2) values, the root mean square error (RMSE), and an estimate of these values as

$$\chi 2 = \sum_{i=1}^{N} \frac{(MRei - MRpi)^2}{N - Z} \text{ .... (3)}$$

$$RMSE = \sqrt{\left[\frac{1}{N}\sum_{i=1}^{N}(MRei - MRpi)^2\right]} \text{ .... (4)}$$

Where, *MRei* is the dimensionless ratio of moisture that was seen, MRpi is the dimensionless moisture ratio that was predicted, N represents the frequency of observations, and Z represents the number of constants in the model.

3.3.2 Calculation of drying's effective moisture diffusivity

The effective moisture diffusivities at various air drying temperatures were estimated using Fick's second rule of diffusion. This regulation has resolved the entire theoretical model for thin layer drying of agricultural products. Given below is the equation for the universal solution of Fick's law, which makes the following assumptions: constant temperature and diffusion coefficient; minimal shrinkage; and only the diffusion process had been used to move moisture.

$$\frac{Mt - Me}{Mo - Me} = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)}\exp(-(2n+1)^2\,\pi^2\,Det\ \div 4L^2) \text{ ... ... (5)}$$

Here,

De is the effective diffusivity in m2/s

L is the slab's half thickness in m,

and T is the time in seconds.

The above equation (5) can be further simplified to only the series of the first term for long periods of drying, which can be written in a logarithmic form as:

$$\ln MR = \ln\left(\frac{8}{\pi^2}\right) - \frac{\pi^2 De}{4L^2} \times t \ldots\ldots\ldots\ldots (6)$$

The equation (6) is used for the determination of effective moisture diffusivity from the slope of ln MR and t (drying time) plot, which is given by:

$$Slope = -\frac{\pi^2 De}{4L^2} \ldots\ldots\ldots\ldots (7)$$

3.3.3 Estimation of Activation Energies

The activation energy is calculated using an Arrhenius type equation, where the temperature depends on effective moisture diffusivity. It is given as

$$De = Deo \exp\left(-\frac{Ea}{RgTa}\right) \ldots\ldots\ldots (8)$$

Where,

Deo, is the effective moisture diffusivity in square metres per second for an infinite temperature.

The activation energy for moisture diffusion in KJ/mol is Ea

Rg is the global constant for gases (8.314 10-3 KJ mol-1 k-1)

The absolute temperature in kelvin is Ta.

3.3.4 B**io-functional activities in tray drying of radish leaves**

The bio-functional activities of different samples were checked. Samples were checked for ascorbic acid , total flavonoids and total phenols.

3.4 Functional properties of radish leaves powders

The physicochemical qualities, structure, content, and molecular conformation of dietary ingredients, as well as the environment in which they are connected and monitored, are frequently linked to the functional properties of powders (Chandra and Samsher.,2013).It includes water absorption capacity (WAC), oil absorption capacity, angle of repose, tapped (compact) density, foaming capacity and bulk density are the parameters which provides an idea of how much powder can be placed in specific packaging material because this attribute includes pores and voids between particles as well (Fazaeli et al., 2012).

3.4.1 Bulk density

According to Twinomuhwezi et al., (2020) approach, the bulk density (BD) was calculated (2020). A 100 ml graduated cylinder containing the 50 g of flour sample was placed inside, and it was tapped 20 to 30 times until there was no longer any volume change. The bulk density was calculated as the sample's weight in relation to its volume.

The bulk density of powder was calculated as follows:

$$\text{Bulk density}\ (\frac{\text{g}}{\text{ml}}) = \frac{W_2 - W_1}{10}$$

Here, W_1= Weight of cylinder only, W_2= The weight of powder and cylinder. The experiment was carried out three times (Bai *et al.,* 2006). Mass of powder = M, Volume of cylinder before tapping = V_0, volume of cylinder after tapping = V_1 .

3.4.2Angle of Repose

The slope that is created by the powder without it collapsing is called the angle of repose. Using the technique described by Brar et al., 2017, the angle of repose was measured. In a nutshell, it can be quantified by determining the sample-created heap's diameter and height (Kaleemullah and Gunasekar, 2002). The sample was held vertically on a horizontal hardwood surface and contained in a plastic cylinder with an inner diameter of 70 mm and a height of 270 mm. To achieve consistent packing and reduce any wall effect, tapping was done during filling. Slowly, the cylinder was lifted above the material so that it could slide about freely and pile up.With the use of a measuring scale, the heap's height (H) and diameter (D) were measured, and the angle of repose () of the maize seed was calculated using the formula below:

$$\text{Angle of repose } (\theta) = \frac{2R}{H}.$$

The flowability of powder can be calculated on the basis of angle of repose by the given criteria

Very free flowing - < 30°, Free flowing - 30°-38°, Fair to passable flow- 38°-45°, Cohesive- 45°-55°, Very cohesive- > 55°.

3.4.3Water absorption capacity (WAC)

The percentage of water bound by grams of flour was used to investigate the water absorption capacity (WAC). The process outlined by Chandra et al. was used to determine it (2015). Briefly, 1 g of the sample was mixed with 10 ml of distilled water and left at room temperature (32 °C) for 30 min. The material was then centrifuged for 30 minutes at 3,000 rpm or 2000 g.

$$\text{Water absorption Capacity (\%)} = \frac{Weight\ of\ hydrated\ residue}{Weight\ of\ dry\ sample} \times\ 100$$

3.4.4 Oil absorption capacity (OAC)

The ability of flour to retain flavour, known as oil absorption capacity (OAC), is crucial when creating food formulations (Ajatta et al., 2016). When utilised in food preparations, the OAC makes the flour excellent for supporting enhancement in tongue feel and flavour. The oil absorption capacity was also determined using the Chandra et al. technique (2015). One gram of the substance was mixed with 10 ml of soybean oil with a specific gravity of 0.9092, allowed to stand for 30 minutes at room temperature (30.2 °C), and then centrifuged for 30 minutes at 300 rpm or 2000 g. The value was shown as a percentage of water and oil bound per gram of the substance.

$$\text{Oil absorption Capacity (\%)} = \frac{Weight\ of\ sample+oil}{Weight\ of\ sample} \times\ 100$$

3.4.5 Foam capacity (FC)

The method described by for calculating foam capacity (Ishara et al., 2002). In a 100ml graduated cylinder, 2g of sample was quickly mixed with 50ml of distilled water. The suspension was then combined and agitated to create foam for five minutes. Foaming capacity (FC), the measure of the volume of foam after 30 seconds after whipping, was calculated using the following formula:

Foaming capacity % = $\frac{V_2-V_1}{V_1} \times 100$ and here,

V_1 – Original volume of sample (ml), V_2 - volume of foam after whipping,

3.5 Structural analysis

3.5.1 Fourier transform infrared (FT-IR) spectroscopy

The Malik and Saini method was used using Tensor FT-IR module (Bruker, USA) to determine the infrared spectra of dried powder (vacuum and tray) (2018). In order to create protein isolate pellets, powder samples were combined 1:100 (w/w) with moisture-free potassium bromide before being compressed in a vacuum hydraulic press. FTIR spectra was recorded in the range of 4000-400 cm^{-1}.

3.5.2 XRD diffraction

X-ray diffraction (XRD) pattern native and detoxified kernel powder protein isolates were obtained using PANalytical- X' pert PRO MRD (Almelo, Netherlands) diffractometer. The samples were scanned for diffractograms over the range of 0°-40° and at a scanning rate of 1.20°/min

3.5.3 LC-MS screening and identification of polyphenolic compounds

The polyphenol rich powder of radish leaves was analysed on Waters Micromass Q-Tof Microsysytem equipped with a C18 column.The system was operated with the flow rate of 1ml/min consisted of (A) H_2O and formic acid (99:1v/v) and (B) acetonitrile and formic acid (99:1v/v). the gradient started at 4% B for 0.5 min and then linear gradient up to 30% B in 60 minutes before returning to an initial condition.The eluate was split after passing the column and 0.3ml/min was directed to the mass spectrometer. Ms testing was carried out in ESI negative ion mode at same chromatographic conditions.The parameters for ESI-MS were : ESI potential source :

4KV, capillary temperature 400°C. Phenolic compounds had been identified in the same sample using a complete scan, data dependent MS_2 from 100 to 800 m/z scanning and selectedreaction monitoring

3.6 Statistical analysis

Analysis of variance test was carried out using commercial statistical package, SPSS ver.

11.5 (SPSS Inc., Chicago, IL, USA). All results of quality parameters were recorded as mean± SD of three replicates. Mean values were compared and significant differences were givenusing Duncan's and LSD test ($p \leq 0.05$).

3.7 Standardization of formulation of radish leaveschutney mix

The dried radish leaves were mixed with the spice mix in different treatment combinations of chutney mix.Mint, anardana, chilli powder, black salt, garlic powder, onion powder, curry leaves, cumin powder, and coriander powder were weighed in the various quantities listed in the table to create a spice mixture (Table 3.2). Four treatment combinations were made using radish leaves powder and spice mix as T0 (0:100), T1 (5:95), T2 (10:90), T3 (15:75), T4 (20:80), and T5 (25:75), as shown in the table, in order to standardize the levels of radish leaves powder and the spice mix in the formulation of radish leaves chutney mix (Table 3.1). These mixtures were made ready for usage by reconstituting them in 1:2 ratios with warm water for sensory evaluation.A group of 10 semi-trained panelists assessed the reconstituted chutneys' sensory quality for a variety of factors, including appearance, colour, flavour, taste, and overall acceptability. According to the method outlined by Sharma and Joshi, the sensory data were gathered on a 9-point hedonic scale, with a maximum score of 9 for "like exceedingly" and a minimum score of 1 for "dislike excessively" (2014).

Table:3.1 Treatment combinations of chutney mix

Formulation	T0	T1	T2	T3	T4	T5
Radish leaves	0	5	10	15	20	25
Spice mix	100	95	90	85	80	75

Table : 3.2 Spice mix composition in radish leaves chutney

Ingredients	Composition (%)
Mint	30
Anardana	10
Chilli powder	15
Black salt	10
Garlic powder	10
Onion powder	10
Curry leaves	5
Cumin	5
Coriander powder	5

3.8 Standardization of formulation of radish leavessoup mix

The dried radish leaves were mixed with the spice mix in different treatment combinations .A soup mix was prepared by weighing the milk powder, carrot grits, sugar black pepper, citric acid , onion powder, garlic powder, chilli powder, starch, salt in the different quantities as mentioned in (Table 3.3).Four treatment combinations were made using radish leaves powder and soup mix as T0 (0:100), T1(0.5:99.5), T2 (1:99), T3(1.5:98.5), and T4(2:98), as indicated in the (Table 3.3), in order to standardize the levels of radish leaves powder and the soup mix in the formulation of radish leaves soup mix.For sensory evaluation, these combinations were prepared by reconstituting with warm water in 1:2 ratios to make them ready to use. The sensory quality of the reconstituted soup was assessed by a group of ten semi-trained panellists based on a variety of factors, including appearance, colour, flavour, and general acceptability. A 9-point hedonic scale was used to collect the sensory data in accordance with Sharma and Joshi's methodology, with a maximum score of 9 denoting "like exceedingly" and a minimum score of 1 denoting "dislike excessively" (2014).

Table :3.3 Treatment combinations of radish leaves based soup mix

Formulation	T0	T1	T2	T3	T4
Radish leaves	0	0.5	1	1.5	2
Soup mix	100	99.5	99	98.5	99

Table 3.4 : Composition of soup mix in radish leaves based soup mix

Ingredients	Composition (g/100g)
Milk powder	25
Carrot grits	5
Sugar	5
Black pepper	5
Citric acid	1
Onion powder	9
Garlic powder	9
Chilli powder	5
Starch	31
Salt	5

3.9 Sensory analysis

3.10 Storage stability studies

To perform the storage/shelf stability investigations, the samples of radish leaves, based chutney mix, and soup mix were packaged in LDPE pouches(50 microns thickness), aluminium pouches(120 microns thickness) and PET jars (350 microns thickness) and heat sealed with a heat-sealing machine. The packaged samples were kept in ambient (15-35°C) storage conditions for 90 days as part of storage tests. Bulk density, true density, porosity, viscosity, TSS, pH, titrable acidity, and pasting characteristics were studied as part of the storage process.

Total soluble solids (TSS)

TSS was calculated using a method described by (Priti and Kandra Prameela 2020). Sugar concentration in fresh or processed food or solutions is determined with a Brix refractometer. Accurately weigh 2 g of the sample. Use 5 mL of distilled water to macerate. Put a drop of the prepared sample on the refractometer's prism, then cover. Record the reading, which shows the soluble solids percentage.

Titratable acidity

(AOAC, 2012) states that 1g of instant soup and chutney mix was dissolved in 100ml of distilled water, and then 1ml of the solution was used to titrate with 0.1N NaOH to an end point while using phenolphthalein indicator. The amount of NaOH used was recorded during the titration.

$$\text{Titratable acidity (\%)} = \frac{\text{Titre} \times \underset{\text{alkali}}{\text{Normality of}} \times \underset{\text{made up}}{\text{volume}} \times \underset{\text{of acid}}{\text{equivalent weight}}}{\text{Volume of sample} \times \text{volume of aliquot} \times 1000} \times 100$$

pH

After calibrating the pH metre with buffers, distilled water was used to rinse the electrode(s) and glass utensils. For the pH analysis, 1g of each sample was precisely measured and added to a 150ml beaker. The washed electrode was inserted into the sample under examination. All of the samples were allowed to arrive in the sealed vials at room temperature. The sample was gently mixed, and the pH value was calculated and recorded to the nearest 0.01.

Viscosity

The viscosities of the soup samples were measured using a digital tabletop Brookfield viscometer (Brookfield DVE viscometer, USA). The soup samples were maintained at 30 °C before analysis. A 150 mL sample was placed in a beaker (70 mm diameter and 125 mm height) and measured for viscosity using spindle no 2 at 12 rpm speed. The results are expressed in poise or $mPaS^{-1}$.

Bulk density

According to the methodology used by Twinomuhwezi et al., (2020) the bulk density (BD) was computed (2020). A 100 ml graduated cylinder containing the 50 g of flour sample was placed inside, and it was tapped 20 to 30 times until there was no longer any volume change. The bulk density was calculated as the sample's weight in relation to its volume.The bulk density of powders were calculated as follows:

$$\text{Bulk density (g/ml)} = \frac{W_2 - W_1}{10}$$

Here, W_1= Weight of cylinder only, W_2= The weight of powder and cylinder. The experiment was carried out three times (Bai et al., 2006).

True density:

By weighing out 10 grammes of the sample, true density was calculated. The liquid displacement method is used to determine the volume exactly. Take a measuring cylinder with a 100 ml capacity and fill it with toluene to the specified level for this. Drop 10 grammes of the sample into the cylinder, then correctly record the volume change. This indicates the sample volume. (AOAC,2004)

$$\text{True density g/ml} = \frac{\text{Weight of sample}}{\text{Volume of sample}}$$

$$\text{Porosity} = \frac{\text{True Density} - \text{Bulk Density}}{\mathit{True\ Density}}$$

$$\text{Porosity \%} = \frac{1 - \text{Bulk density}}{\text{True density}} \times 100$$

CHAPTER 4: RESULTS AND DISCUSSIONS

The variations in moisture content, chlorophyll value, ascorbic acid content, DPPH scavenging properties, overall phenols, and flavonoids of various development stage in green leaves during the storage period in various materials of packaging are introduced in Tables 4.1, 4.2, and 4.3. Moisture content for the examples was observed to reducein the storage period. The moisture content, everything being equal, new younggreens (4 cm), mature greens (8 cm), and over mature greens (12 cm) diminished steadily as the expansion away duration Pace of reduction in moisture content was less in samples packed in LDPE (Table 4.1) trailed by paper and cling for a period of five days.The possible reason for this isthe low water permeability of LDPE which alleviates thetranspiration rate and respiration rate of leaves. However, high permeability of cling and paper bags increases themetabolic activity of fresh leaves which causes high moisture loss. Similar findings were provided by (Reddy et al.,2014) in which Rajagira leaves were stored in polyethylene(LDPE, HDPE), PET jars, muslin cloth and brown paper pouches. In this study, leaves stored in LDPE showed lowest moisture loss (0.29%) as compared to other packaging materials. Also, Koraddi (2005) observed the effect of different packaging materials (brown paper, PET jar) on the moisture loss of carrots, cucumber, fenugreek leaves, coriander and chillies. He observed that although moisture loss was lower in PETjar but decayed rate was increased by 2.62% due to accumulation of moisture and restricted oxygen concentration. Chlorophyll value of examples [young greens(4 cm), mature greens (8 cm), and over mature greens(12 cm)] stuffed for various materials of packaging (Paper, stick, LDPE) was seen diminishingin the duration of storage.Speed of chlorophyll degradation was lower in samples packed in LDPE as compared to paper and cling for a period of five days. This might be due to the accumulation of carbon dioxide and depletion of oxygen concentration inside the atmosphere of LDPE which in turn decrease the yellowing rate of leaves. The loss of chlorophyll pigment is a commonly occurred biochemical change during the storage of leafy vegetables which is characterized by the activity of chlorophyllase enzyme (Yamauchi et al.,1993). Therefore, maintaining the chlorophyll content is an indicator of freshness of fresh produce especially leafy greens. These findingshave been found to be in coherence with Kakade et al. (2015). They also established extension of shelf-life of spinach leaves whenstored in LDPE bags. Spinach leaves were observed freshfor 3 days at room temperature and 14 days at refrigeratedstorage. Also, Lee et al., (2018) checked the effect of LDPE packaging on the shelf-life extension of lettuce. In theirstudy, lettuce packed in LDPE or PP films without any punching holes at 5 °C was found the most desirable forextending the shelf-life.The ascorbic acid substance of all examples [young leaves (4cm), mature leaves (8cm), and over mature leaves (12cm) packed in various materials of packaging (Paper, stick, LDPE) was seen to diminish in the duration of storage time. In any case, there was no critical ($p < 0.05$) change seen in young green leaves put away in chilled environment. Lessening of this level could be because of the oxidation of ascorbic acid in the duration of storage period. Speed of reduction in ascorbic acid substance was fundamentally ($p < 0.05$) more in the examples put away in paper and cling wrap as contrast with tests put away in LDPE. Similar findings were reported by Kaur et al.,(2011)They observed higher level of ascorbic acid in fresh cut spinach packed in LDPE than in PP packed samples. Storage temperature also affected the ascorbic acid content. Higher content of ascorbic acid was seen in the samples put awayin refrigeration as compare to ambient condition. The decline in ascorbic acid during storage conditions has likewise seen in dehydrated fenugreek as well as savoy beet leaves (Negi et al., (2001a) (2001b) and dehydrated curry and drumstick leaves; Singh et al., (2010), and dried out spinach, mint, coriander, amaranthus, and Bengal gram leaves Singh et al., (2003). Flavonoids have a wide range of compound and bioactivity, which includes free radical scavenging activity. Flavonoids are found generally in plant realm and normally in leaves, flower tissues, and pollens. In all of the examples analysed in the current review, the overall flavonoid amount was all together ($p< 0.05$) decreased in all methods. Regardless, fewer differences were seen in example pressed in LDPE than stuffed in paper and cling wrap.The findings are in coherence with Sharma et al., (2018) in which impact of packaging material and storage condition were evaluated on the shelf-life of basil leaves. During the study, it was observed that sample packed in LDPE and stored at 5 °C showed the less changes in flavonoid as compared to sample stored in open trays. Overall phenol amount of all examples was seen to reducein the duration of storage conditions. Complete phenol amount of all examples; new young leaves (4 cm), mature leaves (8 cm), and over mature leaves (12 cm) was diminished progressively with the expansion away duration. Pace of diminishing in complete phenolic content was less in examples stuffed in LDPE followed by cling and paper or a time of 5 days.The possible reason for this trend isthe alleviation in respiration rate due to the modified atmosphere (high CO2 and less O2) formed by the LDPE packaging. Additionally, maintenance antioxidant components were seenhigher in green leaves stored in LDPE in all examples. The pattern can also be related to the maintenance of overall phenolic and flavonoid amounthigher in the specimen packed in LDPE.

From each of the three materials for packing, better maintenance of value was seen in LDPE. Accordingly, it was utilized for packing, and conditions for storage were updated by RSM.

4.1 Optimizing the conditions for storage

4.2 Suitable model

Table 4.4 displays the design matrix for moisture content, chlorophyll content, ascorbic acid content, DPPH scavenging characteristics, overall phenolic content, and total flavonoid content. The 17 experimental runs, each with three independent variables, were created to optimise the storage conditions for radish leaves. In order to evaluate the model's applicability and importance, a second order polynomial equation was used.The model F-value for amount of moisture, chlorophyll value, ascorbic acid, DPPH scavenging properties, overall phenols and total flavonoids were 124.54, 279.27, 88.85, 32.13, 33.26 and 14.39 respectively showing the significance of model. The relevance of model terms is demonstrated by the p-value of 0.05 for model, linear, interaction, and quadratic parameters. All of the response variables showed non-significant lack of fit, demonstrating how well the polynomial model fulfils all of the design criteria. Higher R^2, adjusted R^2, and anticipated R^2 values demonstrated the statistical significance, dependability, and accuracy of the quadratic model.

4.3 Effect of independent variables on response variables

4.3.1 Moisture/Weight loss

Moisture value is among mainboundaries to keep the newness of produce specially in leafy vegetables. Freshly harvested produce are very easy tolose the moisture which results in weight reduction. Henceforth, studying the weight reduction percentage is essential for producein the period of storing. Weight reduction changes depending on vegetable, heat, packing material and the humidity conditions of storing area; Watada et al.,(1987). The results and moisture value are shown in Fig 4.1. The range of moisture content is 72.75 to 94.45%. ANOVA for the fitted moisture model shows significance ($P<0.0001$), whereas the unfit model is not significant ($P>0.5$) (Table 4.5). The coefficient of determination for the response surface regression model on moisture ($R^2=0.9938$) is ideal.

Leaf length makes critical positive quadratic difference ($P<0.0001$) that addresses lesser reaction in middle worth ofleaf length. Moreover, heat conditions of the storing compoundmake a huge negative direct difference ($p<0.05$) addressing decline in the content of moisture satisfied with rise in heat. Not with standing, critical positive quadratic impact ($P<0.0001$) was shown that addresses lesser reaction at the middle worth of leaf length as displayed in Fig 4.1. The conceivable justification for this pattern may be connected with moisture loss through transpiration. These outcomes have relationship with Pandrangi et al., (2004) which detailed the mass reduction in leaves of spinach with expansion in heat. Impact of storing period additionally seen on amount of moisture and negative critical direct impact ($P<0.0001$) was seen that show the amount of moisture in leaves get diminishes with more in time. Nonetheless, critical positive quadratic impact ($P<0.001$) was seen that addresses lesser reaction in middle worth of storing period. Loss of moisture from leaves in the storing period prompts withering in leaves. Loss of water from leaves on happen because of transpiration through stomata Yehoshua et al., (2002). Comparable outcomes were seen by Agüero et al., (2011) in which weight reduction in lettuce leaves expanded with storage period because of loss of moisture. At the point when the impact of leaflength and storing period seen on amount of moisture at the connection, it was observed to give critical ($P<0.05$) negative cooperation. Also, storingheat and periodgave huge ($P<0.01$) negative connection at cooperation point.

$$Moisture\ loss\ (\%) = 77.4 - 1.25\,B - 7.72C - 1.4\,AC - 2.28\,BC + 11.12A^2 + 4.52\,B^2 + 3.22\,C^2$$

4.3.2. Chlorophyll

Chlorophyll is the green variety color present in the plant leaves. Vegetables wealthy in chlorophyll incorporate spinach, beet tops, broccoli, collard greens etc. After the gathering of green vegetables, chlorophyll losshappens. The decline in the value of chlorophyll is related to cell decomposition or potentially senescence that is in many cases utilized assess loss of quality in green vegetables Hodges et al., (2000). In this reportrange of chlorophyll in every one of the plants was seen in 21.02 - 41.26%. ANOVA for the model of value of chlorophyll as fitted gave critical ($P<0.0001$) as well as the absence of fit is non-significant ($P<0.05$) (Table 4.5). Reaction surface relapse model on value of chlorophyll perfectly fits with determination coefficient (R2=0.9972). Leaf length showed huge negative quadratic impact ($P<0.0001$) which addresses increased reaction in middle worth ofleaf length portraying expansion in chlorophyll satisfied with expansion in the length of surrenders to a point then, at that point, decline (Fig. 4.2). These outcomes are in relationship with Albornoz and Cantwell (2015) in that they detailed most noteworthy value of chlorophyll in mid mature leaves. In any case, lessvalue was seen in over-matured leaves of kale. Moreover, heat of the storing conditions showed critical negative quadratic impact ($P<0.0001$) that addresses increased reaction in middle worth of heat of storing conditions comprising decline in chlorophyll value satisfied with increase in heat. Value of chlorophyll reductions with expansion in dehydration heat. Huge decrease in value of chlorophyll during the long haul has likewise been accounted for by Albornoz and Cantwell(2015)

Impact of storing span was additionally seen on value of chlorophyll and negative critical direct impact ($P<0.0001$) was seen that show value of chlorophyll of leaves will diminish with expansion of time. The pattern is connected similarly as time because of the transpiration moisture amount diminishes which advances the deterioration of chlorophyll and speed up leaf decoloring. Comparative perceptions were given by Li et al., (2018) for the storing conditions of green leaves. Comparable outcomes were observed by Raya et al., (2015) in their examination they observed diminishing in the value of chlorophyll in both youthful as well as developed plant leaves of Clinacanthus nutans. Yamauchi and Watada (1993) also noted a decrease in the value of chlorophyll; they noted that the decrease in chlorophyll in parsley increased to 64 percent after 5 days of storage at 200C, from 40 percent during the first 3 days of storage. Finally, there was a negative cooperation with the storage of the leaves.

$$chlorophyll\ content\ (mg/100gm) = 32.99 - 6.43C - 0.52AC - 5.92A^2 + 4.68B^2 + 3.15C^2$$

4.3.4 Ascorbic acid:

One of the many nutrients found in food, ascorbic acid, is one of the most highly labile. It swiftly disintegrates when dried out and is particularly delicate to heating. When this nutrient is exposed to heat and air, it oxidizes fast. Ascorbic acid in this study ranged between (18.31-35.43 percent). Ascorbic acid model fits as critical ($P < 0.0001$) and absence of fit as non-significant ($P < 0.05$) in an ANOVA (Table 4.5). With a determination coefficient of $R^2=0.9913$, the reactive surface relapse model for ascorbic acid provides an excellent fit. Leaf length exhibits a significant negative direct difference ($P<0.01$) showing a decrease in ascorbic acid content with increasing leaf length (Fig. 4.2).Not with standing, huge negative quadratic impact ($P<0.0001$) was seen that addresses bigger reaction at the middle worth of leaf length portraying expansion in ascorbic acid with expansion long of leaves and afterward diminishing. Moreover, critical positive quadratic impact ($P<0.01$) was seen with storage temperature which addresses lower reaction at the middle worth of heat conditions of the storing environment. Outcomes are in relationship with Bergquist et al.,(2006). In their research, they found that immature spinach leaves (*Spinacea oleracea*) stored at 20°C experienced a less pronounced decline in ascorbic acid content than leaves stored at 100°C.

Ascorbic acid content was also affected by storage time, and a negative critical direct impact ($P<0.001$) was observed, indicating that ascorbic acid content in leaves will decrease over time.

A huge decrease in ascorbic acid substance of vegetable leaves in the duration of storing has been accounted for in writing (Gupta et al., 2013), (Joshi et al., 2010) and (Vyankatrao et al., 2014). Impact of leaf length and storage period seen on the ascorbic acid substance in association, it was observed to have critical ($P<0.05$) negative connection. Similar outcomes were seen by Raya et al., (2015). When exposed to longer durations of storage, ascorbic acid in both the young (mean impact of 0.38250a mg/100g) and developed (mean impact of 0.35667amg/100g) leaves of the plant *Clinacanthus nutansa* decreased.Be that as it may, the decrement was continuous in developed leaves when contrasted with more youthful leaves which showed fast decrement.

$$Ascorbic\ acid\ mg/100gm = 32.29 - 1.39A - 2.84C - 0.825AC - 8.35A^2 + 1.21B^2$$

4.3.5 DPPH scavenging property

DPPH scavenging property is connected with cell reinforcement capability of food items. The body produces free radicals, which are anticipated to be combatted by cell reinforcements because they hasten the development of diseases including hyper pigmentation disorder, atherosclerosis, diabetes, and cancer. Results for the DPPH scavenging movement are shown in (Fig. 4.3). According to the DPPH restraint model's fitted importance (P0.0001), DPPH seeking action varies from 33.04 to 42.66 percent ANOVA, and the lack of fit is not significant ($P<0.05$) (Table 4.5). The determination coefficient ($R^2=0.9764$) and the response surface relapse model on DPPH hindrance are excellently matched. With an increase in leaf length, there is a significant positive direct difference ($P< 0.001$) that shows an expansion in the DPPH scavenging property.In any case, critical negative quadratic impact ($P<0.001$) was seen that addresses bigger reaction in middle worth of leaf length. As indicated by Pandajaitan et al ., (2005), the mid leaves had a lot of more elevated amounts of complete phenols and all out flavonoids than youthful and mature leaves. Mixtures are answerable for scavenging property thus; DPPH scavenging property is more in mid-mature condition. In their review, mid development spinach leaves gave more significant amounts of cell reinforcement possibility than young and mature leaves. Moreover, critical positive quadratic impact ($P<0.01$) was shown by storage temperature which addresses lower reaction at the middle worth of heat of the storing conditions.Conceivable justification for less free radicals scavenging property might be the degradation of structure of phenols in view of the exposure to increased heat. These outcomes are in relationship with Madau et al., (2015). Additionally, the impact of the storage duration was observed on the ability of the leaves to remove DPPH, and a negative big direct impact ($P<0.001$) was found, demonstrating that this ability declines over time. Results resemble those of Raya et al., (2015)In their analysis, they found that storage conditions caused a gradual decrease in the DPPH scavenging ability of plant C. nutans passes, and that after only 4 days of storage, the DPPH activity is reduced to nearly 69 %. When the influence of leaf length, storage time, and temperature were examined at the collaboration point, a significant ($P<0.05$) negative relationship was found.

$$DPPH\ scavenging\ activity\ (\%) = 39.73 + 2.11A - 3.03C - 1.11AC - 0.9225BC - 2.45\,A^2 + 1.53B^2$$

4.3.6 Phenols:

Phenols are the plant components which provide different medical advantages from human point of view, for example, mitigating, cancer prevention agent, antiproliferative exercises. The outcomes for the all-out phenolics are displayed in (Fig. 4.4). The total phenolic of radish leaves in this study varies from 563-667 mg GAE/100gm. ANOVA for the fitted phenol model exhibits substantial ($P<0.05$) and non-critical ($P<0.05$) absence of fit. $R^2 = 0.9772$ demonstrates the phenomenal fit of the reaction surface relapse model on phenols. With an increase in leaf length, there is a significant decrease in phenol levels ($P<0.05$), which is represented by the leaf length. Additionally, a significant negative quadratic influence

($P<0.5$) that addresses a larger reaction at the midway value of leaf length was observed. These leaves' stage of growth affects the total phenols. Mid-development stage leaves displayed the highest levels of total phenols in comparison to immature and over-mature leaves, according to M'rabet et al., (2017).The production of auxiliary metabolites, which are absent in young leaves and present in lesser amounts in more established leaves, is the cause of this in mid-mature leaves. Additionally, heat-related storage circumstances have a significant negative straight difference ($p<0.5$) that includes a decrease in total phenols with an increase in heat. Possible explanations for this include phenolics' structural breakdown as a result of increased heat exposure. Additionally, huge negative quadratic impact ($P<0.5$) seen that addresses higher reaction at the middle worth of storing heat. This out comeis in relationship with Rickman et al.,(2007) which detailed quick debasement in phenol value of spinach leaves with expansion in heat. The impact of storage time was also observed on total phenolic content, and a negative significant direct effect ($P< 0.05$) was found, demonstrating that total phenols in leaves decrease with increasing storage time. However, a significant positive quadratic influence ($P<0.5$) was observed that takes into account a lower reaction in the middle of the storage period. It has been suggested that the breakdown of total phenols during storage could possibly be attributed to responsiveness to oxidation. These investigations support the conclusions made by Rickman et al. ,(2007).

$$\text{Total Phenolics}\left(\text{mg}\,\frac{\text{GAE}}{100\text{gm}}\right) = 651.5 - 9.34A - 9.14B - 28.61C + 8.75BC - 39.36A^2 - 8.77B^2 + 9.00C^2$$

4.3.7 Flavonoids

Flavonoids have been accounted for to apply many natural activities like cancer prevention properties, cardioprotective impacts, calming and anticarcinogenic impacts. To be sure, flavonoids act as a well spring of potential bioactive compounds that have promising medical advantages. The outcomes for flavonoids are displayed in (Fig 4.4). The range of flavonoids is (965–1026) (mg quercetin/100gm). ANOVA for the flavonoid model that is fitted demonstrates significance (P< 0.001) while the lack of fit is non-significant (P< 0.05). (Table 4.5). Amazingly, the flavonoid response surface relapse model (R^2=0.9487) matches the coefficient of determination.

Length of leaves showed critical negative quadratic impact (P<0.05) addressing higher reaction at the middle worth of leaf length. Gotten findings are like consequence of Pandajaitan et al ., (2005) which announced that leaves ought to be reaped in mid development stage for customers to profit from the raised degrees of well being advancing flavonoids. Comparative examinations are accounted for by Wongsen et al.,(2013).Besides, heat conditions of the storing environment make a huge negative straight difference (p<0.5) comprising decline in flavonoids with increased heat. Additionally, the impact of storage time was seen on flavonoids, with a negative large linear effect (P< 0.05) showing that the total phenolic content of leaves decreases over time. However, a crucial positive quadratic influence (P<0.5) was observed, which addresses a smaller reaction in the middle of the storage period. The flavonoids were found to demonstrate a strong (P< 0.05) positive correlation at the location when the impact of leaf length and heat storage was observed. However, at the collaboration point, a strong negative correlation between storage temperature and time (P<0.05) was observed.

$$Flavonoids\left(\mathrm{mg}\frac{\mathrm{quercetin}}{100\mathrm{gm}}\right)$$
$$= 1020.27 - 8.63\mathrm{B} - 17.75\mathrm{C} + 12.25\mathrm{AB} - 11.5\ \mathrm{BC} - 14\ A^2 + 1.74\ C^2$$

4.3.8 Selection of optimum condition

Design expert software 7.0 was utilized to enhance the circumstances for radish leaf storage. The length of the leaves (4–12 cm), the storage temperature (4–22°C), the storage time (24–120 hrs), and the free reactions (moisture content, DPPH scavenging activity, ascorbic acid, chlorophyll content, total phenols, and flavonoids) were kept close enough to each other for mathematical improvement.A higher degree of various factors was obtained using the intended function's approach. Ascorbic acid (35.37 mg/100 g), chlorophyll content (48.795 mg/100 g), total phenols (684.025 mg GAE/100 g), and total flavonoid (1040.066 mg quercetin/100 g) were shown to have the highest incentives, with moisture content (94.44 percent), DPPH scavenging action (45.32 percent), length of leaves (8.55 cm), and storage temperature (4°C),

4.3.9 Verification of optimized value

By conducting studies under ideal conditions (leaf length of 8.55 cm, storage temperature of 4°C, and storage time of 24 hours), the health of this model was investigated. Moisture content (94.44 percent), chlorophyll (48.795 mg/100gm), total phenols (684.025 mg GAE/100gm), flavonoids (1040.066 mg quercetin/100gm), ascorbic acid (35.37 mg/100gm), and DPPH scavenging actions are the qualities for the various reactions specified by programming (45.32 percent). (Table 4.6) shows the analysis results we obtained, which are within 94% of the predicted values. This demonstrates the model's plausibility.

Table 4. 1: Treatment combinations with their responses for baby leaves (4 cm) at different temperature.

Leaves /Temp.	Days	Packaging material	Moisture (%)	Ascorbic acid (mg/100g)	Antioxidant (%)	Total phenols (mg GAE/100g)	Flavonoids (mg quercetin/100 g)	Chlorophyll (mg/100g)
4cm (baby leaves) / 4^{o}C	1st day		89.33±0.57^{a}	26.59±2.00^{a}	36.8±1.12^{a}	623.04±12.4^{a}	1023.28±33.8^{a}	27±0.75^{a}
	3rd day	Paper	82.59±0.18^{bq}	25.76±1.06^{abp}	34.26±0.92^{bq}	611.42±6.3^{cq}	981.13±38.4^{bq}	21.05±0.42^{es}
		Cling	85.74+0.44^{bq}	26.23±1.11^{ap}	36.57±1.41^{ap}	619.94±8.9^{abp}	1006.56±22.3^{dp}	25.56±1.92^{bp}
		LDPE	87.08±1.02^{abp}	26.55+1.64^{ap}	36.09±1.75^{ap}	621.35±7.6^{ap}	1021±29.1^{a}	25.75±1.11^{bp}
	5th day	Paper	74.34 ±0.81^{gu}	20.71±2.32^{ds}	31.02±1.04^{cr}	597.03±13.7^{ds}	702±34.1^{et}	19.13±0.56^{ft}
		Cling	81.66±0.64^{fgu}	25.21±1.04^{bq}	34.01±0.96^{bq}	609.01±8.2^{cr}	809±35.6^{cr}	23.2±0.6^{cq}
		LDPE	84.33±0.89^{eftu}	26.19±1.16^{ap}	34.55±1.00^{bq}	619.11±23.2^{abp}	1014±35.4^{ap}	25.03±0.513^{bp}
4cm (baby leaves) / 22^{o}C	1st day		89.33±0.57^{a}	26.59±2.00^{a}	36.8±1.12^{a}	623.04±32.4^{a}	1023.28±33.8^{a}	27±0.75^{a}
	3rd day	Paper	79.80±1.02^{ds}	24.04±1.65^{cr}	33.063±0.12^{cr}	601.33±3.5^{ds}	988.08±32.4^{bq}	21.02±1.11^{dr}
		Cling	83.19+2.63^{ds}	24.12±1.01^{cr}	34.17±1.08^{bq}	611.14±9.1^{bcq}	1001.02±32.1^{ap}	25.01±1.38^{bp}
		LDPE	84.80±1.02^{cr}	24.36+1.76^{cr}	34.57±1.49^{bq}	619.02±5.4^{abpq}	1019±30.5^{ap}	25.25±1.95^{bp}
	5th day	Paper	70.36 ±0.22^{gu}	18.37±1.27^{et}	31.02±1.04^{cr}	597.03±13.7^{ds}	699±24.8^{fu}	17.28±0.98^{gu}
		Cling	77.17±1.48^{dest}	21.18±0.97^{ds}	34.01±0.96^{bq}	609.01±8.2^{cr}	798±28.7^{ds}	19.75±0.2^{ft}
		LDPE	79.29±w0.59^{ds}	23.98±1.11^{cr}	34.55±1.00^{bq}	616.11±23.2^{ap}	1010±23.8^{ap}	22.13±0.23^{dr}

Duncan's multiple range test was used with Sigma Plot 11.0 software (Sigma Plot Software; San Jose, California, USA) to run a two-way ANOVA at $p< 0.05$. Statistics was considered statistically significant for mean values ($p<0.05$). Results are the average of three replicates, and values are the mean ± standard deviation.

Table 4.2: Treatment combinations with their responses of mature radish (8 cm) leaves at different temperature.

Leaves /Temp.	Days	Packaging material	Moisture (%)	Ascorbic acid (mg/100g)	Antioxidant (%)	Total phenols (mg GAE/100g)	Flavonoids (mg quercetin/100 g)	Chlorophyll (mg/100g)
8cm (mature leaves) / 4ºC	1st day		86.96 ± 1.40^{a}	35.43 ± 1.07^{a}	42.66 ± 1.16^{a}	648.11 ± 13.2^{a}	1027 ± 12.1^{a}	41.26 ± 5.21^{a}
	3rd day	Paper	80.15 ± 2.61^{ds}	31.04 ± 1.04^{cq}	38.27 ± 1.00^{cq}	606.12 ± 14.4^{ds}	1012 ± 10.1^{cq}	31.22 ± 2.51^{es}
		Cling	82.02 ± 2.38^{cr}	31.70 ± 0.61^{bcpq}	40.55 ± 1.23^{bp}	611.92 ± 15.8^{ds}	1008 ± 16.20^{cq}	37.34 ± 2.92^{bcpq}
		LDPE	84.19 ± 1.16^{bp}	32.84 ± 1.16^{bp}	41.31 ± 1.47^{bp}	645.27 ± 12.02^{abpq}	1024 ± 17.54^{abp}	39.16 ± 2.12^{abp}
	5th day	Paper	76.46 ± 3.42^{fu}	22.19 ± 2.98^{gt}	36.21 ± 1.16^{ders}	545 ± 13.1^{gv}	929 ± 19.8^{ft}	28.56 ± 1.42^{ft}
		Cling	79.16 ± 2.46^{cr}	27.02 ± 3.54^{es}	40.39 ± 1.29^{bp}	578 ± 13.6^{et}	982 ± 14.5^{dr}	34.02 ± 1.23^{dr}
		LDPE	82.22 ± 2.81^{cq}	31.75 ± 3.23^{bpq}	39.21 ± 1.16^{cq}	634 ± 16.1^{bcqr}	1021 ± 23.4^{c}	36.11 ± 4.04^{cq}
8cm (mature leaves) / 22ºC	1st day		86.96 ± 1.40^{a}	35.43 ± 3.04^{a}	42.66 ± 1.16^{a}	648.11 ± 23.2^{a}	1027 ± 22.1^{a}	41.26 ± 5.21^{a}
	3rd day	Paper	77.43 ± 1.11^{ds}	24.63 ± 4.53^{f}	33.45 ± 1.01^{ft}	626.11 ± 12.9^{cr}	993 ± 6.1^{dr}	30.22 ± 2.17^{e}
		Cling	79.61 ± 1.54^{cr}	30.03 ± 2.71^{cdqr}	37.01 ± 1.58^{dr}	632.19 ± 10.1^{cr}	1005 ± 9.03^{cq}	34.14 ± 1.22^{dr}
		LDPE	81.31 ± 2.06^{cq}	30.62 ± 1.07^{cdqr}	37.31 ± 1.24^{cdqr}	640.02 ± 7.55^{abpq}	1019 ± 5.24^{bcpq}	36.11 ± 1.52^{cq}
	5th day	Paper	68.42 ± 2.09^{fu}	22.41 ± 0.99^{gt}	25.99 ± 1.48^{hv}	544 ± 10.7^{gv}	902 ± 11.7^{gu}	28.56 ± 1.42^{ft}
		Cling	70.16 ± 0.91^{et}	29.02 ± 1.84^{dr}	32.03 ± 1.77^{gu}	556 ± 9.8^{fu}	949 ± 20.1^{es}	30.02 ± 4.23^{es}
		LDPE	72.75 ± 3.47^{et}	29.15 ± 1.66^{dr}	35.52 ± 1.51^{es}	628 ± 13.8^{cr}	1015 ± 14.7^{cq}	36.01 ± 4.04^{cq}

Duncan's multiple range test was used with Sigma Plot 11.0 software (Sigma Plot Software; San Jose, California, USA) to run a two-way ANOVA at p 0.05. Statistics was considered statistically significant for mean values ($p<0.05$). Results are the average of three replicates, and values are the mean ± standard deviation.

Table 4.3: Treatment combinations with their responses of over mature radish leaves (12 cm) at different temperature

Leaves /Temp.	Days	Packaging material	Moisture (%)	Ascorbic acid (mg/100g)	Antioxidant (%)	Total phenols (mg GAE/100g)	Flavonoids (mg quercetin/100 g)	Chlorophyll (mg/100g)
12cm (over mature leaves) / 4ºC	1st day		83.66±1.52^{a}	24.16±2.27^{a}	41.23±1.31^{a}	546.24±18.4^{a}	1017.19±16.5^{a}	35.6±1.34^{a}
	3rd day	Paper	79.33±3.40^{es}	20.75±1.93^{cq}	34.25±0.85^{cr}	519.84±9.6^{bcqr}	974±15.6^{dr}	24.16±1.46^{es}
		Cling	80.48±1.13^{cdqr}	22.03±2.41^{bp}	35.87±2.04^{cr}	524.60±10.7^{bq}	996±17.1^{bp}	32.36±1.98^{abp}
		LDPE	82.62±2.02^{bp}	23.27±3.11^{bp}	40.11±1.91^{ap}	541.11±9.7^{ap}	1010±15.9^{abp}	34.18±1.56^{abp}
	5th day	Paper	74.26±2.02^{gu}	15.90±0.96^{ft}	24.01±0.96^{et}	504.91±8.2^{ds}	917±18.2^{ft}	21.41±1.17^{ft}
		Cling	76.179±2.19^{gu}	19.42±1.64^{cdqr}	31.76±0.63^{ds}	513.84±15.8^{cr}	994±14.1^{bp}	28.30±2.03^{des}
		LDPE	80.01±2.27^{cq}	21.53±1.63^{bp}	33.04±0.52^{cq}	521.04±9.2^{bcr}	1002.50±31.2^{abp}	30.56±0.65^{bp}
12cm (over mature leaves) / 22ºC	1st day		83.66±1.52^{a}	24.16±2.27^{a}	41.23±1.31^{a}	546.24±18.4^{a}	1017.19±26.5^{a}	35.6±1.34^{a}
	3rd day	Paper	75.33±3.40^{es}	16.15±4.13^{es}	31.94±0.85^{ds}	511.84±13.2^{cr}	950±15.6^{es}	19.03±1.81^{es}
		Cling	77.43±1.83^{ft}	19.27±1.19^{cdqr}	34.73±1.48^{cr}	522.31±17.1^{bq}	988±17.1^{cq}	25.36±2.32^{des}
		LDPE	79.62±2.02^{dr}	21.36±1.51^{bp}	37.60±1.34^{bq}	530.31±12.7^{bq}	1006±11.3^{ap}	27.41±2.41^{cq}
	5th day	Paper	70.29±2.02^{gu}	13.01±0.96^{ft}	21.01±0.96^{et}	324.17±11.4^{fu}	909±22.8^{ft}	13.94±1.67^{gu}
		Cling	73.179±2.19^{gu}	16.71±3.14^{es}	28.14±0.63^{ds}	436.11±12.0^{et}	942±14.1^{es}	19.42±1.44^{ft}
		LDPE	75.33±2.27^{es}	18.31±1.63^{dr}	30.11±0.52^{cr}	503.04±14.6^{ds}	966±10.1^{dr}	22.04±2.45^{dr}

Duncan's multiple range test was used with Sigma Plot 11.0 software (Sigma Plot Software; San Jose, California, USA) to run a two-way ANOVA at $p < 0.05$. Statistics was considered statistically significant for mean values ($p<0.05$). Results are the average of three replicates, and values are the mean ± standard deviation.

Table 4.4 : Summary of design of experiment with responses results against factors

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4	Response 5	Response 6
Std	**Run**	**A:Length of leaves**	**B:Storage temp.**	**C:Storage Time**	**Moisture**	**Chlorophyll**	**Ascorbic acid**	**DPPH scavenging activity**	**Total phenols**	**Flavonoids**
		cm	°C	hours	%	mg/100g	mg/100g	%	mg GAE/100g	mg quercetin/100 g
6	1	12	13	24	84.45	35.6	24.16	41.23	623.24	1014.19
4	2	12	22	72	86.62	32.11	21.36	37.6	563.2	991
9	3	8	4	24	86.96	41.63	35.43	42.66	667	1026.0
10	4	8	22	24	76.65	39.26	32.99	37.66	650	1010.24
17	5	8	13	72	74.15	30.75	31.04	38.27	632.99	1012.0
3	6	4	22	72	89.8	25.25	24.36	34.57	600.02	965.01
12	7	8	22	120	72.75	34.56	29.15	35.52	630	973.0
1	8	4	4	72	87.08	25.75	26.55	36.09	621.35	1021.0
14	9	8	13	72	74.16	30.43	30.94	37.87	642.0	1011.27
5	10	4	13	24	83.06	27.00	26.59	36.8	636.04	1019.0
8	11	12	13	120	82.33	27.54	18.31	33.04	576.04	966.64
16	12	8	13	72	75.67	29.99	29.90	38.01	641.5	1005.99
7	13	4	13	120	85.8	21.02	24.04	33.06	601.33	998.45
2	14	12	4	72	82.62	34.18	23.27	41.11	595.01	1001.0
13	15	8	13	72	73.98	31.00	31.10	37.99	640.01	1014.0
11	16	8	4	120	82.22	36.11	31.75	39.21	634.16	1021.0
15	17	8	13	72	80.09	31.61	32.14	39.2	641.09	1015.91

Table 4.5: Analysis of variance (ANOVA) for quadratic model and lack of fit for moisture content, chlorophyll content, ascorbic acid, flavonoids, DPPH scavenging activity phenols as per Box behnken design

Sources of variation	Moisture (%)			Chlorophyll			Ascorbic acid			DPPH (%)			Total phenols			Flavonoids		
	Sum of squares	F-value	p-value	Sum of squares	F-value	p-value	Sum of squares	F-value	p-value	Sum of squares	F-value	p-value	Sum of squares	F-value	p-value	Sum of squares	F-value	p-value
Model	935.36	124.54	<0.0001	474.61	279.27	<0.0001	372.51	88.85	<0.0001	135.35	32.13	<0.0001	11893.03	33.26	<0.0001	6033.07	14.39	0.001
Linear																		
A	0.03	0.0359	0.855	87.97	465.86	0.0638	10.34	22.2	0.0022	23.83	50.92	0.0002	465.75	11.72	0.0111	3.7	0.0794	0.7863
B	8.37	10.03	0.0158	0.913	4.84	0.4468	2.07	4.44	0.0732	2.11	4.51	0.0715	445.79	11.22	0.0123	396.98	8.52	0.0224
C	160.2	191.96	<0.0001	113.99	603.69	<0.0001	22.22	47.69	0.0002	25.36	54.2	0.0002	2258.57	56.85	0.0001	869.19	18.66	0.0035
Interaction																		
AB	1.85	2.22	0.1802	0.6162	3.26	0.1138	0.0196	0.0421	0.8433	0.99	2.12	0.1891	149.82	3.77	0.0933	600.74	12.9	0.0088
AC	7.81	9.36	0.0183	1.08	5.73	0.0479	2.72	5.84	0.0463	4.95	10.58	0.014	39	0.9816	0.3548	182.25	3.91	0.0884
BC	20.7	24.81	0.0016	0.3481	1.84	0.2167	1.17	2.5	0.1576	3.4	7.27	0.0308	306.25	7.71	0.0275	529	1.36	0.0119
Quadratic																		
A^2	520.35	623.53	<0.0001	147.65	781.93	<0.0001	293.36	629.72	<0.0001	25.33	54.13	0.0002	6521.41	164.15	<0.0001	825.5	17.72	0.004
B^2	85.99	103.05	<0.0001	92.15	488.02	<0.0001	6.14	13.19	0.0084	9.82	20.99	0.0025	323.68	8.15	0.0245	27.85	0.598	0.4647
C^2	43.57	52.21	0.0002	41.67	220.66	<0.0001	1.51	3.23	0.1153	0.1987	0.4246	0.5354	341.03	8.58	0.022	12.72	0.273	0.6174
Residual	5.84			1.32			3.26			3.28	2.4		278.11			326.07		
Lack of fit	3.79	2.46	0.2021	0.4705	0.7369	0.5825	0.7391	0.3907	0.7669	2.11		0.2087	222.69	5.36	0.0693	270.26	6.46	0.0517
Pure error	2.05			0.8513			2.52			1.17			55.42			55.8		
Cor total	941.2			475.93			375.77			138.63			12171.14			6359.14		
Parameters used for adequacy of model																		
R^2	0.9938			0.9972			0.9913			0.9764			0.9772			0.9487		
Adjusted R^2	0.9858			0.9937			0.9802			0.946			0.9478			0.8828		
Predicted R^2	0.9322			0.9814			0.958			0.7348			0.7001			0.3063		
C.V (%)	1.1			1.38			2.44			1.8			1.01			0.6792		
Adeq. Precision	29.7496			62.1475			32.5084			19.4832			21.3641			11.6113		

Table 4.6: **Comparison between Predicted and Experimental value at Optimized condition (length of leaves 8.55 cm, storage temperature 4℃ and storage time, 24hours**

	Moisture content (%)	**DPPH scavenging activity (%)**	**Ascorbic acid (mg/100gm)**	**Chlorophyll (mg/100g)**	**Total phenols (mg GAE/100gm)**	**Total flavonoid (mg quercetin/100gm)**
Predicted	94.44	45.32	35.37	48.795	684.025	1040.066
Experimental	86.96	48.65±0.95	33.87±1.75	46.98±0.86	658.43±5.3	1017±8.7

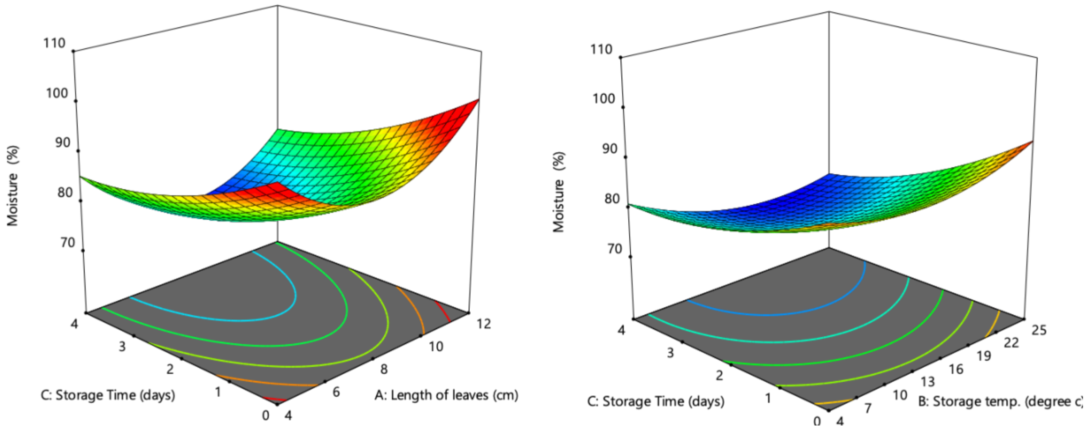

Fig. 4.1: Response surface curve on 3 D plot for Moisture content

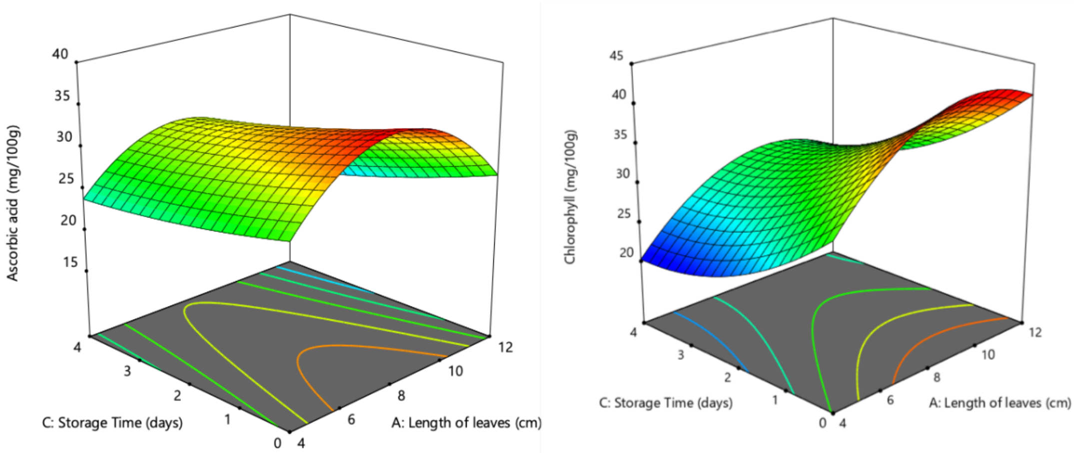

Fig.4.2: Response surface curve on 3 D plot for Ascorbic acid and chlorophyll

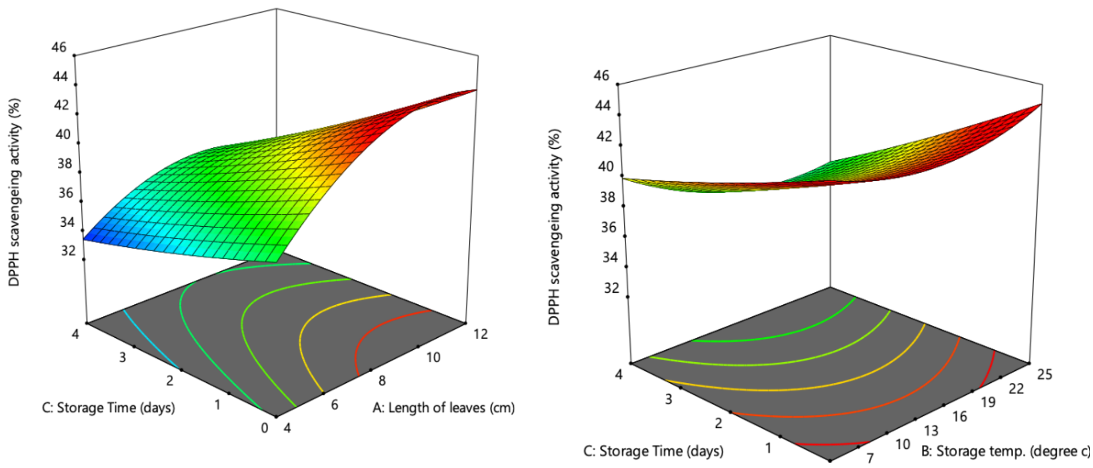

Fig.4.3: Response surface curve on 3 D plot for DPPH scavenging activity

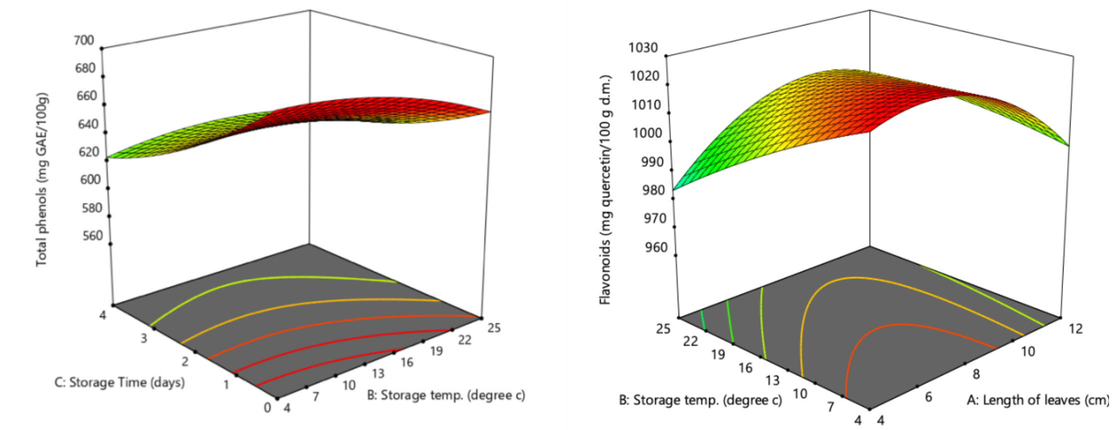

Fig.4.4: Response surface curve on 3 D plot for total phenols and flavonoid content

4.4 Proximate composition of radish leaves(8cm)

Table depicts the proximate composition of radish leaves (8.00 ±0.62 cm) exhibited moisture content (86.96 g/100g), ash content (1.92g/100g), carbohydrates (5.07g/100g), proteins (4.02 g/100g) and fat (0.91g/100g). Similar results were depicted by Joshi and Bains, (2019) who reported the proximate composition of fresh radish leaves (per 100g) contains 2.22 g of protein, 0.51 g fat, 2.77 g carbohydrates and 1.82 g crude fibre .

Table 4.7 Proximate composition of radish leaves

S.No.	Parameter	Values
1	Moisture (g/100g)	86.96±0.16
2	Carbohydrates(g/100g)	5.07±0.29
3	Protein(g/100g)	4.02±0.06
4	Fat(g/100g)	0.91±0.09
5	Ash(g/100g)	1.92±0.11
6	Crude fiber(g/100g)	0.74±0.08
7	Beta-carotene (mg/100g)	3.91±0.18

Values are mean ± standard deviation

4.5 Comparative analysis of Drying curve in tray dying

Figure (4.2) represents the comparison of drying curves (time vs MR) obtained during tray drying of radish leavesat 45°C, 55°Cand 65°C. The differences in moisture content at all the studied temperatures were more given in the first drying stages, However, in the later phase of drying, it showed a falling drying rate.This is due to the resistance offered by the upper hard layer which get formed during the first drying phase. As a result of this layer, moisture diffusion rate get decreases which affect the drying rate in the last drying phase.

Additionally, significant change in total drying time was seenduring the drying at different temperature. The time of drying in tray dryer was 440, 380 and 320 min at 45°C, 55°C, and 65°C respectively. As expected, from the investigated temperature range the time of drying is the longest at 45°C and shortest at 65° C.

The reason behind this trend is that in tray drying method, vapour pressure in sample increases with rise in temperature which leads to greater pressure difference between the sample's inner and outer surfaces. Thus, when greater temperatures were used to dry various natural goods, vegetables, and crops, drying rates significantly increased. Similar drying patterns were seen in the research conducted by various authors, who reported significant increases in drying rates when diverse organic goods, vegetables, and crops were dried at higher temperatures. These included Erenturk et al., (2004) for rosehip, Lee et al., (2004) for chicory root cuts, Madamba et al., (1996) for garlic, and Akpinar (2006) for fragrant rosehip plants.

This is based on thermodynamic behaviour, which results in the same drying performances for different food matrices due to the higher inner vapour pressure driving force.Also, it is apparent that increase in drying temperature significantly decreased the drying time. The justification for this could be, expanded air-drying temperature prompts a superior temperature slope and surface evaporation rate, speeding up moisture diffusion from the middle to the surface. These outcomes arein accordance with another studydone by Akpinar et al., (2003) and, Aksoy et al., (2019) where the reduced drying time was resultedin increase of the air-drying temperature.

4.5 Modelling of drying curve

To illustrate the dehydration changes in biological products, the moisture content data from drying was transformed into a dimensional moisture ratio and fitted to a few simple dehydration models. For the model's fitting requirements, the starting moisture content was considered to be of utmost importance. To fully comprehend the drying process (moisture ratio), 11 mathematical models, including Newton, Page, Henderson and Pabis, Modified Henderson and Pabis, Logarithmic, Singh model, Midillii model, Two term, Two term exponential, and Wang and Singh, were assessed. These models are listed in Table 4.1. The drying experiment's moisture content data were fitted to the parameters, and the parameters' validity was determined by the fitted data's highest R^2, lowest Chi-square, and lowest root mean square error values (RMSE). The R^2, RMSE, and Chi-square values for the tray dryer have been demonstrated to range from 0.884 to 0.998, 0.014 to 0.14, and 0.0002 to

0.009, respectively.Among the twelve models taken into consideration for tray drying, the Midilli model, followed by the Page Model, produced the greatest R^2 values as well as the lowest Chi-square and root mean square error values. This model seems to be the one that best describes how radish leaves dry out.The highest value of the determination coefficient and lowest value of χ^2 and RMSE were found at 55°C temperature. The Midilli model gave a comparatively higher value of R^2 in all the cases, and also RMSE and χ^2 values were lower in this case. Thus, this model may be represented as the most suitable model to study the drying behaviour ofradish in tray dryer. These findings are in line with those of Masud et al., (2021) and Meziane (2011), who found that the Midilli model offered the highest coefficient of determination and the lowest values of χ2and RMSE for modelling the drying kinetics of potato and olive pomace, respectively, by fluidized bed dryer. This showed that, out of the twelve models chosen, these models could explain the drying process more effectively.

Table 4.8: Mathematical expressions that represent fitted dehydration models.

Sl. No.	Mathematical expression	Dehydration Models	Nomenclature
1.	MR= exp(-ktn)	Page	MR - Moisture Ratio t - time, sec a, b, c, g, h, n - constants k,ko,k1 - model coefficients L - half-leaf thickness, mm
2.	MR= exp(-(kt)n)	Modified Page	
3.	MR= exp(-kt)	Newton or Lewis	
4.	MR= a exp(-kt) + (1-a) exp(-kat)	Two Term exponential	
5.	MR= a exp(-kt)	Henderson and Pabis	
6.	MR= a exp(-kt) + c	Logarithmic	
7.	MR= a exp(-kt) + (1-a) exp(-kbt)	Diffusion Approach	
8.	MR= a exp(-k0t) + b exp(-k1t)	Two term	
9.	MR = a exp(-kt) + b exp(-gt) + c exp(-ht)	Modified Henderson and Pabis	
10.	MR= exp(-k(t/L2)n)	Modified Page equation-II	
11.	MR=aexp(-ktn) +b	Midillii model	
12.	MR =exp(- (t/a)^b)	Weibull model	

Table 4.9 Results of the selected models statistical analysis

Sr. No	Model Name	Temperature	R^2	RMSE	χ^2
1	Newton model	45	0.970497	0.094232	0.009250
		55	0.977138	0.072973	0.005547
		65	0.984149	0.053645	0.002998
2	Page model	45	0.971085	0.076163	0.006106
		55	0.997710	0.017973	0.000337
		65	0.954717	0.078203	0.006371
3	Modified page model	45	0.970497	0.094232	0.009250
		55	0.977138	0.072973	0.005547
		65	0.984149	0.053645	0.002998
4	Henderson and Pabis	45	0.958514	0.077422	0.006244
		55	0.969781	0.063414	0.004189
		65	0.980828	0.047294	0.002330
5	Modified Henderson and Pabis	45	0.993318	0.032400	0.001093
		55	0.992790	0.031445	0.001030
		65	0.997076	0.016954	0.000299
6	Logarithmic model	45	0.981427	0.047573	0.002357
		55	0.983783	0.042117	0.001848
		65	0.985280	0.037608	0.001473
7	Singh model	45	0.975339	0.060249	0.003781
		55	0.979700	0.050377	0.002644
		65	0.982012	0.044923	0.002102
8	Midillii model	45	0.996905	0.018421	0.000353
		55	0.998292	0.014452	0.000218
		65	0.996536	0.018301	0.000349
9	Weibull model	45	0.977048	0.101244	0.010678
		55	0.884463	0.146760	0.022436
		65	0.921274	0.132857	0.018387
10	Two term model	45	0.958504	0.077422	0.006244
		55	0.969778	0.063414	0.004189
		65	0.980829	0.047294	0.002330
11	Two term exponential model	45	0.968274	0.068355	0.004867
		55	0.973556	0.058986	0.003624
		65	0.980105	0.050938	0.002703
12	Wang and Singh model	45	0.963574	0.068907	0.004946
		55	0.989840	0.045913	0.002196
		65	0.966846	0.066721	0.004637

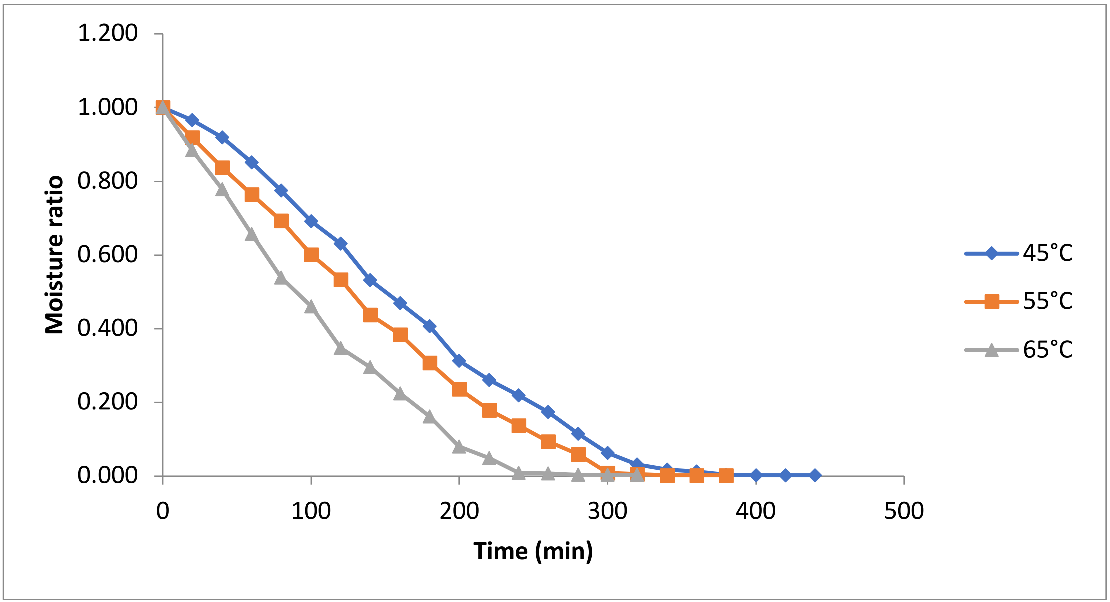

Fig. 4.5 Moisture ratio vs time at different time interval for different temperature

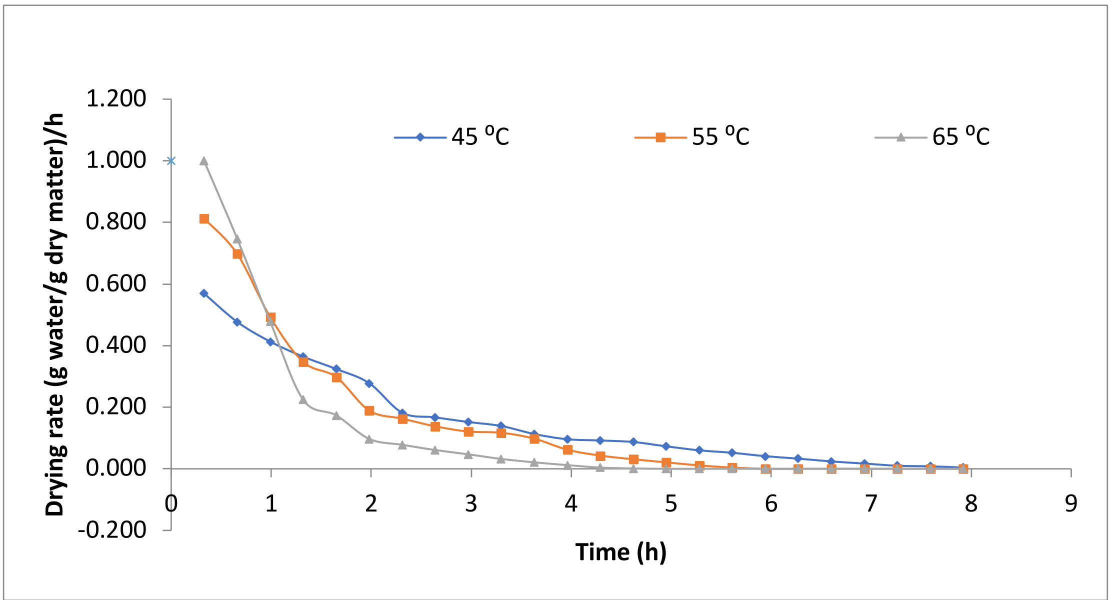

Fig. 4.6 Drying rate vs time at different temperature

4.6 Effect of Temperature on Effective Moisture Diffusivity (D_{eff})

Effective moisture diffusivity (D_{eff})is defined as the rate of movement of water with respect to time during the drying of food. It is quite essential to define effective moisture diffusivity to understand the physical and thermal properties of food products. The obtained D_{eff} values for the radish leaves tray dryerwere observed as 3.74651×10^{-11} m²/s, 4.52562×10^{-11} m²/s and 6.2847×10^{-11}m²/s at 45°C, 55°C, and

65°C respectively. Also, it can be seen from the above-mentioned values that D_{eff} is temperature dependent that increases logarithmically with increase in temperature i.e. lowest at 45°C and highest at 65°C respectively.

The possible reason for this trendcould be the higher water mobility from interior to the surface of food system at higher drying temperature which increases the effective diffusivity of mass transfer (Ruiz-Lopez et al., 2012) and also at the higher temperatures of the molecules of water are loosely attached to the food matrix and hence requires less energy to remove as compared to the low temperatures (Xiong et al., 1992). The highest D_{eff} value was obtained when the purple basil leaves were tray-dried at 55 °C in the study by Altay et al., (2019) and Kaya et al., (2009), who investigated the convective method of drying. This was due to the rapid movement of the water to the surface because of the higher evaporation rate at a higher temperature.Also, drying temperature should not exceed with 70°C as it significantly effect antioxidant compounds, thus degrading the quality. The same pattern for effective moisture diffusivity was also showed by Uribe et al., (2011) and Balzarini et al., (2018) during the drying of pepino and chicory roots respectively by hot air oven and vacuum drying.

4.7 Effect of Temperature on Activation energy (E_a)

Activation energy can be defined as the minimum amount of energy required by the water molecules to surpassenergy barriersduring moisture diffusion from inside to outside of the product. Lower activation energy relates to a quick speed of water expulsion from the product (Resende et al., 2011). Activation energy (Ea) was determined from the incline of the plot ln (Deff) versus the corresponding of temperature,1/(T+273.15). Activation energy (Ea) values for tray driedradish leaves was 23.043 kj/mol at 45°C, 55°C, and 65°C respectively. Similar findings were provided by Doymaz et al.,(2011) and Pardeshi et al., (2009) who reported activation energies of 23.20 and 22.48 kj/mol during the drying of sweet potato and peas.

4.8 Analysis of bioactive compounds in tray dryer

Vitamin C

Vitamin C is taken as an index of nutrient quality of processes; Santos et al., (2008). Vitamin C is portrayed by low warm strength and capacity to oxidize effectively, in this way, each interaction utilizing raised temperature causes loss of Vitamin C when contrasted with the new material.Moreover, the important parameters that influence ascorbic acid degradation during processing was found to be moisture and temperature (Villota et al., 1980).The lower losses of vitamin C was seen at 55°C (23.19 ± 1.76%) as compared 45°C (15.43± 1.96 %) and 65°C (19.36 ±0.94). According to research done in collaboration with Kuljarachanan et al., (2009), drying temperature and moisture content were the main variables influencing how quickly vitamin C degraded in lime residues.

Similar findings were made following the heat processing of broccoli flower buds, strawberries, and aloe vera gel (Miranda et al., 2009 ; Gamboa-Santos et al., 2014; Rybarczyk- Plonska et al., 2014).This could be as a result of the extended exposure period and increased water activity at 45°C, which causes ascorbic acid to be damaged by the oxidation of dehydroascorbic acid to 2,3-diketogulonic acid (Washko et al.,1992).

However, at higher temperature (65°C) thermal degradation of ascorbic acid occur due to thermal sensitivity of compounds. Losses in vitamin C due to thermal degradation have been also reported by Miranda et al., (2010) during the drying of quinoa seeds.

Total phenolic content

The result of this study showed that the temperatures significantly affected the total phenolic content of radish leaves during drying at 45°C, 55°C and 65°C. However, a significant decline in TPC was observed during drying. The TPC content in tray dried sample was 502.78 ± 1.4 , 576.47 ± 1.7 and 537.42 ± 0.99 mg GAE/g at 45°C, 55°C and 65°C respectively. This might be due to the longer exposure time at 45°C which can damage the phenolic compounds (Mrad et al., 2012). However, at higher temperature (65°C) thermal degradation of phenolic compounds occur due to the thermal sensitivity of phenolic compounds. Losses of TPC due to thermal degradation have been also reported by Salve et al., (2020) during the drying of curry leaves.

Total flavonoid content

In this study, drying temperature 45°C, 55°C and 65°C significantly affect the total flavonoid content of radish leaves. The TFC content in tray dried sample was 897.42, 955.47 and 915.78 mg QE/g extract , at 45°C, 55°C and 65°C respectively. However, at lowest temperature 45°C due to prolonged time resulted in depletion of flavonoids whereas at higher temperature the loss in flavonoids might be due to thermal instability of flavonoids. Similar results were observed by Tan et al., (2015) during the drying of Morus alba leaves .

4.9 Proximate composition of radish leaves powder

The tray dried (55°C) radish leaves were analysed for its proximate composition . The moisture content of radish leaves powder was found to be 7.55±0.75 % with protein content as around 11.06±0.11% with higher ash content 9.48±0.19 % whereas crude fiber were reported to be in the range of 17.64±0.82 % (Table 4.10). The data reported by Ankita and Prasad (2015) reported the moisture content of radish leaves powder to be 4.03±0.27 %, protein content around 13% , ash content 12.16±0.40 %and crude fiber as 20.63 ±0.11 %.

Table 4.10 Proximate composition of radish leaves powder

	Parameter	(%)
1	Moisture	7.55±0.75
2	Protein	11.06±0.11
3	Fat	3.35±0.14
4	Ash	9.48±0.19
5	Crude fiber	17.64±0.82
6	Carbohydrate	39.13±0.78

Values are mean ± standard deviation

4.10. Phytochemical composition of radish leaves powder

Table (4.10) depicts the phytochemical composition of radish leaves powder. The study revealed that the tray dried (55°C) radish leaves had Vitamin C (23.19±1.76 mg/100g), total carotenoids (24.73±0.77 mg/100g), flavonoids (903±3.78 mg QE/ 100g), phenols (532.74±4.42) and antioxidants (37.48±1.44%). The results were in coherence with Ankita and Prasad, (2015) where in radish leaf powder reported total carotenoids and flavonoids in the range of 26.85 mg /100 g) and (815.45 mg QE per 100 g) respectively. Among different leaf powders such as carrot, cauliflower and turnip, maximum of the antioxidant activity has been studied in radish leaf (87.04%) with phenolic content of Total (527.61 mg GAE per 100 g)

Table 4.11 Phytochemical composition of radish leaves powder

	Parameter	Values
1	Vitamin C (mg/100g)	23.19±1.76
2	Total carotenoid(mg/100g)	24.73±0.77
3	Flavonoids (mg QE/ 100g)	903±3.78
4	Total phenols (mg GAE/100g)	532.74±4.42
5	Antioxidants (%)	37.48±1.44

Values are mean ± standard deviation

4.9 FTIR evaluation

The FTIR range, which is dependent on the peak value obtained in the infrared radiation region, was used to differentiate the phenolic components in the powder of radish leaves that was obtained under streamlined settings. The FTIR spectrum of powdered radish leaves is shown in (Fig. 4.7), with the quality peaking at 3281.98 cm-1, 2922.09 cm-1, 2360.91 cm-1, 1736.83 cm-1, 1632.71 cm-1, 1546.62 cm-1, 1247.43 cm-1, 1021.60 cm-1, 834.10 cm-1, and 532.66 cm-1, respectively. The extended vibration of the O-H bunch, which delineates the hints of phenolic groups in the powder of radish leaves, was proposed by a solid peak identified at 3453.6 cm1. Peaks obtained at 2939.9 cm-1 correlate to C-H extending, which may very well be caused by CH_2 or CH_3 bunches(Alara et al., 2018).

The prolonged peak at 1726 cm^1 indicates the presence of phenolic acids and may be caused by the presence of carboxyl groups (Lu and Hsieh, 2012). The C=O groups, which were primarily associated with phenols, are assigned the ingestion band at 1632.5 cm1. It was anticipated that the groups at 1408.5 cm and 1231.5 cm would each contain COOH and C-O bunches. When plants are under stress, hydrocarbon bunches in radish leaves are known to have antioxidant effects. A peak obtained at 1057.4 cm1 showed C-O extending, confirming their presence (Singh et al., 2012). In fragrance design, the groups present beneath 924.8 cm1 correspond to the C-H bonds.Subsequently, the examination of radish leaves firmly recommends the presence of utilitarian qualities related with polyphenols.

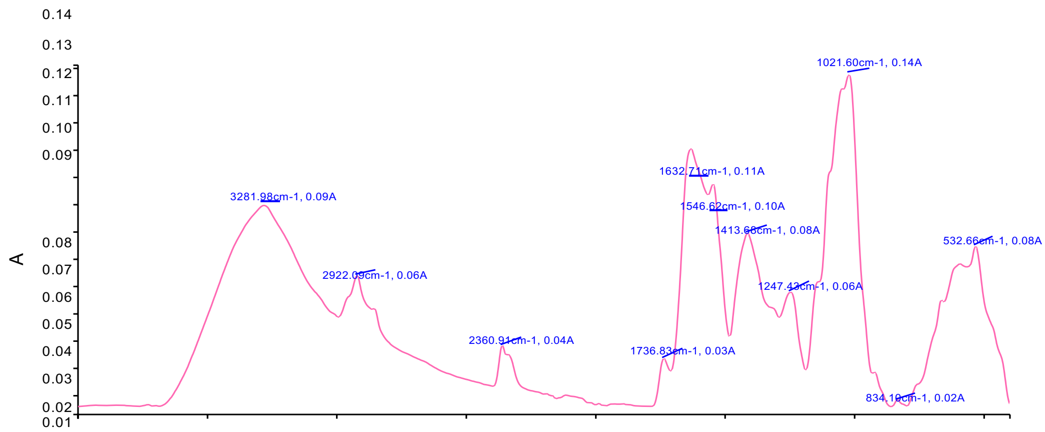

Fig.4.7: FTIR analysis of optimized radish leaves powder

4.10 X-ray diffraction

The effect of drying on radish leaves powder was shown with the help of X-ray diffraction. The X-ray diffractograms of the tray dried sample are given in Fig 4.8). Generally, sharp peaks are shown by crystalline material however, broad background pattern is shown by amorphous product. Major peaks in radish leaves powder sample were observed at 18, 19, 20 and 21° resembling an A type crystalline structure. Whereas the diffraction pattern was also retained after germination. The intensity of peak depends on the differences in electron density between amorphous and crystalline lamellae and amount of ordered semi-crystalline structures. Amorphous material do not give clarity rather the pattern has noise signal smeared peaks or it can have some short order bumps. It can therefore be a suitable option in the formulations of soup and mixes.

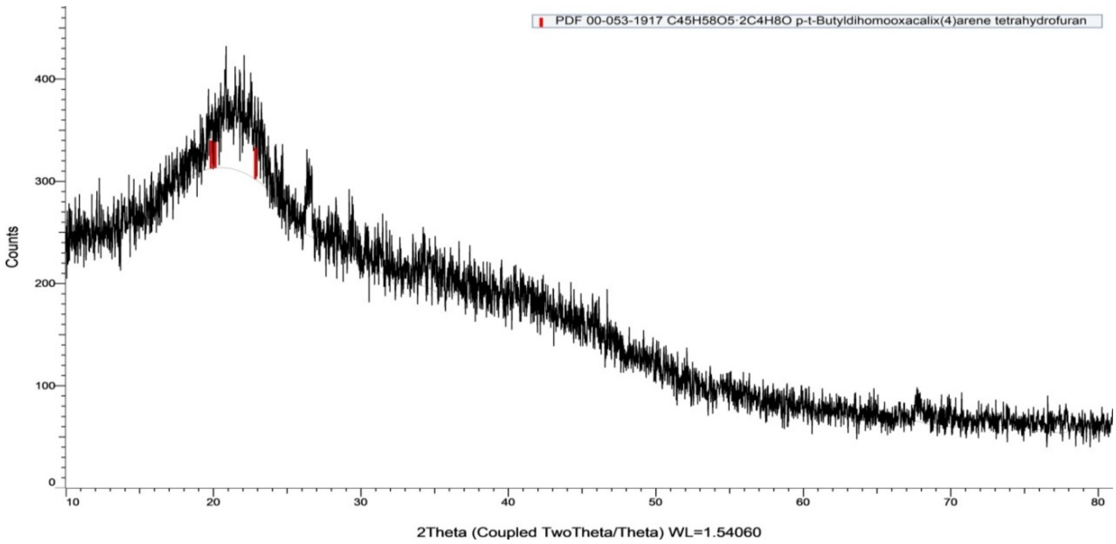

Figure 4.8:XRD pattern of optimized radish leaves powder

4.11 LC-MS screening and identification of polyphenolic compounds

The screening and identification of polyphenols from radish leaves powder were conducted through LC-MS (Table 4.12) .The base peak chromatogram of each sample in positive and negative mode given in figure. Kaempferol-3-*O*-glucoside was was detected at 449 which generated daughter ions at m/z 303 and 176. Similarly Cyanidin-3-*O*-glucuronide was identified at 611 which generated daughter ions at m/z 285 and 303. The results were in coherence with Koley et al., (2017), Bors et al.,(2015) and Ali et al., (2021) who identified the polyphenols in purple radish, white radish and Australian grown herbs respectively.

Table.4.12 Identification of polyphenolic compounds in radish leaves powder

S. No.	Compound name	Molecular formula	RT (min)	Observed (m/z)	LC/MS Product ions
1	Kaempferol-3-*O*- glucoside	$C_{21}H_{20}O_{11}$	1.6	449	303, 176
2	Cyanidin -3-*O*- rhamnoside	$C_{21}H_{21}O_{10}$	12.96	433	287
3	Cyanidin -3-*O*- sophoroside-5-*O*malonylglucoside	$C_{36}H_{43}O_{24}$	13.86	287	859
4	Glucobrassicin	$C_{16}H_{20}N_2O_9S_2$	25.17	364	207
5	Delphinidine-3-O-rutinoside	$C_{27}H_{31}O_1$	31.83	611	287, 449
6	3,4,5,7-Tetrahydroxyisoflavone	$C_{15}H_{12}O_6$	32.64	287	269
7	Luteolin-7-*O*- glucoside	$C_{21}H_{20}O_1$	35.04	535	371
8	Quercetin -3-*O*-xylosyl-glucuronide	$C_{26}H_{26}O_{17}$	36.71	611	285, 303
9	Pelargonidin-3-*O*- feruloyl-diglucoside-5-*O*-glucoside	$C_{42}H_{47}O_{22}$	37.36	933	339,697

Table.4.13 Effect of radish leaf powder's physical characteristics depending on particle size

Particle size	Bulk density (g/cm^3)	WAC (g/g)	WSI %	OHC %	FC %	Angle of repose (°)
50 microns	0.88±0.002^{a}	3.66±0.003^{g}	4.23±0.002^{a}	19.60±0.006g	4.94±0.005^{a}	51.05±0.001^{a}
75microns	0.87±0.003^{a}	3.83±0.005^{f}	4.17±0.004^{b}	20.03±0.002f	4.91±0.006^{b}	50.47±0.007 ^{b}
150microns	0.85±0.004^{bc}	3.95±0.001^{e}	4.12±0.001^{c}	21.09±0.001d	4.47±0.007^{c}	50.05±0.008^{c}
208microns	0.86±0.004^{b}	4.47±0.004^{d}	4.09±0.002^{d}	21.07±0.007e	3.63±0.002^{d}	49.12±0.004^{d}
267microns	0.85±0.001^{b}	4.51±0.001^{c}	4.06±0.005^{e}	22.11±0.005c	3.46±0.005^{e}	48.70±0.009^{e}
416 microns	0.84±0.004^{cd}	4.64±0.005^{b}	3.94±0.006^{f}	23.04±0.006b	2.61±0.003^{f}	48.11±0.003^{f}
625 microns	0.83±0.006^{d}	4.78±0.002^{a}	3.83±0.002^{g}	23.24±0.004a	2.37±0.004^{g}	46.23±0.005^{g}

Duncan's multiple range test was used with Sigma Plot 11.0 software (Sigma Plot Software; San Jose, California, USA) to run a two-way ANOVA at p 0.05. Statistics was considered statistically significant for mean values ($p<0.05$). Results are the average of three replicates, and values are the mean ± standard deviation.

4.12 Distribution of particle size

Particle size is a primarycomponent affecting functionality, handling, processing and marketing of food powders. Bulk density (BD), angle of repose (AOR), water absorption capacity (WAC), oil holding capacity(OHC), foaming capacity (FC) and water solubility index (WSI) are the parameters which are profoundly dependent on particle size and size distribution.The obtained dried samples (8cm leaves) were crushed manually prior to the grinding to powder using a mixer grinder. The particle size distribution was further analysed by sieving through the sieve shaker which contains sieves of different mesh sizes (24 BSS, 36 BSS, 56 BSS, 72 BSS, 100 BSS, 200 BSS, 300 BSS).

4.12.1 Bulk density

Bulk density is a very complex quality characteristic of food powders which influences various functional and economical features. The bulk density with different particle size is shown in (Fig 1) which illustrates that the decrease in particle size resulted in increase of the bulk density. Our results depictedthat as the particle size of radish leaf powder decreases from 625 microns to 50 microns the bulk density increases from 0.842g/cm^3 to 0.883g/cm^3.This may be due to the fact that as the particle size reduced, pore space or void between the particles also reduced resulting in expanded in the bulk density. The results were in alignment with the study conducted by Hong et al., (2021) where bulk density of quercus salicina (blume) leaf powders increases from 0.28 to 0.47 g/ml with decrease in particle size from 90µm to 10µm .Also the similar trend was recorded by Chen et al., (2015) and Zhao et al., (2010) during their studies conducted on red rice.

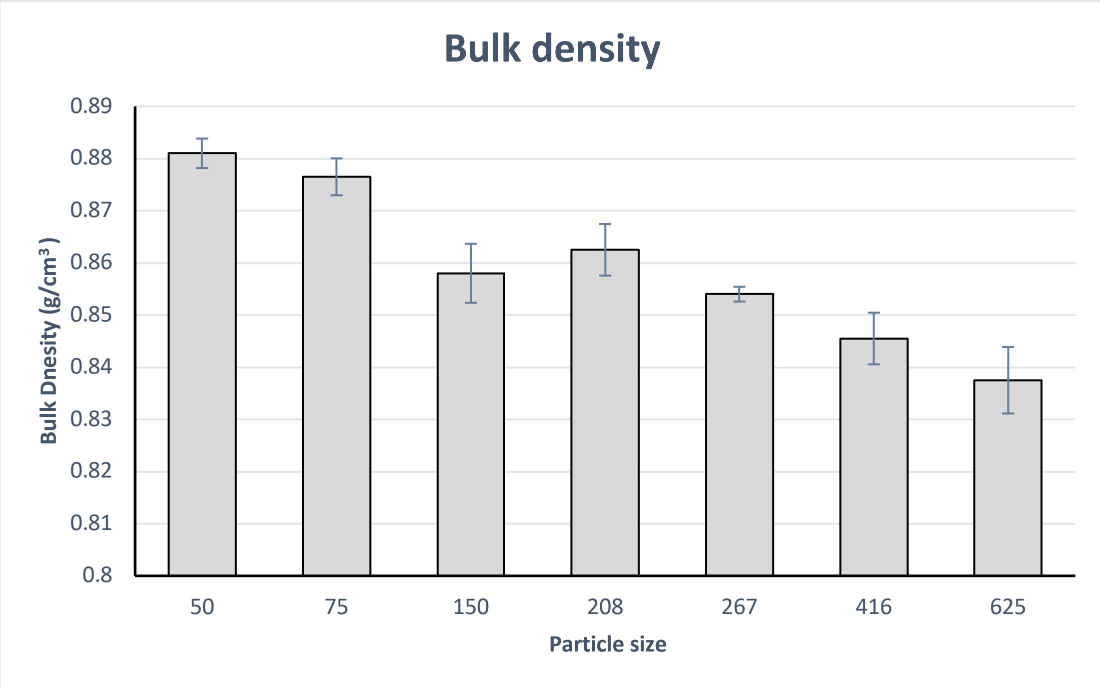

Fig: 4.10: Effect of particle size on bulk density of radish leaves powder

4.12.2 Water absorption capacity and water solubility index

WAC increases with increase in the particle size of the radish leaves which is perhaps associated to the fact that the large pore size of the coarse fractions holds more water in tissue layer spaces and empty pericarp. It ranges between 3.66±0.22 g/g to 4.78 ± 0.13g/g. Similar trend was provided by Onipe et al., (2017) and Chesson et al., (1997) where the WAC decreases with reduction in particle size while studying on wheat bran and wheat straw and wheat grain fractions respectively. The results for the WSI indicated that with reduction in particle size WSI increases from 4.23±0.13% to 3.83±0.14 %.This is because of increased surface area of the fine particles resulted in increase in dispersibility and solubility as compared to the coarser particles. The findings are consistent with Jiang et al., (2017) on the rise in WSI of powdered vaccinium bracteatum thumb leaves.

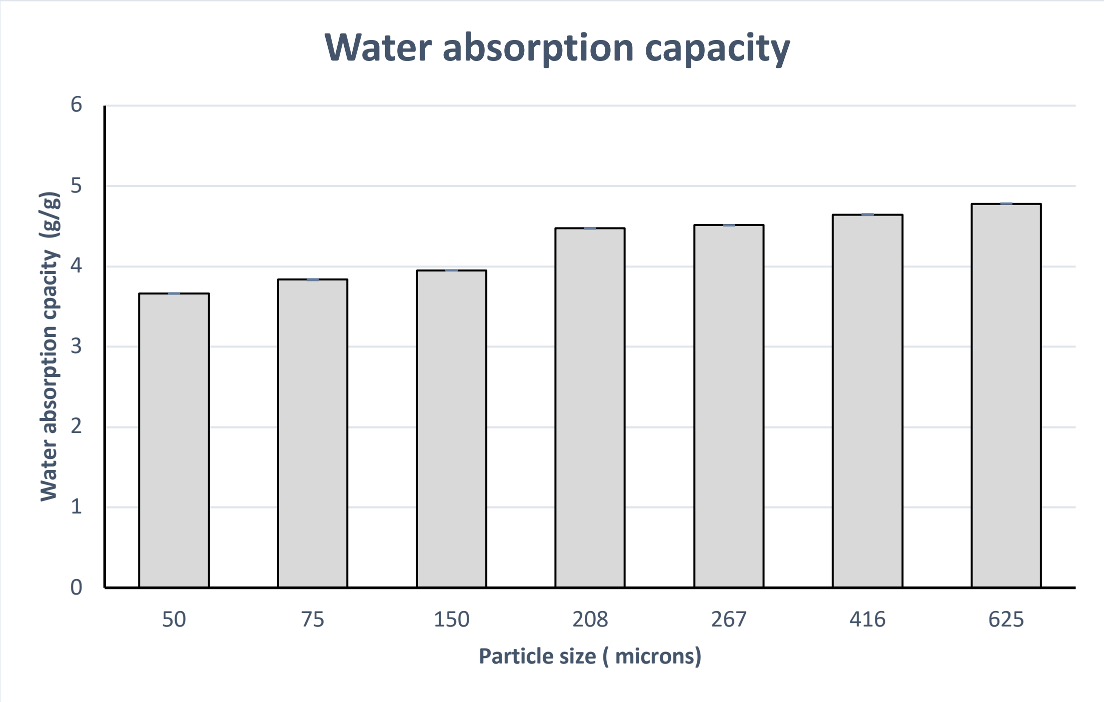

Fig: 4.11 : Effect of particle size on water absorption capacity of radish leaves powder

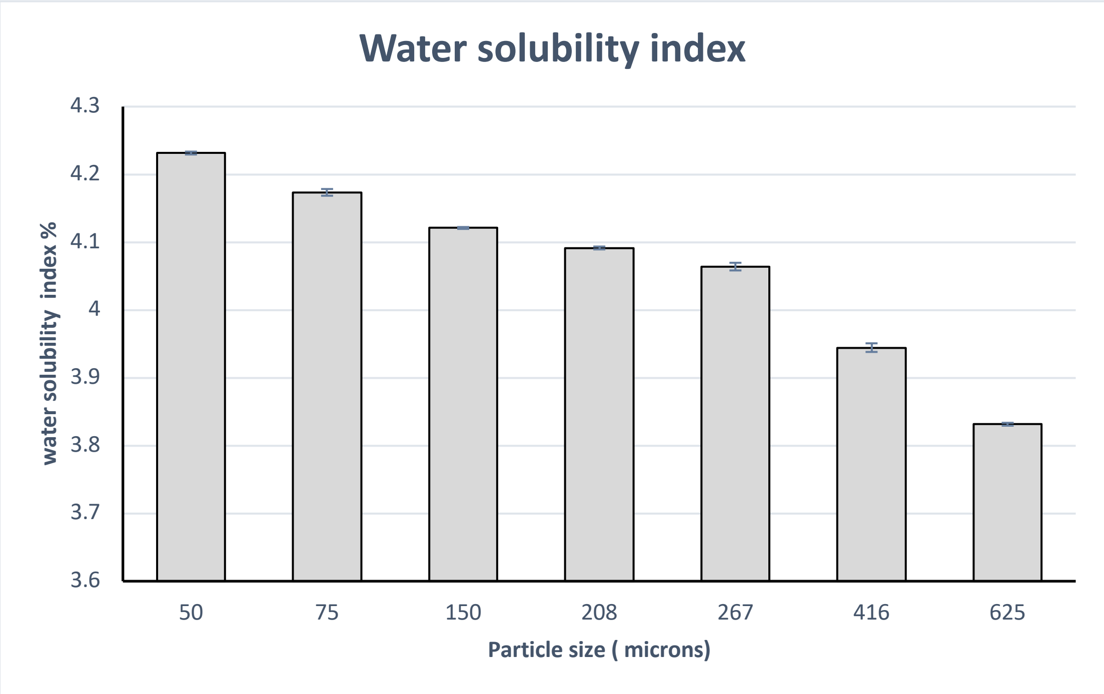

Fig: 4.12: Effect of particle size on water solubility index of radish leaves powder

4.12.3 Oil holding capacity

The oil holding capacity for radish leaf powder with respect to the particle size is mentioned in Figure (4.13) .The values of OHC ranges between 19.60±0.11% to 23.24±0.06.% .It was observed that the OHC decreases with decrease in particle size of radish leaf powder which can be attributed to the fact that the lowest value was obtained for finest fraction (50 microns).This may be due to the reason that the lower protein content leads to the lowest oil holding capacity due to reduced lipophilic tendency. The findings agreed with those of Ahmed et al., (2009) and Walde et al., (2005)

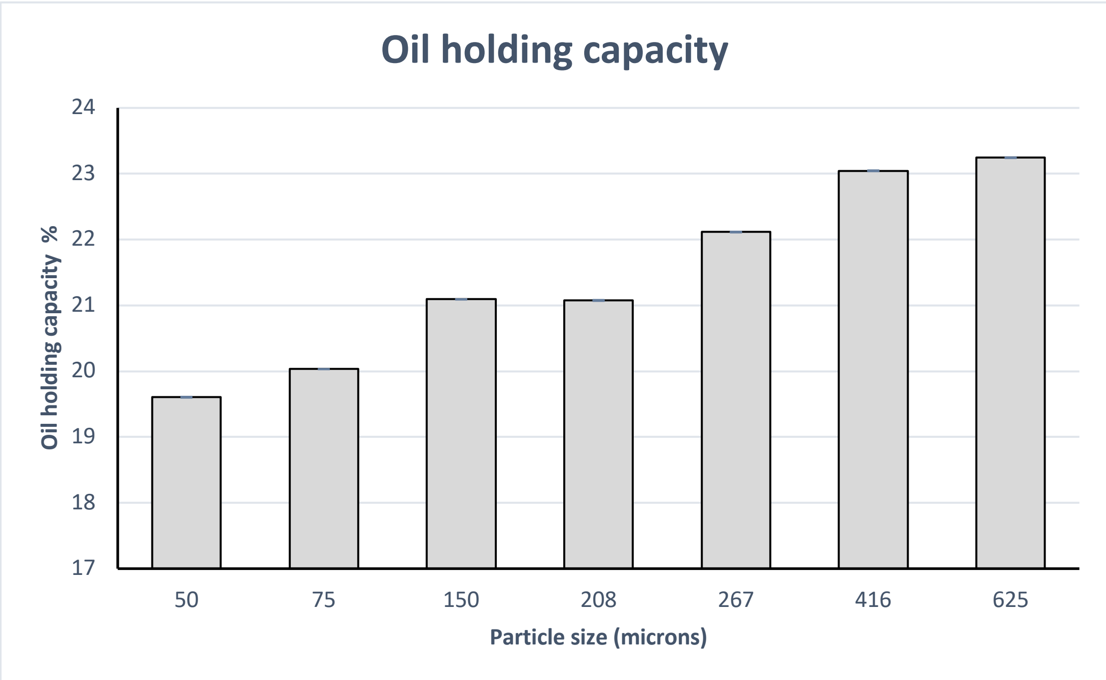

Fig: 4.13: : Effect of particle size on oil holding capacity of radish leaves powder

4.12.4 Foaming capacity

The decrease in foaming capacity was observed with increase in particle size which attributed that the powder having smaller particle size have more foaming capability and less foaming stability as foaming capability and foaming stability sharing an inverse relationship with each other (Kaur et al., 2006). This may be due to the fact that with the decrease into finer particles the accessibility of the proteins to air water interface improves leading to high foaming capacity. Foams are used to improve consistency, appearance and food texture also. Protein molecules that are flexible are associated with good foam ability because they reduce surface tension. On the other hand, highly organised molecules, which resist denaturation, have a low foaming capability (Lattimer and Haub,2010). As the particle size increased, the foaming capacity decreased from 4.94± 0.08 % to 2.38± 0.06 % (Fig.4.14) Our findings were consistent with those of Mugova. K et al., (2021), Martinez et al., (2014), and Banavara et al., (2003) who, in studies on the technological functional properties of Armoured Cricket (Acanthoplus discoidalis) flour, whey powders, and rice flour, respectively, each identified the same trend.

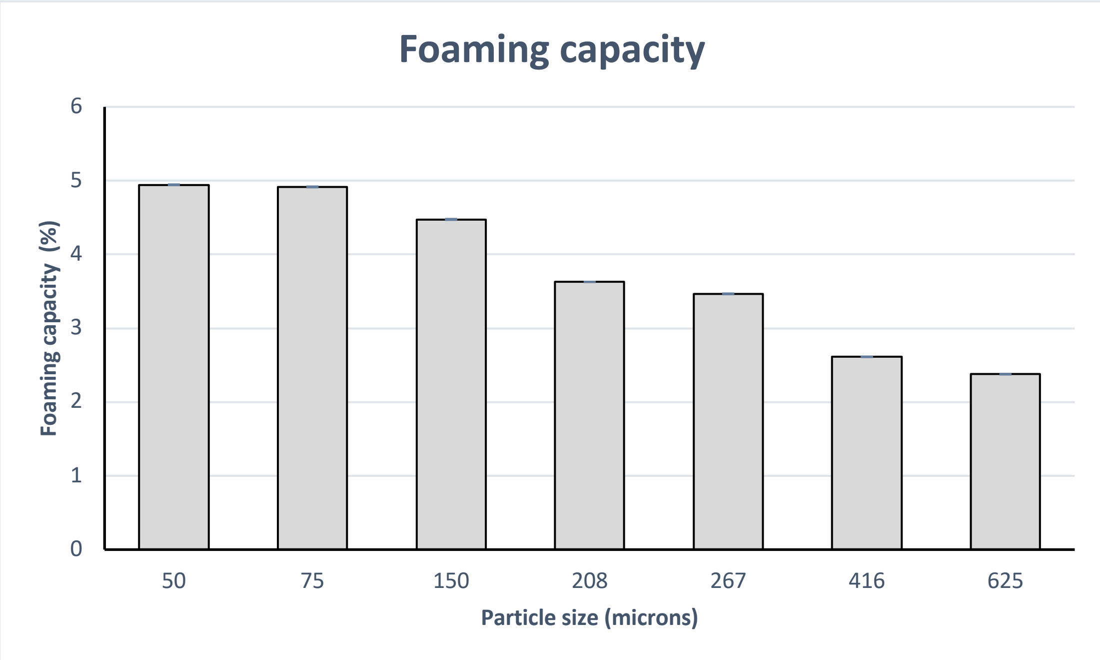

Fig: 4.14 : Effect of particle size on foaming capacity of radish leaves powder

4.12.5 Angle of repose

Angle of repose was found to decrease with increasing particle size of radish leaf powder as shown in figure, rangingbetween 46.23±0.11° to 51.06±0.02°. Finer powders exhibit higher AOR, hence they have lower flowability. On the contrary, large particle size is associated with easy flowing behaviour. This may be due to the fact that the fine powders, may stick together and have higher degree of cohesiveness and lower flowability. The results were found to be in sync with data reported by Abel et al., (2020) on Arabic gum where the decrease in angle of repose was observed with increasing particle size.The trend of angle of repose is shown in (Fig.4.15).

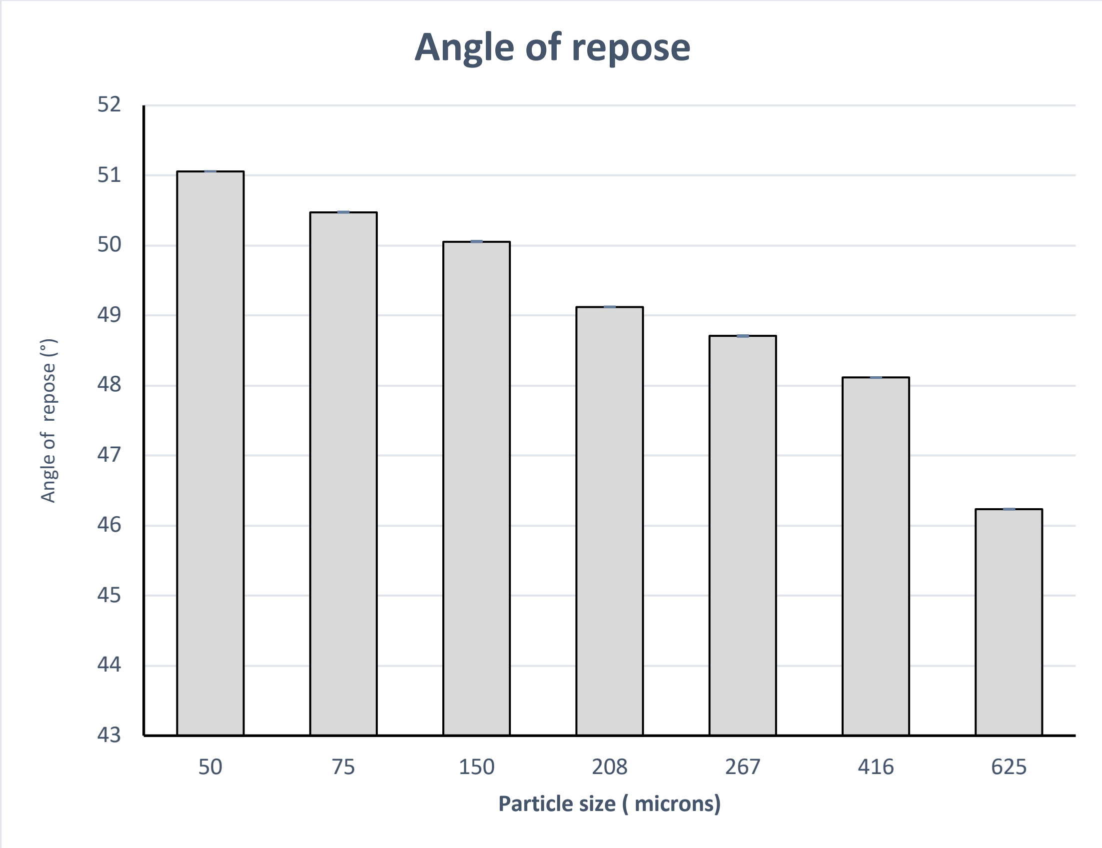

Fig. 4.15 : Effect of particle size on angle of repose of radish leaves powder

4.13 Sensory characteristics of radish leaves based instant chutney powder

Sensory evaluation of different radish leaves based instant chutneypowderwhich were reconstituted with water (1:2) was carried on the preparation day (Fig.4.16). Among different combinations, combination $T_4$was adjudged best on the basis of better sensory score for appearance, taste, flavour and overall acceptability. The overall acceptability of the recipe was initially 7.9±0.22 (Fig. 4.16). However, in case of soup mixreconstituted with warm water (45C) (1:10) combination T_3 was judged best on the basis of on the basis of better sensory score for appearance, taste, flavour and overall acceptability.The overall acceptability of the recipe was initially 8.1±0.35 (Fig. 4.17)

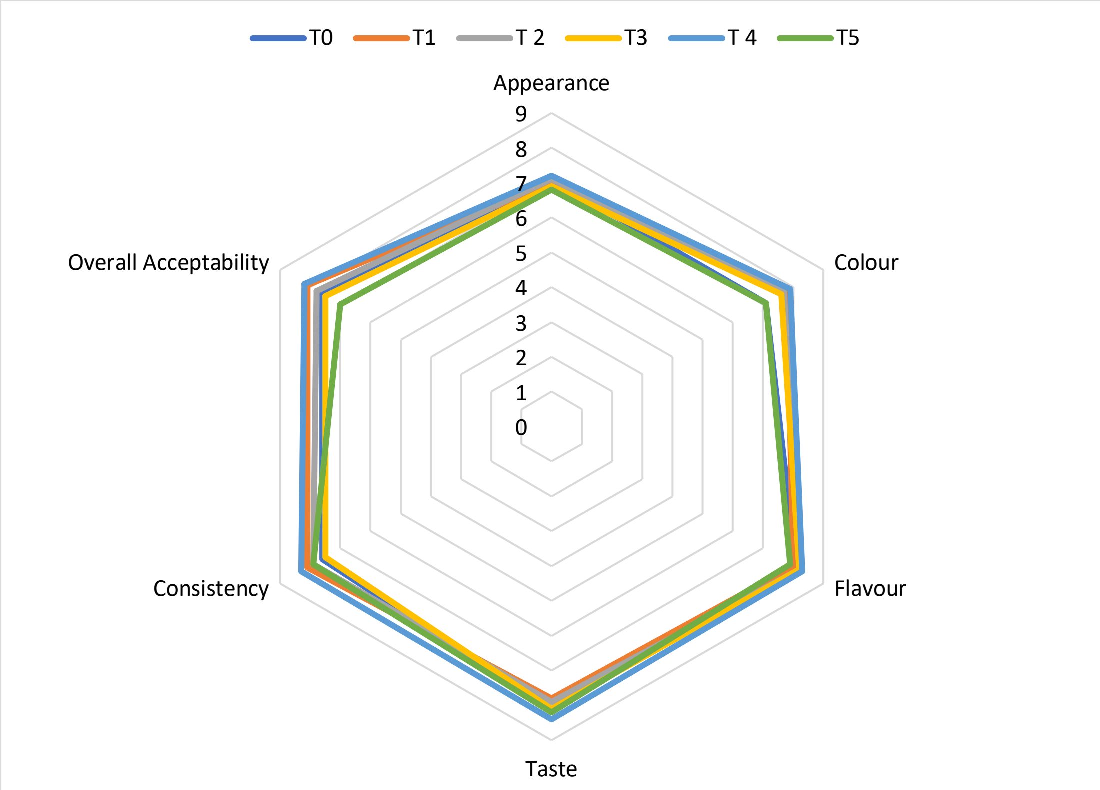

Fig:4.16 Sensory evaluation of radish leaves based chutney mix

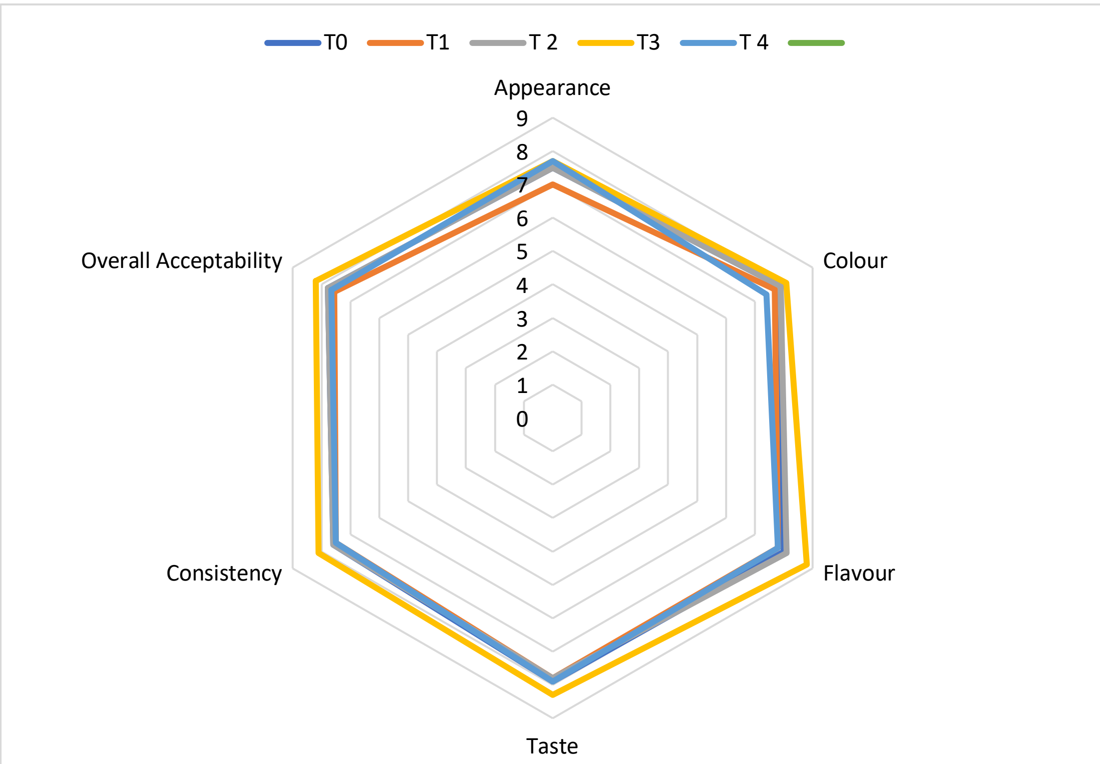

Fig:4.17 Sensory evaluation of radish leaves based soup mix

4.14.Storage

The formulation optimized on the basis of sensory evaluation (Fig. 4.16 and Fig. 4.17) for radish leaves based chutney mix and soup mix was T_4 (20:80:: radish leaves powder : spice mix) and $T_3$1.5:98.5 :: radish leaves powder : spice mix)which was further stored in LDPE, PET jar and aluminium foil pouch at ambient temperature for 6 months. Chemical and functional parameters such as TSS, acidity,pH, bulk density, true density, porosity, viscosity and pasting properties were observed during the storage of chutney mixand soup mix.

4.14.1 Storage analysis of radish leaves based chutney mix

4.14.1.1Total soluble solids

The total soluble solids (TSS) in the chutney mixture made from radish leaves are represented as °Brix. It serves as a measure of sugar content and is primarily brought on by the component's sucrose, glucose, and fructose. (Table 4.12) contains the data that relates to TSS. In this study, the TSS of the chutney mix made with radish leaves increased after 6 months of storage at room temperature in LDPE, PET jars, and aluminium foil pouches. Aluminium pouches experienced the least increase in TSS (52.31±0.02 to 52.89±0.01), whereas LDPE pouches experienced the greatest increase (52.31±0.02 to 53.03±0.06).The increase in total sugar hydrolysis produced by the acid hydrolysis of starch into simple sugars and the production of invert sugar from sucrose may have contributed to the rise in TSS values of the chutney mix formulations during the storage period. Similar tendencies have been observed in RTS produced from bitter gourd, guava, and A. vera-based papaya (Ramachandran and Nagarajan 2014), as well as amla squash blended with lime (Reddy and Chikkasubbanna 2008), and A. vera-based beverage enhanced with mint and ginger (Ramachandran and Nagarajan 2014); Yadav et al., 2013.

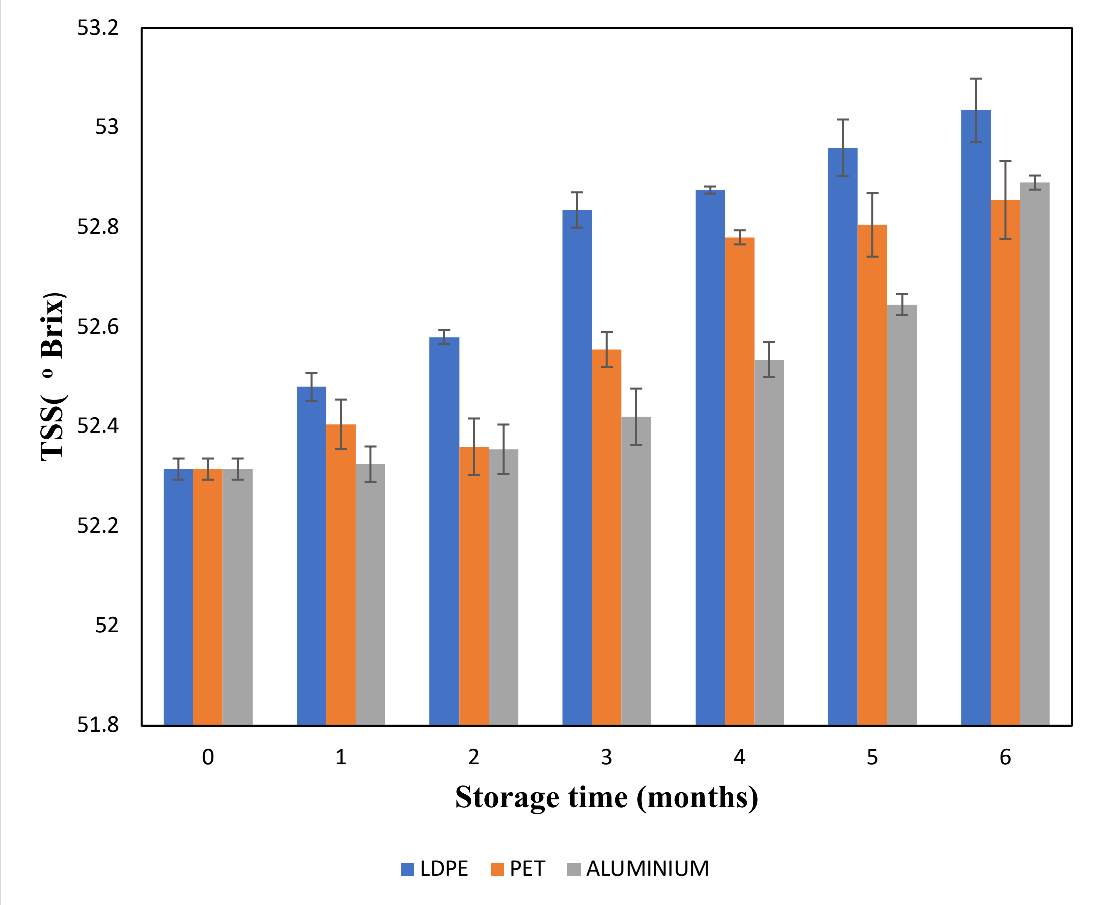

Fig: 4.18 : Effect of storage on TSS of radish leaves based chutney mix stored in different packaging material

4.14.1.2pH

The information got showed that the pH of the chutney blend diminished during the capacity time of a half year. The base lessening in pH was seen in the radish leaves based chutney blend put away in aluminium lined pouches (3.63±0.02to 2.81±0.04) and greatest reduction in pH was seen in the radish leaves based chutney blend plan put away in LDPE pockets (3.63±0.0 to 2.56±0.06) during the capacity time frame. The increased sharpness observed in this study may be attributed to the pH decreasing during the storage time. Comparative findings have been explained by (Tandon et al., 1983; Sandhu et al., 2001). Banana and sapota RTS (Hamaran and Amutha, 2007) and bael-guava RTS (Nidhi et al., 2008) have also been used to explain the pH reduction. Since it possesses antibacterial qualities and the low pH genuinely inhibits the growth of pathogenic microbes, the ongoing pH decline may be seen as a good factor.

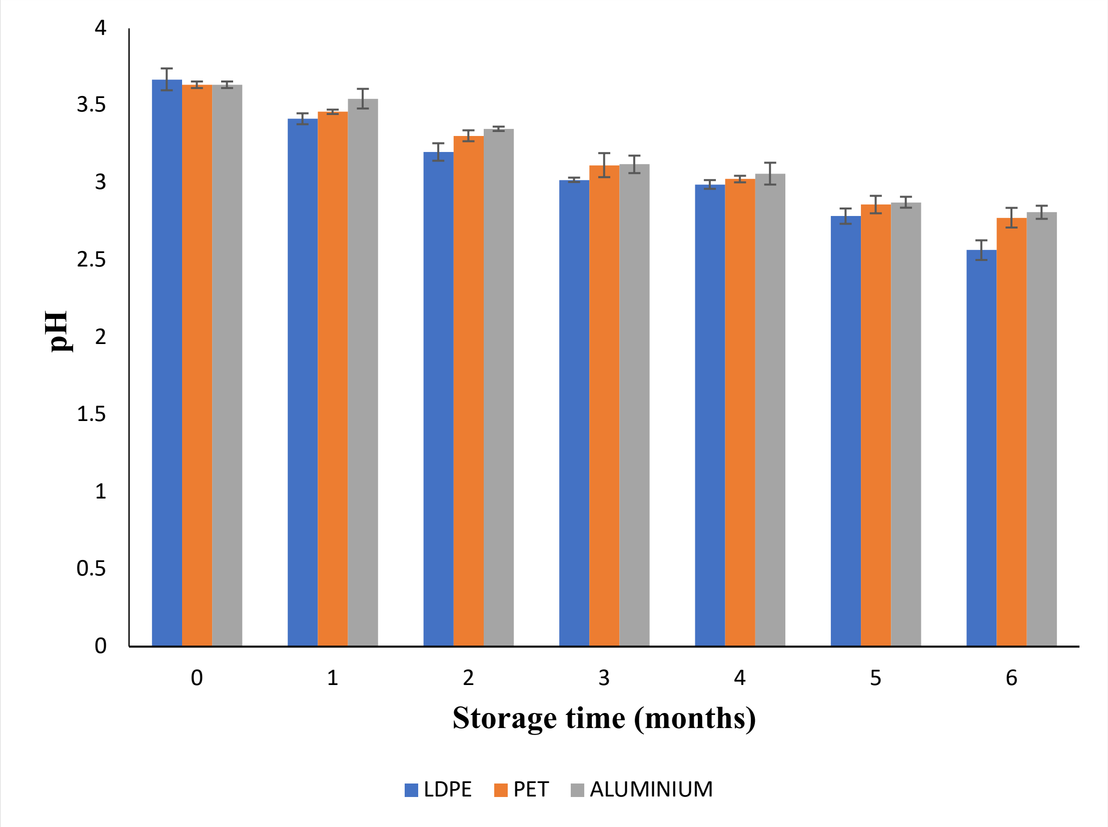

Fig: 4.19 Effect of storage on pH of radish leaves based chutney mix stored in different packaging material

4.14.1.3Titratable acidity

The data on titratable acidity (Table 4.12) revealed that the titratable acidity of the radish leaf-based chutney blend stored in various packing materials increased significantly ($P<0.05$) with time. The chutney mixture stored in LDPE pouches experienced the greatest increase in titratable acidity, followed by PET holders and aluminium walled pouches individually.The chutney blend put away in aluminium lined pouches displayed least expansion in acidity from (1.02±0.02% to 2.68±0.03 %) while chutney blend put away in LDPE pouches showed most extreme increment going from (1.02±0.02% to 3.44±0.05) in titratable sharpness. The expansion in acidity may be because of development of organic acids by decomposition of sugars during storage period. Comparable outcomes were accounted for by Sarker et al., (2014) in tomato powder and Bhatt et al.,(2020) in moment tomato soup blend powder.

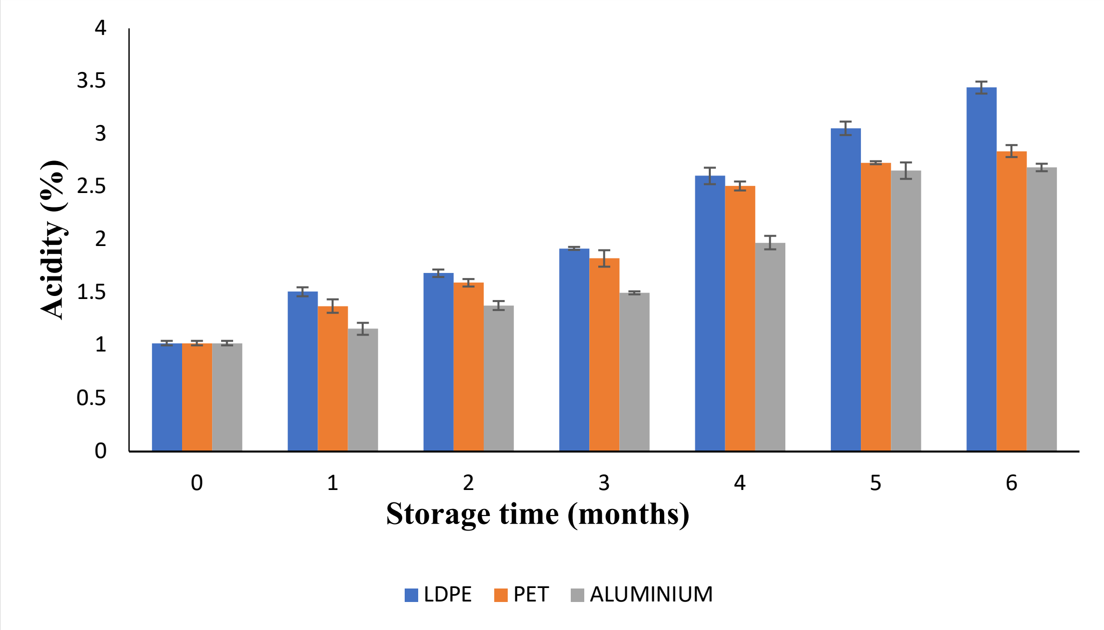

Fig: 4.20 Effect of storage on titratable acidity of radish leaves based chutney mix stored in different packaging material

4.14.1.4 Viscosity of chutney mix:

Storage had a significant effect on viscosity of chutney mix. The values varied between 811 ± 3.535 to 737 ± 7.071 poise. Decrease in viscosity could be attributed to softening of tissues as a result of alteration in macromolecular structure of polymeric substance forming cell walls (Rudra et al., 2008). The decrease in viscosity with increase in storage period was also citedby Ahmed et al., (2004) in coriander leaf puree

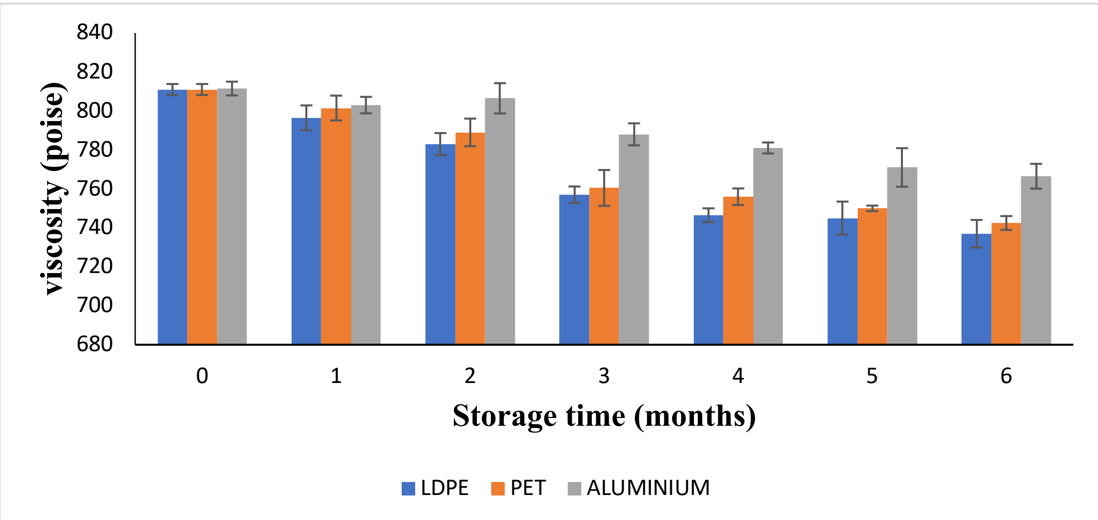

Fig: 4.21 : Effect of storage on viscosity of radish leaves based chutney mix stored in different packaging material

Table 4.14: Effect of storage on the physical and chemical characteristics of a chutney made with radish leaves and kept in various packaging materials

Packaging material	(Months)	TSS	pH	Acidity	Viscosity
Aluminium	0	52.31 ± 0.02^{j}	3.63 ± 0.02^{a}	1.02 ± 0.02^{m}	811 ± 3.535^{a}
	1	52.32 ± 0.03^{ij}	3.54 ± 0.06^{ab}	1.16 ± 0.05^{l}	803 ± 4.242^{ab}
	2	52.35 ± 0.04^{i}	3.35 ± 0.01^{c}	1.38 ± 0.04^{k}	806 ± 7.778^{ab}
	3	52.42 ± 0.05^{hi}	3.12 ± 0.05^{efg}	1.5 ± 0.01^{j}	788 ± 5.656^{de}
	4	52.53 ± 0.03^{fg}	3.06 ± 0.07^{fgh}	1.97 ± 0.06^{g}	781 ± 2.828^{ef}
	5	52.64 ± 0.02^{e}	2.87 ± 0.03^{hijk}	2.65 ± 0.07^{de}	771 ± 9.899^{fg}
	6	52.89 ± 0.01^{bc}	2.81 ± 0.04^{jk}	2.68 ± 0.03^{d}	766 ± 6.363^{gh}
PET	0	52.31 ± 0.02^{j}	3.63 ± 0.02^{a}	1.02 ± 0.02^{m}	811 ± 3.535^{a}
	1	52.40 ± 0.04^{hij}	3.46 ± 0.01^{abc}	1.37 ± 0.03^{k}	801 ± 6.363^{abc}
	2	52.36 ± 0.05^{ij}	3.30 ± 0.03^{cde}	1.59 ± 0.02^{ij}	789 ± 7.071^{cde}
	3	52.55 ± 0.03^{efg}	3.11 ± 0.07^{fg}	1.82 ± 0.07^{h}	760 ± 9.192^{ghi}
	4	52.78 ± 0.01^{d}	3.02 ± 0.02^{fghi}	2.51 ± 0.04^{f}	756 ± 4.242^{hij}
	5	52.80 ± 0.06^{cd}	2.86 ± 0.05^{ijk}	2.73 ± 0.01	750 ± 1.414^{ik}
	6	52.85 ± 0.07^{cd}	2.77±0.06k	2.84 ± 0.05^{c}	742 ± 3.535^{k}
LDPE	0	52.31 ± 0.02^{j}	3.63 ± 0.02^{a}	1.02 ± 0.02^{m}	$811\ \pm3.535^{a}$
	1	52.48 ± 0.03^{gh}	3.41 ± 0.03^{bc}	1.51 ± 0.04^{j}	796 ± 6.363^{bcd}
	2	52.58 ± 0.01^{ef}	3.20 ± 0.05^{def}	1.68 ± 0.03^{i}	783 ± 5.656^{ef}
	3	52.83 ± 0.04^{cd}	3.02 ± 0.01^{fghi}	1.92 ± 0.01^{gh}	757 ± 4.242^{hij}
	4	52.87 ± 0.00^{bcd}	2.99 ± 0.02^{ghij}	2.60 ± 0.07^{ef}	746 ± 3.535^{jk}
	5	52.96 ± 0.05^{ab}	2.78 ± 0.04^{k}	3.05 ± 0.06^{b}	745 ± 8.485^{jk}
	6	53.03 ± 0.06^{a}	2.56 ± 0.06^{l}	3.44 ± 0.05^{a}	737 ± 7.071^{k}

Duncan's multiple range test was used with Sigma Plot 11.0 software (Sigma Plot Software; San Jose, California, USA) to run a two-way ANOVA at p 0.05. Statistics was considered statistically significant for mean values ($p<0.05$). Results are the average of three replicates, and values are the mean ± standard deviation.

4.14.1.5 Bulk Density of chutney mix

Bulk density of radish leaves-based chutney mix powder increased with the storage period of six months. The bulk density of the chutney mix stored in different packaging materials viz., aluminium pouches (0.455±0.005 to 0.541±0.007 g/cm^3), LDPE jars (0.455±0.005 to 0.555±0.005 g/cm^3) and LDPE (0.455±0.005 to 0.598±0.005 g/cm^3). The increased weight of the particles brought on by the absorption of moisture during storage may result in a rise in bulk density (Chauhan and Patil, 2013). Probiotic Sohiong powder (Vivek et al., 2020), honey powder (Samborska et al., 2015), and mango milk powder (Chauhan and Patil, 2013) all showed an increase in bulk density with increasing moisture content.

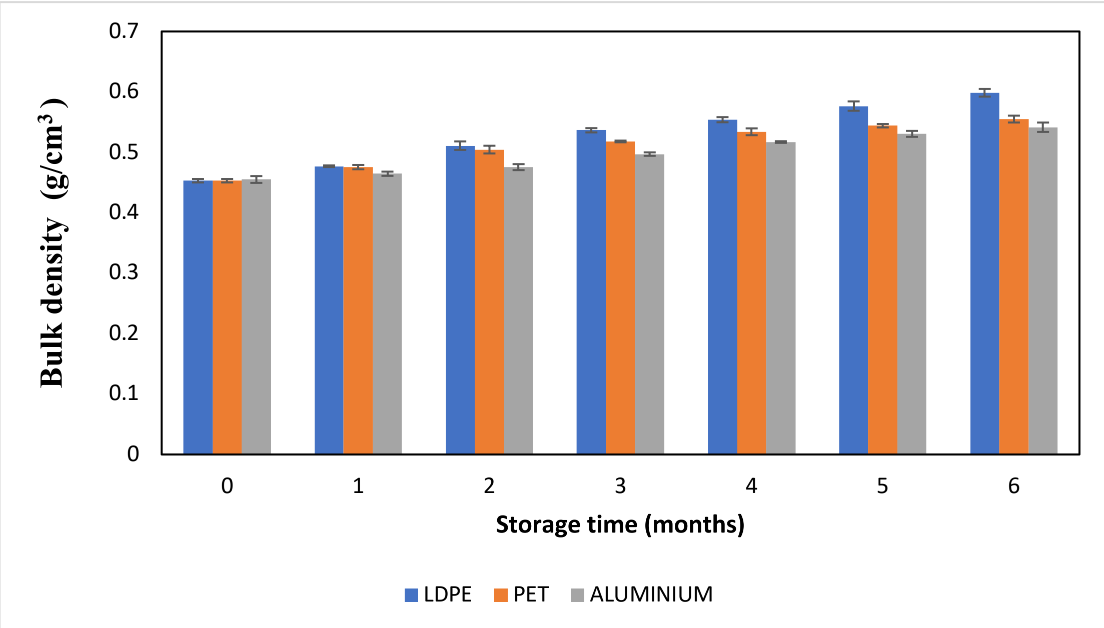

Fig: 4.22 Effect of storage on bulk density of radish leaves based chutney mix stored in different packaging material

4.14.1.6 True Density of chutney mix

True density values of chutney mix stored in all packaging materials were in a range of 1.102±0.002 to 1.332 ±0.012 g/cm^3 (Table 4.15) which were same as to that of spray-dried whole cow milk powder (~1.280 g/cm^3) and camel milk powder (1.238–1.275 g/cm^3) (Chen, 1994; Ho et al., 2019).The powder's structure and moisture content have an effect on its actual density. The crystalline powder often has a greater actual density than the amorphous one because the atoms in crystalline lattices are arranged with the least amount of space between them, which results in a decrease in volume (Yang et al., 2012).

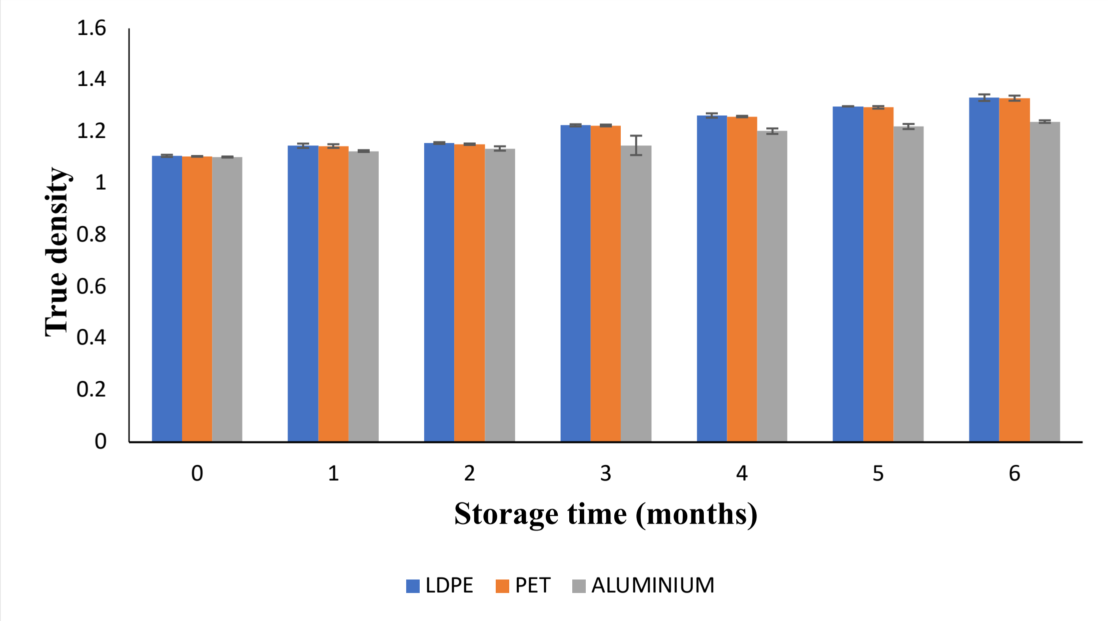

Fig: 4.23 : Effect of storage on true density of radish leaves based chutney mix stored in different packaging material

4.14.1.7 Porosity of chutney mix

Porosity of chutney mix ranges from 0.591 ± 0.001 to 0.541 ± 0.041. The decrease in porositymay be because of decrease in surface exposure of the particles to moisture and air of the dried product. Similar results were reported by Sunil and Venkatachalapthy (2017) in foxtail millet.

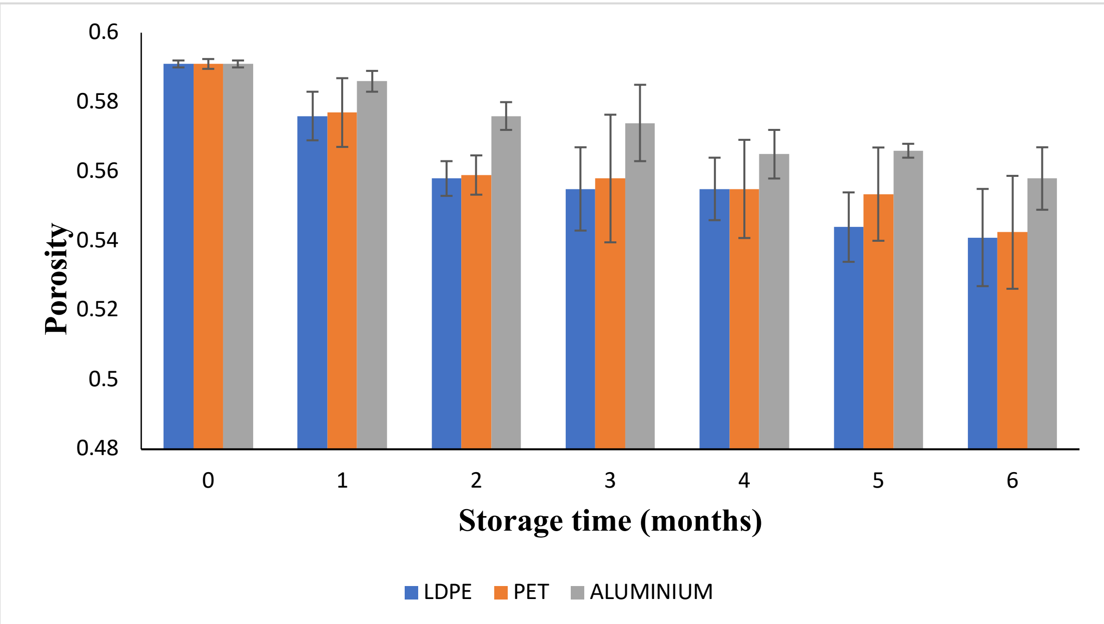

Fig: 4.24 : Effect of storage on porosity of radish leaves based chutney mix stored in different packaging material

Table 4.15:Effect of storage on the functional qualities of a chutney mixture made with radish leaves that was kept in various packaging materials

Packaging material	Storage intervals (months)	Bulk density	True density	Porosity
Aluminium	0	0.455±0.005^{k}	1.102±0.002^{j}	0.591±0.001^{a}
	1	0.464±0.003^{k}	1.125±0.003^{i}	0.576±0.007^{b}
	2	0.475±0.004^{j}	1.135±0.008^{hi}	0.558±0.005^{de}
	3	0.497±0.002^{i}	1.136±0.001^{h}	0.555±0.012^{ef}
	4	0.517±0.001^{g}	1.202±0.010^{f}	0.555±0.009^{ef}
	5	0.530±0.004^{f}	1.220±0.009^{e}	0.544±0.010^{f}
	6	0.541±0.007^{e}	1.239±0.004^{d}	0.541±0.014^{f}
PET	0	0.455±0.005^{k}	1.102±0.002^{j}	0.591±0.001^{a}
	1	0.475±0.003^{j}	1.145±0.007^{gh}	0.577±0.007^{a}
	2	0.504±0.006^{hi}	1.152±0.002^{g}	0.559±0.009^{def}
	3	0.518±0.001^{g}	1.224±0.003^{e}	0.558±0.018^{de}
	4	0.534±0.005^{ef}	1.259±0.002^{c}	0.555±0.014^{ef}
	5	0.544±0.002^{de}	1.294±0.004^{b}	0.553±0.013^{ef}
	6	0.555±0.005^{c}	1.330±0.009^{a}	0.542±0.016^{f}
LDPE	0	0.455±0.005^{k}	1.102±0.002^{j}	0.591±0.001^{a}
	1	0.477±0.001^{j}	1.146±0.008^{gh}	0.586±0.003^{a}
	2	0.511±0.007^{gh}	1.156±0.003^{g}	0.576±0.004^{b}
	3	0.536±0.003^{ef}	1.225±0.004^{e}	0.574±0.011^{b}
	4	0.554±0.004^{cd}	1.263±0.008^{c}	0.565±0.007^{c}
	5	0.576±0.007^{b}	1.298±0.001^{b}	0.566±0.002^{c}
	6	0.598±0.006^{a}	1.332±0.012^{a}	0.558±0.009^{de}

Duncan's multiple range test was used with Sigma Plot 11.0 software (Sigma Plot Software; San Jose, California, USA) to run a two-way ANOVA at p 0.05. Statistics was considered statistically significant for mean values (p<0.05). Results are the average of three replicates, and values are the mean ± standard deviation.

4.14.2 Storage analysis of radish leaves based soup mix

4.14.2.1 TSS (Total Soluble Solids) of soup mix

Total soluble solids in radish leaves-based soup mix are presented in Table (4.16). The TSS increased significantly (P>0.05) for all the formulations stored in the different packaging materials viz. aluminum pouches, PET containers and LDPE pouches.

However, maximum increases in TSS from (5.05 ± 0.01 to 5.47 ± 0.05° Brix) was observed in the soup mix stored in LDPE pouches followed by PET containers (5.05 ± 0.01 to 5.35 ± 0.03°Brix) and aluminum pouches (5.05± 0.01 to 5.28 ± 0.06° Brix) respectively. In a ready-to-serve aloe vera lemon functional drink, (Kausar et al., 2016) observed a similar growing tendency (Jemziya and Mahendran 2018) in therapeutic ready-to-serve (RTS) prepared from a blend of cabbage. The increase might result due to the transformation of polysaccharides (starch) to simple sugars in the presences of organic acids.

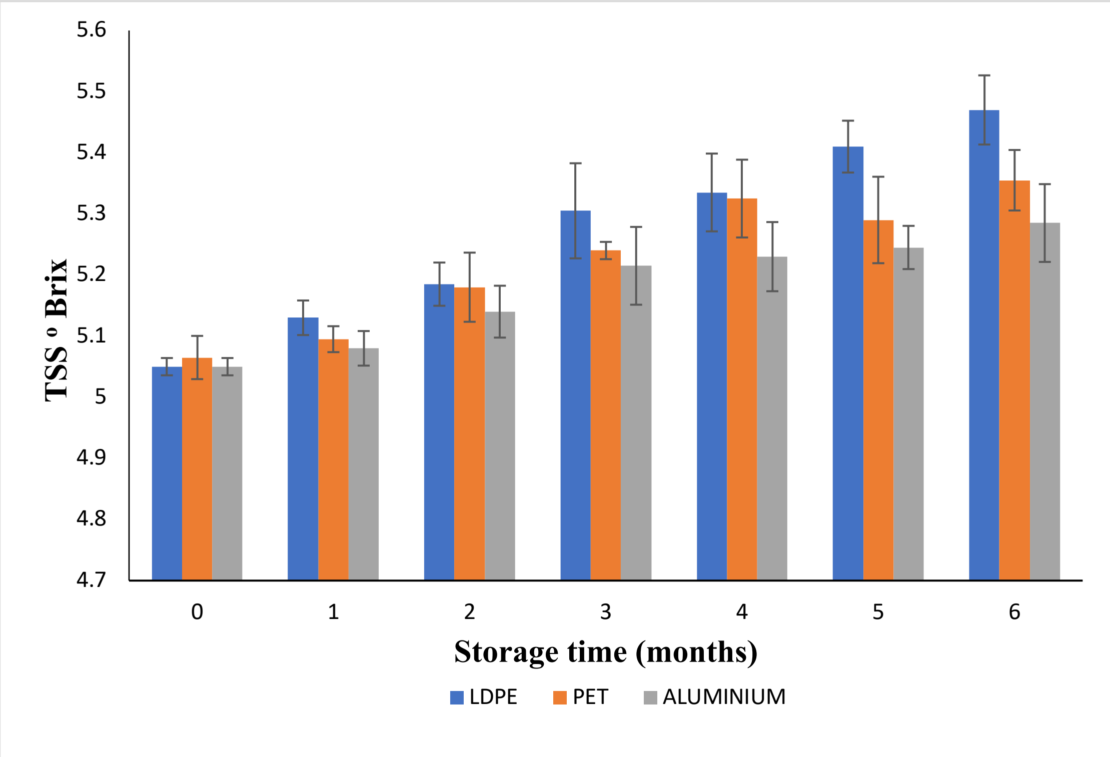

Fig: 4.25 : Effect of storage on TSS of radish leaves based soup mix stored in different packaging material

4.14.2.2 pH of soup mix

The pHof radish leaves-based soup mix decreased over the period of six months when stored in different packaging materials i.e., aluminum pouches (5.92 ± 0.02 to 5.73 ± 0.07) PET containers (5.92 ± 0.02 to 5.76 ± 0.05) and LDPE pouches(5.92 ± 0.02 to 5.64 ± 0.05). the decrease in pH may be resulted of decrease in ascorbic acid due to oxidation by an enzyme ascorbic oxidase. Our results agreed with those observations by (Giordano et al.,2000) and Sarker et al.,2014) in storage of tomato powder in different packaging materials.

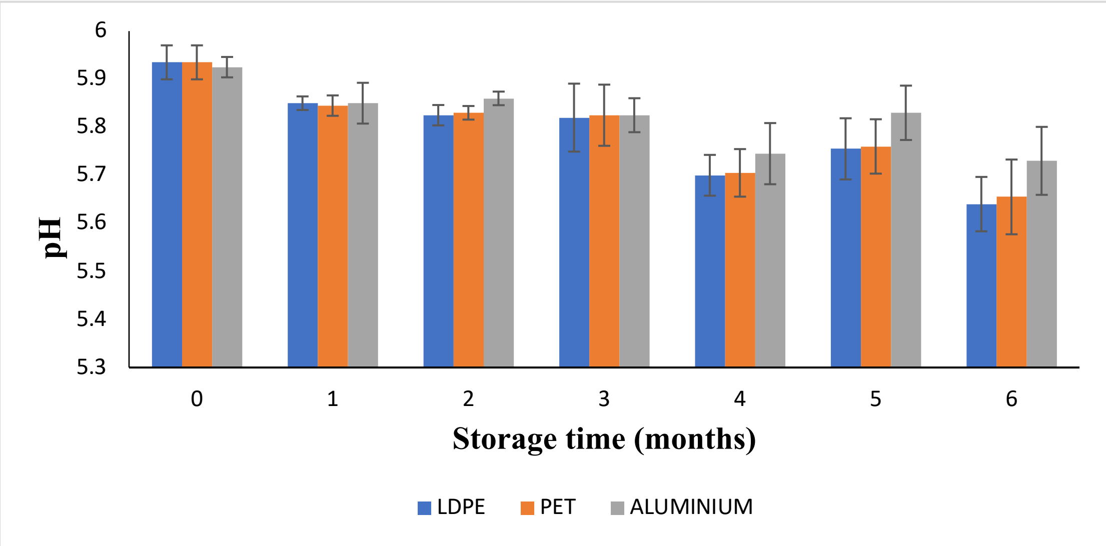

Fig: 4.26 : Effect of storage on the pH of a soup base made with radish leaves that was kept in various types of packaging

4.14.2.3 Titratable acidity of soup mix

During storage, there was a general upward trend in the titratable acidity of the soup mix made from radish leaves, which may have been caused by the conversion of lactose to lactic acid and the production of organic acids from the breakdown of carbohydrates.However, with respect to the packaging material maximum increase in titratable acidity was observed in soup mix stored in LDPE, where the increase in acidity from 2.81 ± 0.041 % to 3.31 ± 0.03 % was observed over the period of storage for 6 months whereas soup mix was stored in aluminum pouches exhibited minimum increase in acidity 2.81 ± 0.04 % to 3.12 ± 0.05 %. The findings are in correlation with (Yadav et al., 2013) where the increased acidity observed in whey-based banana herbal beverage with incorporate of *Mentha arvensis* extract (0 to 4 %).

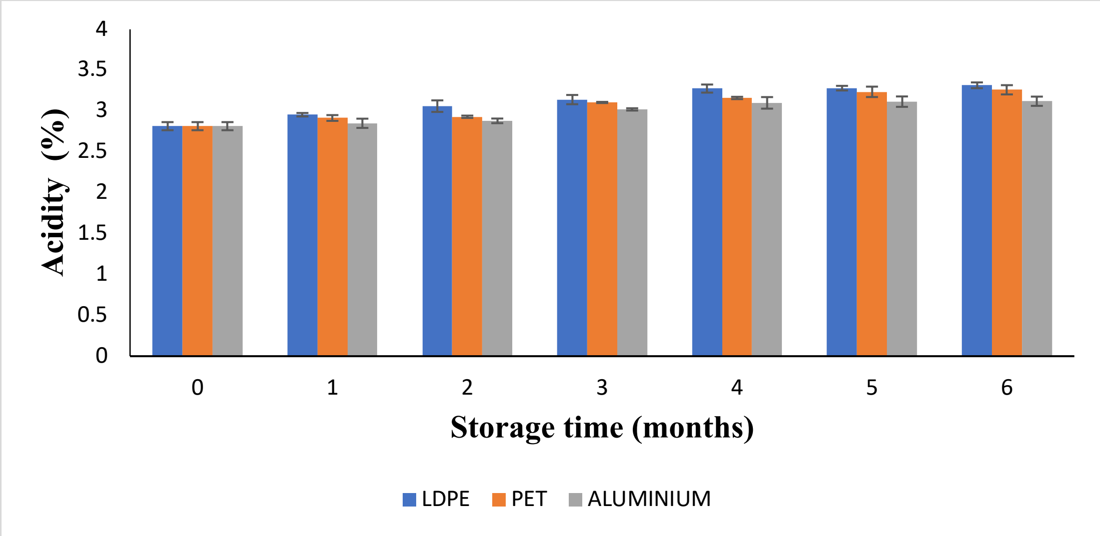

Fig: 4.27:Effect of storage on titratable acidity of radish leaves based soup mix stored in different packaging material

4.14.2.4 Viscosity of soup mix:

In soup making, viscosity is an index of thickness (Ikegwu et al., 2009). It provides desirable body to the soup mix. The data predicted significant decrease ($p \leq 0.05$)in viscosity of soup mixfor all the samples stored in aluminum pouches, PET containers and LDPE. The values arein the range of 8.55 ± 0.03 to 7.89 ± 0.05 poise (Fig. 4.28). The decrease may be associated with increase in density of powder mix and also breakdown of macromolecules forming cell walls.The results are in agreement with Kashyap et al., (2016).

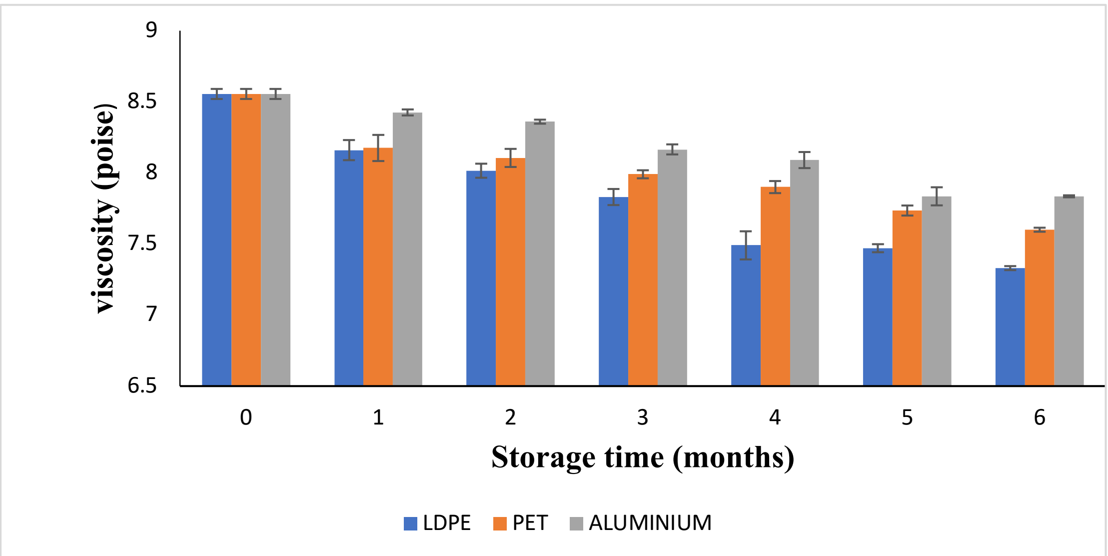

Fig:4.28: Effect of storage on viscosity of radish leaves based soup mix stored in different packaging material

Table 4.16:Effect of storage on the physical and chemical properties of radish leaf chutney and kept in various packaging materials

Packaging material	(Months)	T SS	pH	Acidity	Viscosity
Aluminium	0	5.05±0.01^{i}	5.92±0.02^{a}	2.81±0.04^{j}	8.55±0.03^{a}
	1	5.08±0.02^{hi}	5.85±0.04^{ab}	2.85±0.05^{ij}	8.42±0.02^{b}
	2	5.14±0.04^{ghi}	5.86±0.01^{ab}	2.88±0.02^{hij}	8.36±0.01^{b}
	3	5.21±0.06^{efg}	5.82±0.03^{abc}	3.02±0.01^{defghi}	8.16±0.03^{c}
	4	5.23±0.05^{defg}	5.74±0.06^{bcde}	3.10±0.07^{bcdef}	8.09±0.05^{cde}
	5	5.24±0.03^{cdefg}	5.83±0.05^{abc}	3.11±0.06^{bcdef}	7.83±0.06^{gh}
	6	5.28±0.06^{cdef}	5.73±0.07^{cde}	3.12±0.05^{bcde}	7.83±0.01^{gh}
PET	0	5.05±0.01^{i}	5.92±0.02^{a}	2.81±0.04^{j}	8.55±0.03^{a}
	1	5.09±0.02^{hi}	5.84±0.03^{abc}	2.91±0.03^{ghij}	8.17±0.09^{c}
	2	5.18±0.05^{fgh}	5.83±0.01^{abc}	2.93±0.01^{fghij}	8.10±0.06^{cd}
	3	5.24±0.01^{cdefg}	5.82±0.06^{abc}	3.10±0.00^{bcdefg}	7.99±0.02^{ef}
	4	5.32±0.06^{bcde}	5.70±0.04^{d}	3.16±0.01^{abcd}	7.90±0.04^{fg}
	5	5.29±0.07^{cdef}	5.76±0.05^{bcd}	3.23±0.06^{abc}	7.73±0.03^{h}
	6	5.35±0.03^{bc}	5.65±0.07^{de}	3.26±0.05^{ab}	7.60±0.01^{i}
LDPE	0	5.05±0.01^{i}	5.92±0.02^{a}	2.81±0.04^{j}	8.55±0.03^{a}
	1	5.13±0.02^{ghi}	5.85±0.01^{a}	2.95±0.02^{efghij}	8.16±0.07^{c}
	2	5.18±0.03^{fgh}	5.82±0.02^{abc}	3.06±0.07^{cdefgh}	8.01±0.04^{de}
	3	5.30±0.07^{bcde}	5.82±0.07^{ab}	3.14±0.05^{abcde}	7.83±0.05^{g}
	4	5.33±0.06^{bcd}	5.70±0.04^{de}	3.27±0.04^{ab}	7.49±0.09^{j}
	5	5.41±0.04^{ab}	5.75±0.06^{bcd}	3.28±0.02^{ab}	7.47±0.02^{j}
	6	5.47±0.05^{a}	5.64±0.05^{e}	3.31±0.03^{a}	7.33±0.01^{k}

Duncan's multiple range test was used with Sigma Plot 11.0 software (Sigma Plot Software; San Jose, California, USA) to run a two-way ANOVA at p 0.05. Statistics was considered statistically significant for mean values ($p<0.05$). Results are the average of three replicates, and values are the mean ± standard deviation.

4.14.2.5 Bulk density of soup mix:

Regardless of the packaging materials employed, the bulk density of the soup mix made from radish leaves rose during the course of a 6-month storage period. The soup mix held in LDPE pouches showed the greatest increase in bulk density (0.814 0.007 to 0.814 0.007), followed by PET containers (0.817 0.006 to 0.817 0.006) and aluminium pouches (0.755 0.003 to 0.809 0.004). Increased cohesiveness brought on by moisture absorption during storage could be the source of the increase in bulk density. The findings were consistent with research on spray-dried tamarind pulp powder done by Muzaffar and Kumar (2015) and Chauhan and Patil (2013).

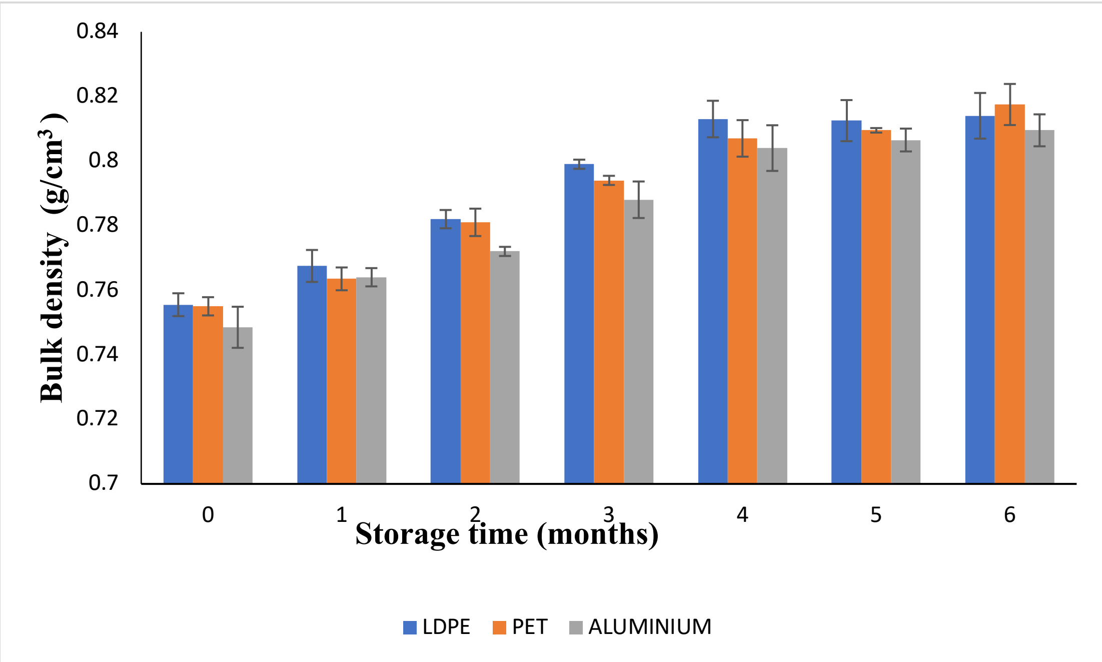

Fig: 4.29 :Effect of storage on bulk density of radish leaves based soup mix stored in different packaging material

4.14.2.6 True density of soup mix:

The true density of radish leaves based soup mix were in range of 1.425 ± 0.004 to 1.533 ± 0.007, stored in aluminium pouches, PET containers and LDPE pouches for over a period of 6 months. This might be resultant of increase in moisture during storage. Powar et al.,(2018) and Rao et al., (2020) have also stated increase in true density during studies conducted in finger millet and barnyard millet respectively.

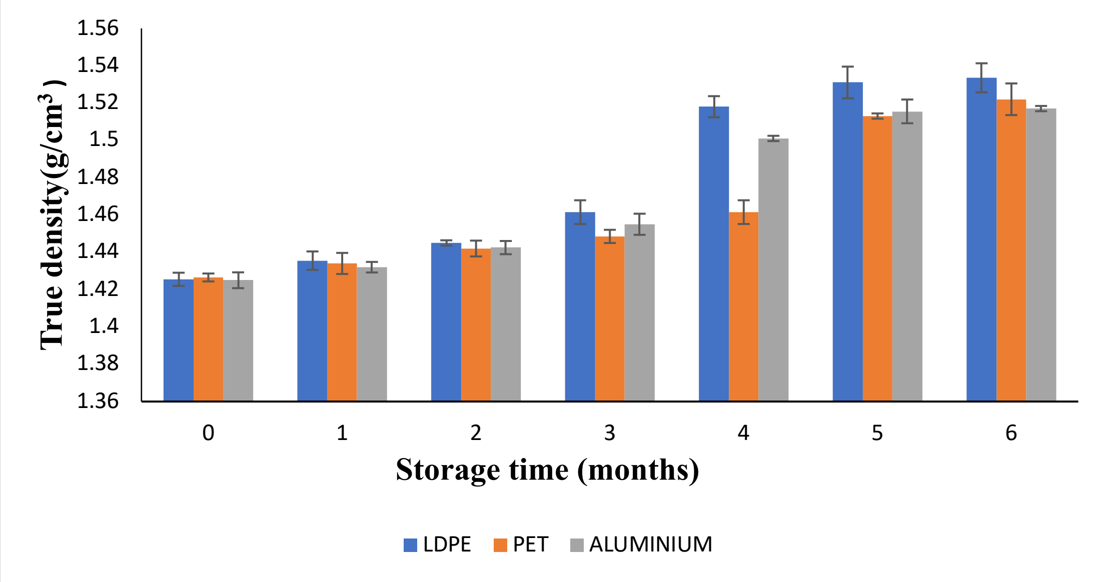

Fig: 4.30 : Effect of storage on True density of radish leaves based soup mix stored in different packaging material

4.14.2.7 Porosity of soup mix:

Porosity is an index of quality parameter. Good flow properties are provided by high density and low porosity. Porosity of soup mix ranges from 0.475 ± 0.03 to 0.465 ± 0.001. The decrease in porosity may be attributed to decrease in exposure of surface area to light, moisture and air of the dried product. In the case of kodo millet, similar findings were reported by Swami and Swami (2010). Good flow properties are provided by high density and low porosity.

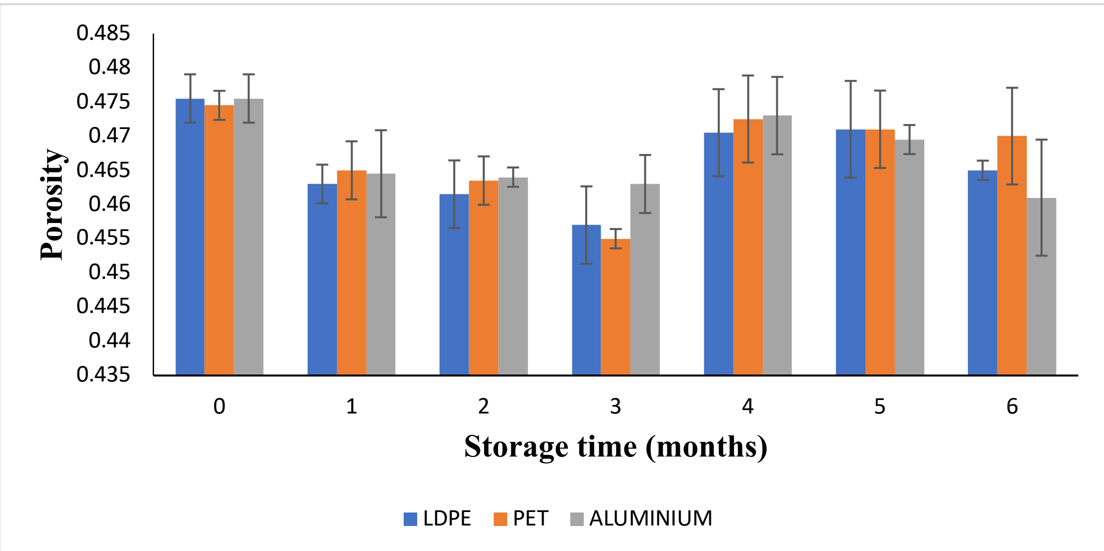

Fig: 4.31 : Effect of storage on porosity of radish leaves based soup mix stored in different packaging material

Table 4.17:Effect of storage on the functional qualities of a soup base made with radish leaves that was kept in various packaging types

Packaging material	Storage intervals (months)	Bulk density	True density	Porosity
Aluminium	0	0.755 ± 0.003^{ij}	1.425 ± 0.004^{j}	0.475 ± 0.003^{a}
	1	0.764 ± 0.002^{hi}	1.432 ± 0.002^{ij}	0.464 ± 0.006^{bcd}
	2	0.772 ± 0.001^{gh}	1.442 ± 0.003^{ghi}	0.464 ± 0.001^{bcd}
	3	0.788 ± 0.005^{ef}	1.455 ± 0.005^{ef}	0.463 ± 0.004^{bcd}
	4	0.804 ± 0.007^{bcd}	1.501 ± 0.001^{d}	0.473 ± 0.005^{abc}
	5	0.806 ± 0.003^{abc}	1.515 ± 0.006^{c}	0.469 ± 0.002^{c}
	6	0.809 ± 0.004^{abc}	1.517 ± 0.001^{c}	0.461 ± 0.008^{cd}
PET	0	0.755 ± 0.003^{ij}	1.426 ± 0.002^{j}	0.475 ± 0.003^{a}
	1	0.763 ± 0.003^{hu}	1.434 ± 0.005^{ij}	0.465 ± 0.004^{abc}
	2	0.781 ± 0.004^{fg}	1.442 ± 0.004^{ghi}	0.463 ± 0.003^{bcd}
	3	0.794 ± 0.001^{de}	1.448 ± 0.003^{fg}	0.455 ± 0.001^{d}
	4	0.807 ± 0.005^{abc}	1.461 ± 0.006^{e}	0.472 ± 0.006^{abc}
	5	0.809 ± 0.000^{ab}	1.513 ± 0.001^{c}	0.471 ± 0.005^{abc}
	6	0.817 ± 0.006^{a}	1.522 ± 0.008^{c}	0.470 ± 0.007^{abc}
LDPE	0	0.755 ± 0.003^{ij}	1.426 ± 0.002^{j}	0.475 ± 0.003^{a}
	1	0.767 ± 0.004^{h}	1.435 ± 0.004^{j}	0.463 ± 0.002^{bcd}
	2	0.782 ± 0.002^{f}	1.445 ± 0.001^{efg}	0.461 ± 0.004^{cd}
	3	0.799 ± 0.001^{cd}	1.461 ± 0.006^{e}	0.457 ± 0.005^{d}
	4	0.813 ± 0.005^{ab}	1.518 ± 0.005^{c}	0.470 ± 0.006^{abc}
	5	0.813 ± 0.006^{ab}	1.531 ± 0.008^{ab}	0.471 ± 0.007^{abc}
	6	0.814 ± 0.007^{ab}	1.533 ± 0.007^{a}	0.465 ± 0.001^{abc}

Duncan's multiple range test was used with Sigma Plot 11.0 software (Sigma Plot Software; San Jose, California, USA) to run a two-way ANOVA at p 0.05. Statistics was considered statistically significant for mean values ($p<0.05$). Results are the average of three replicates, and values are the mean ± standard deviation.

CHAPTER 5 : CONCLUSION AND FUTURE SCOPE

The present study on "**Characterization, functionality and utilization of radish leaves for its value addition**" was undertaken to explore the possibilities for future use of radish leaves as an economical wellspring of supplements and wellbeing helping bioactive mixtures, that will assist in expanding the dietary benefit of the food and will with giving a better choice to the shopper on the lookout. Despite their great nutritional value and health-promoting qualities, radish leaves are generally considered to be waste, with the exception of the less developed regions of the world. The addition of bio-waste as a result of handling creates a major threat to not only mankind but also the environment. Therefore, it is necessary to take appropriate waste reduction strategies that will also benefit society.

In this study, work has been completed to maintain the freshness of radish leaves for a longer period of time without affecting its quality metrics. The storing conditions were improved using a box-behnken design.Leaf lengths of 8.55cm stored at 4 °C were observed to exhibit higher quality upkeep.In addition, leaves were dried at 45, 55, and 65 °C, and the moisture content data from the drying experiment were fitted to the fitted parameters. Validation of the fitted parameters was determined based on the fitted parameters with the highest R2, and the fitted parameters with the lowest values of Chi-square (χ 2) and root mean square error (RMSE). The tray dryer's R2, RMSE, and χ2 values have been reported to range from 0.884 to 0.998, 0.014 to 0.14, and 0.0002 to 0.009, respectively. The Midilli model, followed by the Page Model, gave the highest R2 values, as well as the lowest Chi-square and root mean square error values, among the twelve models considered for tray drying.

The results about the drying of radish leaves seemed to best fit this model. At a temperature of 55 °C, the coefficient of determination had the highest value while the RMSE and χ2 had the lowest values. In all situations, the R2 value was significantly higher for the Midilli model, and the RMSE and χ2 values were also lower in this instance.The dried leaves powder was analysed for it powder properties on the basis of sieve size, 50 microns sieve exhibited best results.

The powder wasthen formulated into soup and chutney mixes which were then stored into different packaging materials (aluminium pouches, PET containers and LDPE pouches) over the period of six months under ambient conditions. During the storage of instant mixes, aluminium pouch packed product gave best results analysed on the basistheir functional properties.

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