TO STUDY THE EFFECT OF NANOENCAPSULATION ON THE GROWTH BEHAVIOUR AND STORAGE LIFE OF PROBIOTICS IN THE NUTRICEREALS BASED BEVERAGES

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Food Science and Technology

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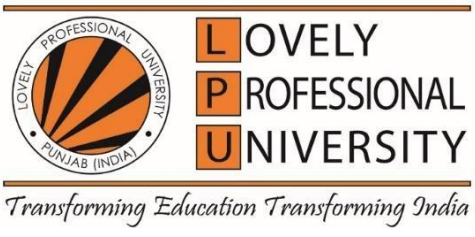


LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

August, 2025

DECLARATION

I, hereby declare that the presented work in the Project entitled "**To study the effect of nanoencapsulation on the growth behaviour and storage life of probiotics in the nutricereals based beverages**" in fulfillment of the degree of **Doctor of Philosophy** is an outcome of research work carried out by me under the supervision of Dr Mukul Kumar, Associate Professor, Department of Food Technology of School of Agriculture, Lovely Professional University, for the award of degree of Ph.D Food Science and Technology.

Date: 12/08/2025

Sneha K (11919638)

I certify that the above statement made by the student is correct to the best of my knowledge and belief.

Date: 12/08/2025

Place: Phagwara, Punjab (India)

Supervisor- Dr. Mukul Kumar

Signature of External Examiner

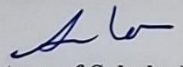
Co-Supervisor – Dr. Ashwani Kumar

Institute of Food Technology (Assistant Professor)

Bundelkhand University, Jhansi

DECLARATION

I, hereby declared that the presented work in the thesis entitled "**To study the effect of nanoencapsulation on the growth behaviour and storage life of probiotics in the nutricereals based beverage**" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of **Dr. Mukul Kumar**, working as **Associate Professor**, in the **Department of Food Technology and Nutrition/School of Agriculture**, of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled **To study the effect of nanoencapsulation on the growth behaviour and storage life of probiotics in the nutricereals based beverage**" submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the **Department of Food Technology and Nutrition/School of Agriculture**, is a research work carried out by **Sneha K., 11919638** is bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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List of Abbreviations

Abbreviation	Expansion
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Collaboration
B1	Beverage with nanoencapsulated *L. plantarum*
B2	Beverage with nanoencapsulated *L. acidophilus*
B1C	Beverage with free cells of *L. plantarum*
B2C	Beverage with free cells of *L. acidophilus*
BV	Biological Value
C1-C5	Composition1 -Composition5
CCD	Central Composite Design
CFU	Colony Forming Units
CM	Conventional Millet flour
CMC	Carboxy Methyl Cellulose
cP	Centipoise
CV	Coefficient of Variation
DLS	Dynamic Light Scattering
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EE	Encapsulation efficiency
EDTA	Ethylenediaminetetraacetic Acid
EM	Extruded Millet flour
FAO	Food and Agriculture Organization
FE-SEM	Field Emission-Scanning Electron Microscopy
FS	Ferrous Sulfate
FSSAI	Food Safety and Standard Authority of India
FT	Freeze-Thaw
g	Gram
GAE	Gallic acid equivalent
GM	Germinated Millet flour
HDL	High-Density Lipoprotein
HC	Heat-Cool
hrs	Hours

INR	Indian Rupee
ISAPP	International Scientific Association for Probiotics and Prebiotics
KC	κ-carrageenan
kDa	Kilodalton
Kg	Kilogram
L	Litre
LA	Lactic acid
LDL	Low-Density Lipoprotein
LC-MS	Liquid Chromatography-Mass spectroscopy
Log CFU	Logarithm Colony Forming Units
mg	Milligram
ml	Millilitre
mm	Millimetre
MRS	De Man, Rogosa and Sharpe
mV	Millivolt
NE1-NE6	Nanoemulsion 1 – Nanoemulsion 6
NDRI	National Dairy Research Institute
nm	Nanometre
PDI	Poly disparity Index
pH	Potential of Hydrogen
PMG	Homopolysaccharide
rpm	revolutions per minute
RSM	Response Surface Methodology
SD	Standard Diet
S.D.	Standard deviation
SEM	Scanning Electron Microscopy
TA	Tannic acid
TAC	Total Antioxidant Capacity
TPC	Total Phenolic content
TS	Total solids
TSS	Total Soluble Solids
WHO	World Health Organization

WPI	Whey Protein Isolate
˚C	degree Celsius
µl	Microlitre
µm	Micrometre

Abstract

A nanoemulsion is a dispersion system comprising two immiscible fluids, commonly oil and water, stabilized by an emulsifier in nanoscale (<200nm). Nanoencapsulation is the process of encapsulating essential component in a protective casing in nanoscale or with nanoparticles. In this study, the probiotic strains of lactobacillus are nano-encapsulated and used to develop a synbiotic drink by incorporating in a nutricereals-based beverage prepared from germinated finger millet, pearl millet, and buckwheat malt. The nanoemulsions were prepared by emulsifying κ -carrageenan (KC) (0.25%, 0.5% and 1.0%) and carboxy methyl cellulose (CMC) (0%, 0.5% and 0.25%) with 1% sunflower oil containing 1 and 10% Tween 80 as emulsifier. The nanoemulsion with 1% KC and had droplet size of 186.80 nm, polydispersity index (PDI) of 0.243 and zeta potential of -17.47 mV. The FE-SEM analysis of the prepared nanoemulsion confirmed the smooth, uniform sphere-like shape. The nanoemulsion was added with *Lactobacillus plantarum* NCDC 685 and *Lactobacillus acidophilu*s NCDC 600 individually to obtain 11.90 Log CFU/ ml and 11.86 Log CFU/ml. The encapsulation efficiency of *L. acidophilu*s NCDC 600 in the freeze-dried nanoemulsion was 76.33%, which was significantly ($p<0.05$) higher than the encapsulation efficiency of 74.28% for *L. plantarum* NCDC 685. The viability of nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 had lower decrease of 9.66% and 9.25%, respectively, when exposed to 0.3% bile for 2 hrs which were significantly higher than free cells of 32.29% and 22.69%, respectively. After 120 min of exposure to pH 2, the nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 had cells maintained significantly ($p<0.05$) higher viability of 7.12 Log CFU/ml and 7.64 Log CFU/ml, respectively. A gradual and steady release from the nanoencapsulation matrix was observed over 6 hrs of incubation by end of which, a significant ($p<0.05$) release of 90.12% and 89.69% was observed for *L. plantarum* NCDC 685 (8.29 Log CFU/g) and *L. acidophilus* NCDC 600 (8.857 Log CFU/g), respectively. The germination of nutricereals were studied with soaking time of 16 and 24 hrs, germination temperature of 22 ˚C, 26 ˚C and 30 ˚C for 24, 48 and 72 hrs of germination. Highest TPC was with germination at 30 ˚C for finger millet (28.63 mg GAE/100g) after 24 hrs of germination, at 22 ˚C after 24 hrs of germination for pearl millet (28.63 mg GAE/100g) and at 22 ˚C after 72hrs germination with soaking for 16 hrs for buckwheat (56.55 mg GAE/100g), where the grains were soaked for 16 hrs and dried at 60 ˚C. Highest antioxidant activity was with germination at 22 ˚C for finger millet (23.06 % inhibition of

DPPH) and pearl millet (28.24 % inhibition of DPPH) after 24 hrs of germination and while buckwheat (53.51 % inhibition of DPPH) was after 72 hrs of germination, where the grains were soaked for 16 hrs and dried at 60 ˚C. The lowest tannin content was at 30 ˚C for finger millet (6.49 mg TA/100g) and buckwheat (9.39 mg TA/100g) after 72 hrs of germination while for pearl millet (4.01 mg TA/100g) the lowest tannin content was at 22 ˚C after 24 hrs germination with 24 hrs of soaking and drying at 80 ˚C. Prebiotic effect was highest with *Lactobacillus plantarum*, for finger millet (9.53 Log CFU/g) germinated at 30 ˚C, pearl millet (9.26 Log CFU/g) germinated at 26 ˚C and buckwheat (9.86 Log CFU/g) germinated at 22 ˚C after 72 hrs of germination for all the grains and were soaked for 16 hrs and dried at 60 ˚C. The optimal germination conditions included, a soaking time of 16 hrs for all grains, germination temperature of 30 ˚C for finger millet, 26 ˚C for pearl millet, and 22 ˚C for buckwheat, followed by 72 hrs of germination and drying at 60 ˚C. The nutricereal extract with pearl millet, finger millet and buckwheat in the proportions of 80:15:5, was found to have highest overall acceptability of 6.85. The nutricereal beverage was formulated using a combination of nutricereal malt extract and skimmed milk, analysed for total solids, total phenolic content, tannin content, prebiotic effect, and overall acceptability using response surface methodology (RSM) central composite design (CCD). The optimized ratio of nutricereal malt extract to skimmed milk was determined to be 60:40. The optimized beverage has TS of 21.27%, TPC of 25.99 mg GAE/100ml, tannin content of 17.48 mg TA/100 ml, prebiotic effect of 11.43 Log CFU/ml and overall acceptability of 6.90. Probiotic viability with nanoencapsulates revealed that the beverages containing 1% nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 showing adequate viable counts, meeting the standards set by FAO/WHO for probiotic products of 8.429 Log CFU/ml and 9.547 Log CFU/ml, respectively. The initial viability for beverages with free *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 significantly ($p<0.05$) by 21st day of storage to 6.19 Log CFU/ml and 5.88 Log CFU/ml, respectively, while the nanoencapsulated forms had decreased significantly ($p<0.05$) on 36th day of storage to 5.97 Log CFU/ml and 6.16 Log CFU/ml, respectively. The titratable acidity for beverages with free *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 significantly ($p<0.05$) increased to 0.110 % TA and 0.107 % LA. However, pH for beverages with free *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 significantly ($p<0.05$) decreased to 5.73 and 5.33, respectively by 21st day of storage, while, their nanoencapsulated forms had significantly ($p<0.05$) decreased at 36th day of storage to 5.16 and 5.23, respectively. Nanoencapsulation was found to improve the sensory stability of

probiotic beverages, with the overall acceptability of beverages with free *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 were 6.97 and 6.74, respectively on 21st day of storage, while, their nanoencapsulated forms had significantly ($p<0.05$) decreased on 36th day of storage to 6.59 and 6.55, respectively. The estimated cost for a 200 ml bottle of the optimized nanoencapsulated probiotic beverage is Rs. 35.72 with the nanoencapsulation estimated at Rs. 4.37 /g.

Abbreviations: KC: κ -carrageenan; **CMC:** Carboxy Methyl Cellulose; **PDI**: Polydispersity Index; **FE-SEM:** Field Emission-Scanning Electron Microscopy; **CFU**: Colony Forming Units; **TPC:** Total Phenolic Content; **GAE:** Gallic Acid Equivalent; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **TA:** Tannic Acid; **RSM:** Response Surface Methodology; **CCD:** Central Composite Design;

Key words: Nanoencapsulation, nanoemulsion, droplet size, PDI, zeta potential, FE-SEM, stability, germination, finger millet, pearl millet, buckwheat, nutricereal, *L. plantarum, L. acidophilus,* probiotic beverage, titratable acidity, pH, prebiotic effect, sensory analysis.

Chapter 1

INTRODUCTION

Probiotics are beneficial bacteria that are consumed in the form of supplements or through food. The commonly consumed probiotics for clinical purposes are species from the genera Lactobacillus and Bifidobacterium, (Das et al., 2020). Probiotics by definition are living organisms and have to be administered at an adequate quantity of viable bacteria to the host to promote health (Salminen et al., 2021). The primary selection criteria for probiotics also include their ability to survive gastric acidity and bile toxicity, their significance in surviving and growing in the gastrointestinal environment, and their ability to contribute to promote health (Jinendiran et al., 2019). Various environmental factors, such as pH, mechanical action and temperature, significantly affect the viability and survival of probiotics during processing, storage, and passage through the gastrointestinal tract, and be released in a controlled manner at the target site in the lower gastrointestinal tract to confer health benefits (Razavi et al., 2021). Encapsulation technology is gaining prominence in the food and pharmaceutical industries for preserving and precisely delivering sensitive ingredients. Probiotics can be encapsulated using various cost-effective biomaterials to enhance their stability under challenging conditions (Abbas et al., 2022). Encapsulating probiotics presents a promising approach to enhance their viability and effectiveness within the human body (Yao et al., 2020). Various encapsulation techniques, including microencapsulation, nanoencapsulation, and liposome encapsulation, have been explored as methods to protect probiotic bacteria from the harsh conditions of the stomach and facilitate their targeted release in the digestive tract with nanoencapsulation emerging as a promising approach (Roobab et al., 2020, Zhu and Huang, 2019). Nanoencapsulation utilizes biopolymers such as alginate, chitosan, gelatin, whey protein isolate, cellulose derivatives, and vegetable gums to encapsulate probiotic cells, providing protection from external influences and enhancing their survivability (Afzaal et al., 2019). Furthermore, nanoencapsulation can enhance the adherence and colonization of probiotics in the human gastrointestinal tract, thereby augmenting their effectiveness in promoting health benefits (Xu et al., 2022).

Traditional encapsulation techniques such as spray-drying, extrusion, freeze-drying, and emulsification enhance probiotic tolerance to adverse conditions. Comparatively, freeze-drying is gentler but still poses challenges, highlighting the need for careful consideration

of microencapsulation techniques in preserving probiotic viability (Xu et al., 2022). In this research, nanoencapsulation is achieved through a combination of nanoemulsification and freeze-drying techniques. This approach merges the advantages of emulsification, facilitating uniform distribution, with the gentler conditions of freeze-drying, which minimize damage to encapsulated materials. A nanoemulsion represents a nanoscale dispersion system comprising two immiscible fluids, commonly oil and water, stabilized by an emulsifier. Renowned for its adaptability and effectiveness, nanoemulsion serves as an exceptional delivery system (Dhiman et al., 2021). Nanoemulsions possess a unique advantage stemming from their small droplet size, typically falling within the range of 10 to 200 nm. This feature enhances surface area and consequently improves the stability and bioavailability of active ingredients (Kumar et al., 2019). Physical encapsulation methods often require sophisticated equipment, and considerable energy input, and chemical approaches may involve expensive metal precursors and the use of harmful or toxic chemical solvents. Nanoencapsulation, though offers significant advantages in enhancing viability and stability of probiotics, it also poses several practical challenges. The physical encapsulation methods often require sophisticated equipment, and considerable energy input, and chemical approaches may involve expensive metal precursors and the use of harmful or toxic chemical solvents (Taouzinet et al., 2023). Due to its specific parameters required to obtain complete encapsulation, scale up for commercial production without compromising the efficiency, yield, or particle uniformity is difficult (Lavanya et al., 2024). The nanoencapsulation in food system also involves certain challenges, such as preserving the is nutritional efficiency while it is still manufactured with economic and practical processing techniques suitable for large scale production (Sahoo et al., 2021). Similarly, there is a lack of comprehensive data on the toxicological effect and long-term impacts of components of the nanoparticles that may cross biological barriers and accumulate in the body which raises safety concerns (Jagtiani et al., 2022). The encapsulating agent has a direct influence on the particle morphology, diameter, and permeability, while also ensuring effective protection for microbial cells against environmental factors and demonstrating satisfactory control over the release process (Rodrigues et al., 2020). Extensive research has explored various materials for use in conjunction with probiotics encapsulation where, polysaccharides and protein-based materials have surfaced as the prevailing options. Food-grade polymers like gelatin, alginate, chitosan, carrageenan, pectin, and carboxymethyl cellulose (CMC) are commonly observed in encapsulation techniques (Sun et al., 2023). Carrageenans are naturally occurring sulphated polymers

extracted commercially from red algae. Kappa-carrageenan has the ability to form double-helix structures through interaction with bivalent ions, leading to increased viscosity of polymer solutions and protection of the ionic groups (Madruga et al., 2020). Encapsulation of probiotics, particularly on nanoparticles like nano-chitosan, offers a viable solution for safe delivery and synergistically improved the antioxidant and antibacterial properties of the probiotics, showing promise for controlling pathogenic bacteria (Al-Hazmi and Naguib, 2023).

Current consumers prioritize personal health and expect their food to offer both nutritional value and potential illness prevention benefits and diverse food carriers for probiotics have been explored for this purpose. These carriers encompass dairy products like cheese, yogurt, and ice cream, as well as non-dairy alternatives such as meat, fruits, cereals, and chocolate (Yoha et al., 2021). The production and consumption of functional beverages have garnered significant attention, and the market is experiencing growth (Srikaeo, 2020). Beverages serve as an ideal medium for delivering nutrients and bioactive compounds to the body while enhancing their bioavailability including probiotics (Ignat et al., 2020). Cereal-based beverages incorporating germinated cereals have garnered attention as potential functional foods enriched with probiotics, delivering dual benefits to consumers (Ignat et al., 2020). Plant based beverages are low in saturated fats, free of cholesterol and lactose making it suitable for people who cannot consume bovine milk. Also, they contain fibre and phytochemicals (Silva et al., 2020). The malting process consists of three main stages, namely steeping where the grains are soaked in water to rehydrate, followed by germination to promote the sprouting process, and finally drying, to stop germination and ensure their storage over an extended period (Cash, 2021). This is followed by mashing which involves dissolving the naturally water-soluble components, followed by enzymatic hydrolysis, which breaks down complex components into simpler and then the process is concluded with the separation of the dissolved components (Montanari, et al., 2005). The germination process promotes enzymatic activity initiates the breakdown of proteins, carbohydrates, and lipids into simpler forms, along with activating proteases responsible for protein degradation (Sruthi and Rao, 2021). Additionally, the activation of these hydrolytic enzymes results in the degradation of starch, non-starch polysaccharides, and proteins, leading to the accumulation of oligosaccharides and amino acids (Kumari et al., 2024). Germination of millets has been shown to potentially to enhance the functional attributes of various millet varieties. Consequently, the changes in the nutritional attributes

of different millet varieties through germination could position them as potential healthy foods capable of either inhibiting or preventing disease incidences (Bhat et al., 2021). Moreover, the germination process of cereals enhances their nutritional value and bioavailability, rendering them an optimal substrate for probiotic growth. The inclusion of probiotics in these beverages not only enhances their functional properties but also augments potential health benefits, particularly in improving gut health, strengthening the immune system, and promoting overall well-being (Srikaeo, 2020). Additionally, the incorporation of germinated cereals enriches the beverages' nutritional profile with essential vitamins, minerals, and fiber (Ignat et al., 2020). The use of millets and pseudo-cereals in the development of probiotics gives the additional benefits of prebiotics and make them synbiotic (Kumar et al., 2020a). The prebiotic oligosaccharides found in millets and pseudocereals offer a valuable source of specific nutrients capable of promoting the proliferation of beneficial gut microflora, including *bifidobacteria* and *lactobacilli* (Chen et al., 2021). Prebiotic oligosaccharides such as xylooligosaccharides, fructooligosaccharides, and inulin are commonly found in various millets and pseudocereals (Torbica et al., 2022). The prebiotic oligosachharides such as xylobiose and xylooligosaccharides present in finger millet have the potential to promote the growth of probiotic lactobacillus species (Kumar et al., 2020a). Finger millet, commonly known as ragi in India, has garnered recognition as a nutritious and versatile cereal, often hailed as a functional food. Renowned for its richness in essential nutrients such as calcium, iron, and vitamin D, finger millet emerges as a compelling choice for promoting bone health (Jagati et al., 2021). White finger millet probiotic beverage has been found to have potential to serve as an excellent alternative to conventional probiotic foods for strict vegans and lactose-intolerant individuals, offering a nutritious and functional beverage option (Navyashree et al., 2022). Overall, finger millet stands as a nutrient-rich grain offering a plethora of health benefits. Its versatility allows for incorporation into various dietary forms, including beverages, breakfast cereals, and groats, providing individuals with diverse avenues to integrate its nutritional advantages into their diets. Pearl millet grain is esteemed for its remarkable nutritional composition, characterized by substantial levels of proteins, fats, and essential vitamins, including vitamin A and various B vitamins. Furthermore, it boasts low starch levels, augmented fiber content, and elevated concentrations of iron and zinc, essential minerals vital for various physiological functions (Srivastava et al., 2020b). When malted finger millet was combined with toned milk and inoculated with *Lactobacillus helveticus*, it exhibited a superior probiotic effect compared

to milk alone. This suggests that the addition of malted finger millet enhanced the probiotic activity of the beverage. Such findings underscore the potential of incorporating malted finger millet into dairy products as a means to augment their probiotic properties, offering potential health benefits to consumers (Chaudhary and Mudgal, 2020). Pearl millet flour underwent lactic acid fermentation using *L. plantarum* across a range of temperatures, durations, and pH levels. The initial microbial count in unfermented pearl millet increased significantly post-fermentation, demonstrating the efficacy of *L. plantarum* in enhancing microbial population. Specifically, the total viable count rose from its initial level, as did the count of Lactic Acid Bacteria. These findings underscore the potential of pearl millet as a substrate for probiotic fermentation with *L. plantarum*, suggesting its suitability for producing fermented products with enhanced microbial activity (Srivastava et al., 2024).

The study is focused on preparation of nutricereal malt-based beverage with nutricereal malt prepared by malting and mashing the grains. The incorporation of probiotics into nutricereal-based beverages, particularly those enriched with germinated millets and pseudocereal such as finger millet, pearl millet and buckwheat, offer a novel avenue to deliver functional foods with enhanced nutritional and health-promoting properties. This research also contributes to the development of innovative dietary solutions that support gut health and overall well-being.

Chapter 2

REVIEW OF LITERATURE

2.1. Probiotics

Probiotics consist of live microorganisms that, when consumed in sufficient quantities, offer health advantages. Commonly utilized strains include Lactic acid bacteria, bifidobacteria, and the yeast *Saccharomyces boulardii*. Additionally, research is ongoing to explore the probiotic potential of various bacterial strains like Enterococcus, Streptococcus, and Bacillus, among others (Anee et al., 2021). Probiotic as a term is a word meaning "for life" and it is currently used to describe a group of bacteria when administered in sufficient quantity, provide beneficial effects for humans and animals (FAO/WHO, 2012). The International Scientific Association for Probiotics and Prebiotics (ISAPP) defined probiotics as "live microorganisms, that when administered in adequate amounts, confer a health benefit on the host" (Salminen et al., 2021). Probiotics have been found to prevent and improve digestive disorders, allergic disorders, and Clostridium difficile–associated diarrhoea and some inflammatory bowel disorders in adults. Probiotics may also be useful as coadjutants in the treatment of metabolic diseases such type 2 diabetes, non-alcoholic fatty liver disease, metabolic syndrome, and obesity (Sharifi-Rad et al., 2020).

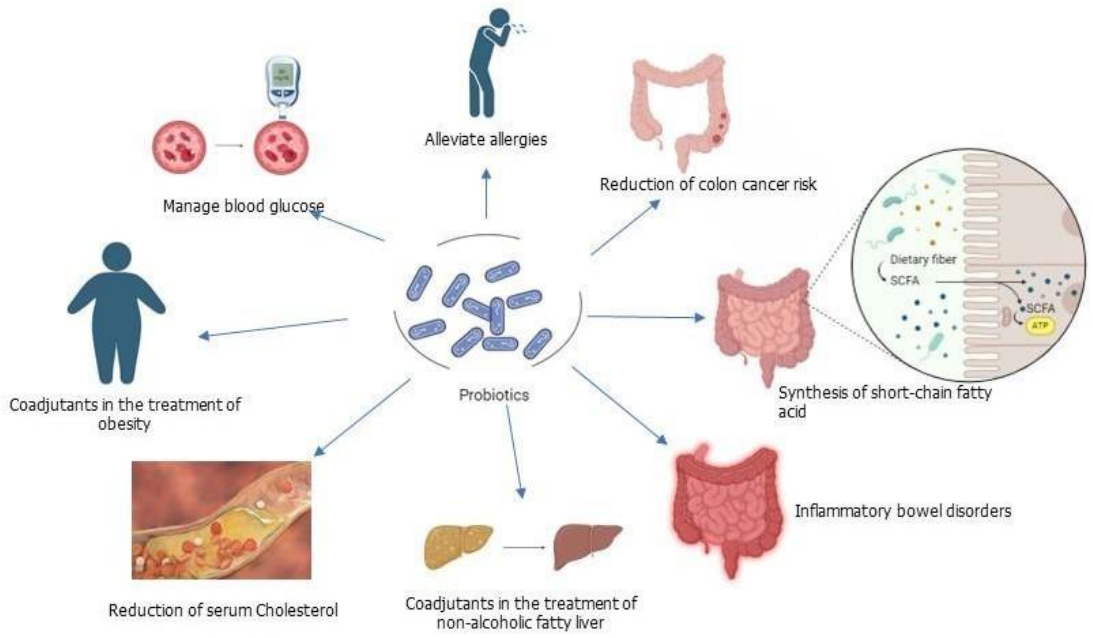


Figure. 2.1. Potential health benefits of probiotics in human health

A probiotic product should ideally contain a viable cell count of probiotic microorganisms exceeding 10^6 colony forming units per ml or g. Various factors can impact the viability of probiotics in food products during processing and storage. These factors may be intrinsic, including pH, titratable acidity, oxygen levels, water activity, and the presence of salt, sugar, hydrogen peroxide, bacteriocins, artificial additives, or processing-related treatments. Additionally, extrinsic factors such as fermentation conditions, incubation temperature, heat treatments, cooling and storage conditions, packaging materials, production scale, and the microbiological characteristics of the probiotics, such as the specific strain or inoculation rate, can also influence probiotic viability (El-Sohaimy and Hussain, 2023).

The effect of the consumption of indigenous probiotic *Lactobacillus plantarum* Dad-13 powder was studied in overweight adults having a BMI equal to or greater than 25. The study revealed no significant change in cholesterol and triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and the LDL/HDL ratio. A significant decrease in the average body weight and BMI was observed with the group administered with probiotic decrease from 84.54 ± 17.64 kg to 83.14 ± 14.71 kg and 33.10 ± 6.15 kg/m^2 to 32.57 ± 5.01 kg/m^2, respectively while there was no change in the placebo group (Rahayu et al., 2021).

In a randomized, double-blind, placebo-controlled study, the effects of probiotic yogurt containing *L. acidophilus* La5 and *Bifidobacterium lactis* Bb12 on oxidative stress biomarkers in patients with metabolic syndrome were evaluated. Forty-four participants, comprising 22 males and 22 females aged 20 to 65 years, were divided into treatment and control groups, with each group consuming 300 g/d of probiotic or regular yogurt, respectively, for 8 weeks. Serum levels of uric acid, oxidized low-density lipoprotein, malondialdehyde, and total antioxidant capacity were assessed before and after the trial. Probiotic yogurt consumption led to a significant reduction in serum uric acid levels and a notable increase in total antioxidant capacity. Additionally, a positive correlation was observed between uric acid levels and insulin concentration, as well as the homeostasis model assessment of insulin resistance, alongside an inverse relationship with insulin sensitivity. These findings suggest that probiotic yogurt consumption may enhance insulin sensitivity, thereby potentially mitigating oxidative stress and lowering uric acid levels, although further research is warranted for conclusive determinations (Rezazadeh et al., 2021).

2.2. Encapsulation

2.2.1. Definition

Encapsulation involves enveloping a core material within a protective shell to achieve desired characteristics, including preservation, controlled release, targeted delivery, environmental protection, corrosion inhibition, stability, and toxicity mitigation. This is applicable in various sectors such as food, pharmaceuticals, cosmetics, and environmental engineering (Pasarkar et al., 2023).

Encapsulated particles are categorized as nanocapsules, microcapsules, and macrocapsules based on their size, where nanocapsules are <0.2 µm, microcapsules are 0.2–5000 µm, and macrocapsules are >5000 µm (Pateiro et al., 2021). Solid nanoparticles are categorized as either nanospheres or nanocapsules, constituting dispersed particles ranging in size from 10 to 1,000 nm (Lima et al., 2022). The nano size of the capsules is beneficial in modifying the organoleptic properties of food without compromising on the added value, aid in processability and increase the stability during storage. Nanoencapsulation has also been found to increase the bioavailability of the encapsulated active component (Pateiro et al., 2021). Nanoencapsulation using nanoemulsions involves dispersing active ingredients in a system of tiny droplets, typically ranging from 10 to 200 nanometers, to improve their stability and bioavailability (Banasaz et al., 2020).

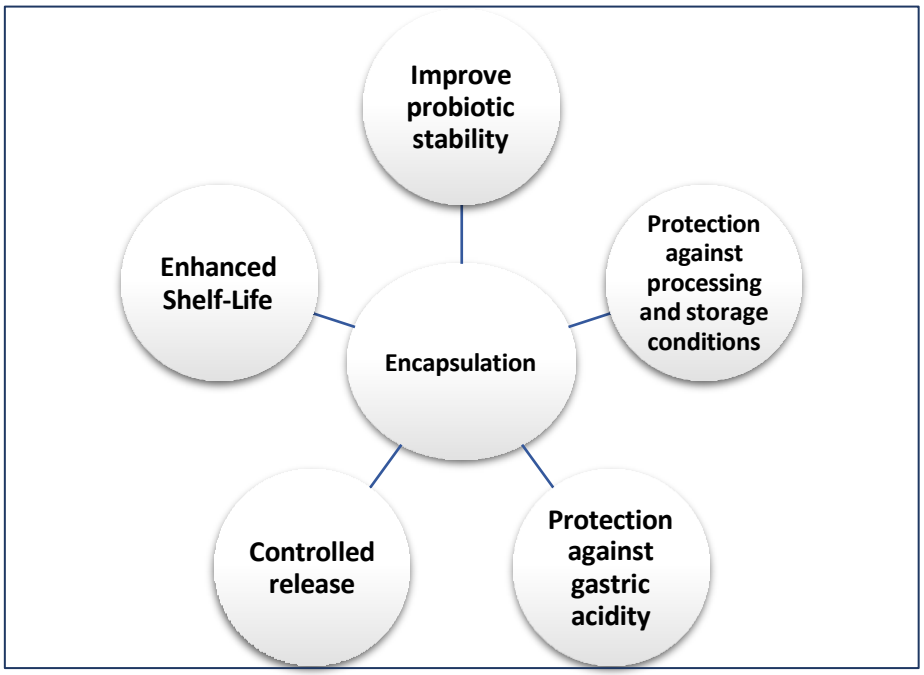


Figure. 2.2. Key benefits of encapsulating probiotics.

In a study, the viability and stability of *L. acidophilus* was found to be enhanced by nanoencapsulating with chitosan. It was also found that the survival of the probiotic was improved by nanoencapsulation in simulated gastric and intestinal environment (Ebrahimnezhad et al., 2017). *L. paracasei* KS-199 nanoencapsulated at 842 nm using alginate has shown enhanced thermal stability, enhanced viability and survivability in simulated gastric juices and in kefir, without changing the texture or other characteristics of kefir (Yilmaz et al., 2020). Although nanoencapsulation is discussed, there is inconsistency in the reported size ranges and lack of clarity on standardization methods across studies. Nanoencapsulation of *Lactobacillus brevis* with sodium alginate by electrospinning in a yogurt drink called ayran, apple juice and orange juice were studied to find that the nanoencapsulation has increased the survival ratio of the bacteria without decreasing the pH of the beverage (Mohaisen et al., 2019). In another study, a Wurster fluidized bed coater was utilized to apply double coatings to *L. acidophilus*, aiming to enhance its resistance to simulated gastric conditions and increase heat stability during bread baking. The first coating layer consisted of Xanthan (0.5%, 1%, and 1.5% w/v) and alginate (0.5%, 1%, and 1.5% w/v), with microcapsules coated in 1% alginate exhibiting the highest relative survival in simulated gastric conditions, thus chosen as the first layer. The second layer comprised chitosan (0.5%, 1%, and 1.5% w/v) and gellan (0.5%, 1%, and 1.5% w/v). Encapsulation efficiency decreased with increasing levels of alginate and xanthan, with microcapsules containing 1% xanthan showing significantly higher encapsulation yield. The presence of 1% alginate in the wall matrix significantly improved acid resistance, while 0.5% chitosan in the outer layer increased resistance to heat treatment at 90˚C for 15 min. Scanning electron microscopy (SEM) results revealed that chitosan provided a smoother surface, crucial for cell protection. Evaluation in bread demonstrated that 1% chitosan in the outer layer enhanced probiotic survivability both immediately post-baking and 24 hrs later, suggesting the effectiveness of alginate and chitosan in probiotic bread production (Mirzamani et al., 2021). This study systematically evaluates different coating compositions and concentrations, providing data on encapsulation yield, acid resistance, heat stability, and probiotic viability post-processing. It also notes a decrease in encapsulation efficiency with higher polymer concentrations but does not delve deeply into optimizing the balance between efficiency and functional performance such as resistance to change in pH and temperature.

The literature provides a broad overview of encapsulation and its relevance across sectors such as food, pharmaceuticals, cosmetics, and environmental engineering. The functional advantages of nanoencapsulation are clearly articulated, particularly in food applications. These include improved bioavailability, better organoleptic properties, enhanced stability, and compatibility with food matrices. Most findings are based on *in vitro* simulations, with minimal to no mention of *in vivo* studies that would validate the biological efficacy and bioavailability of encapsulated probiotics in human or animal models. While some these studies claim no adverse sensory impact, detailed sensory analysis or consumer acceptance studies are lacking to support this claim systematically. Though encapsulation of probiotics show promising results, the literature lacks analysis of economic feasibility, scalability of encapsulation techniques, and potential challenges in commercial manufacturing settings.

2.2.2. Nanoemulsion

An emulsion characterized by droplets exceeding a mean diameter of 200 nm is commonly termed a conventional emulsion. Conversely, an emulsion featuring droplets with a mean diameter below this threshold is denoted as a nanoemulsion, offering distinct advantages in various applications (Choi and McClements, 2020). Nanoemulsion systems exhibit versatility in encapsulating, safeguarding, enhancing bioavailability, and precisely releasing delicate functional components. They hold significant promise for transporting nutraceuticals, probiotics, as well as enhancing the delivery of flavours and colours, thereby opening avenues for diverse applications in the food and pharmaceutical industries (Islam et al., 2022). The manufacturing techniques employed for nano-emulsions significantly influence droplet size and, consequently, the stability of the emulsion system through process parameters and composition (Sarheed et al., 2020). Nanoemulsions within the 50-200 nm size range yield a transparent solution, whereas those with sizes up to 500 nm exhibit a milky or cloudy appearance, delineating a distinct visual contrast based on droplet size distribution (Mushtaq et al., 2023). Nanoemulsions are created by reducing the size of larger emulsion droplets into nano-sized droplets through the application of external forces, such as pressure or energy. This process enhances the physicochemical and sensory characteristics of nanoemulsion, offering advantages in various applications, including food, pharmaceuticals, and cosmetics (Sneha and Kumar, 2021). Nanoemulsions, being kinetically stable systems, do not necessitate the inclusion of a cosurfactant, unlike microemulsions. This stability is attributed to the dominant Brownian motion of droplets, which counteracts tendencies toward sedimentation and coalescence (Barradas and de

Holanda e Silva 2021). The minute droplet size characteristic of nanoemulsions effectively mitigates sedimentation, creaming, flocculation, and phase separation phenomena during storage. By virtue of their small size, nanoemulsion droplets exhibit enhanced stability, which prevents coalescence and aggregation, thereby maintaining the homogeneity and uniformity of the system over prolonged periods of storage (Patel et al., 2022). High-energy emulsification techniques demand substantial mechanical force input to produce uniform droplets, employing methods such as high-pressure homogenization (HPH), microfluidization, and ultrasonication. These processes are instrumental in generating nanoemulsions with consistent droplet size distributions (Wilson et al., 2022). Nanoemulsions produced through low-energy methods, harnessing the internal chemical energy of the system, known as the chemical potential of components. The energy released during emulsification drives the process in this method. Typically, low-energy methods entail gentle stirring at a lower rate, typically around 1600 rpm, resulting in reduced energy consumption compared to high-energy methods. This approach leverages the inherent chemical dynamics of the system to create nanoemulsions efficiently and effectively (Safaya and Rotliwala, 2020).

The literature provides a strong foundational overview of nanoemulsion systems, highlighting their structural uniqueness, stability, and functional applications across industries. However, it remains largely theoretical and general, with minimal experimental or formulation-specific evidence. There is a need for a deeper exploration of formulation variables, and real-world stability data to bridge the gap between theoretical potential and practical implementation.

2.2.3. Emulsifiers used for production of nanoemulsion

Nanoemulsions, being thermodynamically unstable, require the addition of surfactants or emulsifiers before the emulsification process. These substances enable the formation of nanoemulsions that remain kinetically stable for a desired duration, facilitating their use in various applications (Banasaz et al., 2020). Emulsions consist of three essential components: an oil or oleaginous liquid, a water or aqueous liquid, and a suitable emulsifier (Espinoza-Leandro et al., 2023). The emulsifier, which can be a low-molecular-weight surfactant, protein, peptide, or lipid, plays a crucial role in emulsion formation. Its addition is vital for reducing the interfacial tension between the oil and water phases, facilitating the formation of small droplets. Additionally, the emulsifier contributes to the stabilization of

nanoemulsions by providing steric hindrance and promoting repulsive electrostatic interactions, thereby enhancing the overall stability of the system (Sneha and Kumar, 2021). Nanoemulsions are usually formulated with surfactant concentrations of 5–10%, contrasting with micro-emulsions that are prepared with more than 20% (Sarheed et al., 2020). Selection of an appropriate emulsifier is crucial for effective emulsification and long-term stability of the emulsion. Both high-energy and low-energy methods for nanoemulsion formation rely on the emulsifier and are influenced by factors like temperature fluctuations and surfactant concentration. Typically, low-energy methods necessitate the inclusion of co-surfactants to aid in the creation of nanoemulsions (Gazolu-Rusanova et al., 2020). Emulsifiers utilized in nanoemulsion formulations can be categorized as natural or synthetic based on their source. Natural emulsifiers include proteins, polysaccharides, phospholipids, and saponins, while synthetic emulsifiers consist of small-molecule surfactants like Tweens and Spans (Sneha and Kumar, 2021).

The literature provides a comprehensive introduction to nanoemulsion systems, emphasizing their unique structural features, high kinetic stability, and wide-ranging applications in sectors such as food, pharmaceuticals, and cosmetics. It successfully outlines the distinguishing characteristics of nanoemulsions compared to conventional and microemulsions, along with an overview of both high-energy and low-energy preparation methods. The advantages associated with reduced droplet size, such as improved stability, enhanced bioavailability, and prevention of physical separation, are clearly articulated. However, there is a noticeable gap in data related to product stability and performance under actual storage and usage conditions. In order to address the practical application of nanoemulsions, further studies are required on providing empirical evidence, refining formulation techniques, and evaluating system behaviour in real-world scenarios.

2.2.4. Nanoencapsulation material

The polymers that are complex molecules made up of multiple repetitions of one or more units, referred to as monomers. They can be categorized into three groups: natural, synthetic, and semi-synthetic. These substances serve as suitable encapsulating materials for biomolecules and must possess specific characteristics such as favourable rheological properties, stability, inertness towards the encapsulated material, solubility in safe solvents, lack of hygroscopicity and controlled release capabilities. Biopolymers, which are natural polymers, are derived from plants, animals, and microorganisms. They consist primarily of

proteins such as gelatin, collagen, and albumin; polysaccharides including starch, cellulose , agarose and chitosan; as well as lipids like paraffin, beeswax and stearic acid (da S. Pereira et al., 2021).

Carrageenans are sulphated polysaccharides extracted from different types of red algae. They consist of high-molecular-weight hydrophilic compounds made up of alternating units of d-galactose and 3,6-anhydro-galactose connected by alternating α-1,3 and β-1,4-glycosidic bonds. With an ester-sulphate content ranging between 15% and 40%, carrageenans are classified as anionic polysaccharides. Initially employed as a thickening agent in the food industry, their gelling, emulsifying, and stabilizing properties have led to their use across various other areas (Pacheco-Quito et al., 2020). Nanoencapsulated grapefruit essential oil was created using carrageenan and the o/w single emulsion solvent evaporation method with a concentration of 0.5% w/v, aiming to enhance its bioactivity and improve stability. The resulting particles formed a visibly cloudy emulsion without any signs of phase separation, indicating stability. The average diameter size of the nanoencapsulated particles was less than 100 nm, and their zeta potential ranged from -20 to -40 mV (Gupta et al., 2023). In a research, d-limonene-κ-carrageenan nanoparticles were synthesized using an electrospray method, combining d-limonene and κ-carrageenan in a single step. The nanoparticles exhibited spherical morphology, with an encapsulation efficiency reaching 97%. These nanoparticles demonstrated pH-dependent release behaviour in vitro. Increasing the κ-carrageenan concentration enhanced the photostability and thermostability of d-limonene, with over 85% of d-limonene preserved after 120 min of UV-light exposure in nanoparticles containing 0.5% κ-carrageenan. This approach offers a promising method for the encapsulation of sensitive bioactive agents like d-limonene, potentially enhancing their stability and controlled release characteristics (Fani et al., 2022). Nutraceutical delivery systems utilizing carrageenan encompass a diverse array of formulations including, hydrogels, nanoparticles, complexes, emulsions, microcapsules, aerogels, microbeads, and nanotubes. The efficacy of these systems is influenced by various factors such as the type of carrageenan, biopolymer ratios, crosslinking agents, sequence of material addition, and pH conditions, highlighting the importance of meticulous formulation design and optimization (Dong et al., 2021).

Biopolymers, are presented as effective materials for nanoencapsulation due to their natural origin, biodegradability, and functional properties such as gelling, emulsifying, and stabilizing capabilities. The structural and functional attributes that make natural polymers

suitable for encapsulation, such as their biodegradability, gelling ability, and compatibility with various active agents are highlighted. Studies have demonstrated the potential of carrageenan-based nano formulations to enhance the stability, bioactivity, and controlled release of sensitive compounds with promising results in terms of particle size, encapsulation efficiency, and physicochemical stability. Additionally, the literature acknowledges the broad applicability of carrageenan in diverse delivery systems, emphasizing the influence of formulation variables on performance. However, while these findings underscore the theoretical potential and experimental promise of carrageenan-based systems, the discussion lacks in vivo validation and long-term stability data under real storage and application conditions. Moreover, the scalability of these nanoencapsulation techniques for industrial use is not addressed.

2.2.5. Nanoencapsulation

Freeze-drying is crucial for achieving high encapsulation efficiency and stability of nano-capsules, particularly for heat-sensitive food ingredients and bioactive compounds. This technique is increasingly favoured for nanoencapsulation due to its efficient drying process. The characteristics of the nanoparticles produced via freeze-drying depend on the emulsification methods or other encapsulation techniques used to reduce droplet size to the nanoscale (Tahir et al., 2021). Lyophilization through freeze drying, stands as a viable method for enhancing the stability of nanoparticle-based carriers. Ideally, a successful lyophilizate should retain the physical and chemical attributes of the original product, manifesting as a visually appealing product with rapid reconstitution, minimal residual moisture, and prolonged stability. Resuspended nanoparticle-based carriers should exhibit ease of dispersion without substantial alterations in particle size, size distribution, or encapsulant integrity. Achieving these objectives necessitates meticulous optimization of both the formulation and lyophilization process, along with careful consideration of storage conditions for the resultant lyophilized product (Degobert and Aydin, 2021).

A study assessed the efficacy of whey protein isolate in combination with maltodextrin and gum Arabic as a delivery system for encapsulating Citrus reticulata essential oil. Molecular docking simulated the interaction between Citrus reticulata essential oil and whey protein isolate (WPI). Results indicated significant improvements in physicochemical characteristics and storage stability of formulations containing WPI. The formulation exhibited optimal results including encapsulation efficiency of 92.08%, highest glass

transition temperature of 79.11 ˚C, high crystallinity of 45.58%, thermal stability with < 5% mass loss at 100 ˚C, and superior antioxidant activity with the lowest peroxide value post-storage (Mahdi et al., 2021). Kiran et al., (2023), investigated the impact of protein-based nanoencapsulation on the viability and stability of probiotic *Lactobacillus rhamnosus* under digestion conditions and in a model food system. Protein-based nanoparticles were first prepared using the pH cycling method, followed by nanoencapsulation of *L. rhamnosus*. Whey protein and zein protein were utilized for individual encapsulation of the probiotics. The nanoencapsulated probiotics were characterized by particle size analysis, SEM, and in vitro assays. Subsequently, both free and nanoencapsulated probiotics were incorporated into model yogurt and subjected to microbiological and sensory evaluations. Nanoencapsulation with both whey and zein proteins significantly improved the stability and viability of *L. rhamnosus*. The particle sizes of the whey and zein nanoencapsulated probiotics ranged from 96 to 100 nm, with encapsulation efficiencies of 96% and 87%, respectively. SEM images revealed the irregular spherical structure of both zein and whey nanoparticles. In vitro analysis revealed that whey nanoencapsulation exhibited the highest viability in simulated gastric conditions and simulated intestinal conditions, followed by zein nanoencapsulation, while free probiotics exhibited the lowest viability.

The ability of freeze-drying to preserve the physicochemical properties and reconstitution capacity of nanoparticles underscores its relevance in food and pharmaceutical applications. Studies have demonstrated that the success of this method relies heavily on precise control of both formulation and lyophilization conditions. Despite these promising findings, there is a lack of long-term storage studies and real food system validations beyond model systems. Furthermore, the scalability and cost-effectiveness of these nanoencapsulation techniques under industrial conditions remain largely unexplored.

2.3. Nutricereals-based beverages

Sethi et al., (2016), states that, “plant-based milk alternatives are fluids that results from breakdown (size reduction) of plant material (cereals, pseudo-cereals, legumes oilseeds, nuts) extracted in water and further homogenisation of such fluids, results in particle size distribution in range of 5-20 μm which imitates cow’s milk in appearance and consistency”. People are switching or preferring plant-based milk products due to various reasons, such as, lactose intolerance, milk-protein allergy, or choice of diet, as in veganism, among others (Chalupa-Krebzdak et al., 2018).

A nutricereal based beverage prepared using germinated finger millet, oats and water (15:10:75), mixed with double toned milk in the ratio 47:53 was studied for its probiotic activity with *L. acidophilus.* The initial probiotic viability of 6.38±0.03 log CFU/ml increased to 7.21±0.03 log CFU/ml after 15 days of storage at 4±1˚C (Kumar et al., 2020b) The potential of brown rice as a plant-based milk alternative were explored in a study, by the use of *L. acidophilus* and *Streptococcus thermophilus*, was found to have adequate probiotic viability and improved sensory properties compared to unfermented product (Ndife et al., 2019). A study with cashew nut milk inoculated with *Bifidobacterium animalis* was found to be an adequate medium for a probiotic plant-based beverage without any significant change in its sensory properties when stored at 4˚C for a period of 30 days (Bruno et al., 2020).

With plant-based beverages gaining popularity due to dietary restrictions, health concerns, and lifestyle preferences, several studies have demonstrated the feasibility of incorporating probiotics into plant-based beverages derived from cereals, pseudo-cereals, and nuts. These literature support the potential of plant-based matrices in developing functional probiotic beverages. However, most of the studies are limited to short-term storage evaluations and specific strains of probiotics. There is a lack of comparative analysis across different plant substrates and probiotic combinations, as well as limited insight into the mechanisms influencing probiotic stability and sensory acceptability over longer periods. Broader investigations involving diverse plant ingredients, extended shelf-life studies, and in vivo validation of health benefits would strengthen the foundation for commercial applications.

2.3.1. Finger millet

The protein content of finger millet ranges from 5.60 to 12.70%, with prolamin being the predominant protein component, comprising 24.6 to 36.2%. The essential amino acids constitute 44.7% of the total amino acid content in finger millet (Abioye et al., 2022). Essential amino acids in finger millet include phenylalanine, histidine, isoleucine, leucine, lysine, methionine, and threonine at 4.1 to 5.2, 2.2, 4.3, 6.6 to 9.5, 2.2, 2.5 to 3.51 and 3.4 to 4.2 g/100 g, respectively. Non-essential amino acids found in finger millet include aspartic acid at 6.5 to 7.90 g/100 g, glutamic acid at 20.3 to 27.1 g/100 g, alanine at 6.1 to 6.2 g/100 g, arginine at 2.77 to 4.5 g/100 g, cystine at 1.7 to 2.5 g/100 g, glycine at 2.14 to 4.0 g/100 g, proline at 7.0 to 9.9 g/100 g, serine at 3.6 to 5.1 g/100 g, and tyrosine at 2.79 to 3.6 g/100 g (Ramashia et al., 2019; Jagati et al., 2021). The potassium, magnesium,

calcium, phosphorus, chromium, iron, and zinc content of 18 varieties of finger millet in Africa ranged from 279 to 688.51, 279.99 to 294.38, 241.67 to 466.67, 169.42 to 321.67, 0.55 to 1.29, 7.93 to 19.39, and 31.30 to 56.63 mg/100g, respectively. Likewise, beta-carotene, Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B6, and Vitamin B9 content varied from 0.003 to 0.01, 7.90 to 13.95, 4.08 to 12.98, 0.41 to 5.74, 0.49 to 3.89, and 0.87 to 6.40 mg/100g, respectively (George et al., 2023).

In a study, finger millet underwent a soaking period of 24 hrs followed by germination for durations of 24, 48, and 72 hrs at room temperature (23.2 ± 2.6˚C) and relative humidity (37.91 ± 8.17%). Subsequently, the germinated finger millet was oven-dried and processed into four distinct flour samples for analysis. The results revealed notable changes in various parameters with increasing germination duration. Specifically, as the germination period lengthened, there was an increase in shoot length, germination percentage, germination loss, total titratable acidity, and protein content. Conversely, a decrease was observed in the pH value, ash content, and fat content. The pH value declined from 6.43 to 5.97, while the ash content decreased from 2.41 to 1.67 mg/100 g, and the fat content reduced from 2.41 to 1.67 mg/100 g. These findings suggest that germination significantly influences the biochemical composition of finger millet, leading to alterations in its nutritional profile and physicochemical properties (Yenasew and Urga 2023). In the study conducted on finger millet varieties, it was found that malting for 60 hrs significantly influenced the bioaccessibility. The bioaccessibility values for selected minerals, such as, potassium, magnesium, calcium, phosphorus, chromium, iron and Zinc were found to increase from 69.33, 30.00, 31.77, 29.00, 5.29, 10.83 and 24.829% to 89.53, 49.28, 60.41, 69.40, 12.9, 59.84, and 66.89%, respectively. Additionally, for beta-carotene, vitamins B1, B2, B3, B6, and B9, the bioaccessibility values after malting were, beta-carotene, Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B6, and Vitamin B9 increased from 55, 67.39, 63.25, 74.79, 25.34, and 52.25% to 71, 68.47, 69.58, 96.03, 66.71, and 60.91%, respectively (George et al., 2023).

The addition of malted and heated finger millet flour at 20% in milk, followed by fermentation using a culture comprising *Streptococcus thermophilus* MTCC 5460 and probiotic strain *Lactobacillus helveticus* MTCC 5463 at 2%, significantly enhanced the product's probiotic, antimicrobial, antioxidant, and antidiabetic potential in comparison to the control. This highlights the beneficial impact of incorporating finger millet into dairy products, offering enhanced functional properties and potential health benefits (Chaudhary

and Mudgal, 2020). White finger millet is renowned for its nutritional richness, making it an ideal candidate for developing functional foods. A study was designed to create a vegan white finger millet probiotic beverage and optimize its formulation using the Box-Behnken design. The formulation's physicochemical and nutritional properties were extensively studied. Optimal conditions for white finger millet probiotic beverage were identified as 14% white finger millet flour, 1% microbial inoculum, and 5% sugar, resulting in a beverage with a total soluble solid (TSS) of 15.4 ˚Brix, pH of 4.33, microbial load of 9.45 log (CFU/ml), and viscosity of 43.32 cP, with a high sensory score of 8.34 and desirability of 0.975. The white finger millet probiotic beverage was found to contain 11.91% carbohydrates, 1.46% protein, 0.89% fat, and 0.632% crude fibre. Rheological studies indicated that white finger millet probiotic beverage is a shear-thinning fluid, which could enhance its palatability (Navyashree et al., 2022). The better survivability of *Lactobacillus casei* in finger millet beverage has been observed after 5 weeks of storage to be 9.05 log CFU/ml (Fasreen et al., 2017).

Finger millet has been extensively recognized for its nutritional and functional value. Multiple studies have highlighted how germination and malting processes enhance the bioaccessibility of micronutrients and alter the biochemical composition to improve digestibility. Studies on the use of finger millet in probiotic and functional beverages further reinforce its versatility, demonstrating enhanced viability of probiotic strains, improved antioxidant and antimicrobial activity, and desirable sensory and rheological properties. Notably, probiotic beverages formulated with finger millet have achieved high microbial viability during storage and have been well-accepted by consumers. However, the variability in nutritional composition across different varieties and treatments remains underexplored, and there is limited information on the long-term stability and consumer acceptability of such beverages under commercial conditions.

2.3.2. Pearl millet

The composition of pearl millet includes 2.70±0.31% minerals, 11.86±0.14% protein, 4.28±0.08% fat, 60.32±3.45% carbohydrates, and 11.76±0.64% dietary fibre (Sachdev et al., 2023). The nutritional profile of 87 diverse Pearl millet germplasms, comprising both landraces and commercial varieties, was evaluated. The findings revealed significant variability in various components including total carbohydrates consisting of, 50.37 to 63.25 g/100g of starch, 19.26 to 27.90 g/100g of amylose, 0.58 to 1.53 g/100g of sucrose,

0.32 to 0.75 g/100g of glucose, 1.49 to 3.52 g/100g of resistant starch and 1.53 to 3.22 g/100g of total soluble sugars. The protein content was in the range of 8.07 to 18.15 g/100 g and the total dietary fibre was in the range of 7.68 to 16.18 g/100 g. The lipids and fatty acids profile of pearl millet incudes, total lipid, palmitic, linoleic, oleic, and stearic acid content in the range of 5.24 to 9.99, 20.30 to 32.49, 32.11 to 46.91 and 3.28 to 7.91 g/100g, respectively. The phytic acid, phenols and raffinose family oligosaccharides contents of pearl millet were found to be in the range of 0.54 to 1.43 g/100 g, 0.04 to 0.21 g/100 g and 0.27 to 2.08 mmol/100 g, respectively (Tomar et al., 2021). The total mineral content of pearl millet encompasses phosphorus, calcium, iron, zinc, copper, and manganese contents, which range from 450 to 990 mg/100g, 10.00 to 60.00 mg/100g, 8.3 to 9.90 mg/100g, 5.3 to 7.70 mg/100g, 1.8 mg/100g, and 2.3 mg/100g, respectively (Adegbola et al., 2023). The other micronutrient contents include, 132 µg of total carotenoids, 240 µg of thiamine, 200 µg riboflavin, 2.9 mg of niacin and 45.5 µg of folic acid (Mallesh et al., 2021; Paschapur et al., 2021).

Germinated pearl millet has been found to an adequate prebiotic source as it promotes the probiotic growth. The nutritional value and its bioavailability were also found to have been increased in the process (Kumari et al., 2024). Theodoro et al., (2024) investigated the impact of consuming pearl millet whole grain pre-cooked conventional, germinated, and extruded flours on iron metabolism and antioxidant capacity in rats. After an initial 28-day period on an iron-free standard diet (SD iron-free), the rats were divided into four groups: ferrous sulfate (SD + FS), conventional millet flour (SD + CM), germinated millet flour (SD + GM), and extruded millet flour (SD + EM), for 21 days. Compared to the SD + FS group, the SD iron-free group exhibited increased expression of ferroportin, hephaestin, and ferritin genes, along with elevated malondialdehyde (MDA) concentration. The SD + GM group displayed heightened expression of DcytB, ferroportin, and hephaestin genes. The SD + EM group demonstrated increased transferrin concentration and serum iron, albeit reducing liver total antioxidant capacity (TAC). The SD + CM group showed increased ferritin expression. All test groups exhibited enhanced iron metabolism and reduced oxidative stress compared to the SD + FS group, indicating the potential of millet for improving iron bioavailability, particularly through germination, while maintaining antioxidant capacity similar to conventional millet.

In recent study, the chemical composition of a water-insoluble homopolysaccharide (PMG) derived from pearl millet, along with its prebiotic attributes, was thoroughly examined. The

findings revealed PMG as a glucan with an approximate molecular weight of 361 kDa, featuring a backbone composed of (1 → 3) α-d-glucopyranosyl residues. Notably, the hydrolytic susceptibility of PMG to salivary and pancreatic α-amylase was determined to be 1.75±0.34 and 1.99±0.18 %, respectively. Furthermore, the evaluation of PMG's prebiotic potential demonstrated a favourable prebiotic score with both *L. acidophilus* and *L. brevis* of 0.446 ± 0.031 and 0.427 ± 0.016, respectively (Mondal et al., 2023). Pearl millet flour (100 g) underwent lactic acid fermentation using *L. plantarum* under varied conditions of temperature (30–50 ˚C), time (4–30 h), and pH (3–7). Initial total viable count in unfermented pearl millet was 1.41±0.31 Log CFU/g, rising substantially to 9.34±0.17 Log CFU/g post-fermentation. Similarly, the Lactic Acid Bacteria count increased from <10 CFU/g in unfermented samples to 9.28±0.61 Log CFU/g following probiotic fermentation with *L. plantarum*. This signifies the effectiveness of fermentation in enhancing microbial population and underscores the potential of pearl millet as a substrate for probiotic fermentation with *L. plantarum* (Srivastava et al., 2024). Rice and pearl millet milk fortified with pumpkin and sesame seed milk has been studied for their probiotic effects using *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* BB-12 which has recorded an improvement in its colour, flavour and overall acceptability with viable cell count up to 15 days (Hassan et al., 2012).

Pearl millet stands out as a nutritionally dense grain with considerable variability in its macronutrient and micronutrient composition across different varieties, making it a valuable candidate for functional food development. Germination and fermentation processes have shown to improve its nutrient bioavailability, iron metabolism, antioxidant capacity, and prebiotic effects. In vivo studies using animal models further validate its potential in enhancing iron absorption and mitigating oxidative stress, while prebiotic assays confirm the utility of specific polysaccharide fractions in supporting probiotic growth. However, despite these promising outcomes, the sensory and physicochemical impacts of different processing techniques, particularly in multi-ingredient systems, warrant more systematic exploration to support commercial application and consumer acceptability.

2.3.3. Buckwheat

The crude protein content of buckwheat is 146.6 g/kg with a protein digestibility of 68.97±4.42, biological value (BV) of 86.33±7.88, protein efficiency ratio 2.69±0.25 and

net protein value of 59.77±8.87 (Vršková et al., 2013). Buckwheat is a good source of B-Vitamins, such as, thiamine (2.2–3.3 µg/g dry matter), riboflavin (10.6 µg/g dry matter), niacin (18 µg/g dry matter), pantothenic acid (11 µg/g dry matter), B6 (pyridoxine, 1.5 µg/g dry matter), and vitamin C (50µg/g dry matter) (Bastida et al., 2019). The mineral content of common buckwheat includes, copper content of 6.39 to 7.76 mg/Kg, manganese content of 8.90 to 10.99 mg/Kg, iron content of 24.22 to 29.99 mg/Kg, zinc content of 47.65 to 63.07 mg/Kg, magnesium content of 1367 to 1543 mg/Kg, calcium content of 772 to 1139 mg/Kg, sodium content of 11.77 to 16.74 mg/Kg, potassium content of 5157 to 6228 mg/Kg, and phosphorus content of 3643 to 3861 mg/Kg (Podolska et al., 2021). The flavonoids in common buckwheat grains were found have rutin, vitexin, quercetin and kaempferal content of 4.069 to 13.236, 0.010 to 0.212, 0.034 to 1.438 and 0.005 to 0.063 mg/100g, respectively (Vollmannová et al., 2021).

In a study, buckwheat grains were subjected germination process at 30˚C for 48 hrs, preceded by 12 hrs of soaking in an incubator, and subsequently dried at 60˚C. This germination resulted in notable enhancements in moisture, protein, and crude fibre content, in the ranges of 11.03% to 12.77%, 10.22% to 12.14%, and 0.92% to 1.44%, respectively. Concurrently, mineral levels, including sodium and potassium, exhibited an increase of 65.48% and 20.90%, respectively. Moreover, the germination process induced elevations in total and reducing sugars, by 76.08 and 46.77%, respectively, juxtaposed with a reduction in starch content 8.35% (Shreeja et al., 2021).

A novel probiotic beverage was developed using varying concentrations of germinated and ungerminated pearl millet flour in green gram milk, alongside sugar and cardamom. Following inoculation with *Lactobacillus acidophilus* and subsequent incubation, characterization during a 21-day storage period revealed higher acidity in germinated flour variants. Probiotic counts ranged from 8.19 to 8.7^{7} × 107 and 8.04 to 8.52 × 10^{7} log CFU/ml for germinated and ungerminated flour beverages, respectively. Increased flour concentration correlated with enhanced antioxidant activity and polyphenol content, with vitexin and isovitexin identified as primary polyphenolic compounds via Liquid Chromatography-Mass spectroscopy (LC-MS) analysis. The optimal sensory attributes were associated with 0.5 % germinated millet flour, indicating its potential as a non-dairy probiotic beverage (Ghoshal et al., 2024).

Buckwheat demonstrates notable nutritional quality, highlighted by its high crude protein content, favorable digestibility, and rich composition of essential vitamins, minerals, and bioactive flavonoids such as rutin and quercetin. Germination has been shown to significantly enhance its protein, fibre, mineral, and sugar contents while reducing starch levels, suggesting improved functional properties and nutrient bioavailability. Furthermore, the integration of germinated buckwheat and millet flours into probiotic beverage formulations has led to promising outcomes in terms of microbial viability, antioxidant activity, and sensory appeal. However, most studies rely heavily on compositional analysis and controlled experimental setups. There is a lack of comprehensive human clinical trials to validate physiological benefits, particularly in terms of digestibility, glycemic response, and long-term probiotic viability. Moreover, comparative studies addressing the differential impact of germination parameters on bioactive compound retention are limited, and there is minimal insight into scalability and consumer acceptance across broader demographic segments.

Chapter 3

HYPOTHESIS OF THE STUDY

Nanoencapsulation of probiotic cultures could improves their stability, viability, and functionality, optimizing their performance under varying environmental and processing conditions. Nanoencapsulation through nanoemulsion preparation would be cost effective utilising available resources in comparison to other methods. A standardized process for developing a nutricereal-based composite beverage could potentially result in a nutritionally rich, organoleptically acceptable product with desirable physicochemical properties. The concentration of nanoencapsulated probiotic cultures in the nutricereal-based composite beverage would significantly affects its sensory attributes, physicochemical properties, and probiotic viability. Storage duration, significantly influence the physicochemical stability, sensory quality, and microbiological viability of the probiotic beverage over time.

Chapter 4

OBJECTIVES OF THE STUDY

The major objectives of the proposal are:

1. To optimize the process for the development of nanoencapsulated probiotic cultures.
2. To standardize the process for the development of nutricereals based composite beverage.
3. To study the effect of inoculation of nutricereal based composite beverage with different concentrations of nanoencapsulated probiotic cultures.
4. To study the effect of storage conditions on the physicochemical, sensorial and microbiological viability and quality characteristics of the probiotic beverage

Expected Outcome

Expected outcomes upon completing the thesis include:

1. Nanoencapsulation of probiotic culture will be developed and optimized.
2. Standardized process of nutricereals based composite beverage will be developed
3. The optimal concentrations of nanoencapsulated probiotic cultures in the nutricereal based composite beverage shall be determined.
4. Database for nutritional, physicochemical, sensorial and microbiological viability and quality characteristics of the probiotic beverage will be generated.

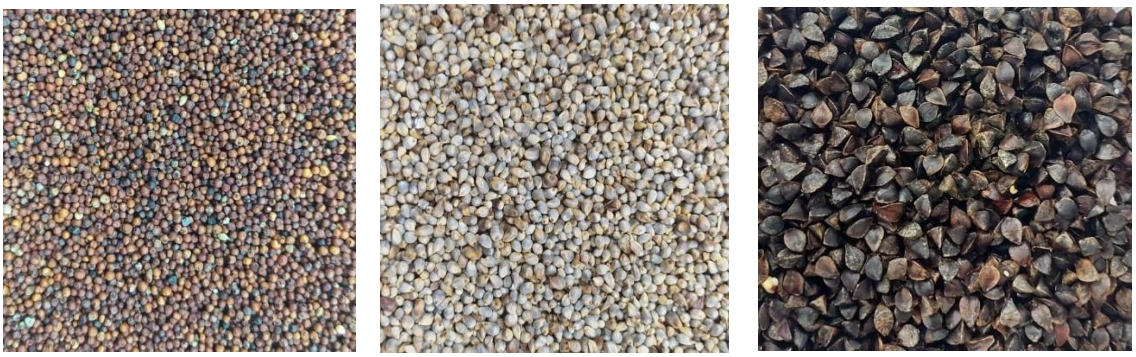
Chapter 5

MATERIALS AND METHODS

describes the materials used and the procedures followed to carry out the experimental work. It includes information on the selection and preparation of ingredients, the techniques applied for analysis, and the overall approach used to conduct the experiments. The studies were carried out in the Department of Food Science and Technology, Lovely Professional University, Phagwara, Punjab. The materials used, experimental methods and analytical techniques employed have been described under the following heads:

5.1. Materials

The soy lecithin, carboxymethyl cellulose (CMC), dihydrogen phosphate – monobasic, dihydrogen phosphate – dibasic, NaCl, Folin-Ciocalteu reagent, sodium tungstate, Phosphomolybdic acid, phosphoric acid, sodium carbonate, methanol, ethanol, EDTA (Ethylenediaminetetraacetic acid), sodium hydroxide, and phenolphthalein were purchased from Loba Chemicals, Mumbai, Maharashtra, India and were obtained from Lovely Professional University, Phagwara, Punjab, India. Gallic acid, tannic acid, MRS broth, MRS agar and DPPH (2,2-diphenyl-1-picrylhydrazyl were from HiMedia, obtained from Lovely Professional University, Phagwara, Punjab, India. Bile salt powder was obtained from TM Media, Delhi, India. Food grade κ-carrageenan (KC) was purchased from Urban platter, Mumbai, Maharashtra, India. Food grade Tween 80/ polysorbate 80 (E433) was purchased from Ases Chemical Works, Jodhpur, Rajasthan, India. Sunflower oil, sugar, finger millet, pearl millet and Nestlé a+ Slim Milk (0.2% fat) were procured from local market, Phagwara, Punjab, India. Buckwheat was procured from NutriBuck, Raigarh, Chhattisgarh, MP, India. Bacterial cultures of *Lactobacillus plantarum* 685 and *Lactobacillus acidophilus* 600 were obtained from NDRI, Karnal, Haryana, India.



a) b) c)

Figure. 5.1. Cleaned grains (a) finger millet, (b) pearl millet, and (c) buckwheat.

5.2. Nanoemulsion preparation

The nanoemulsion preparation procedure was conducted following a modified methodology outlined by (Dafe et al., 2017).

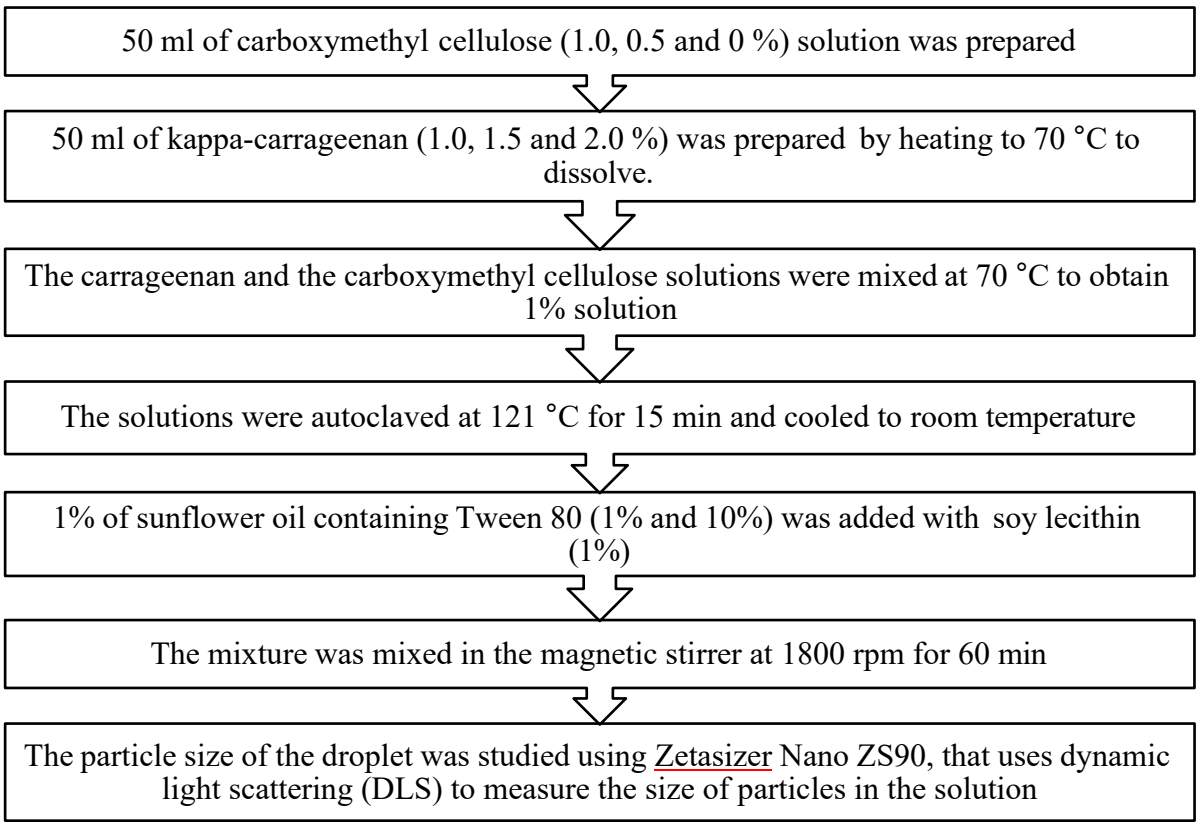


Figure. 5.2. Flowchart for preparation of the nanoemulsion

Table 5.1. The variables studied for the preparation of the nanoemulsion

Treatment	κ - carrageenan (w/v %)	CMC (w/v %) (w/v %)	Tween 80 (% of oil)	Soy Lecithin (w/v %)	Sunflower oil (v/v %)
NE1	0.5	1.5	1	1	1
NE2	1.0	1.0	1	1	1
NE3	2.0	-	1	1	1
NE4	0.5	0.5	10	1	1
NE5	1.0	1.0	10	1	1
NE6	2.0	-	10	1	1

5.3. Nanoemulsion Characterization

5.3.1. Droplet size, polydispersity index (PDI) and zeta potential

The droplet size, PDI, and zeta potential of the emulsions were studied using Zetasizer Nano ZS90, from Amil Ltd., New Delhi, India, that uses dynamic light scattering (DLS) to measure the size of droplets in the solution (Ibrar et al., 2022).

5.3.2. FE-SEM Analysis

The size and surface morphology of the nanoemulsions were characterized using Field Emission-Scanning Electron Microscopy (FE-SEM) employing JOEL SM-7610F Plus EDS: OXFORD EDS LN2 free, Au coater: JOEL Smart Coater microscope. The nanoemulsion was spread on a glass slide to form a thin film and allowed to dry. Subsequently, a thin layer of gold particles was applied to the surface of the dried nanoemulsion to enhance conductivity and improve imaging quality before they were examined (Keykhasalar et al., 2020).

5.3.3. Preparation of probiotic nanoencapsulates

5.3.3.1. Preparation of bacterial cells in bulk

The bacterial cell preparation procedure was conducted following the methodology outlined by Ebrahimnezhad et al., (2017).

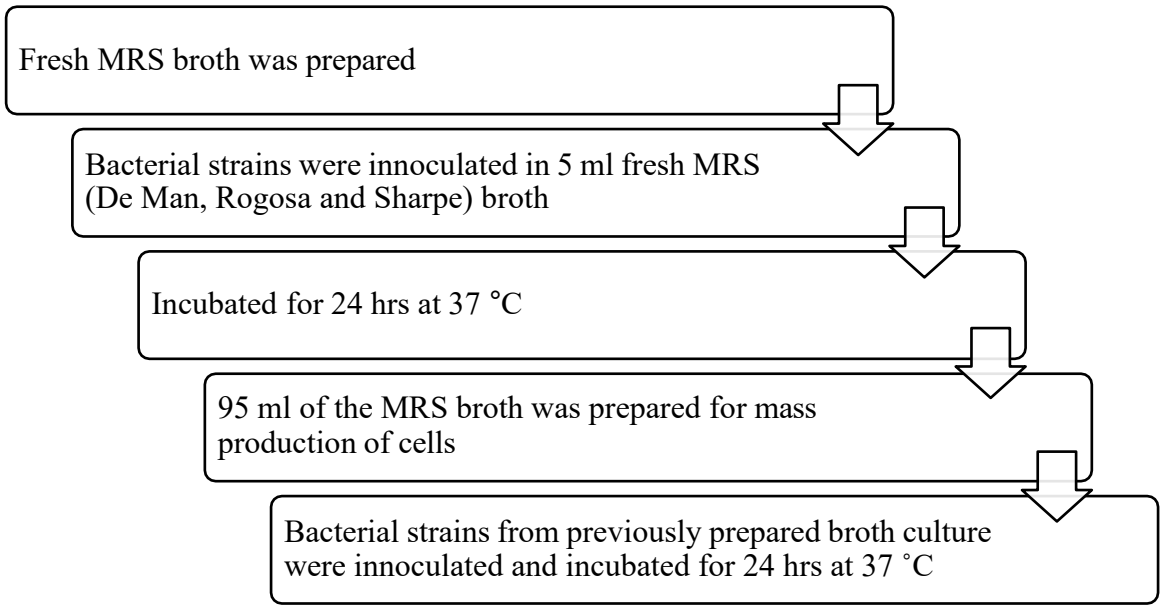


Figure. 5.3. Flowchart for preparation of bacterial cell for the nanoencapsulation

5.3.3.2. Cell extraction

The cell extraction method is based on study by Ebrahimnezhad et al., (2017).

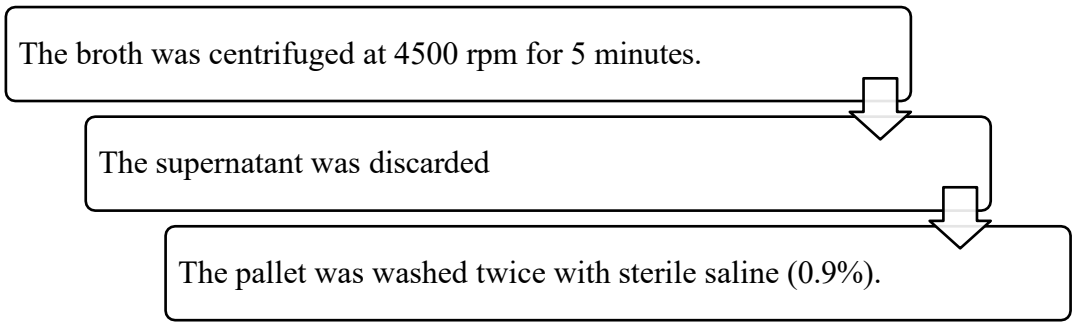


Figure 5.4. Flowchart for bacterial cell extraction for nanoencapsulation

5.3.3.3. Nanoencapsulation

The bacterial pellet was added to the prepared nanoemulsion and stirred for 15 min in the magnetic stirrer to disperse the bacteria into the emulsion to obtain >11 Log CFU/ml of emulsion (Dafe et al., 2017). This was then freeze dried to obtain the encapsulated powder.

5.3.4. Encapsulation efficiency

The freeze-dried powder was rehydrated to the initial volume and heated mildly (not more than 40 ˚C) to completely dissolve. The CFU in the initial nanoemulsion and the freeze-dried powder was studied by spread plate method on Lactobacillus MRS agar after serial

dilution upto10^{11}. The encapsulation efficiency (EE%) was calculated using the formula, and expressed in percentage of encapsulation (Dafe et al., 2017).

$$\text{EE } \% = \frac{Log\ CFU\ before\ encapsulation - Log\ CFU\ after\ encapsulation}{Log\ CFU\ before\ encapsulation} \times 100$$

5.3.5. Efficiency of encapsulation against intestinal conditions

5.3.5.1. Bile tolerance

The bile tolerance of encapsulated cells were assessed with method by Feng et al., (2020).

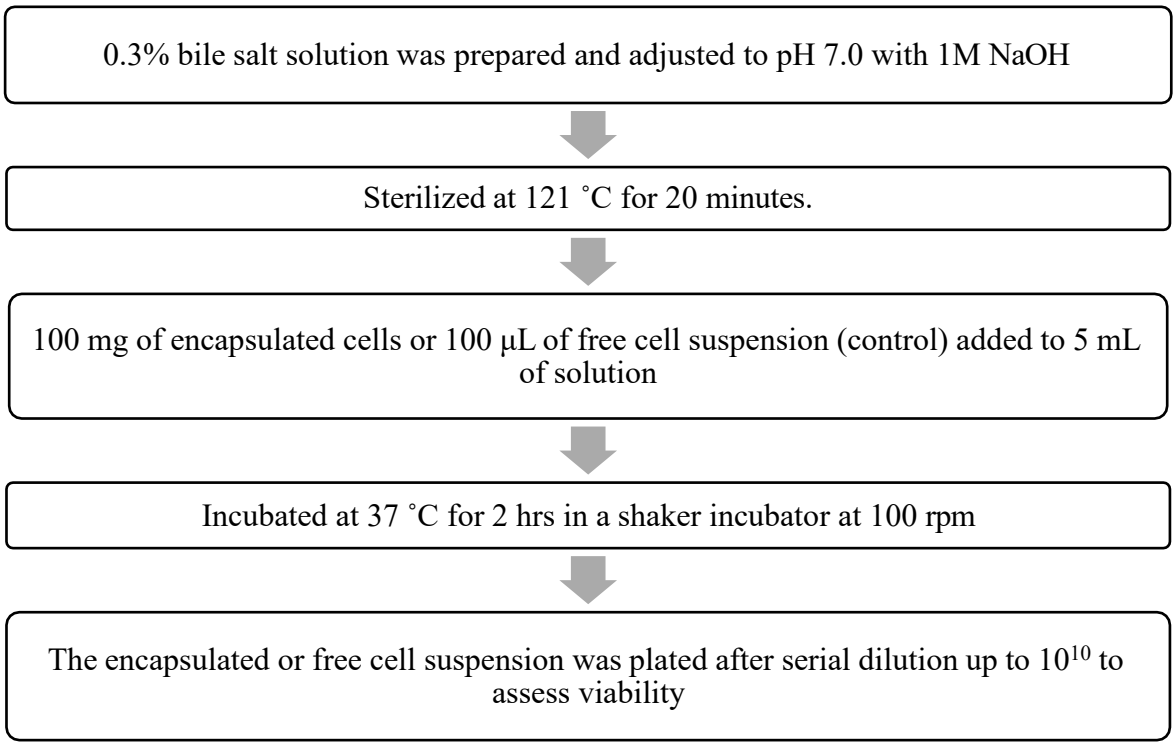


Figure. 5.5. Flowchart for bile tolerance test for nanoencapsulates

5.3.5.2. Acid tolerance

The acid tolerance of encapsulated cells were assessed with method given by Adhikari et al., (2000).

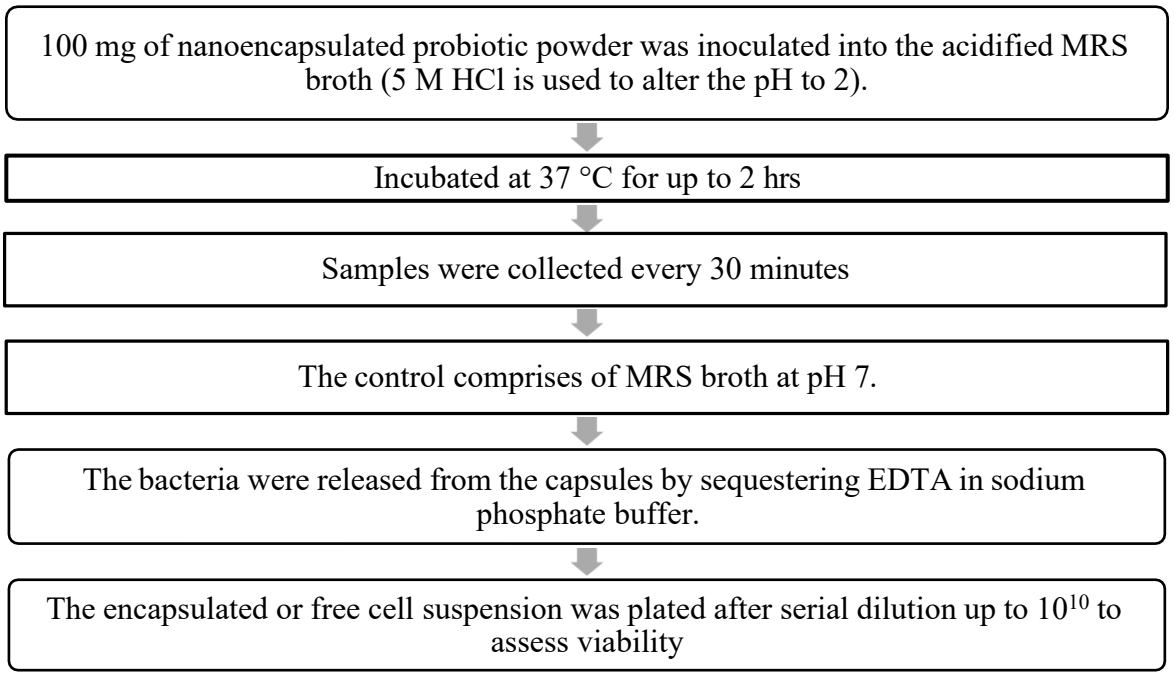


Figure. 5.6. Flowchart for acid tolerance test for the nanoencapsulates

5.3.6. Release study

The release of nanoencapsulated cells were assessed with method given by Dafe et al., (2017).

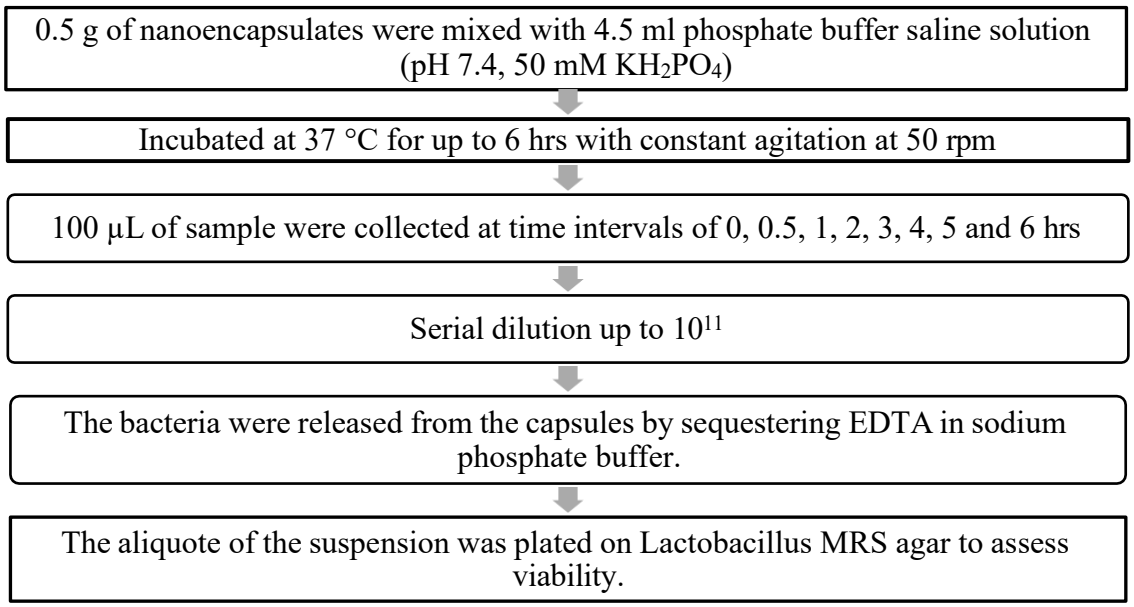


Figure. 5.7. Flowchart for release study of the nanoencapsulates

5.4. Optimization of germination

5.4.1. Germination of grains

The grains were soaked at 25 ˚C for 16 hrs. They were then further germinated at 22, 26 and 30 ˚C for 24, 48 and 72 hrs in seed germinator and the humidity maintained at 67%. The grains were germinated in on a container covered with filter paper. The germinated grains were dried in tray dryer at 60 and 80 ˚C for further analysis.

5.4.2. Radicle length

The Radicle length of the germinating grains were measured after 24, 48 and 72 hrs of germination in mm.

5.4.3. Total Phenolic Content (TPC)

1g sample was extracted using 10 ml of 80% methanol at room temperature for 30 min., and centrifuged at 4000 rpm for 15 min. The supernatant was used for further estimation (Zieliński and Kozłowsk, 2000). The total phenolic content was estimated colorimetrically using Folin-Ciocalteu assay from methanolic extract of the sample (Rocchetti et al., 2018). The calibration curve was prepared with a standard gallic acid, and the results are expressed as gallic acid equivalent per 100g sample.

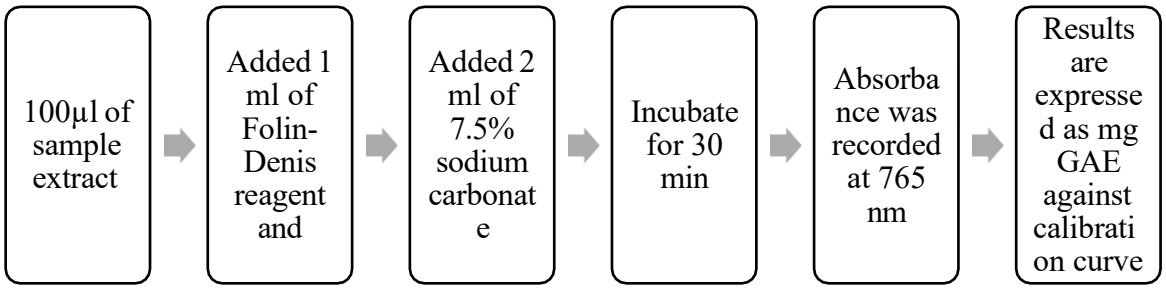


Figure. 5.8. Flowchart for estimation of total phenolic content

5.4.4. Antioxidant activity

The antioxidant activity was estimated by DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity (Agunbiade et al., 2022).

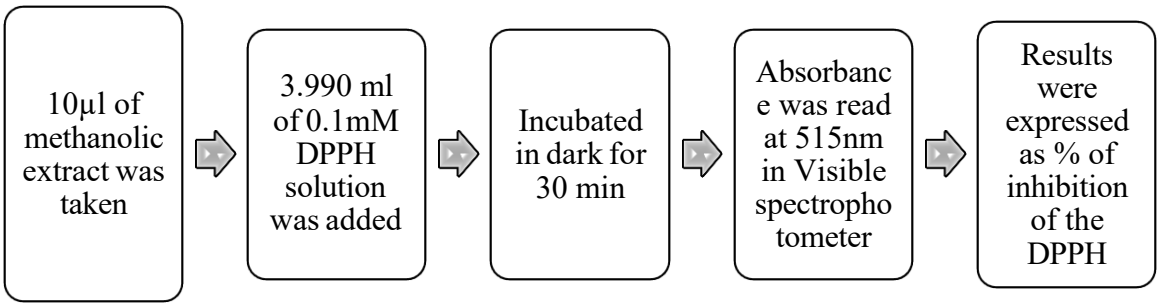


Figure. 5.9. Flowchart for estimation of antioxidant activity

$$\% \text{ inhibition of DPPH} = \frac{Absorbance\ of\ control - Ansorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

5.4.5. Tannin Content

The tannin content was estimated using Folin-Denis reagent, with a standard curve of tannic acid (Gull et al., 2016).

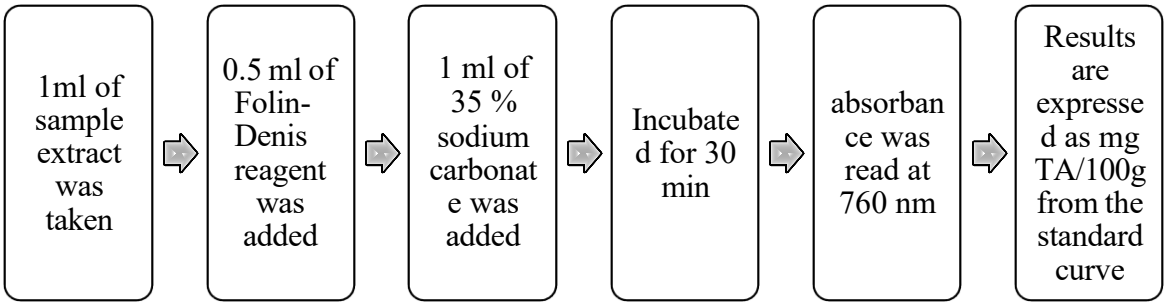


Figure. 5.10. Flowchart for estimation of tannin content

5.4.6. Prebiotic effect

The prebiotic effect was studied by modified method of Budhwar et al., (2020). 0.5 g of ground sample was mixed with 10 ml of distilled water and autoclaved at 121 ˚C, 15 psi for 15 min. The cooled mixture was inoculated with the probiotic bacteria *L. acidophilus* NCDC 600 and *L. plantarum* NCDC 685 at 10^6 dilution and incubated at 37 ˚C for 24 hrs. The bacterial count was enumerated with Lactobacillus DeMan–Rogosa–Sharpe (MRS) agar medium. The fermented samples were serial diluted up to 10^9 and pour plated. The petri plates were incubated at 37 ˚C for 24 hrs and the results were expressed as Log CFU/g sample.

5.5. Optimization of nutricereal beverage

5.5.1. Preparation of malt extract

Malted pearl millet, finger millet and buckwheat were individually ground into coarse flour. According to preliminary investigations, five different ratios of pearl millet, finger millet, and buckwheat were prepared, in the ratio of 50:30:20 (C1), 60:25:15 (C2), 70:20:10 (C3), 80:15:5 (C4) and 90:10:0 (C5), as outlined in Table 3.2. Water was added to the flour at a ratio of 3:1, and the mixture was mashed at various temperature to facilitate enzymatic hydrolysis of the starch, following established procedures (Kumar et al., 2020a). The mashing involved holding the mixture for 30 min at different temperatures of 45 ˚C (enables the proteases to break down protein), 52 ˚C (enables the activity of other proteolytic enzymes), 63 ˚C (enables starch hydrolysis by β-amylase), 72 ˚C (enables starch hydrolysis by α-amylase) and finally held at 78 ˚C for 30 min to inactivate the amylolytic enzymes (Montanari et al., 2005).

Table. 5.2. The preliminary study for optimization of malt extract with germinated pearl millet, finger millet, buckwheat and water

Ingredient	Combinations of Treatment				
	C1	C2	C3	C4	C5
Pearl millet	50	60	70	80	90
Finger millet	30	25	20	15	10
Buckwheat	20	15	10	5	0
Water	300	300	300	300	300

After the mashing process, the liquid was filtered using muslin cloth. The resulting malt extract samples were evaluated for sensory attributes using a sensory profiling scale ranging from 1 to 9, where 9 represented maximum intensity and 1 indicated the absence of the attribute being evaluated. Parameters considered during sensory analysis included appearance, consistency, flavour, and bitterness with overall acceptability calculated. Based on the sensory evaluations, the most well-received malt extract was chosen for further investigation.

5.5.2. Preparation of functional beverage

Initial experiments were conducted to establish the acceptable ranges of malt drink and skimmed milk ratios for sensory preferences. Subsequently, optimization of the functional

drink formulation was performed using response surface methodology (RSM). The key product variables included malt drink (40–60%) and skimmed milk (40–60%), while sugar was held constant at 6.5 g/100 ml. Dependent factors under consideration comprised total solids, total phenolic content, tannin content, prebiotic effect and overall acceptability, assessed through sensory evaluations. To optimize the functional drink, 13 trials were conducted as given in Table 5.3. Based on the obtained responses, the software predicted the optimal combinations of nutricereal malt extract and skimmed milk to create a functional drink with maximum total solids, total phenols, prebiotic effect, overall acceptability, and minimal tannin content. This approach ensured the development of a product that meets both nutritional and sensory requirements.

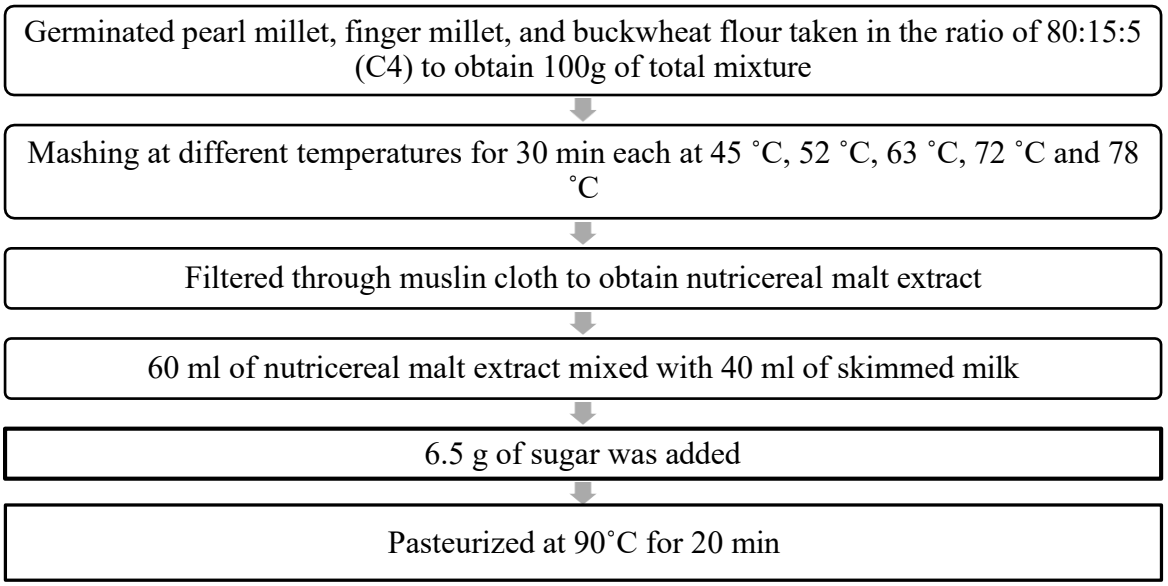


Figure. 5.11. Flowchart for preparation of the optimized beverage

Table. 5.3. The software predicted the optimal combinations of nutricereal malt extract and skimmed milk

		Factor 1	Factor 2	Response 1	Response 2	Response 3	Response 4	Response 5
Standard	Run	A: nutricereal malt extract (ml)	B: Skimmed milk (ml)	Total Solids (%)	TPC (mg GAE/100ml)	Tannin (mg /TA100ml)	Prebiotic effect (Log CFU/ml)	Overall acceptability
8	1	50	64.14					
2	2	60	40					
6	3	64.14	50					
9	4	50	50					
13	5	50	50					
4	6	60	60					
12	7	50	50					
3	8	40	60					
11	9	50	50					
1	10	40	40					
5	11	35.85	50					
7	12	50	35.85					
10	13	50	50					

5.6. Inoculation of probiotic nanocapsules in the beverage

The nanocapsules were added to 100ml of prepared and pasteurised beverage at a concentration of 0.5, 1, 1.5 and 2%. The beverages were mixed gently to disperse the nanocapsules through the liquid, sealed and stored at 4˚C.

5.7. Enumeration of probiotic bacteria of the beverage

The beverages were treated with EDTA in sodium phosphate buffer to dissolve the nanoencapsulation coating (Adhikari et al., 2000) and diluted to 10^6 to 10^9. This was then plated on Lactobacillus MRS agar media by pour plate method and incubated condition at 37˚C for 24 hours.

5.8. Sensory Analysis of prepared probiotic beverage

The developed probiotic plant-based beverage were evaluated for sensory attributes with 30 panellists. The samples were rated on nine –point hedonic scale for their appearance, taste, texture, flavour and overall acceptability in the following the order of '1' denoting 'dislike extremely' to '9' denoting 'like extremely'.

5.9. Proximate analysis of the prepared beverage

5.9.1. Moisture

The moisture content was estimated according to AOAC method no. 945.38 (. Approximately 5 g of the sample were weighed into clean, dry, pre-weighed crucibles. The crucibles and their contents were dried in a moisture extraction oven at 110 ˚C for 4 hrs. The samples were then cooled in desiccators and reweighed. This drying process was repeated until a constant weight was achieved (AOAC International, 2002).

5.9.2. Protein

The protein content of the beverage was assessed using the Kjeldahl method. This involved determining the total nitrogen content, which was then used to calculate the total protein percentage. A conversion factor of 6.25 was applied to convert the nitrogen content to protein percentage (Ahern et al., 2023). 5 g of the sample was weighed and transferred to 800 ml Kjeldahl flask. To the flask, 0.5 g of copper sulphate, 15 g of potassium sulphate, and 40 ml of concentrated sulfuric acid were added, along with two to three glass beads. The flask was placed in the digestion chamber and heated gently at a low flame until the

mixture boiled steadily and turned pale blue. After cooling, 500 ml of water was added, followed by granulated zinc and sodium hydroxide solution to make the mixture alkaline. The flask was connected to a distillation apparatus, and 150 ml of distillate was collected. The distillate was then titrated with 0.1 N sodium hydroxide after adding 5 drops of methyl red indicator. A blank titration was also performed.

5.9.3. Fat

For the sample preparation, an appropriate volume of the milk-based drink, typically 10-25 ml, was measured. 1 ml of ammonia solution was added to the sample to liberate the fat, and the mixture was shaken well. Following this, 10 ml of hydrochloric acid was added and the mixture was shaken until the sample was thoroughly digested. The digested sample was then transferred to a Mojonnier flask to which 10 ml of ethyl ether was added for extraction. The flask was capped and shaken vigorously for 1 min, followed by the addition of 10 ml of petroleum ether, which was again shaken vigorously for 1 min. The flask was then centrifuged to separate the layers, and the ether layer was carefully decanted into a pre-weighed evaporation dish. The extraction process was repeated with another 10 ml of ethyl ether and 10 m of petroleum ether to ensure complete fat extraction, and the ether extracts were combined in the same evaporation dish. The evaporation dish was placed in a water bath or on a hot plate to evaporate the ether solvents. Once the solvents had evaporated, the dish was dried in an oven at 100-105˚C to remove any residual moisture, then cooled in a desiccator and weighed (AOAC International, 2016). The fat content was determined using the formula:

$$\text{Fat (\%)} = \frac{Weight\ of\ fat\ residue}{Weight\ of\ sample} \times 100$$

5.9.4. Ash

5 g of samples were taken in crucibles and dried in an oven until they reached a constant weight. Then were charred on a hot plate till the samples were completely black, Subsequently, the samples were transferred into a muffle furnace using tongs and ashed at 550˚C for 4 hrs until ash was obtained. The samples were then removed from the furnace, cooled in a desiccator, and reweighed (AOAC International, 2002). The percentage of ash was calculated according to the following formula:

$$\text{Ash (\%)} = \frac{Weight\ of\ ash\ (g)}{Weight\ of\ sample\ (g)} \times 100$$

5.9.5. Crude fibre

10 ml of the beverage was dried and weighed. The enzymatic digestion was initiated by adding 50 ml of phosphate buffer solution (6.9 pH) to the sample, and heated to 95-100 ˚C after which α-amylase is introduced into the mixture. This was incubated for 30 min to gelatinize and hydrolyze the starch. After cooling the mixture to 60 ˚C, the pH was adjusted to 7.5, protease was added, and the mixture was incubated for an additional 30 min to digest the proteins. The pH was then adjusted to 4.5, amyloglucosidase was added, and the mixture was incubated for another 30 min to further hydrolyze any remaining starches. Filtration was carried out by passing the mixture through a pre-weighed crucible containing filter paper, and the residue was washed with hot water, ethanol, and acetone to remove soluble components. The residue was dried in an oven at 105 ˚C until a constant weight was achieved, then cooled in a desiccator, and the crucible with the dried residue was weighed. Finally, the dried residue was incinerated in a muffle furnace at 525 ˚C for 5 hrs, cooled in a desiccator, and the crucible with the ash was weighed (AOAC International, 2016). The fibre content was calculated with the following formula:

$$\text{Crude Fiber (\%)} = \frac{Weight\ of\ residue\ after\ drying - Weight\ of\ residue\ after\ ashing}{Weight\ of\ sample} \times 100$$

5.9.6. Carbohydrate

The carbohydrate content of the sample was determined by difference, calculated as the remainder when the percentages of moisture, fat, protein, fibre, and ash were subtracted from 100% (Frances et al., 2023). The formula used was:

$$\text{Carbohydrate (\%)} = 100 - (\text{moisture \%} + \text{fat \%} + \text{protein \%} + \text{fibre \%} + \text{ash \%})$$

5.9.7. Reducing sugar

The Lane and Eynon method for determining reducing sugars in the samples involved a titration process (FSSAI, 2016). First, a known volume of the sample was mixed with Fehling's solution, composed of Fehling's A (copper sulphate solution) and Fehling's B (alkaline tartrate solution). This mixture was heated to boiling. A standard glucose solution was titrated against the Fehling's mixture until a colour change indicated the end point, typically the formation of a brick-red precipitate of cuprous oxide (Cu_2O). The volume of

glucose solution required to achieve this end point was noted. The same procedure was then followed with the beverage sample, allowing the reducing sugar content to be calculated by comparing the titration volumes. The results were expressed as a percentage of reducing sugars in the sample.

5.9.8. Water activity

The determination of water activity in food samples, specifically following the guidelines of the Food Safety and Standards Authority of India (FSSAI, 2016) method number 01.061, involved measuring the equilibrium relative humidity of the sample using a water activity meter. First, a representative and homogeneous sample of the food item was prepared and placed in the sample cup of the water activity meter. The instrument was calibrated using standard solutions with known water activities to ensure accuracy. The sample cup was then placed in the chamber of the water activity meter, which was sealed to allow the sample to equilibrate to the chamber's environment. The instrument measured the equilibrium relative humidity, which directly correlated to the sample's water activity. The water activity value was displayed on the meter and recorded for further analysis. This method provided a precise measure of water activity, essential for evaluating the stability and microbial growth potential of food products.

5.9.9. Minerals

The mineral contents of the test samples were determined using the dry ash extraction method. 20 grams of each sample were incinerated to ash following the procedure for ash determination. The resulting ash was dissolved in 100 ml of dilute hydrochloric acid (1M HCl) and then diluted to 100 ml in a volumetric flask using distilled water. This solution was used for the subsequent mineral analyses for calcium, iron, phosphorus, potassium and magnesium (Frances et al., 2023).

5.9.9.1. Calcium

Calcium contents of the test sample was determined by the EDTA complex isometric titration. 20 ml of each extract was dispersed into a conical flask and panels of the masking agents, hydroxytannin, hydrochlorate, and potassium cyanide was added followed by 20ml of ammonia buffer (pH 10.0). A pinch of the indicator-Ferrochrome black was added and

the mixture was shaken very well (Frances et al., 2023). It was titrated against 0.02N EDTA solution. The calcium contents were calculated using the formulae below:

$$\text{Calcium (mg/100 g)} = \frac{Titre\ value \times 0.4008 \times 1000}{Volume\ of\ sample\ extract} \times 100$$

5.9.9.2. Iron

The concentrations of iron (ppm) were analyzed using an atomic absorption spectrophotometer at a wavelength of 243 nm, and the concentration in mg/100 g was determined using the following equation (Frances et al., 2023):

$$\text{Iron (mg/100g)} = \frac{concentration(ppm) \times Dilution\ factor \times 1000}{Weight\ of\ sample\ (g)} \times 100$$

5.9.9.3. Phosphorus

A 20 ml sample solution was put in a 100 ml volumetric flask. The solution was neutralized with ammonia and nitric acid solution (1:2). Twenty (20) ml of vanadate molybdate reagent was added and diluted to the mark. It was allowed to stand for ten min and absorbance read at 470nm in the ultra violet region and the mineral concentration in mg/100 g was calculated using the following equation (Frances et al., 2023):

$$\text{Phosphorus (mg/100g)} = \frac{concentration(ppm) \times Dilution\ factor \times 1000}{Weight\ of\ sample\ (g)} \times 100$$

5.9.9.4. Potassium

The concentration of potassium (ppm) was analyzed using UV- spectrophotometer at a wavelength of 766.5 nm, and the concentration in mg/100 g was calculated using the following equation (Frances et al., 2023):

$$\text{Potassium (mg/100g)} = \frac{concentration(ppm) \times Dilution\ factor \times 1000}{Weight\ of\ sample\ (g)} \times 100$$

5.9.9.5. Magnesium

10 ml of the sample filtrate was pipetted into 250 ml conical flask after which 25 ml of ammonia buffer solution was added into the conical flask and was properly mixed. Then a pinch of Erichrome black T indicator was added and titrated with 0.02N of EDTA until the colour of the solution change (Frances et al., 2023).

$$\text{Magnesium (mg/100 g)} = \frac{Titre\ value \times 0.2432 \times 1000}{Volume\ of\ sample\ extract} \times 100$$

5.9.9.6. Zinc

25g of the dried sample was weighed into a clean silica dish to which, 25 ml of 20% sulfuric acid was added and thoroughly. The contents were dried on a steam bath or in an oven at 110 ˚C.The dish was then heated with a soft flame until all volatile or combustible matter was removed. The dish was then transferred to a furnace set at 250 ˚C, with the temperature gradually increased to 500 ˚C, ashing for 6 to 8 hrs. The dish was cooled, and 1 ml HNO3 and 10 ml water were added and heated until the ash dissolved. The solution was then transferred to a 50 ml volumetric flask, and the dish was heated with 10 ml HCl and the solution was again transferred to the same flask and diluted with water. The absorbance of zinc in the sample was measured in atomic absorption spectrophotometer at a wavelength of 213.9 nm (FSSAI, 2016).

$$\text{Zinc (mg/100 ml)} = \frac{Absorbance\ \ of\ \ sample}{Slope\ of\ standard\ curve\ (m)\times 1000} \times 100$$

5.9.9.7. Sodium

10 ml of sample was weighed and digested by adding nitric acid and heating to remove organic matter and release sodium ions into solution. The digested sample was cooled and diluted with deionized water to 100 ml. The flame atomic absorption spectrophotometer was calibrated using standard sodium solutions of known concentrations. The digested sample solution was aspirated into the flame, and the absorbance was measured at a 589 nm. The absorbance readings were compared to the calibration curve prepared from the sodium standards to determine the sodium concentration in the sample (AOAC, 1990).

$$\text{Sodium (mg/100 ml)} = \frac{Absorbance\ of\ sample - Absorbance\ of\ blanck}{Slope\ of\ standard\ curve\ (m)} \times \text{Dilution factor}$$

5.9.10. Total phenolic content (TPC)

The total phenolic was performed according to Zieliński and Kozłowsk, (2000), and Rocchetti et al., (2018) as described previously in Section 5.4.3.

5.9.11. Antioxidant activity

The antioxidant activity was performed according to Agunbiade et al., (2022), as described previously in Section 5.4.4.

5.9.12. Tannin content

The tannin content was performed according to Gull et al., (2016), as described previously in Section 5.4.5.

5.9.13. Titratable acidity

An appropriate volume of the beverage sample (10 ml) was measured and transferred to a clean Erlenmeyer flask and diluted with 25 ml distilled water. 2-3 drops of phenolphthalein indicator were added to the sample in the Erlenmeyer flask. This was titrated against 0.1 N NaOH solution while continuously shaking until the endpoint was reached. The endpoint was indicated by a persistent pink colour that lasted for at least 30 seconds (AOAC International, 2016). The titratable acidity was then calculated using the following formula:

$$\text{Titaratable acidity (\%)} = \frac{Volume\ of\ NaOH \times 0.1 \times 90.08 \times Dilution\ factor}{Volume\ of\ sample \times 1000} \times 100$$

5.9.14. pH

The determination of pH was done by the use of Labtronics, Microprocessor pH meter (LT-49), after calibrating with pH 4 and 7 buffer solutions.

5.9.15. Sensory evaluation

The developed probiotic plant-based beverages are to be evaluated for sensory attributes with 30 panellists. They are to be seated in individual sensory booths. The samples will be rated on nine –point hedonic scale for their appearance, consistency, flavour, and bitterness with overall acceptability calculated in the following the order of '1' denoting 'dislike extremely' to '9' denoting 'like extremely'.

5.10. Shelf-Life Study

The product prepared were stored at refrigerated temperature of 4 ˚C. The beverage was tested periodically every 3 days until a significant change was observed. The beverage was tested for change in pH, titratable acidity and total plate count for viable cells.

5.11. Cost analysis

The production cost of the functional drink was determined by calculating the expenses related to the raw materials. Additionally, losses incurred during the malting process were accounted for and incorporated into the total cost of the nutricereals.

5.12. Statistical Analysis

Statistical analysis was conducted to evaluate the experimental data using appropriate tools to ensure accuracy and reliability of the results. Microsoft Excel was used for basic descriptive statistics, including the calculation of mean, standard deviation, and graphical representation of data trends. The results were further visualized using graphs and charts to interpret relationships, trends, and variations effectively. Analysis of Variance (ANOVA) was performed to determine the significance of the independent variables and their interactions on the responses. One-way ANOVA was used for sensory of nutricereal malt extract and prepared beverages, and proximate analysis. Two-way ANOVA was used for Droplet size, PDI and zeta potential of nanoemulsions, Bile and acid tolerance, release study, radicle length, TPC, antioxidant activity, tannin content, prebiotic effect, and shelf-life study. Response Surface Methodology (RSM) was applied through Design-Expert software to optimize the development of the nutricereal-based beverage. These tools collectively facilitated a comprehensive and systematic analysis of the data, ensuring the research objectives were achieved.

Chapter 6

RESULTS AND DISCUSSIONS

The present study was conducted in Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara, Punjab. India.

6.1. Nanoemulsion characterization

6.1.1. Droplet size

Table 6.1. and Figure 6.1 shows the respective droplet size of nanoemulsion prepared with κ -carrageenan (KC) and carboxy methyl cellulose (CMC) are given in. The different concentrations of nanoemulsions were prepared, NE1 (0.25% KC), NE2 (0.5% KC) and NE3 (1.0% KC) with 1% Tween 80, where different concentrations of nanoemulsions were also prepared, NE4 (0.25% KC), NE5 (0.5% KC) and NE6 (1.0% KC) with 10% Tween 80. The emulsions prepared with 1% tween 80 had droplet size of 564.63 (NE1), 552.40 (NE2) and 446.13 nm (NE3) which decreased significantly ($p<0.05$) to 314.00 (NE4), 301.87 (NE5) and 186.80 nm (NE6) with 10% of the emulsifier. In a study, increase in the surfactant to oil ratio within the range of 5% to 25% showed a significant reduction ($p<0.05$) in droplet size (Akram et al.,2021). The size of droplets in nanoemulsions significantly ($p<0.05$) decreased as the concentration of tween 80 from 1% to 10% of same composition of polymers and the results are depicted in Figure 6.1. This reduction in droplet size can be attributed to the enhanced emulsifying ability of Tween 80 at higher concentrations, leading to finer droplet sizes due to improved emulsification and stabilization process. It was observed that increasing the concentration of KC leads to a significant reduction ($p<0.05$) in droplet size across the nanoemulsion formulations suggests that KC is suitable as a stabilizing agent, contributing to the formation of smaller droplets. This is possibly due to its ability to enhance emulsification and stabilize the interface between the oil and water phases (Li et al., 2020; Jiang et al., 2021). The emulsion NE6 with particle size (186.80 nm) less 200 nm can only be considered as nanoemulsion.

In another study, the mean size of nanoemulsions formed using natural emulsifiers ranged from approximately 161.80 nm to 143.67 nm when the concentration of the emulsifier was 1%. Whereas the average size significantly ($p<0.05$) decreased to the size range of 113.43 nm to 127.50 nm as the concentration of the biopolymer increased to 2% (Oliyaei et al., 2022). The emulsion prepared using kappa carrageenan and methyl cellulose found to

decrease the droplet size with the increase in the concentration of kappa carrageenan (Lee et al., 2023). The CMC had lesser effect on droplet size as compared to KC. NE3 and NE6 which contains no CMC, exhibited significantly ($p<0.05$) smaller droplet size compared to NE1, NE2, NE4 and NE5 with comparable KC concentrations. This suggests that CMC plays a crucial role in stabilizing the nanoemulsions, which showed, the impact on droplet size was significantly low as compared to KC. Similar trend was observed in another study where, the droplet size of nanoemulsion prepared with CMC and olive oil increased with increase in the CMC concentration from 0 to 0.75% (Arancibia et al., 2016).

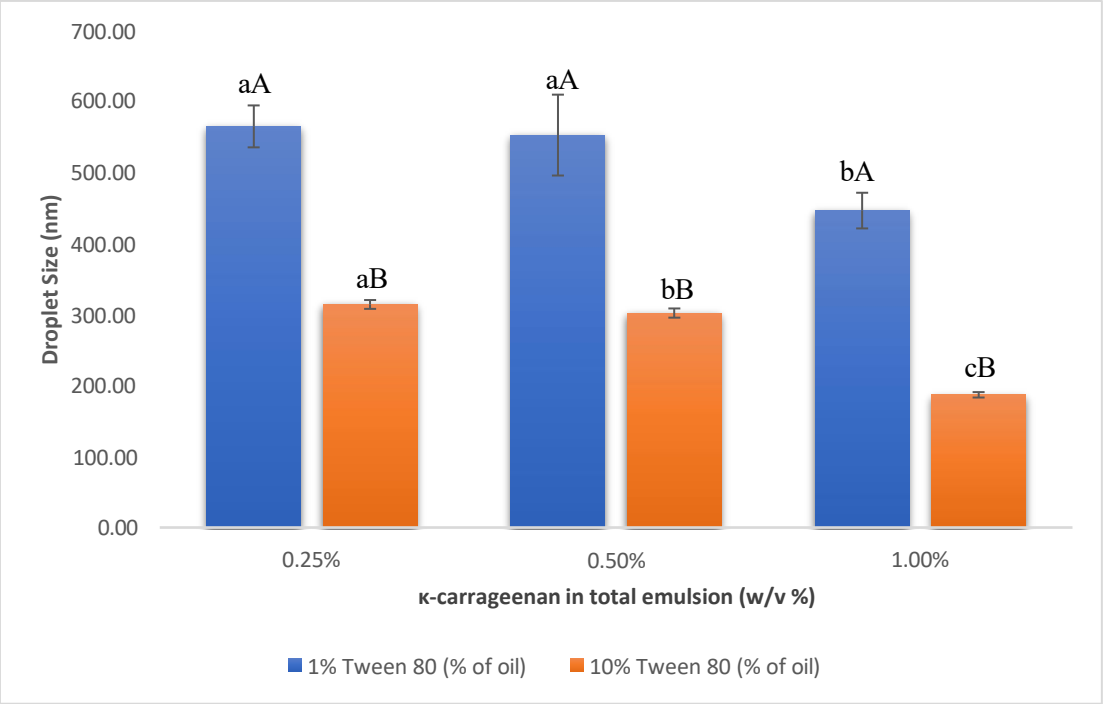


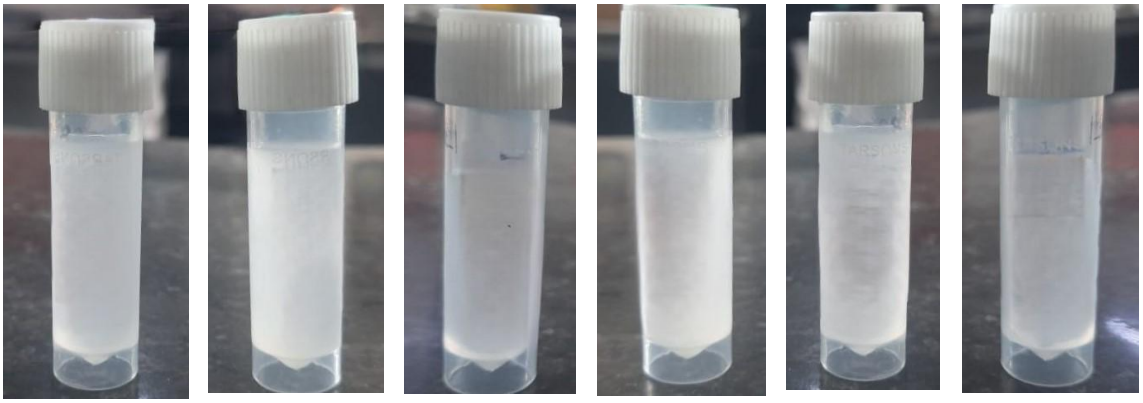
Figure. 6.1. Droplet size of the emulsions prepared with different concentrations of κ -carrageenan and Tween 80 (nm)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis.[a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-B]Mean within rows with different superscript are significantly ($p<0.05$) different

Table. 6.1. The droplet size of the emulsions prepared with different concentrations of κ -carrageenan and Tween 80 (nm)

κ-carrageenan in total emulsion (w/v %)	1% Tween 80 (% of oil)	10% Tween 80 (% of oil)
0.25	564.63±29.50^{aA}	314.00±6.20^{aB}
0.50	552.40±56.93^{aA}	301.87±6.47^{bB}
1.00	446.13±25.10^{bA}	186.80±3.82^{cB}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis.$^{a-c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-B}$Mean within rows with different superscript are significantly ($p<0.05$) different



(a) (b) (c) (d) (e) (f)

Figure. 6.2. Depicted emulsion samples (a) NE1, (b) NE2, (c) NE3, (d) NE4, (e) NE5, and (f) NE6

6.1.2. Poly disparity index (PDI)

The PDI in a nanoemulsion refers to the variability in droplet sizes within the formulation. Lower PDI indicates higher uniformity in droplet size, which is desirable for a nanoemulsion formulation aiming for consistent performance (Jadhav et al., 2020). The droplet size distribution in an emulsion can be influenced by the composition of the emulsion system and adjustments made to homogenization conditions (Choi and McClements, 2020). Table 6.2. and Figure 6.3. represents PDI values for NE1 to NE6 formulations (0.243 to 0.728). Emulsions prepared with 1% Tween 80 showed significantly ($p<0.05$) higher PDI of 0.527 to 0.728 as compared with 10% Tween 80 (0.243 to 0.475) as depicted in Table 6.2. The PDI significantly ($p<0.05$) increased with increase in KC concentrations (0.25 to 0.5%) was 0.665 to 0.728 for emulsion prepared with 1% Tween 80. Similarly, it significantly ($p<0.05$) decreased to 0.527 with 1% KC. Similar trend was

observed for emulsion prepared with 10% Tween 80, where PDI significantly ($p<0.05$) decreased with increase in KC from 0.25 to 0.5% of (0.309 to 0.475). However, PDI value of emulsion with 1% KC significantly ($p<0.05$) decreased to 0.243. Emulsions exhibit better dispersion and higher stability when the PDI falls within the range of 0.2 to 0.5. Within this range, the distribution of droplet sizes is more concentrated, leading to improved dispersion effects. An elevated PDI value signifies a more widely scattered droplet size distribution, potentially making the emulsion prone to delamination or precipitation. Conversely, a lower PDI, with similar droplet sizes, can lead to enhanced stability (Shi et al., 2021). The formulations NE1 (0.665), NE2 (0.728), showed significantly ($p<0.05$) higher PDI values compared to NE3 (0.527) and NE4 (0.309), and NE5 (0.475), showed significantly ($p<0.05$) higher PDI values than NE6 (0.243), indicating a broader distribution of droplet sizes. The PDI value of emulsion prepared with 1% Tween was 0.665 (NE1), 0.728 (NE2) and 0.527 (NE3) that was significantly ($p<0.05$) higher than emulsion prepared with 10% Tween of 0.309 (NE4), 0.475 (NE5) and 0.243 (NE6), respectively, for KC concentrations of 0.25, 0.5 and 1%. While still within an acceptable range for stability, the slightly higher PDI values showed less uniform distribution of droplet sizes. Which increase the likelihood of coalescence and Ostwald ripening over time, potentially impacting the overall stability of the nanoemulsions, albeit to a lesser extent compared to formulations with higher PDI values (Alhasso et al., 2023). A broader droplet size distribution within the emulsions for NE4, NE5, and NE6, prepared with 10% Tween 80, had lower PDI, indicating a more uniform droplet size distribution and enhanced stability with NE6 having lowest PDI and highest stability. This suggests that higher emulsifier concentrations contribute to better homogeneity in droplet sizes within the emulsions.

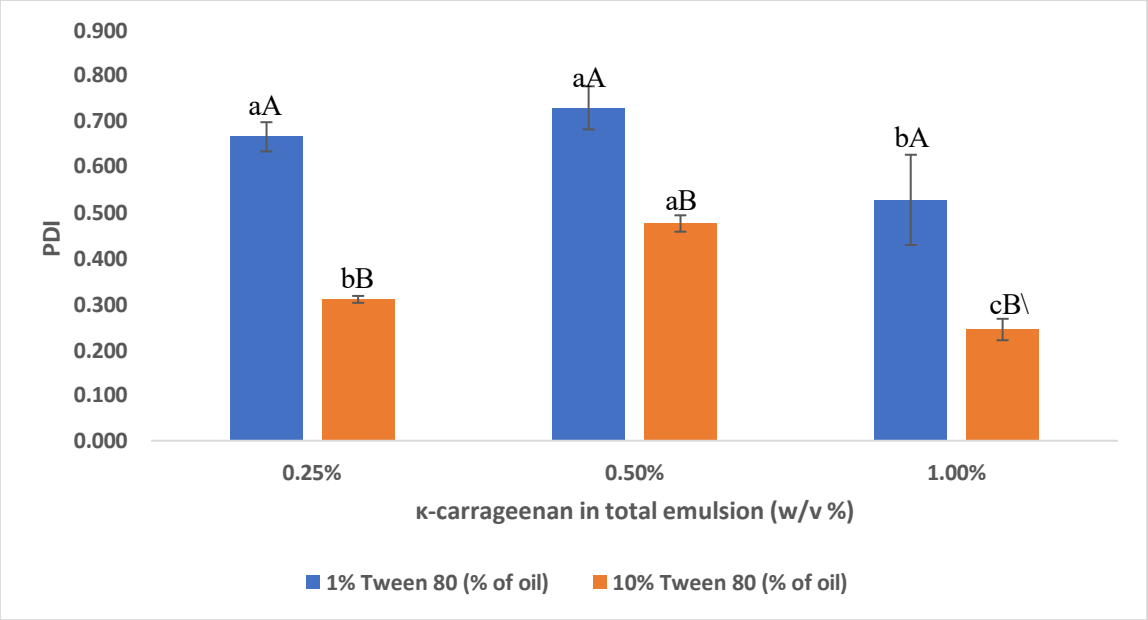


Figure. 6.3. Polydispersity index (PDI) of the emulsions prepares with different concentrations of polymers and Tween 80

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis.[a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-B]Mean within rows with different superscript are significantly ($p<0.05$) different

Table. 6.2. The polydispersity index (PDI) and the zeta potential of the emulsions prepares with different concentrations of polymers and Tween 80

κ-carrageenan in total emulsion (w/v %)	1% Tween 80 (% of oil)		10% Tween 80 (% of oil)	
	PDI	Zeta potential (mV)	PDI	Zeta potential (mV)
0.25	0.665±0.032^{aA}	-50.00±5.02^{aB}	0.309±0.008^{bB}	-22.50±0.44^{bA}
0.50	0.728±0.047^{aA}	-56.07±1.17^{aB}	0.475±0.018^{aB}	-27.00±1.03^{cA}
1.00	0.527±0.099^{bA}	-53.43±0.51^{aB}	0.243±0.023^{cB}	-17.47±0.51^{aA}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis.[a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-B]Mean within rows with different superscript are significantly ($p<0.05$) different

6.1.3. Zeta potential

Zeta potential is a measure of the electrostatic repulsion between droplets in a dispersion, influencing the stability of the colloidal system. A higher magnitude of zeta potential indicates greater repulsion between droplets, leading to enhanced stability. A zeta potential < 30 mV is considered indicative of a stable suspension system. Emulsions prepared with 10% Tween 80 showed significantly ($p<0.05$) lower zeta potential of, -17.47 to -27.00 mV as compared with 1% Tween 80 (-50.00 to -56.07 mV) as depicted in Table 6.2. and Figure 6.4. The least stable emulsion is NE6 with 10% tween and prepared with KC alone of -17.47 mV. The zeta potential for emulsion prepared with 1% Tween 80 significantly ($p<0.05$) increased with increase in KC concentrations from 0.25 to 0.5% (-50.00 to -56.07 mV) for emulsion prepared with 1% Tween 80. Similarly, it significantly ($p<0.05$) increased to -53.43 mV with 1% KC. Similar trend was observed for emulsion prepared with 10% Tween 80, where zeta potential significantly ($p<0.05$) increased with increase in KC from 0.25 to 0.5% (-22.50 to -27.00 mV). However, significantly ($p<0.05$) decreased to -17.47 mV for KC concentration of 1%. A higher absolute value indicates greater stability and reduces the likelihood of aggregation (Shen et al., 2021). The most stable emulsions were where the KC and CMC were in equal quantity. The observed significant ($p<0.05$) decrease in zeta potential values with increasing KC concentration might suggest a significant ($p<0.05$) decrease in surface charge density, possibly due to the shielding effect of KC molecules on the droplet surface.

The results indicate that the concentration of emulsifier (Tween 80) significantly ($p<0.05$) influence the droplet size, PDI, and zeta potential of the prepared nanoemulsions. However, higher concentrations of Tween 80 led to smaller droplet sizes, lower PDIs, and zeta potentials, which shows improved homogeneity and decreased stability within the nanoemulsions.

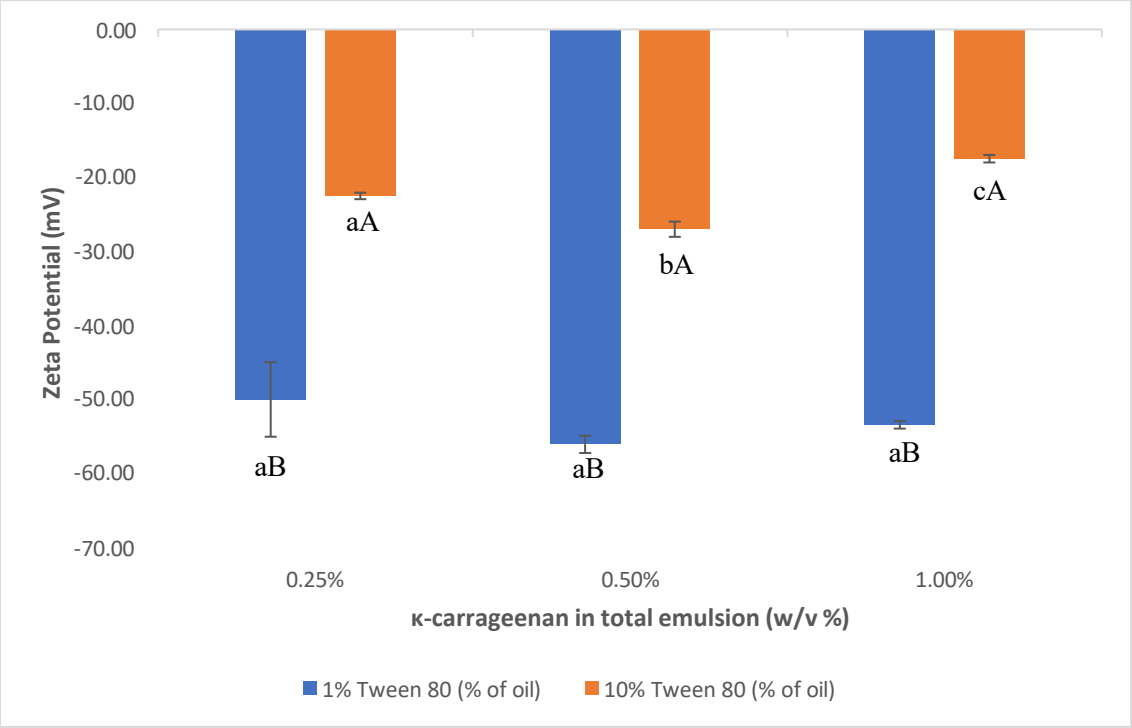


Figure. 6.4. Zeta potential of the emulsions prepares with different concentrations of polymers and Tween 80

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis.[a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-B]Mean within rows with different superscript are significantly ($p<0.05$) different

6.2. FE-SEM

The morphology of the prepared nanoemulsion was examined using Field Emission-Scanning Electron Microscopy (FE-SEM) as given in Figure 6.5., the morphology of the nanoemulsion droplets (Alhamdany et al., 2021). The average droplet size of the selected nanoemulsion (NE6) was 128.89 nm. The nanoemulsion droplets are relatively uniform in size, with most droplets being spherical or near-spherical. The uniformity in droplet size is indicative of a stable nanoemulsion system (Shi et al., 2022). The droplets predominantly exhibit a smooth sphere-like shape, which is typical for nanoemulsions. The spherical shape of lipid nanodroplets has been found to be better in controlled drug release as the spherical particles have minimal contact surface with the aqueous environment and the longest pathway for drug movement within the nanoparticle (Jafarifar et al., 2022).

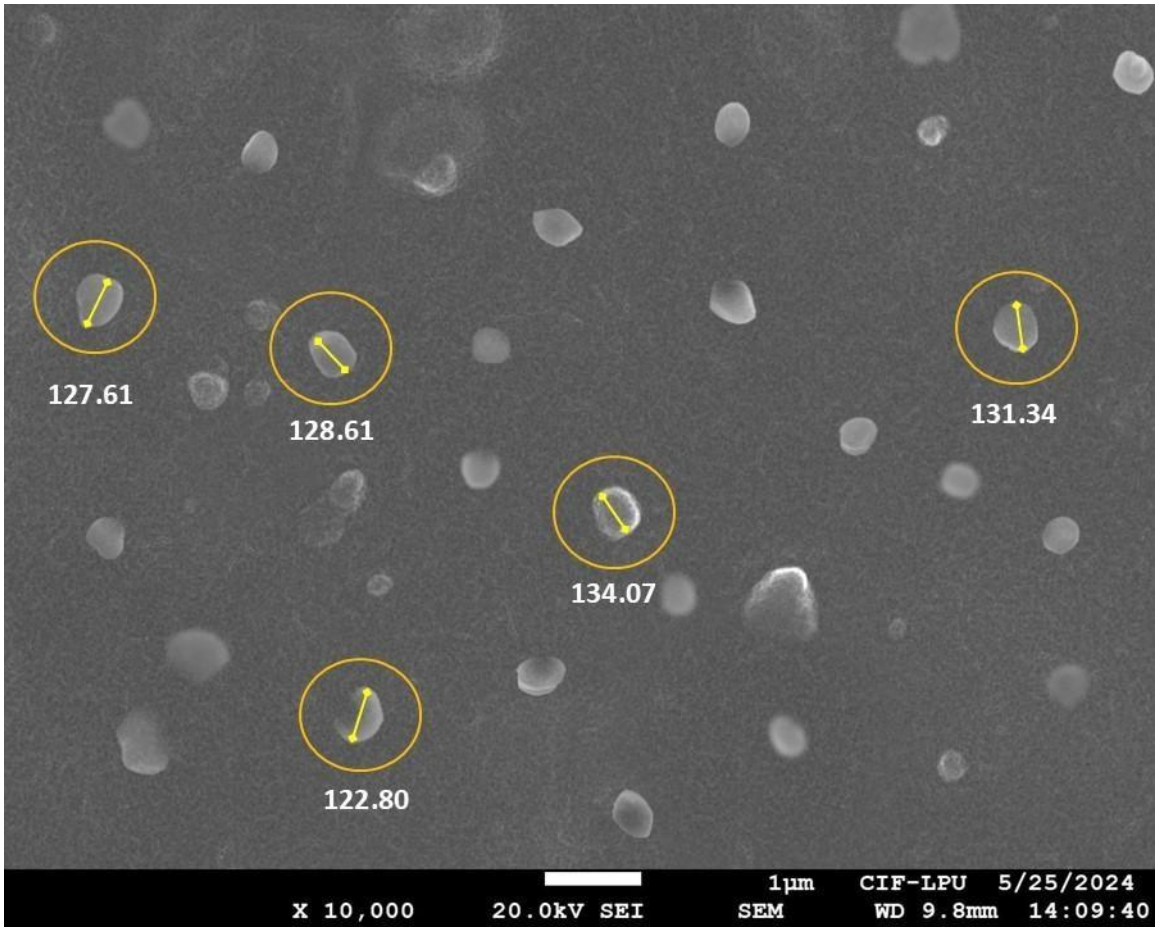


Figure. 6.5. Field Emission-Scanning Electron Microscopy (FE-SEM) of the prepared κ -carrageenan nanoemulsion at 10000X

6.3. Encapsulation efficiency

The encapsulation efficiency (EE) was representing the proportion of microorganisms remain viable throughout the encapsulation process. It helps in evaluating the effectiveness of encapsulation techniques, particularly in preserving the integrity and functionality of the encapsulated microorganisms (de Araújo Etchepare et al., 2020). The weight of the of *L. plantarum* NCDC 685 pellet was 0.39 g and *L. acidophilus* NCDC 600 pellet was 0.36 g. The encapsulation efficiency of *L. acidophilus* NCDC 600 in freeze dried nanoemulsion was 76.33%, which was significantly ($p<0.05$) higher than the encapsulation efficiency of *L. plantarum* NCDC 685 of 74.28%. Similarly, in a previous study, the microencapsulation of *L. plantarum* using biopolymer CMC and KC has been found to have a significant ($p<0.05$) increase in encapsulation efficiency (94.7%) with increase in KC content (1.5%) (Dafe et al., 2017). The lower encapsulation efficiency could be due the loss of viable cells during the freezing process.

6.4. Efficiency of encapsulation against intestinal conditions

6.4.1. Bile tolerance

The viability of nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 was shown in Table 6.3.and Figure 6.6. The study observed the viability of nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 significantly ($p<0.05$) decreased from 9.94 8.98 (9.66% decrease) and 10.61 to 9.63 Log CFU/g (9.25% decrease), respectively, at the bile concentration of 0.3% after 2 hrs of incubation. The free cells had significantly higher decrease ($p<0.05$) of 32.29% and 22.69% for *L. plantarum* NCDC 685 and *L. acidophilus NCDC* 600, respectively. In a study, nanoencapsulated *L. acidophilus* NCDC 600 and *Lactobacillus rhamnosus* were exposed to 0.2% bile, where the survivability significantly ($p<0.05$) decreased from 9.97 Log CFU/g to 8.85 Log CFU/g after 5 min of incubation, which further significantly ($p<0.05$) decreased to 7.15 Log CFU/g after 120 min of incubation. While, the free cells had significantly ($p<0.05$) decreased from 10.05 to 4.17 Log CFU/g after 5 min of incubation after which no live cells were found (Atraki and Azizkhani, 2021).

The viable cells had significant ($p<0.05$) decrease in the nanoencapsulated cells showed better tolerance to the bile concentrations in comparison. Bile salts are potent surfactants, and exposure to bile is highly toxic to probiotic *Lactobacillus* species, affecting their survival and activity (Kusada et al., 2021). The decrease in viable cell count when exposed to 0.3% bile is primarily caused by the disruptive effects of bile salts on the bacterial cell membrane. Bile salts, which are detergents, can solubilize the lipids and proteins in the cell membrane, leading to increased permeability and cell lysis (Zhou et al., 2019). These results indicate that the nanoencapsulation process significantly ($p<0.05$) increased the bile tolerance of the cells, enabling them to withstand exposure to bile salts at concentrations commonly encountered in the gastrointestinal tract. However, this tolerance suggests the potential of nanoencapsulation as a protective strategy for improving the survival and functionality of probiotic microorganisms in the harsh conditions of the digestive system.

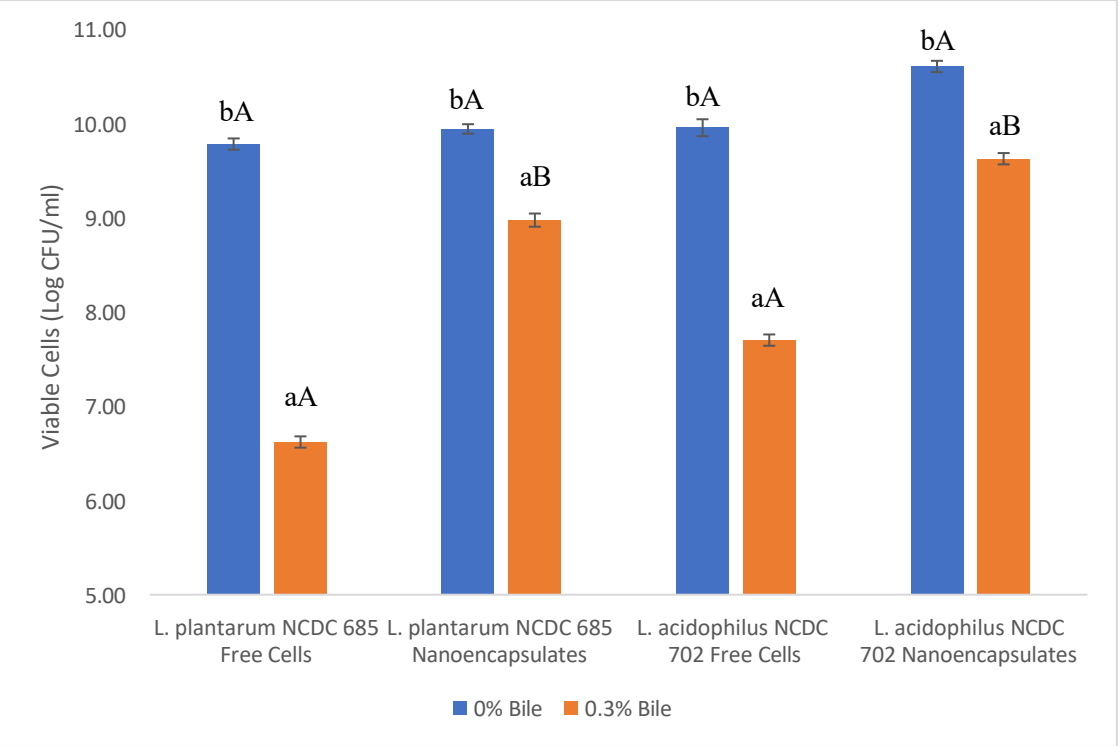


Figure. 6.6. Survival of the free and nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 in simulated bile concentrations of 0 and 0.3% for 2 hrs (Log CFU/ml)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-b]Mean within column with different superscript are significantly ($p<0.05$) different; [A-B]Mean within rows with different superscript are significantly ($p<0.05$) different

Table. 6.3. Survival of the free and nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 in simulated bile concentrations of 0% and 0.3% for 2 hrs (Log CFU/ml)

Bile	*L. plantarum* NCDC 685		*L. acidophilus* NCDC 600	
	Free Cells	Nanoencapsulates	Free Cells	Nanoencapsulates
(%)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)
0.0	9.78 ± 0.06^{bA}	9.94 ± 0.05^{bA}	9.96 ± 0.09^{bA}	10.61 ± 0.06^{bA}
0.3	6.62 ± 0.06^{aA}	8.98 ± 0.07^{aB}	7.70 ± 0.06^{aA}	9.63 ± 0.06^{aB}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-b]Mean within column with different superscript are significantly ($p<0.05$) different; [A-B]Mean within rows with different superscript are significantly ($p<0.05$) different

6.4.2. Acid tolerance

The free cells of *L. plantarum* NCDC 685 at pH 2 showed no viable cells after 120 min of incubation whereas, *L. acidophilus* NCDC 600 had lowest viable count of 3.54 Log CFU/ml as represented in the Table 6.4. and Figure 6.7. Whereas, the nanoencapsulated cells had significantly ($p<0.05$) lower decrease when exposed to lower pH it showed viable count of 7.116 and 7.65 log CFU/ml, *L. plantarum* NCDC 685 and *L. acidophilus NCDC* 600, respectively after 120 min of incubation. A study found that three strains of *L. acidophilus* isolated from dahi exhibited a reduction of 49.55% to 56.81% after 5 hrs of incubation at pH 2 (Farid et al., 2021). In another study, microencapsulated *L. acidophilus* and free cells were exposed to pH 2 for 2 hrs, where the survivability of free cells decreased significantly from 9.05 Log CFU/g to 3.16 Log CFU/g while the encapsulated cells had survivability of 7.5 Log CFU/g after 2 hrs from the initial count of 8.98 (Arepally et al., 2020). In a study, nanoencapsulated *L. acidophilus* and *Lactobacillus rhamnosus* were exposed to pH 2.5 – 3, where the survivability significantly ($p<0.05$) decreased from 9.97 Log CFU/g to 8.95 Log CFU/g after 60 min of incubation However with increase in incubation time (120 min.) significantly ($p<0.05$) decreased to 8.39Log CFU/g after 120 min of incubation. While, the free cells had decreased from 10.05 to 6.11 Log CFU/g after 60 min of incubation and no live cells were found 120 min of incubation (Atraki and Azizkhani, 2021). However the exposure time increasing, the free cells experienced a significant ($p<0.05$) decline in viability, and *L. acidophilus* NCDC 600 reached levels below detection at the 120 min as depicted in Figure 6.7. In contrast, the nanoencapsulated cells showed remarkable resilience, maintaining significantly ($p<0.05$) higher viability even after prolonged exposure to lower pH of 2. The *L. acidophilus* NCDC 600 had better acid tolerance than *L. plantarum* NCDC 685 in both free and nanoencapsulated forms. The nanoencapsulation significantly ($p<0.05$) enhances the acid tolerance of both *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600. Therefore, *L. acidophilus* NCDC 600 showed significantly ($p<0.05$) good acid resistance than *L. plantarum* NCDC 685 in both forms. These findings showed the protective effect of nanoencapsulation against stomach pH, which showed positive effect on the survival and viability of *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 during gastrointestinal transit and thereby improving its efficacy as a probiotic agent.

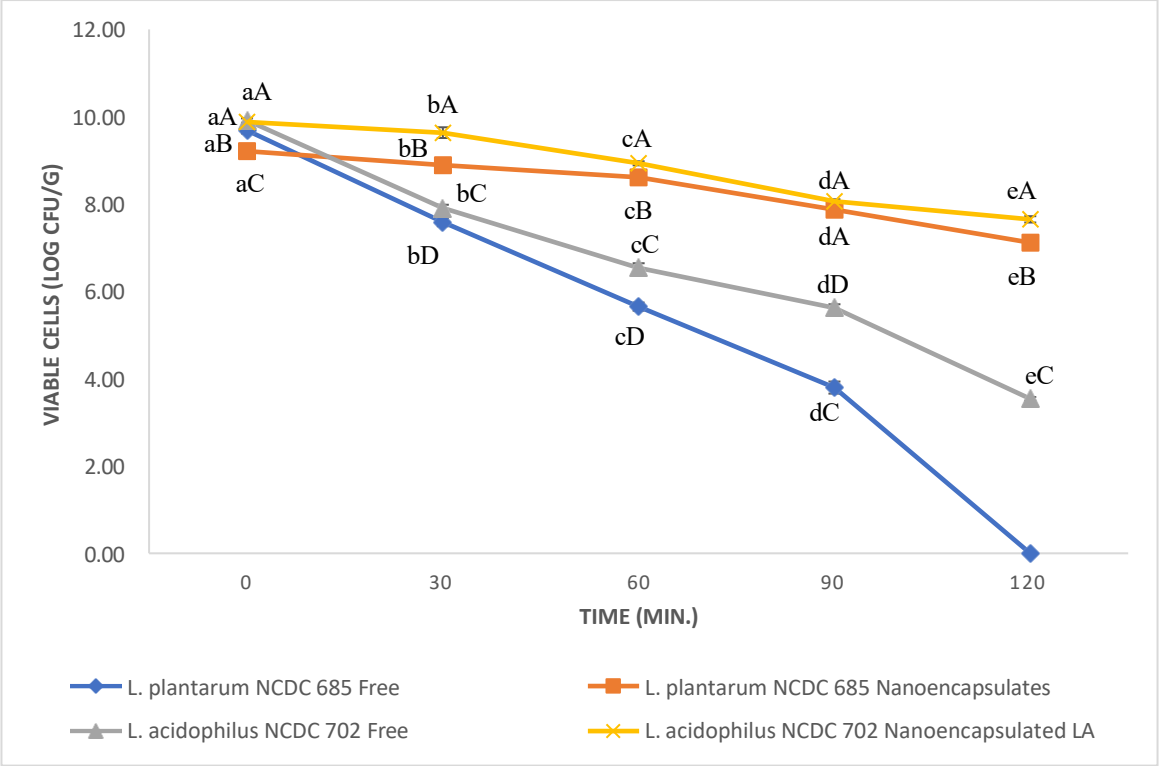


Figure. 6.7. Survival of the free and nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 at pH 2.0

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a-e}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-D}$Mean within rows with different superscript are significantly ($p<0.05$) different

Table. 6.4. The survival of free and nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 at pH 2.0 (Log CFU/ml)

Incubation Time	*L. plantarum* NCDC 685		*L. acidophilus NCDC* 600	
	Free cell	Nanoencapsulated	Free cell	Nanoencapsulated
(Min.)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)
0	9.69±0.07^{aB}	9.21±0.037^{aC}	9.92±0.06^{aA}	9.88±0.08^{aA}
30	7.58±0.09^{bD}	8.89±0.091^{bB}	7.91±0.08^{bC}	9.635±0.12^{bA}
60	5.65±0.09^{cD}	8.61±0.091^{cB}	6.54±0.11^{cC}	8.933±0.05^{cA}
90	3.80±0.14^{dC}	7.87±0.147^{dA}	5.62±0.09^{dB}	8.060±0.05^{dA}
120	-	7.12±0.053^{eB}	3.54±0.04^{eC}	7.649±0.07^{eA}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a-e}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-D}$Mean within rows with different superscript are significantly ($p<0.05$) different

6.5. Release study

Table 6.5 and Figure 6.8. represents gradual and steady release of *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 from the nanoencapsulation matrix was observed over the period of 6 hrs of incubation. A significant ($p<0.05$) release of cells was observed at the end of 6 hrs of 8.29 (90.12%) and 8.86Log CFU/g (89.69%) of *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600, respectively as depicted in Figure 6.5. Similar trend was observed by Dafe et al., (2017), for microencapsulated *L. plantarum*. This release pattern suggests that the nanoencapsulation system provides controlled and sustained release of *L. plantarum* NCDC 685 over an extended period. Such controlled release characteristics are desirable for probiotic delivery systems, as they ensure prolonged exposure of the probiotic cells to the target site within the gastrointestinal tract, potentially enhancing their efficacy in conferring health benefits.

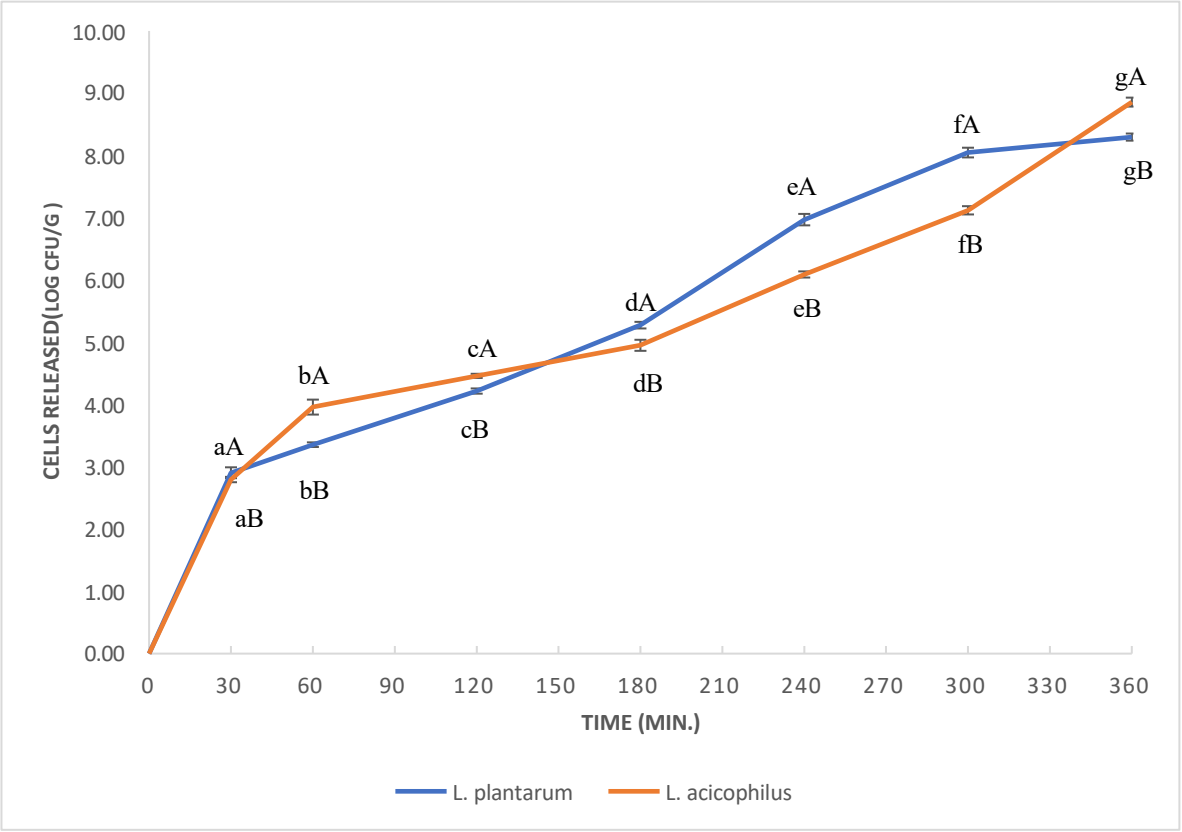


Figure. 6.8. Release profile of the nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 cells in buffered solution at pH 7.4

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis.[a-g]Mean within column with different superscript are significantly ($p<0.05$) different; [A-B]Mean within row with different superscript are significantly ($p<0.05$) different

Table. 6.5. The release profile of the nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 cells in buffered solution at pH 7.4

Incubation time (Min.)	*L. plantarum* NCDC 685 (Log CFU/g)	*L. acidophilus* NCDC 600 (Log CFU/g)
0	-	-
30	2.90 ± 0.09^{aA}	2.80 ± 0.04^{aB}
60	3.36 ± 0.04^{bB}	3.96 ± 0.12^{bA}
120	4.22 ± 0.04^{cB}	4.46 ± 0.03^{cA}
180	5.28 ± 0.05^{dA}	4.95 ± 0.09^{dB}
240	6.97 ± 0.09^{eA}	6.21 ± 0.06^{eB}
300	8.05 ± 0.08^{fA}	7.12 ± 0.07^{fB}
360	8.29 ± 0.06^{gB}	8.86 ± 0.07^{gA}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a\text{-}g}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A\text{-}B}$Mean within row with different superscript are significantly ($p<0.05$) different

6.6. Optimization of germination

6.6.1. Radicle length

Table 6.6. represents finger millet (Figure 6.9) and pearl millet (Figure 6.10) demonstrating the improved growth at the highest germination temperature of 30˚C as compared with lower temperatures 22˚C and 26˚C. Conversely, buckwheat exhibited optimal growth at the lowest temperature of 22˚C as shown in Table 6.6 and Figure 6.11. Whereas radicle length significantly ($p<0.05$) increased with both germination time and temperature for finger millet. The initial radicle growth of finger millet was observed at 48 hrs under 22˚C, measuring 2.0 and 1.0 mm. Similarly for the grains soaked at 16 hrs and 24 hrs, respectively, showed significant ($p<0.05$) increase to 3.6 and 2.2 mm over the soaking time. At 26˚C, radicle growth of grains soaked for 16 hrs, commenced earlier at 24 hrs (2.8 mm) and reached 19.2 mm by 72 hrs, indicating a significant ($p<0.05$) increase in growth with increase in temperature. Similar trend was observed for grains soaked for 24 hrs, where the initial radicle length after 24 hrs of germination was 3.4 mm which significantly ($p<0.05$) increased to 19.6 mm. At 30˚C, the maximum radicle growth of finger millet was 29.2 mm after 16 hrs of soaking, which showed a significant ($p<0.05$) increase, where finger millet soaked for 24 hrs had radicle length of 19.4 mm.

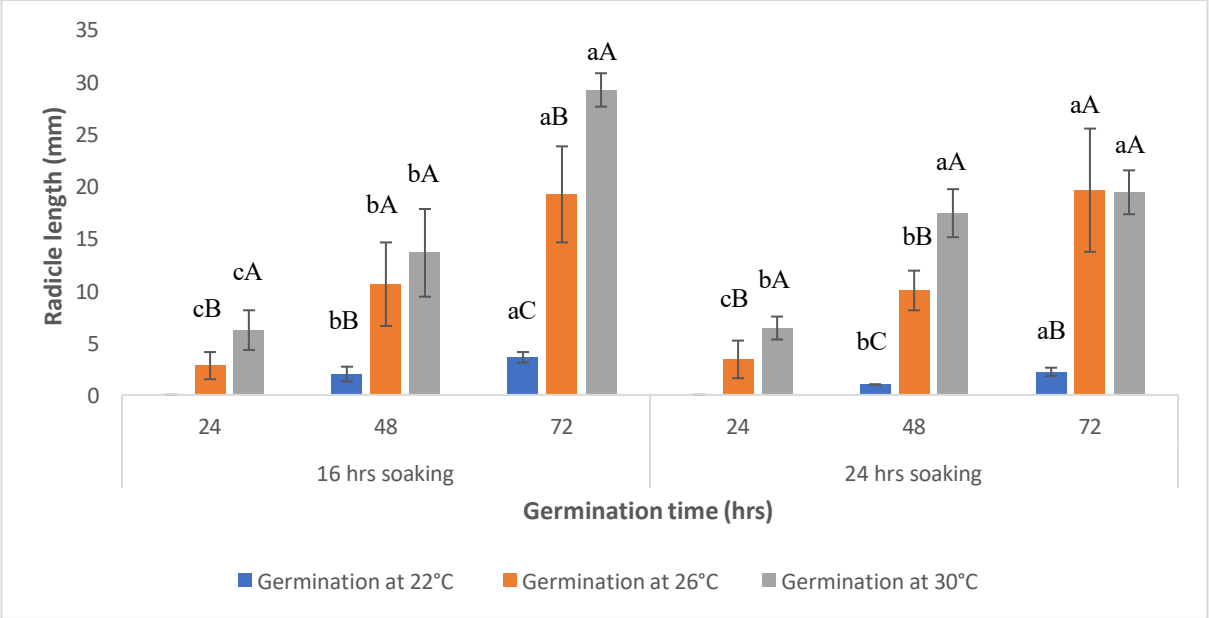


Figure. 6.9. Radicle length of the germinated finger millet grains at different conditions (mm)

Data are expressed as mean ±S.D. (n=5). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different

For pearl millet, radicle growth began at 24 hrs with measurements of 0.4, 4.0 and 8.8 mm at 22, 26 and 30˚C, respectively, for grains soaked for 16 hrs and 1.2, 8.6 and 4.8 mm for pearl millet soaked for 24 hrs, which continued to increase significantly ($p<0.05$) by 72 hrs (4.0±1.0, 14.2±3.7 and 19.6±4.8 mm for 16 hrs soaking and 5.0±0.7, 20.8±5.8 and 29.0±7.5 mm for 24 hrs soaking). Pearl millet had initial radicle length of 0.4, 4.0 and 8.8 mm at 22˚C, 26˚C and 30˚C, respectively after 24 hrs of germination after being soaked for 16 hrs which significantly ($p<0.05$) increased to 4.0, 14.2, and 19.6 after 72 hrs of germination. However, grains soaked for 24 hrs had initial radicle length of 1.2, 8.6 and 4.8 mm after 24 hrs of germination which significantly ($p<0.05$) increased to 5.0, 20.8 and 29.0 mm after 72 hrs of germination.

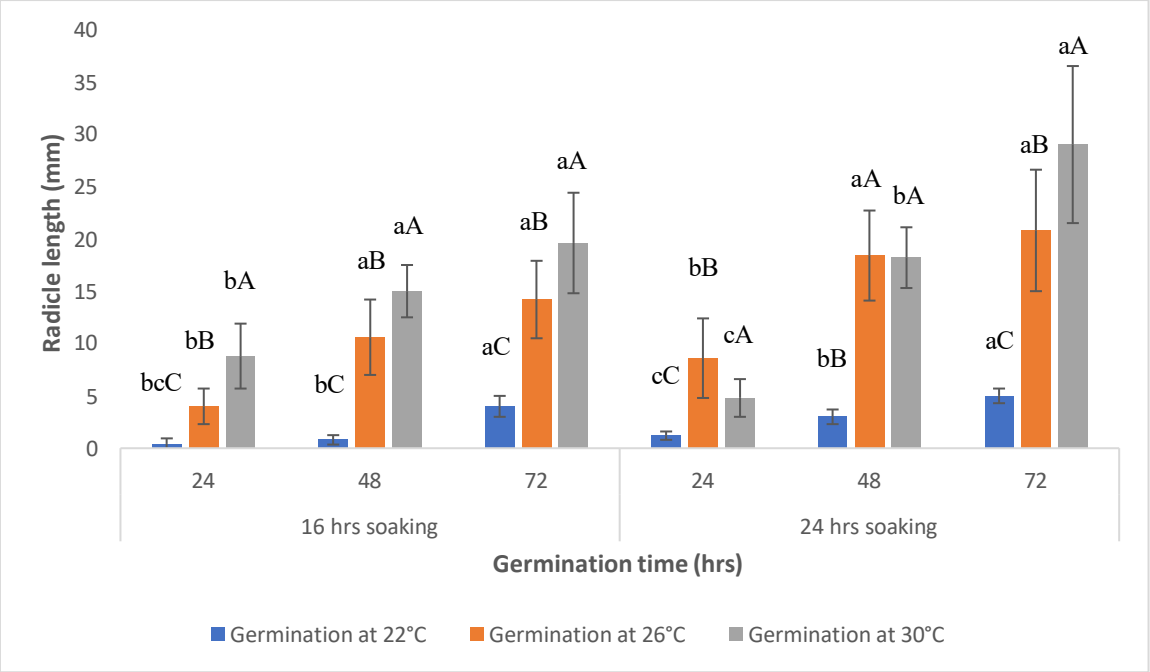


Figure. 6.10. Radicle length of the germinated pearl millet grains at different conditions (mm)

Data are expressed as mean ±S.D. (n=5). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different

In contrast, buckwheat's initial growth was limited, for buckwheat soaked for 16 hrs showing no growth at 22˚C and 30˚C at 24 hrs, but it showed significant ($p<0.05$) increases at 48 hrs (1.2, 0.4 and 1.4 mm) and 72 hrs (5.0, 2.0 and 2.6 mm) across the temperatures. The buckwheat grains soaked at for 24 hrs which showed no growth after 24 hrs of germination at 22˚C and 26˚C, while 30˚C radicle length (0.2 mm), significantly ($p<0.05$) increased to 2.0, 1.8 and 3.0 mm after 72 hrs of germination.

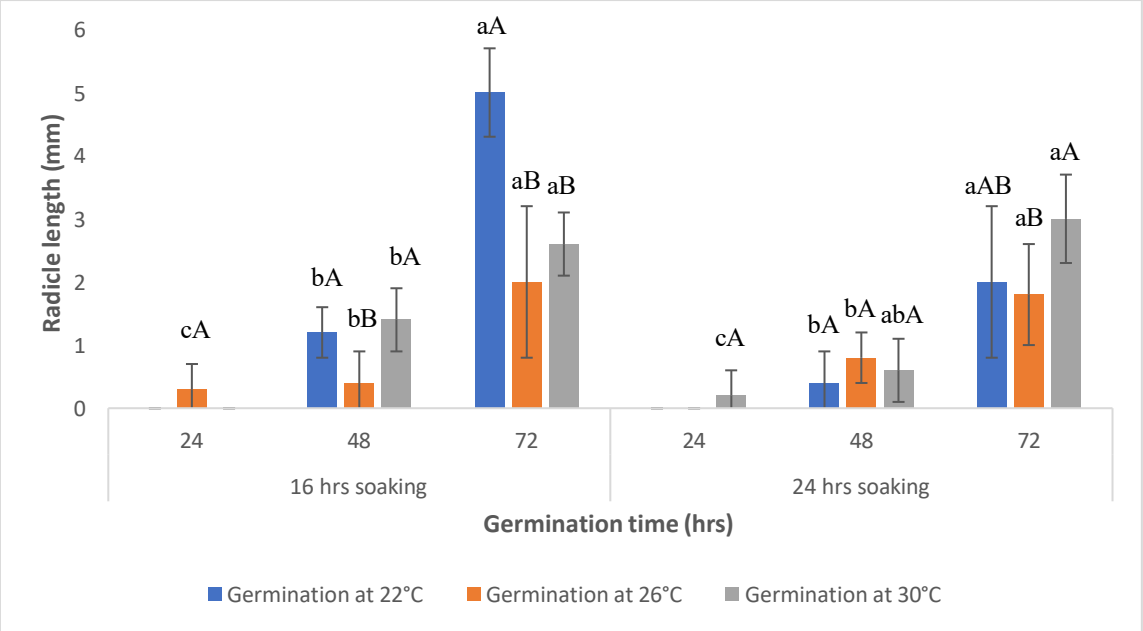


Figure. 6.11. Radicle length of the germinated buckwheat grains at different conditions (mm)

Data are expressed as mean ±S.D. (n=5). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different

The observed variations in growth can be attributed to temperature-induced changes in enzyme activity that affect germination (Keil et al., 2021). Generally, higher temperatures facilitate seed germination by breaking dormancy, while lower temperatures may suppress this process. However, different plant species exhibit varying optimal temperatures for seed germination, and some crops may achieve higher germination rates at lower temperatures (Kumar and Anand, 2021). Prolonged exposure to elevated temperatures can adversely affect seed germination. However, high temperatures often result in increased respiration rates and reduced metabolic activity within the seed embryo, which diminishes the energy available for the germination process. Additionally, elevated temperatures can induce water stress, further hindering the germination of seeds (Krishnan et al., 2012).

a) b) c)

Figure. 6.12. Representation the germinated (a) finger millet at 30˚C for 72 hrs after 16 hrs of soaking, (b) pearl millet at 30˚C for 72 hrs after 24 hrs of soaking, (c) buckwheat at 22˚C for 72 hrs after 16 hrs of soaking.

Table 6.6. The radicle length of the germinated Finger millet, pearl millet and buckwheat grains at different conditions (mm)

Soaking time (hrs)	Germination temperature / Germination time (hrs)	Finger millet			Pearl millet			Buckwheat		
		22 ˚C (mm)	26 ˚C (mm)	30 ˚C (mm)	22 ˚C (mm)	26 ˚C (mm)	30 ˚C (mm)	22 ˚C (mm)	26 ˚C (mm)	30 ˚C (mm)
16	24	0	$2.8\pm1.3b^{cB}$	6.2 ± 1.9^{cA}	0.4 ± 0.5^{bcC}	4.0 ± 1.7^{bB}	8.8 ± 3.1^{bA}	0	0.3 ± 0.4^{cA}	0
	48	2.0 ± 0.7^{bB}	10.6 ± 4.0^{bA}	13.6 ± 4.2^{bA}	0.8 ± 0.5^{bC}	10.6 ± 3.6^{aB}	15.0 ± 2.5^{aA}	1.2 ± 0.4^{bA}	0.4 ± 0.5^{bB}	1.4 ± 0.5^{bA}
	72	3.6 ± 0.5^{aC}	19.2 ± 4.6^{aB}	29.2 ± 1.6^{aA}	4.0 ± 1.0^{aC}	14.2 ± 3.7^{aB}	19.6 ± 4.8^{aA}	5.0 ± 0.7^{aA}	2.0 ± 1.2^{aB}	2.6 ± 0.5^{aB}
24	24	0	3.4 ± 1.8^{cB}	6.4 ± 1.1^{bA}	1.2 ± 0.4^{cC}	8.6 ± 3.8^{bB}	4.8 ± 1.8^{cA}	0	0	0.2 ± 0.4^{cA}
	48	1.0 ± 0.0^{bC}	10.0 ± 1.9^{bB}	17.4 ± 2.3^{aA}	3.0 ± 0.7^{bB}	18.4 ± 4.3^{aA}	18.2 ± 2.9^{bA}	0.4 ± 0.5^{bA}	0.8 ± 0.4^{bA}	0.6 ± 0.5^{abA}
	72	2.2 ± 0.4^{aB}	19.6 ± 5.9^{aA}	19.4 ± 2.1^{aA}	5.0 ± 0.7^{aC}	20.8 ± 5.8^{aB}	29.0 ± 7.5^{aA}	2.0 ± 1.2^{aAB}	1.8 ± 0.8^{aB}	3.0 ± 0.7^{aA}

Data are expressed as mean ±S.D. (n=5). Two-way ANOVA used for statically analysis. $^{a\text{-}c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A\text{-}C}$Mean within rows with different superscript are significantly ($p<0.05$) different

6.6.2. Total Phenolic Content (TPC)

Table 6.7. and Figure 6.13. represents the total phenolic content of the finger millet. The TPC of finger millet soaked for 16 hrs and dried at 60˚C significantly ($p<0.05$) decreased from 27.18 mg GAE/100g after 24 hrs of germination to 16.72 mg GAE/100g after 72 hrs at 22˚C. Similarly at 26˚C and 30˚C, the TPC significantly ($p<0.05$) decreased from 24.15 mg GAE/100g and 28.63 mg GAE/100g at 24 hrs to 14.46 mg GAE/100g and 13.74 mg GAE/100g after 72 hrs of germination, respectively. With the increase in soaking time to 24 hrs, TPC followed the declining trend with the increase in germination time, while it was significantly ($p<0.05$) higher than for the samples soaked for 16 hrs. After 72 hrs of germination, TPC for grains soaked for 24 hrs and dried at 60˚C significantly ($p<0.05$) decreased to 16.27 mg GAE/100g at 22˚C, 14.01 mg GAE/100g at 26˚C, and 17.63 mg GAE/100g at 30˚C. The decrease in the phenolic content was also corresponding to the increase in the germination temperature and time. The initial decrease after 24 hrs of germination was significantly ($p<0.05$) lower at 30˚C than 26˚C and 22˚C (28.63, 27.18 and 24.15 mg GAE/100g). While, after 72 hrs of germination the decrease was significantly ($p<0.05$) higher at 30˚C than 26 and 22˚C (13.74, 14.46 and 16.72 mg GAE/100g). The increase in drying temperature from 60˚C to 80˚C decreased the TPC of finger millet. Finger millet soaked for 16 hrs, TPC after 72 hrs of germination decreased to 16.43 mg GAE/100g at 22˚C, 13.55 mg GAE/100g at 26˚C, and 14.53 mg GAE/100g at 30˚C after 72 hrs of germination when dried at 80˚C. Similarly, for grains soaked for 24 hrs, the TPC decreased from 17.30 mg GAE/100g at 22˚C to 16.22 mg GAE/100g at 30˚C after 72 hrs. According to previous studies, the phenolic content of finger millet decreased from 1.80 to 0.38g/100 g (Krishnan et al., 2012). In another study, total phenolic content had a significant reduction of about 50% was observed after germination of 48 hrs (Hithamani and Srinivasan, 2014). Studies have also found the phenolic content to increase with germination of finger millet (Sunil et al., 2017). The reduction in TPC can be attributed to the activity of the polyphenol oxidase enzyme, which breaks down free polyphenols through an oxidation reaction (Zhou et al., 2021).

Table. 6.7 Total phenolic content of the finger millet at various germination conditions (mg GAE/100g)

Drying temperature	Soaking time (hrs)	Germination temperature	22 ˚C	26 ˚C	30 ˚C
		Germination time (hrs)	(mg GAE/100g)	(mg GAE/100g)	(mg GAE/100g)
60 ˚C	16	24	27.18 ± 0.77^{aB}	24.15 ± 0.13^{aC}	28.63 ± 0.04^{aA}
		48	17.49 ± 0.13^{bC}	21.11 ± 0.36^{bB}	21.90 ± 0.04^{bA}
		72	16.72 ± 0.06^{bA}	14.46 ± 0.04^{cB}	13.74 ± 0.11^{cC}
	24	24	21.30 ± 0.04^{aC}	23.84 ± 0.04^{aB}	30.43 ± 0.04^{aA}
		48	18.21 ± 0.06^{bC}	21.83 ± 0.04^{bB}	24.51 ± 0.04^{bA}
		72	16.27 ± 0.22^{cB}	14.01 ± 0.04^{cC}	17.63 ± 0.07^{cA}
80 ˚C	16	24	27.18 ± 0.04^{aB}	24.46 ± 0.07^{aC}	28.36 ± 0.04^{aA}
		48	17.18 ± 0.36^{bC}	21.65 ± 0.04^{bB}	22.54 ± 0.04^{bA}
		72	16.43 ± 0.04^{cA}	13.55 ± 0.06^{cC}	14.53 ± 0.40^{cB}
	24	24	21.59 ± 0.04^{aC}	25.23 ± 0.06^{aB}	29.68 ± 0.04^{aA}
		48	18.75 ± 0.04^{bC}	23.35 ± 0.36^{bB}	23.82 ± 0.04^{bA}
		72	17.30 ± 0.04^{cA}	12.91 ± 0.04^{cC}	16.22 ± 0.10^{cB}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a-c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-C}$Mean within rows with different superscript are significantly ($p<0.05$) different

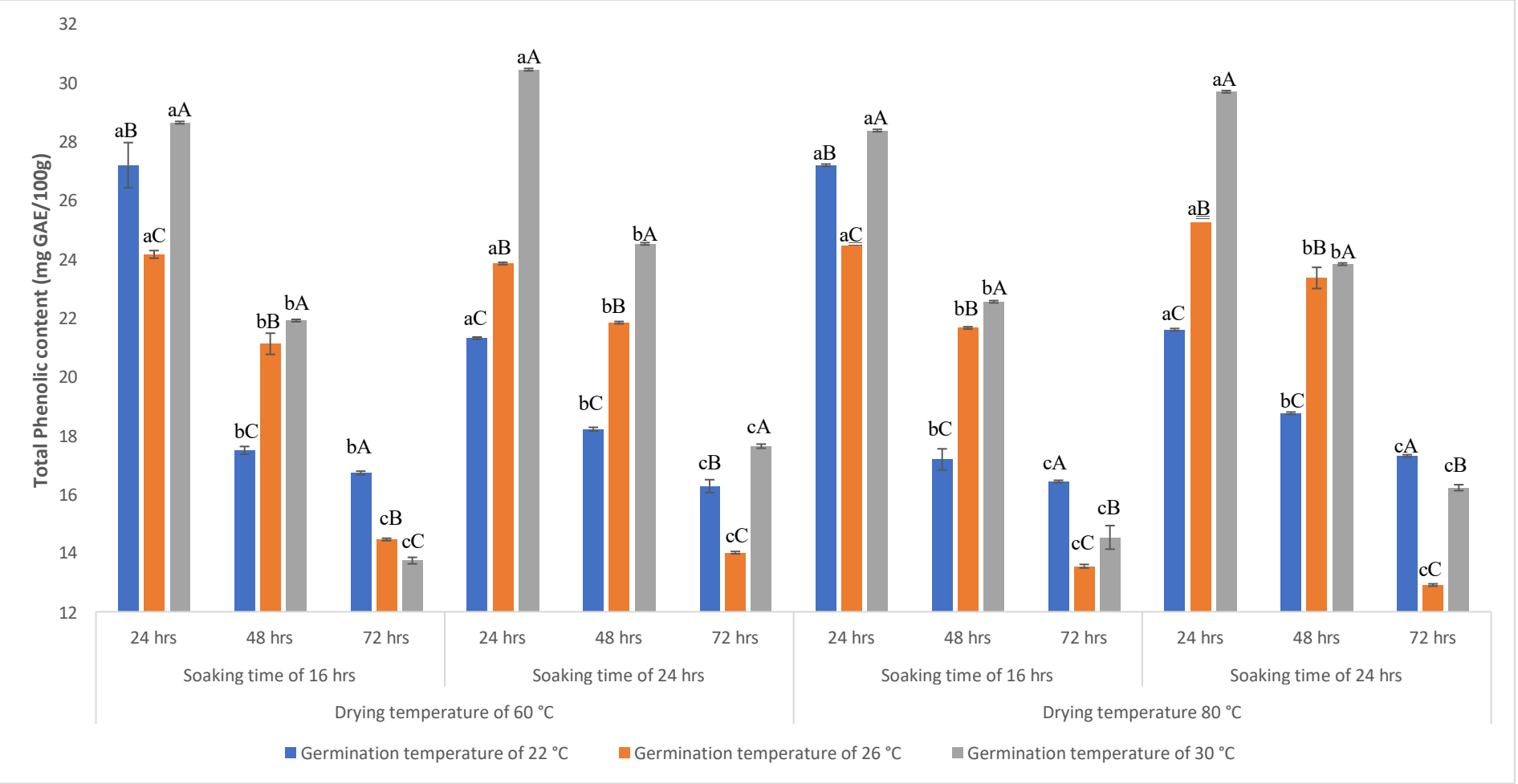


Figure. 6.13. Total phenolic content of the finger millet at various germination conditions (mg GAE/100g)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different

The TPC of pearl millet are represented in the Table 6.8. and Figure 6.14. Pearl millet germinated for 24 hrs had a TPC of 30.72 mg GAE/100g at 22˚C, at drying temperature of 60˚C and a soaking time of 16 hrs, which significantly ($p<0.05$) decreased to 26.89 mg GAE/100g at 26˚C and 24.17 mg GAE/100g at 30˚C. While, with increase in germination time to 72 hrs, TPC significantly ($p<0.05$) decreased to 26.96 mg GAE/100g at 22˚C, 18.44 mg GAE/100g at 26˚C and 16.92 mg GAE/100g at 30˚C. This trend indicates that higher germination temperatures, especially 30˚C, increased the reduction in phenolic compounds. Similarly, with soaking time of 24 hrs, the TPC after 24 hrs of germination significantly ($p<0.05$) decreased to 23.88 mg GAE/100g at 22˚C and 21.95 mg GAE/100g at 30˚C from pearl millet soaked for 16 hrs. After 72 hrs of germination, TPC significantly ($p<0.05$) decreased further, to 19.93 mg GAE/100g at 22˚C and 15.60 mg GAE/100g at 30˚C. The pearl millet soaked for 16 hrs and dried at 80˚C had TPC of 29.37 mg GAE/100g after 24 hrs of germination at 22˚C, which significantly ($p<0.05$) decreased to 26.62 mg GAE/100g at 26˚C and 23.78 mg GAE/100g at 30˚C. Similarly, after 72 hrs of germination, TPC significantly ($p<0.05$) decreased to 25.42±0.06 mg GAE/100g at 22˚C, 18.25 mg GAE/100g at 26˚C and 16.70±0.02 mg GAE/100g at 30˚C. Similar trend was observed for pearl millet soaked for 24 hrs, where TPC significantly ($p<0.05$) decreased from 23.59 mg GAE/100g at 22˚C after 24 hrs of germination to 15.44 mg GAE/100g at 30˚C after 72 hrs. The decrease was consistent with both the increase in the germination temperature and time, unlike in finger millet. The drying temperature does not have significant effect on the TPC content. Bhati et al., (2016) reported that the total phenolic content of pearl millet significantly ($p<0.05$) decreased with germination. Obadina et al., (2017), found a significant decrease of the total phenolic content from 169.90 to 130.20 mg/100g sample after 96 hrs of germination at 25˚C. The findings of Singh et al., (2017) also reported a decrease in the phenolic content in pearl millet which could be due to leaching of polyphenols at the period of soaking and increase in enzymatic activity during germination. The activity of enzymes such as polyphenol oxidase and hydrolytic enzymes during germination can contribute to the loss of phenolic compounds (Taylor and Duodu, 2015).

Table. 6.8. Total phenolic content of the pearl millet at various germination conditions (mg GAE/100g)

Drying temperature	Soaking time (hrs)	Germination temperature /time	22 ˚C	26 ˚C	30 ˚C
		Germination time (hrs)	(mg GAE/100g)	(mg GAE/100g)	(mg GAE/100g)
60 ˚C	16	24	30.72±0.33^{aA}	26.89±0.02^{aB}	24.17±0.06^{aC}
		48	29.66±0.04^{bA}	22.17±0.04^{bB}	21.71±0.02^{bC}
		72	26.96±0.15^{cA}	18.44±0.02^{cB}	16.92±0.02^{cC}
	24	24	23.88±0.02^{aB}	24.65±0.02^{aA}	21.95±0.02^{aC}
		48	20.74±0.06^{bB}	22.95±0.02^{bA}	20.34±0.02^{bC}
		72	19.93±0.08^{cA}	17.86±0.02^{cB}	15.60±0.06^{cC}
80 ˚C	16	24	29.37±0.08^{aA}	26.62±0.02^{aB}	23.78±0.02^{aC}
		48	28.81±0.04^{bA}	21.55±0.02^{bB}	21.23±0.04^{bC}
		72	25.42±0.06^{cA}	18.25±0.02^{cB}	16.70±0.02^{cC}
	24	24	23.59±0.04^{aB}	24.71±0.02^{aA}	21.29±0.07^{aC}
		48	19.25±0.02^{bC}	20.28±0.02^{bA}	19.93±0.02^{bB}
		72	17.88±0.02^{cA}	16.87±0.04^{cB}	15.44±0.02^{cC}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a-c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-C}$Mean within rows with different superscript are significantly ($p<0.05$) different

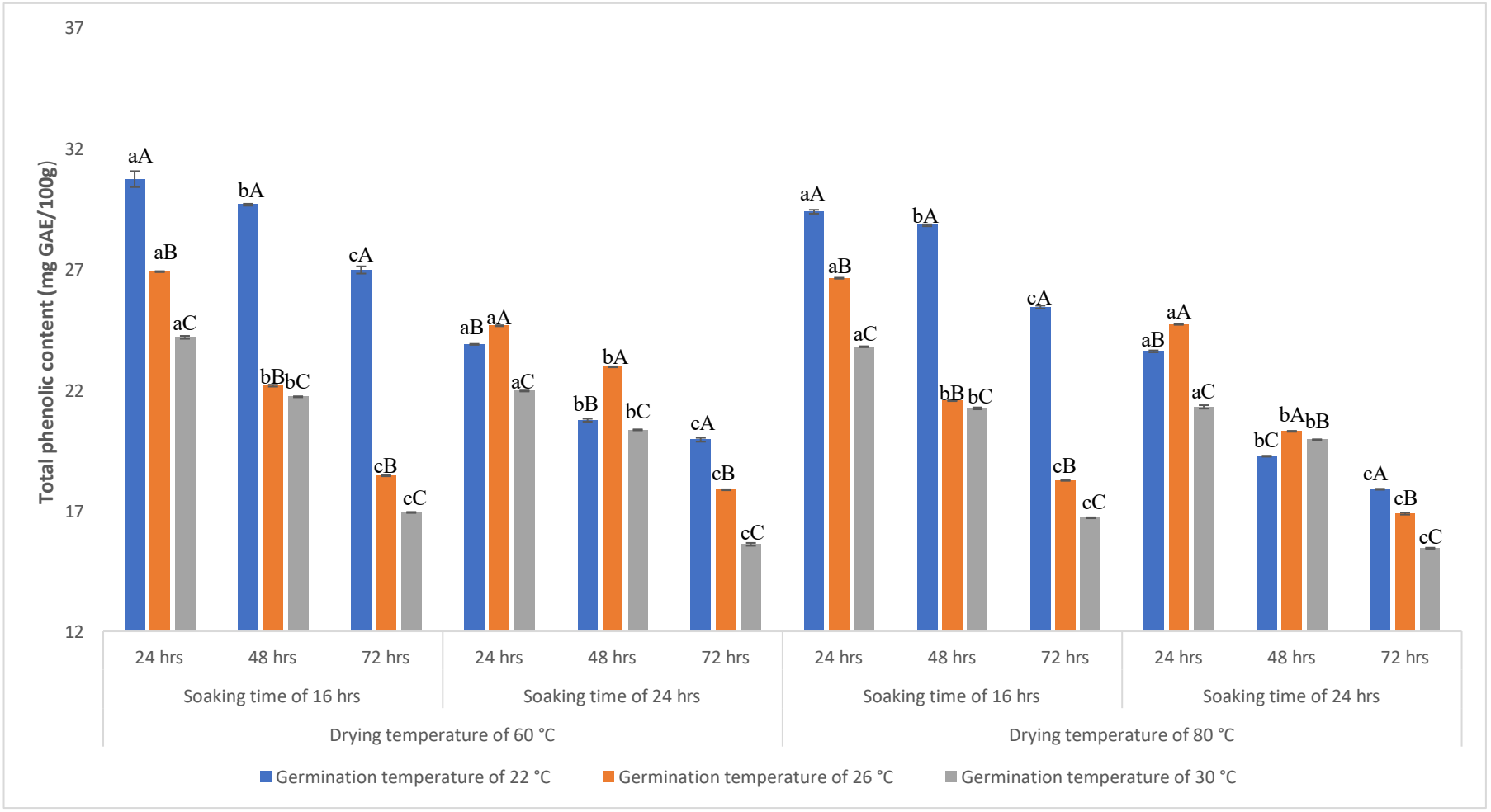


Figure. 6.14. Total phenolic content of the pearl millet at various germination conditions (mg GAE/100g)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different;

Table 6.9. and Figure 6.15. represents the total phenolic content of the buckwheat at various germination conditions. Buckwheat germinated for 24 hrs had a TPC of 54.03 mg GAE/100g at 22˚C, 50.53 mg GAE/100g at 26˚C, and 27.65 mg GAE/100g at 30˚C at drying temperature of 60˚C and a soaking time of 16 hrs, which significantly ($p<0.05$) increased 56.56 mg GAE/100g at 22˚C, 54.63 mg GAE/100g at 26˚C, and 42.85 mg GAE/100g at 30˚C, after 72 hrs of germination. The data indicates that lower germination temperatures (22˚C and 26˚C) lead to higher TPC retention, especially after extended germination times. Similarly increasing the soaking time to 24 hrs also showed significant ($p<0.05$) increase in TPC with germination time, while it was lower than buckwheat soaked for 16 hrs. After 72 hrs of germination, TPC significantly ($p<0.05$) increased to 58.05 mg GAE/100g at 22˚C, 44.30 mg GAE/100g at 26˚C, and 42.27 mg GAE/100g at 30˚C from 39.56 mg GAE/100g at 22˚C, 22.77 mg GAE/100g at 26˚C and 27.61 mg GAE/100g at 30˚C. The buckwheat dried at 80˚C had significantly ($p<0.05$) lower TPC compared to buckwheat dried at 60˚C. Similarly, buckwheat germinated for 24 hrs and dried at 80˚C had TPC of 16.58 mg GAE/100g at 22˚C, and 20.30 mg GAE/100g at 26˚C, which significantly ($p<0.05$) increased after 72 hrs of germination, of 21.94 mg GAE/100g at 22˚C and 22.33 mg GAE/100g at 26˚C. There is significant ($p<0.05$) decrease in TPC with for buckwheat dried at 80˚C than at 60˚C (19.54 and 58.05 mg GAE/g, respectively) for buckwheat soaked for 24 hrs and germinated for 72 hrs at 22˚C. Similar trend was observed in a study where the red and white quinoa sprouts dried at 60˚C (17.57 and 13.94 mg GAE/g, respectively) had a significant ($p<0.05$) decrease of phenolic content than sprouts dried at 45˚C (28.79 and 15.15 mg GAE/g, respectively) (Złotek et al., 2019). According to Terpinc et al., (2016), the total phenolic content of buckwheat had increased with the germination time. Živković et al., (2021), also found a similar trend of decreasing phenolic content after 24 hrs and a gradual increase further due to the leaching during soaking process. The total phenolic content of foxtail millet and proso millet has also been found to increase with germination (Ko et al., 2011). It was also observed that phenolic content decreased with the increase in soaking time. The total phenolic content was increased in buckwheat due to the release of polyphenols from the polysaccharides cell wall during the soaking treatment, which led to the softening of the tissue structure of the grain (Sunil et al., 2017; Karki and Kharel, 2012). However, the TPC increases with germination time, were also decreases with increase in germination temperature. The TPC of buckwheat after 72 hrs of germination at 22, 26 and 30˚C was 58.05, 44.30 and 42.27 mg GAE/100g, respectively, showing a significantly ($p<0.05$) decreasing trend. As low temperatures have

been observed to enhance the production of phenolic content by stimulating the synthesis of phenylalanine ammonia-lyase, whereas high temperatures may impact the activity of enzymes involved in phenolic synthesis (Guo et al., 2011).

Table. 6.9. Total phenolic content of the buckwheat at various germination conditions (mg GAE/100g)

Drying temperature	Soaking time (hrs)	Germination temperature	22 ˚C	26 ˚C	30 ˚C
		Germination time (hrs)	(mg GAE/100g)	(mg GAE/100g)	(mg GAE/100g)
60 ˚C	16	24	54.03 ± 0.04^{cA}	50.53 ± 0.13^{cB}	27.65 ± 0.12^{cC}
		48	55.69 ± 0.04^{bA}	53.10 ± 0.04^{bB}	41.42 ± 0.13^{bC}
		72	56.56 ± 0.04^{aA}	54.63 ± 0.19^{aB}	42.85 ± 0.13^{aC}
	24	24	39.56 ± 0.04^{cA}	22.77 ± 0.19^{cC}	27.61 ± 0.13^{cB}
		48	49.04 ± 0.04^{bA}	38.48 ± 0.13^{bB}	38.05 ± 0.17^{bC}
		72	58.05 ± 0.04^{aA}	44.30 ± 0.12^{aB}	42.27 ± 0.10^{aC}
80 ˚C	16	24	16.58 ± 0.07^{cB}	20.30 ± 0.04^{cA}	20.34 ± 0.04^{aA}
		48	17.47 ± 0.11^{bC}	20.57 ± 0.19^{bA}	18.85 ± 0.04^{cB}
		72	21.94 ± 0.06^{aB}	22.33 ± 0.04^{aA}	20.18 ± 0.04^{bC}
	24	24	17.20 ± 0.07^{cB}	17.18 ± 0.04^{cB}	19.10 ± 0.04^{cA}
		48	19.37 ± 0.04^{bAB}	19.35 ± 0.07^{bB}	19.50 ± 0.07^{bA}
		72	19.54 ± 0.04^{aB}	19.56 ± 0.07^{aB}	20.41 ± 0.04^{aA}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a\text{-}c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A\text{-}C}$Mean within rows with different superscript are significantly ($p<0.05$) different

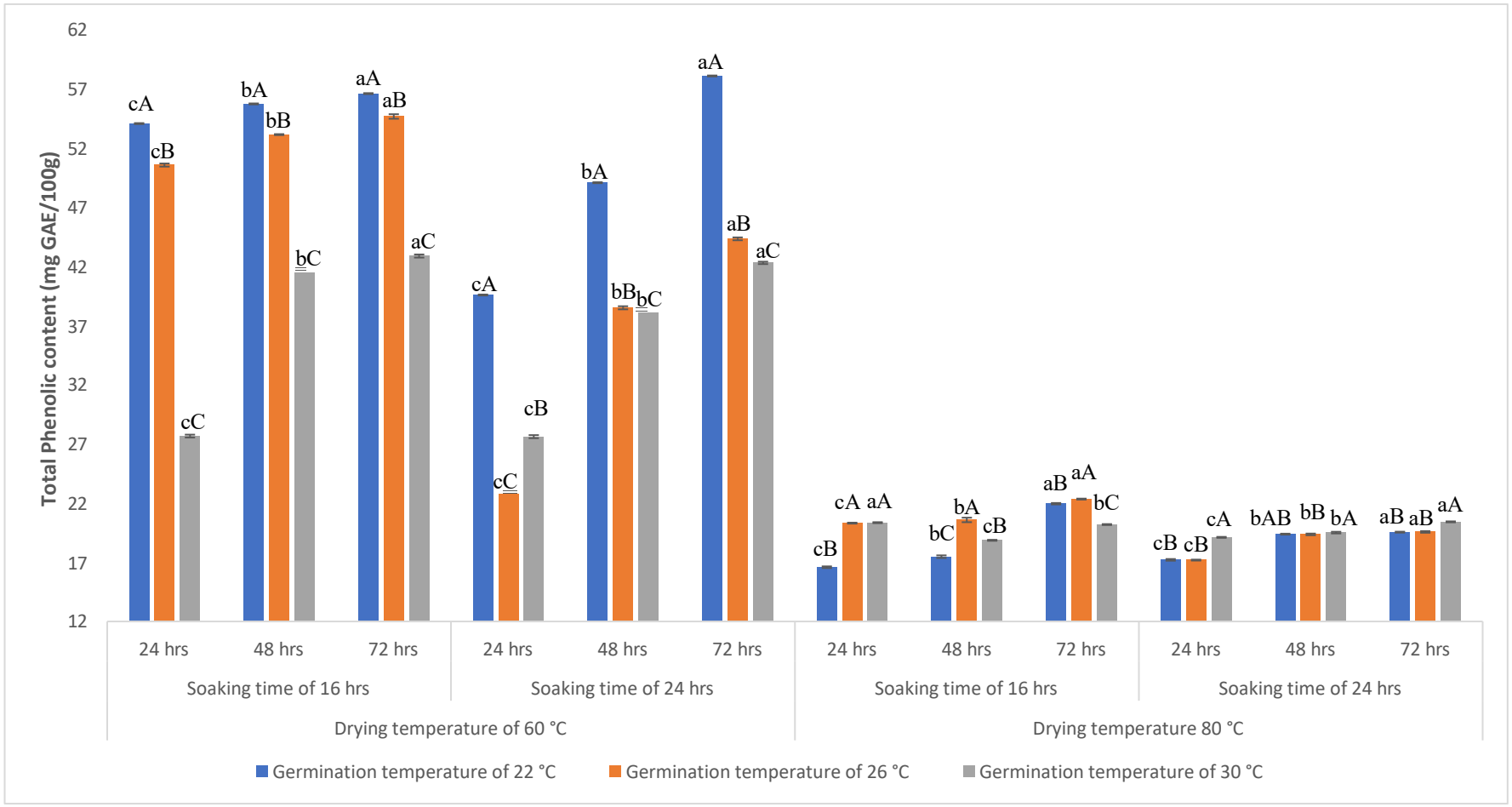


Figure. 6.15. Total phenolic content of the buckwheat at various germination conditions (mg GAE/100g)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different;

6.6.3. Antioxidant activity

The antioxidant activity observed in germinated finger millet as shown in Table 6.10. and Figure 6.16. In study, it was observed finger millet soaked for 16 hrs and germinated at 22˚C (23.07 % inhibition of DPPH) was significantly ($p<0.05$) higher as compared with 26˚C and 30˚C (18.31 % inhibition of DPPH and 14.59 % inhibition of DPPH) for 24 hrs, when dried at 60˚C. Similarly, it observed with an increase in temperature 30˚C, antioxidant activity (13.69 and 12.79 % inhibition of DPPH) showed a significantly ($p<0.05$) declining trend at 48 hrs and 72 hrs as compared with 22˚C (16.74 % inhibition of DPPH and 14.70 % inhibition of DPPH) and 26˚C (16.75 % inhibition of DPPH and14.32 % inhibition of DPPH). It was also found that the antioxidant activity was significantly ($p<0.05$) decreasing with the increase in soaking time, where, finger millet soaked for 24 hrs and germinated at 22˚C, 26˚C and 30˚C had antioxidant activity of 12.67 % inhibition of DPPH, 14.54 % inhibition of DPPH and 5.08 % inhibition of DPPH after 72 hrs of germination and dried at 60˚C. The germinated finger millet soaked for 24 hrs, germinated at 22˚C, 26˚C and 30˚C and dried at 80˚C showed significant ($p<0.05$) decrease with antioxidant activity of 11.22 % inhibition of DPPH, 14.29 % inhibition of DPPH and 12.09 % inhibition of DPPH after 72 hrs of germination. Karki and Kharel (2012), showed a significant ($p<0.05$) decrease in antioxidant with the germination of finger millet. The lowest antioxidant activity was found in grains germinated at 30˚C of 3.97 % inhibition of DPPH, 1.51 % inhibition of DPPH and 1.27 % inhibition of DPPH after 24, 48 and 72 hrs. The reduction of antioxidant activity is due to the decrease in phenolic components during the germination process (Zhou et al., 2021).

Table. 6.10. Antioxidant activity of the finger millet at various germination conditions (% inhibition of DPPH)

Drying temperature	Soaking time	Germination temperature	22 ˚C	26 ˚C	30 ˚C
		Germination time (hrs)	(% inhibition of DPPH)	(% inhibition of DPPH)	(% inhibition of DPPH)
60 ˚C	16	24	23.07 ± 0.12^{aA}	18.31 ± 0.10^{aB}	14.59 ± 0.05^{aC}
		48	16.74 ± 0.12^{bA}	16.75 ± 0.10^{bA}	13.69 ± 0.05^{bB}
		72	14.70 ± 0.12^{cA}	14.32 ± 0.10^{cB}	12.79 ± 0.05^{cC}
	24	24	20.20 ± 0.10^{aB}	21.35 ± 0.07^{aA}	4.78 ± 0.83^{aC}
		48	14.66 ± 0.20^{bB}	20.67 ± 0.07^{bA}	4.60 ± 0.08^{aC}
		72	12.67 ± 0.10^{cB}	14.54 ± 0.12^{cA}	5.08 ± 0.13^{aC}
80 ˚C	16	24	21.43 ± 0.06^{aA}	15.40 ± 0.09^{aB}	13.52 ± 0.14^{aC}
		48	18.35 ± 0.06^{bA}	15.14 ± 0.09^{bB}	13.68 ± 0.14^{aC}
		72	11.22 ± 0.12^{cC}	14.29 ± 0.09^{cA}	12.09 ± 0.14^{bB}
	24	24	21.29 ± 0.16^{aA}	19.95 ± 0.12^{aB}	4.69 ± 0.15^{cC}
		48	16.21 ± 0.11^{bB}	17.80 ± 0.12^{bA}	4.01 ± 0.14^{bC}
		72	12.35 ± 0.06^{cA}	12.52 ± 0.12^{cA}	5.71 ± 0.14^{aB}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a\text{-}c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A\text{-}C}$Mean within rows with different superscript are significantly ($p<0.05$) different;

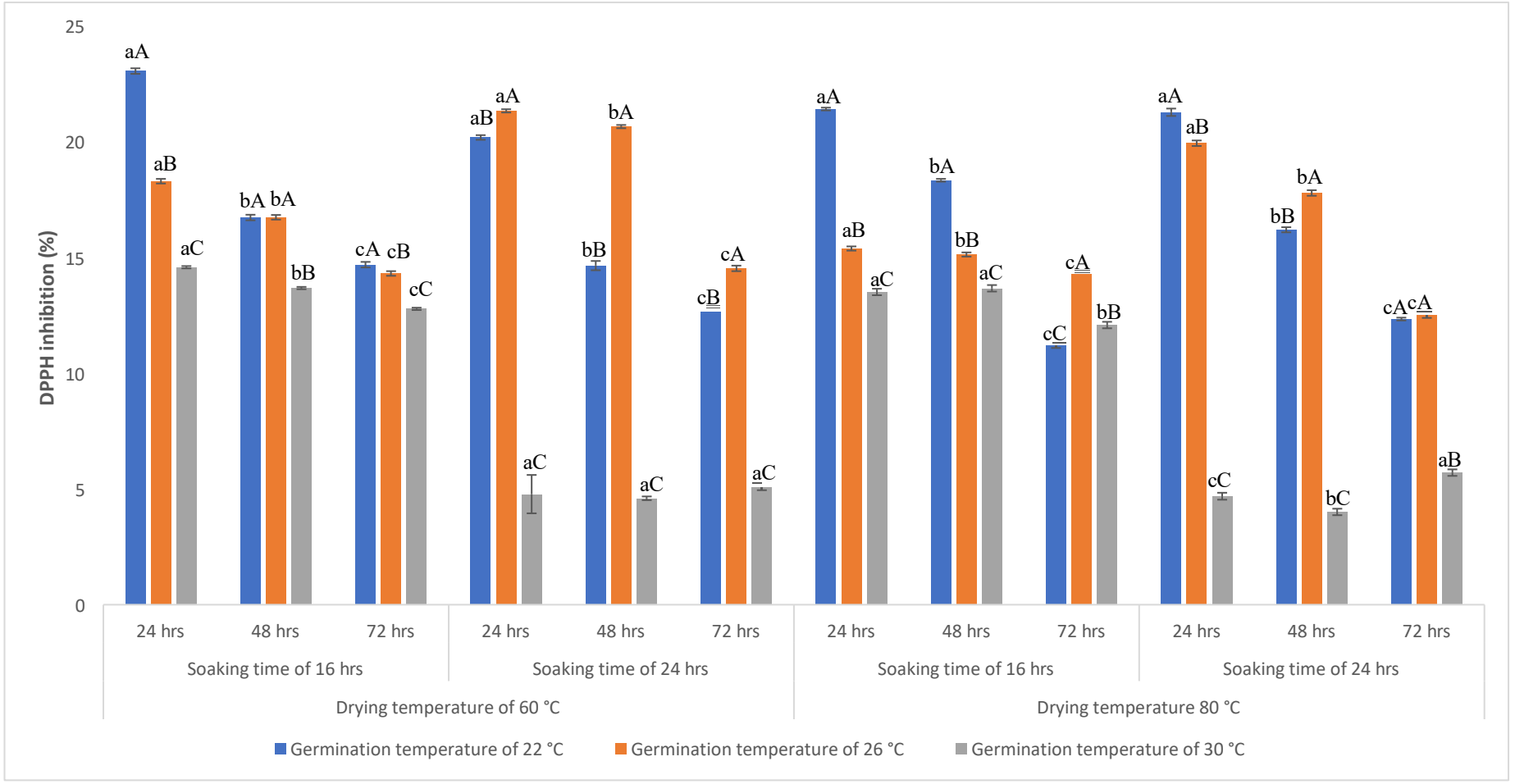


Figure. 6.16. Antioxidant activity of the finger millet at various germination conditions (% inhibition of DPPH)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different;

The Table 6.11 and Figure 6.17. shows the antioxidant activity of pearl millet, given as the % inhibition of DPPH. Antioxidant activity in pearl millet germinated at 22˚C (20.89 % inhibition of DPPH) significantly($p<0.05$) decreased at 26˚C (19.82 % inhibition of DPPH) and 30˚C (19.62 % inhibition of DPPH) after 24 hrs of germination for grains soaked for 16 hrs and dried at 60˚C. However, the antioxidant activity at 26˚C was non-significantly ($p<0.05$) decreased at 30˚C. While with increase in germination time to 72 hrs, antioxidant activity significantly ($p<0.05$) decreased to 17.65 % inhibition of DPPH at 22˚C, 17.14 % inhibition of DPPH at 26˚C and 17.91 % inhibition of DPPH at 30˚C. Similarly, pearl millet soaked for 24 hrs showed a similar trend. Antioxidant activity in pearl millet germinated at 22˚C (20.08 % inhibition of DPPH) significantly($p<0.05$) decreased at 26˚C (19.21 % inhibition of DPPH) and 30˚C (18.71 % inhibition of DPPH) after 24 hrs of germination for grains soaked for 26 hrs and dried at 60˚C. Where, the antioxidant activity at 26˚C was non-significantly ($p<0.05$) decreased at 30˚C. While with increase in germination time to 72 hrs, antioxidant activity significantly ($p<0.05$) decreased to 18.56 % inhibition of DPPH at 22˚C, 18.23 % inhibition of DPPH at 26˚C and 17.45 % inhibition of DPPH at 30˚C. After 24 hrs of germination, the antioxidant activity was 20.85 % inhibition of DPPH at 22˚C and 19.33 % inhibition of DPPH at 30˚C, which was lower than the corresponding values at 60˚C drying. As germination time increased to 72 hrs, the antioxidant activity continued to decline, with values reaching 17.41 % inhibition of DPPH at 22˚C and 16.69 % inhibition of DPPH at 30˚C. This trend suggests that higher drying temperatures, like 80˚C, cause greater degradation of antioxidants. Similarly, pearl millet germinated for 24 hrs and dried at 80˚C showed antioxidant activity of 20.85 % inhibition of DPPH at 22˚C, 19.41 % inhibition of DPPH and 19.33 % inhibition of DPPH at 30˚C, which significantly ($p<0.05$) decreased after 72 hrs of germination, to 17.41 % inhibition of DPPH at 22˚C, 16.81 % inhibition of DPPH at 26˚C and 16.69 % inhibition of DPPH at 30˚C. Singh et al. (2017) investigated in research that the antioxidant activity of finger and pearl millet was decreased corresponding to the decrease of the total phenolic content. The decline in antioxidant activity of finger and pearl millet was higher with an increase in the germination temperature. This could be attributed to the increase in germination, which causes enzymatic activity that reduces the antioxidant components, such as the total phenols. The antioxidant activity decreased with an increase in the germination temperature. This trend was similar to that of the total phenolic content had an initial decrease after 24 hrs and an increase in DPPH inhibition from there on (Živković et al., 2021).

Table. 6.11. Antioxidant activity of the pearl millet at various germination conditions (% inhibition of DPPH)

Drying temperature	Soaking time (hrs)	Germination temperature /time	22 ˚C	26 ˚C	30 ˚C
		Germination time (hrs)	(% inhibition of DPPH)	(% inhibition of DPPH)	(% inhibition of DPPH)
60 ˚C	16	24	20.89±0.21^{aA}	19.82±0.18^{aB}	19.62±0.05^{aB}
		48	19.69±0.32^{bA}	18.51±0.68^{bB}	18.90±0.29^{bAB}
		72	17.65±0.12^{cA}	17.14±0.18^{cB}	17.91±0.14^{cA}
	24	24	20.08±0.42^{aA}	19.21±0.06^{aB}	18.79±0.08^{aB}
		48	19.01±0.09^{bA}	18.08±0.06^{bB}	17.97±0.13^{bB}
		72	18.56±0.12^{bA}	18.23±0.37^{bA}	17.45±0.13^{cB}
80 ˚C	16	24	20.85±0.33^{aA}	19.41±0.24^{aB}	19.33±0.06^{aB}
		48	19.56±0.09^{bA}	17.83±0.24^{bB}	17.81±0.27^{bB}
		72	17.41±0.08^{cA}	16.82±0.09^{cB}	16.69±0.06^{cB}
	24	24	19.45±0.05^{aA}	18.40±0.12^{aB}	17.65±0.26^{aC}
		48	18.99±0.12^{bA}	17.34±0.23^{bB}	16.97±0.15^{bC}
		72	18.15±0.29^{cA}	16.06±0.20^{cB}	16.45±0.14^{cB}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a-c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-C}$Mean within rows with different superscript are significantly ($p<0.05$) different;

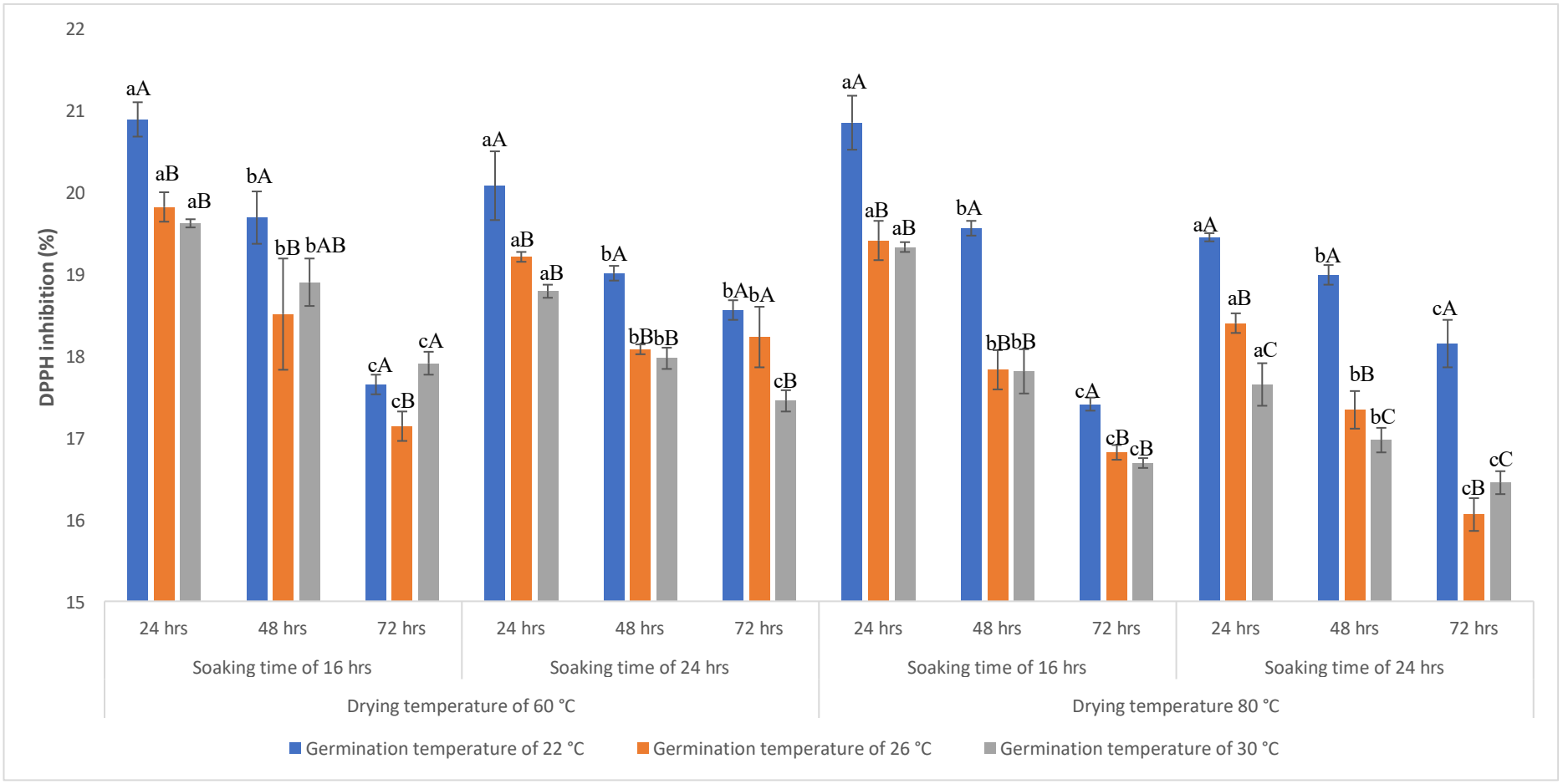


Figure. 6.17. Antioxidant activity of the pearl millet at various germination conditions (% inhibition of DPPH)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different;

Table 6.12. and Figure 6.18. represents the antioxidant activity of buckwheat germinated at different conditions. Buckwheat germinated for 24 hrs had antioxidant activity of 28.25 % inhibition of DPPH at 22˚C, 24.7 3 inhibition of DPPH % at 26˚C, and 17.31 % inhibition of DPPH at 30˚C, at drying temperature of 60˚C and a soaking time of 16 hrs, which significantly ($p<0.05$) increased 53.51 % inhibition of DPPH at 22˚C, 53.33 % inhibition of DPPH, at 26˚C, and 34.28 % inhibition of DPPH at 30˚C, after 72 hrs of germination. Similarly, with increasing the soaking time to 24 hrs also showed significant ($p<0.05$) increase in antioxidant activity with germination time. Antioxidant activity of buckwheat after 72 hrs of germination, significantly ($p<0.05$) increased to 51.06 % inhibition of DPPH at 22˚C, 46.45 % inhibition of DPPH, at 26˚C, and 30.72 % inhibition of DPPH at 30˚C from 27.51 % inhibition of DPPH at 22˚C, 27.59 % inhibition of DPPH, at 26˚C, and 16.29 % inhibition of DPPH at 30˚C. The buckwheat dried at 80˚C had significantly ($p<0.05$) lower antioxidant activity compared to buckwheat dried at 60˚C. Where, buckwheat soaked for 16 hrs, germinated for 24 hrs and dried at 80˚C showed antioxidant activity of 27.86 % inhibition of DPPH at 22˚C, 23.93 % inhibition of DPPH at 26˚C, and 16.83 % inhibition of DPPH at 30˚C, which significantly ($p<0.05$) increased after 72 hrs of germination, to 52.54 % inhibition of DPPH at 22˚C, 51.70 % inhibition of DPPH, at 26˚C, and 33.37 % inhibition of DPPH at 30˚C. However, the antioxidant activity of buckwheat significantly ($p<0.05$) decreased with increase in the germination temperature from 28.25 % inhibition of DPPH at 22˚C, to 24.73 % inhibition of DPPH at 26˚C, and 17.31 % inhibition of DPPH at 30˚C for grains soaked for 16 hrs and dried at 60˚C. Beitane et al., (2018), reported antioxidant activity of buckwheat was decrease after 24 hrs of germination, which increased after 48h. The phenolic compound synthesis increased the antioxidant activity during the germination process.

Table. 6.12. Antioxidant activity of the buckwheat at various germination conditions (% inhibition of DPPH)

Drying temperature	Soaking time (hrs)	Germination temperature	22 ˚C	26 ˚C	30 ˚C
		Germination time (hrs)	(% inhibition of DPPH)	(% inhibition of DPPH)	(% inhibition of DPPH)
60 ˚C	16	24	28.25±0.06^{cA}	24.73±0.16^{cB}	17.31±0.10^{cC}
		48	42.25±0.06^{bA}	41.08±0.16^{bB}	26.14±0.12^{bC}
		72	53.51±0.06^{aA}	53.33±0.80^{aA}	34.28±0.06^{aB}
	24	24	27.51±0.45^{cA}	27.59±0.05^{cA}	16.29±0.12^{cB}
		48	36.15±0.17^{bA}	34.69±0.05^{bB}	24.24±0.06^{bC}
		72	51.06±0.17^{aA}	46.45±0.34^{aB}	30.72±0.12^{aC}
80 ˚C	16	24	27.86±0.06^{cA}	23.93±0.26^{cB}	16.83±0.06^{cC}
		48	42.17±0.11^{bA}	39.93±0.09^{bB}	25.31±0.06^{bC}
		72	52.54±0.06^{aA}	51.70±0.15^{aB}	33.37±0.06^{aC}
	24	24	26.25±0.06^{cA}	22.14±0.06^{cB}	15.51±0.04^{cC}
		48	35.56±0.06^{bA}	30.00±0.12^{bB}	24.18±0.04^{bC}
		72	50.19±0.12^{aA}	43.06±0.06^{aB}	30.03±0.04^{aC}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a-c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-C}$Mean within rows with different superscript are significantly ($p<0.05$) different;

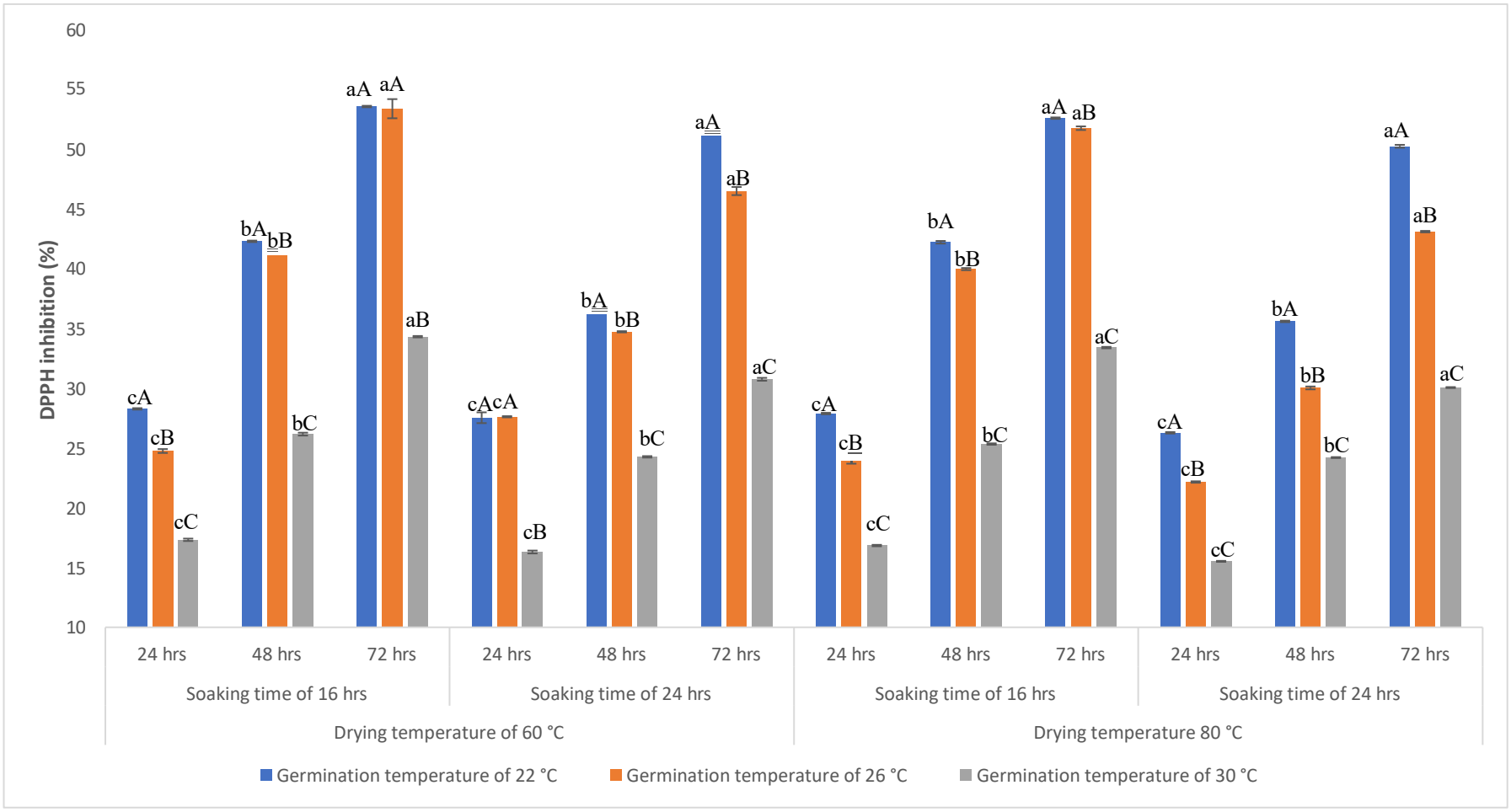


Figure. 6.18. Antioxidant activity of the buckwheat at various germination conditions (% inhibition of DPPH)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different;

6.6.4. Tannin content

The tannin content of the germinated finger millet is represented in Table 6.13., and Figure 6.19. The finger millet dried at 60˚C, with soaking time of 16 hrs, the tannin content at 22˚C, 26˚C, and 30˚C after 24 hrs of germination was 21.61 mg TA/100g, 20.50 mg TA/100g, and 17.65 mg TA/100g, respectively. With the increase in germination time to 48 hrs, tannin content significantly ($p<0.05$) decreased to 17.31 mg TA/100g at 22˚C, 15.62 mg TA/100g at 26˚C, and 10.30 mg TA/100g at 30˚C. Which, after 72 hrs of germination, significantly ($p<0.05$) decreased to 7.12 mg TA/100g at 22˚C, 14.65 mg TA/100g at 26˚C, and 10.06 mg TA/100g at 30˚C. This shows a clear trend of decreasing tannin levels with increasing germination time, with the most substantial reduction occurring at 30˚C germination after 72 hrs. Similarly, with increasing soaking time to 24 hrs, the initial tannin content after 24 hrs of germination was 19.73 mg TA/100g, 19.39 mg TA/100g, and 17.99 mg TA at 22˚C, 26˚C, and 30˚C germination temperatures, after 48 hrs of germination, significantly ($p<0.05$) decreased to 17.27 mg TA/100g at 22˚C, 14.02 mg TA/100g at 26˚C, and 12.04 mg TA/100g at 30˚C. Where, after 72 hrs of germination, significantly ($p<0.05$) decreased to 11.46 mg TA/100g at 22˚C, 9.39 mg TA/100g at 26˚C, and 7.31 mg TA/100g at 30˚C. The longer soaking and germination time contributes to significantly ($p<0.05$) reduction in tannin content, with 30˚C being the most effective germination temperature for tannin reduction. Similar trend was observed at drying temperature of 80˚C. Finger millet after 24 hrs of germination soaking time of 16 hrs, the tannin content at 22˚C, 26˚C, and 30˚C was 21.46 mg TA/100g, 19.05 mg TA/100g, and 17.12 mg TA/100g, respectively. Where significantly ($p<0.05$) decreased to 11.56 mg TA/100g at 22˚C, 9.68 mg TA/100g at 26˚C, and 7.89 mg TA/100g at 30˚C, after 72 hrs of germination. With a soaking time of 24 hrs and drying at 80˚C, the tannin content after 24 hrs of germination was 19.44 mg TA/100g at 22˚C, 17.94 mg TA/100g at 26˚C, and 17.16 mg TA/100g at 30˚C. Which, after 72 hrs, significantly ($p<0.05$) decreased to 9.15 mg TA/100g at 22˚C, 9.00 mg TA/100g at 26˚C, and 6.49 mg TA/100g at 30˚C. In a previous study, the tannin content of finger millet was found to decrease significantly ($p<0.05$) after germination, while it increased in pearl millet (Abioye et al., 2018). This decrease in tannin content is due to the decrease in polyphenols during the germination process (Abioye et al., 2018; Karki et al., 2012).

Table. 6.13. Tannin content of the finger millet at various germination conditions (mg TA/100g)

Drying temperature	Soaking time (hrs)	Germination temperature	22 ˚C	26 ˚C	30 ˚C
		Germination time (hrs)	(mg TA/100g)	(mg TA/100g)	(mg TA/100g)
60 ˚C	16	24	21.61 ± 0.15^{aA}	20.50 ± 0.08^{aB}	17.65 ± 0.08^{aC}
		48	17.31 ± 0.08^{bA}	15.62 ± 0.30^{bB}	10.30 ± 0.15^{bC}
		72	14.65 ± 0.15^{cA}	10.06 ± 0.08^{cB}	7.12 ± 0.15^{cC}
	24	24	19.73 ± 0.15^{aA}	19.39 ± 0.08^{aB}	17.99 ± 0.15^{aC}
		48	17.27 ± 0.16^{bA}	14.02 ± 0.08^{bB}	12.04 ± 0.15^{bC}
		72	11.46 ± 0.15^{cA}	9.39 ± 0.08^{cB}	7.31 ± 0.08^{aC}
80 ˚C	16	24	21.46 ± 0.15^{aA}	19.05 ± 0.08^{aB}	17.12 ± 0.15^{aC}
		48	17.21 ± 0.17^{bA}	15.62 ± 0.17^{bB}	10.01 ± 0.15^{bC}
		72	11.56 ± 0.08^{cA}	9.68 ± 0.08^{cB}	7.89 ± 0.08^{cC}
	24	24	19.44 ± 0.15^{aA}	17.94 ± 0.17^{aB}	17.16 ± 0.22^{aC}
		48	16.39 ± 0.15^{bA}	13.01 ± 0.08^{bB}	10.40 ± 0.09^{bC}
		72	9.15 ± 0.15^{cA}	9.00 ± 0.15^{cB}	6.49 ± 0.08^{cC}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a-c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-C}$Mean within rows with different superscript are significantly ($p<0.05$) different;

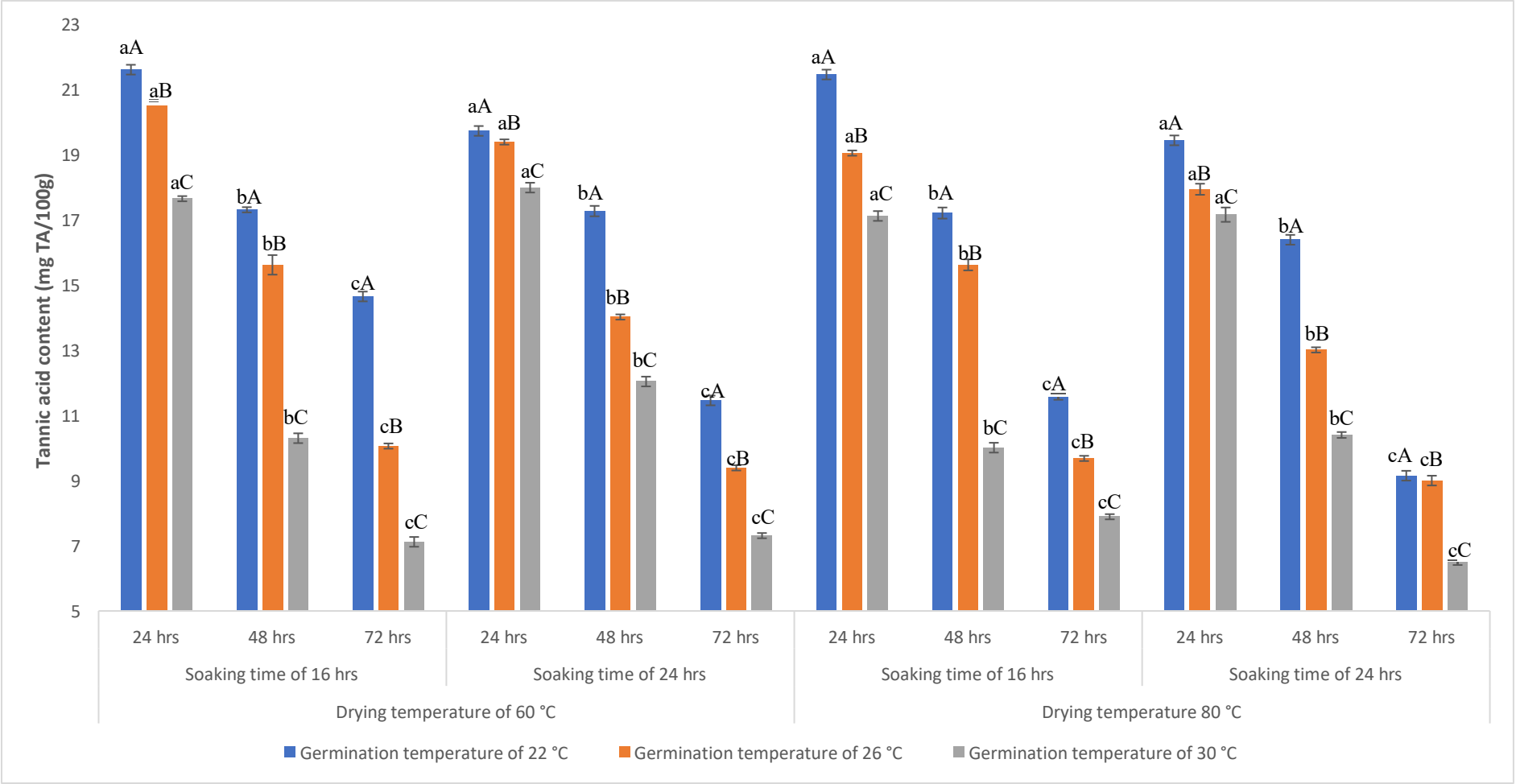


Figure. 6.19. Tannin content of the finger millet at various germination conditions (mg TA/100g)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly (*p<0.05*) different; [A-C]Mean within rows with different superscript are significantly (p<0.05) different;

The tannin content of the germinated pearl millet is represented in Table 6.14. and Figure 6.20. The pearl millet dried at 60˚C, with soaking time of 16 hrs, the tannin content at 22˚C, 26˚C, and 30˚C after 24 hrs of germination was 12.79 mg TA/100g, 14.32 mg TA/100g, and 14.72 mg TA/100g, respectively. With the increase in germination time to 48 hrs, tannin content significantly ($p<0.05$) increased to 13.69 mg TA/100g at 22˚C, 16.75 mg TA/100g at 26˚C, and 16.74 mg TA/100g at 30˚C. Which, after 72 hrs of germination, significantly ($p<0.05$) increased to 14.59 mg TA/100g at 22˚C, 18.31 mg TA/100g at 26˚C, and 23.07 mg TA/100g at 30˚C. Similarly, with increasing soaking time to 24 hrs, the initial tannin content after 24 hrs of germination was 5.08 mg TA/100g, 14.54 mg TA/100g, and 12.67 mg TA at 22˚C, 26˚C, and 30˚C germination temperatures, after 48 hrs of germination, non-significantly ($p<0.05$) decreased to 4.60 mg TA/100g at 22˚C and significantly ($p<0.05$) increased to 20.62 mg TA/100g at 26˚C, and 14.66 mg TA/100g at 30˚C. Where, after 72 hrs of germination, non-significantly ($p<0.05$) increased to 4.78 mg TA/100g at 22˚C, and significantly ($p<0.05$) increased to 21.35 mg TA/100g at 26˚C, and 20.20 mg TA/100g at 30˚C. The increase in soaking and germination time showed significant ($p<0.05$) reduction in tannin content. Similar trend was observed at drying temperature of 80˚C. Pearl millet after 24 hrs of germination and soaking time of 16 hrs, the tannin content at 22˚C, 26˚C, and 30˚C was 12.09 mg TA/100g, 14.29 mg TA/100g, and 11.22 mg TA/100g, respectively. Which significantly ($p<0.05$) increased to 13.52 mg TA/100g at 22˚C, 15.44 mg TA/100g at 26˚C, and 18.35 mg TA/100g at 30˚C, after 72 hrs of germination. With an increased soaking time of 24 hrs and drying at 80˚C, the tannin content after 24 hrs of germination was 4.01 mg TA/100g at 22˚C, 12.52 mg TA/100g at 26˚C, and 12.35 mg TA/100g at 30˚C. Which, after 72 hrs, significantly ($p<0.05$) increased to 5.71 mg TA/100g at 22˚C, 19.95 mg TA/100g at 26˚C, and 21.29 mg TA/100g at 30˚C. Tannin content of finger millet decreased with germination time, and the tannin content of pearl millet increased with germination. The tannin content was found to have a significant reduction ($p<0.05$) of about 30% after germination of 48 hrs (Hithamani and Srinivasan, 2014). This could be due to the enzymatic hydrolysis of polyphenols and tannins induced by the germination process (Taylor and Duodu, 2015; Abioye et al., 2018). The tannin content of pearl millet is more concentrated in its seed coat and is not involved in the germination process, thereby increasing the tannin content (Kulla et al., 2021).

Table. 6.14. Tannin content of the pearl millet at various germination conditions (mg TA/100g)

Drying temperature	Soaking time	Germination temperature	22 ˚C	26 ˚C	30 ˚C
		Germination time (hrs)	(mg TA/100g)	(mg TA/100g)	(mg TA/100g)
60 ˚C	16	24	12.79 ± 0.05^{cC}	14.32 ± 0.10^{cB}	14.70 ± 0.12^{cA}
		48	13.69 ± 0.05^{bB}	16.75 ± 0.10^{bA}	16.74 ± 0.12^{bA}
		72	14.59 ± 0.05^{aC}	18.31 ± 0.10^{aB}	23.07 ± 0.12^{aA}
	24	24	5.08 ± 0.13^{aC}	14.54 ± 0.12^{cA}	12.67 ± 0.10^{cB}
		48	4.60 ± 0.08^{aC}	20.67 ± 0.06^{bA}	14.66 ± 0.20^{bB}
		72	4.78 ± 0.83^{aC}	21.35 ± 0.06^{aA}	20.20 ± 0.10^{aB}
80 ˚C	16	24	12.09 ± 0.14^{bB}	14.29 ± 0.09^{cA}	11.22 ± 0.12^{cC}
		48	13.68 ± 0.14^{aC}	15.14 ± 0.09^{bB}	18.35 ± 0.06^{bA}
		72	13.52 ± 0.13^{aC}	15.40 ± 0.09^{aB}	21.43 ± 0.06^{aA}
	24	24	4.01 ± 0.14^{bB}	12.52 ± 0.12^{cA}	12.35 ± 0.06^{cA}
		48	4.69 ± 0.15^{cC}	17.80 ± 0.12^{bA}	16.21 ± 0.11^{bB}
		72	5.71 ± 0.14^{aC}	19.95 ± 0.12^{aB}	21.29 ± 0.16^{aA}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a-c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-C}$Mean within rows with different superscript are significantly different ($p<0.05$) different

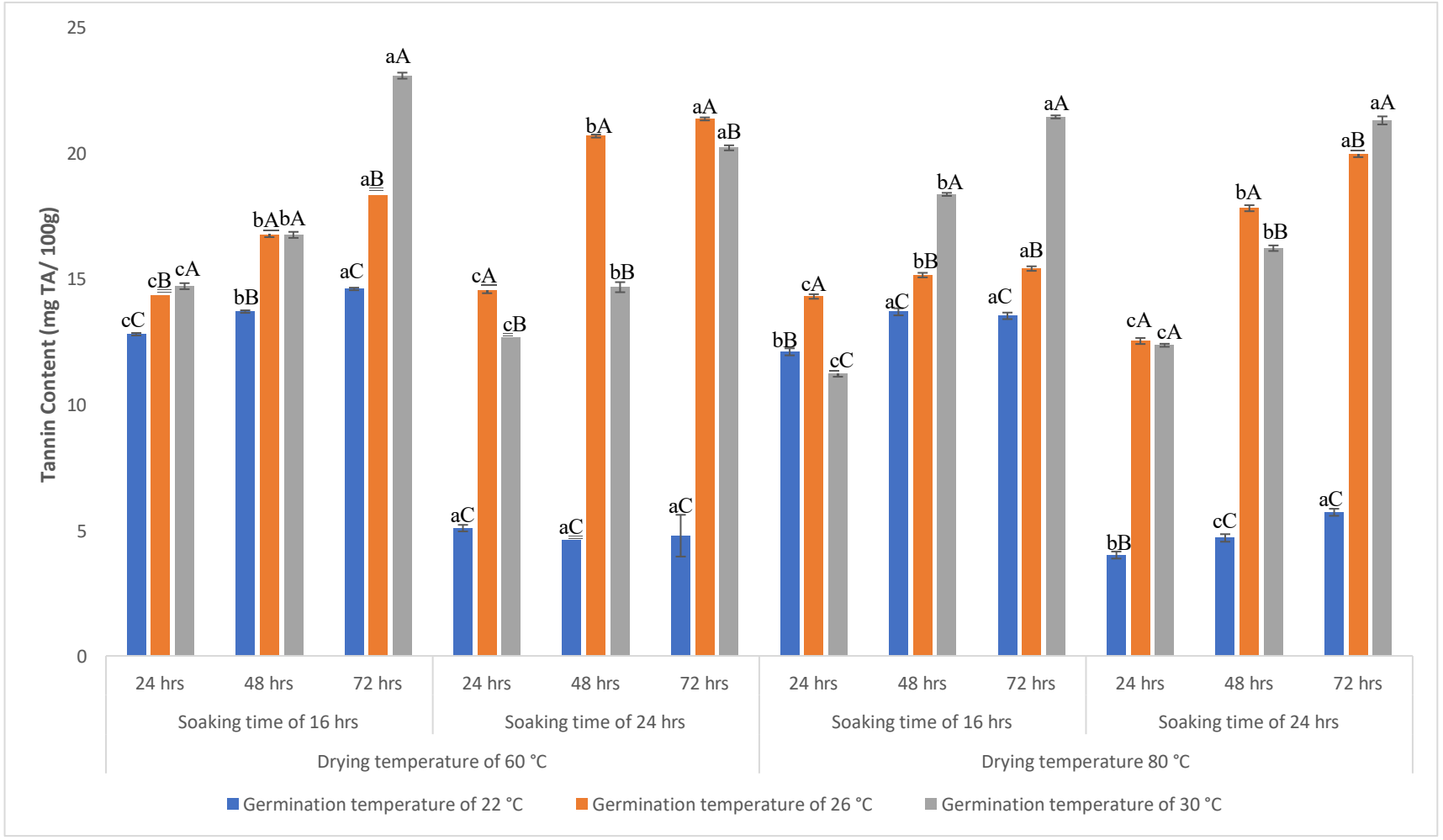


Figure. 6.20. Tannin content of the pearl millet at various germination conditions (mg TA/100g)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different

The tannin content of the germinated buckwheat is represented in Table 6.15. and Figure 6.21. The buckwheat dried at 60˚C, with soaking time of 16 hrs, the tannin content at 22˚C, 26˚C, and 30˚C after 24 hrs of germination was 23.20 mg TA/100g, 22.43 mg TA/100g, and 18.76 mg TA/100g, respectively. With the increase in germination time to 48 hrs, tannin content significantly ($p<0.05$) decreased to 20.55 mg TA/100g at 22˚C, 18.66 mg TA/100g at 26˚C, and 17.94 mg TA/100g at 30˚C. Which, after 72 hrs of germination, significantly ($p<0.05$) decreased to 18.95 mg TA/100g at 22˚C, 16.54 mg TA/100g at 26˚C, and 15.62mg TA/100g at 30˚C. Similarly, with increasing soaking time to 24 hrs, the initial tannin content after 24 hrs of germination was 19.53 mg TA/100g, 18.52 mg TA/100g, and 17.74 mg TA at 22˚C, 26˚C, and 30˚C germination temperatures, after 48 hrs of germination, significantly ($p<0.05$) decreased to 17.65 mg TA/100g at 22˚C, 15.62 mg TA/100g at 26˚C, and 15.33 mg TA/100g at 30˚C. Which, after 72 hrs of germination, significantly ($p<0.05$) decreased to 16.49 mg TA/100g at 22˚C, 13.73 mg TA/100g at 26˚C, and 10.79 mg TA/100g at 30˚C. The increase in soaking and germination time showed significant ($p<0.05$) reduction in tannin content. Similar trend was observed at drying temperature of 80˚C. Buckwheat after 24 hrs of germination and soaking time of 16 hrs, the tannin content at 22˚C, 26˚C, and 30˚C was 14.51 mg TA/100g, 14.22 mg TA/100g, and 13.88 mg TA/100g, respectively. Which significantly ($p<0.05$) decreased to 11.27 mg TA/100g at 22˚C, 11.46 mg TA/100g at 26˚C, and 10.21 mg TA/100g at 30˚C, after 72 hrs of germination. With an increased soaking time of 24 hrs and drying at 80˚C, the tannin content after 24 hrs of germination was 12.82 mg TA/100g at 22˚C, 13.83 mg TA/100g at 26˚C, and 12.49 mg TA/100g at 30˚C. Which, after 72 hrs, significantly ($p<0.05$) decreased to 10.64 mg TA/100g at 22˚C, 10.84 mg TA/100g at 26˚C, and 9.39 mg TA/100g at 30˚C. The increase in the tannin content also increased with the germination temperature. Buckwheat has been found to have a high decrease in tannin content with germination (Kumari et al., 2022). The reduction of tannin content in finger millet and buckwheat may be due to the binding of protein by tannin in the grain, leaching of tannin during soaking, and other metabolic enzymes of germination (Owheruo et al., 2018). The Joint FAO/WHO Food Standards Program specifies that the tannin content in whole grains should not exceed 0.5% (500 mg/100g) on a dry weight basis, while for decorticated sorghum grains, it should be less than 0.3% (300 mg/100g) on a dry weight basis (Sharma et al., 2021).

Table. 6.15. Tannin content of the buckwheat at various germination conditions (mg TA/100g)

Drying temperature	Soaking time (hrs)	Germination temperature	22 ˚C	26 ˚C	30 ˚C
		Germination time (hrs)	(mg TA/100g)	(mg TA/100g)	(mg TA/100g)
60 ˚C	16	24	23.20 ± 0.15^{aA}	22.43 ± 0.08^{aB}	18.76 ± 0.08^{aC}
		48	20.55 ± 0.08^{bA}	18.66 ± 0.08^{bB}	17.94 ± 0.08^{bC}
		72	18.95 ± 0.08^{cA}	16.54 ± 0.00^{cB}	15.62 ± 0.08^{cC}
	24	24	19.53 ± 0.08^{aA}	18.52 ± 0.08^{aB}	17.74 ± 0.08^{aC}
		48	17.65 ± 0.08^{bA}	15.62 ± 0.22^{bB}	15.33 ± 0.17^{bB}
		72	16.49 ± 0.08^{cA}	13.73 ± 0.08^{cB}	10.79 ± 0.08^{cC}
80 ˚C	16	24	14.51 ± 0.15^{aA}	14.22 ± 0.15^{aB}	13.88 ± 0.08^{aC}
		48	13.16 ± 0.22^{bB}	13.59 ± 0.22^{bA}	11.66 ± 0.08^{bC}
		72	11.27 ± 0.17^{cA}	11.46 ± 0.15^{cA}	10.21 ± 0.08^{cB}
	24	24	12.82 ± 0.08^{aB}	13.83 ± 0.22^{aA}	12.49 ± 0.15^{aC}
		48	11.61 ± 0.15^{bB}	13.01 ± 0.17^{bA}	11.17 ± 0.15^{bC}
		72	10.64 ± 0.30^{cA}	10.84 ± 0.22^{cA}	9.39 ± 0.08^{cB}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a-c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-C}$Mean within rows with different superscript are significantly different ($p<0.05$)

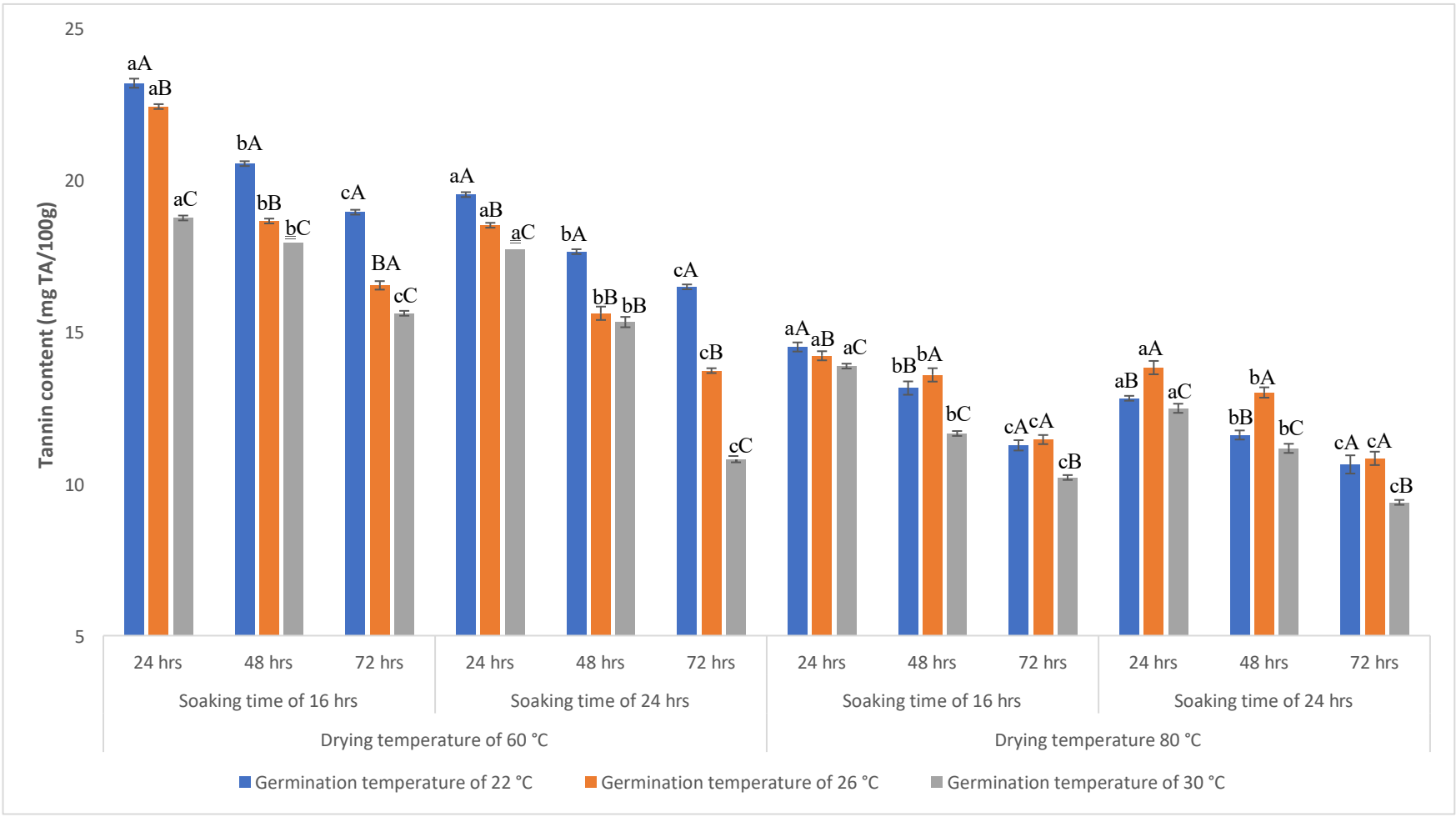


Figure. 6.21. Tannin content of the buckwheat at various germination conditions (mg TA/100g)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different

6.6.5. Prebiotic effect

The prebiotic effect increased with increase in germination time of finger millet with both *L. acidophilus* NCDC 600 and *L. plantarum* NCDC 685 as shown in Table 6.16. and Figure 6.22. With the 16 hrs soaking time and germination at 30˚C, *L. plantarum* NCDC 685 had significantly ($p<0.05$) increased prebiotic effect from 8.93 Log CFU/g at 24 hrs to 9.53 log CFU/g at 72 hrs of germination. Similarly, *L. acidophilus* NCDC 600 had significant ($p<0.05$) increase in prebiotic effect from 7.44 Log CFU/g at 24 hrs to 8.07 Log CFU/g at 72 hrs under the same conditions. This trend was observed at all soaking times and temperatures, indicating that extended germination enhances the prebiotic compounds that selectively stimulate the growth of the bacteria. Increase in germination temperature also favoured the prebiotic effect. After 72 hrs of germination with a 16-hour soak, the prebiotic effect of *L. plantarum* NCDC 685 were 9.14 Log CFU/g, 9.55 Log CFU/g, and 9.53 Log CFU/g at 22˚C, 26˚C, and 30˚C, respectively. *L. acidophilus* NCDC 600 showed a similar trend, with counts of 8.06 Log CFU/g, 9.013 Log CFU/g, and 8.07 Log CFU/g at the respective temperatures of 22˚C, 26˚C, and 30˚C. This indicates that increase in temperatures (26-30˚C) during germination are responsible for significantly ($p<0.05$) increasing the prebiotic compounds that promote the growth of these probiotic strains. Soaking time had a less definite effect compared to germination time and temperature. However, finger millet soaked for 24 hrs resulted in slightly higher prebiotic effect than the grains soaked for 16 hrs at the same germination time and temperature. Finger millet germinated for 72 hrs at 26˚C, had prebiotic effect of 9.55 Log CFU/g with a 16-hour soak against 9.54 Log CFU/g with a 24-hour soak with *L. plantarum* NCDC 685. This indicates that longer soaking may enhance the availability of prebiotic compounds, but the effect is minor. The enzymatic hydrolysis of the starch polysaccharides and proteins during germination results in synthesis of oligosaccharides and amino acids that are favourable for the probiotic growth, thereby increasing prebiotic effect with germination (Kumari et al., 2024).

Table. 6.16. Prebiotic effect of the germinated Finger millet dried at 60 ˚C (Log CFU/g)

Soaking time (hrs)	Germination temperature	*L. plantarum* NCDC 685			*L. acidophilus* NCDC 600		
		22 ˚C	26 ˚C	30 ˚C	22 ˚C	26 ˚C	30 ˚C
	Germination time (hrs)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)
16	24	8.74 ± 0.03^{bC}	8.82 ± 0.01^{cB}	8.93 ± 0.01^{cA}	7.99 ± 0.03^{bcB}	8.34 ± 0.03^{cA}	7.44 ± 0.10^{cC}
	48	9.09 ± 0.01^{bC}	9.28 ± 0.01^{bA}	9.21 ± 0.01^{bB}	8.03 ± 0.02^{abB}	8.64 ± 0.01^{bA}	7.83 ± 0.04^{bC}
	72	9.14 ± 0.02^{aB}	9.55 ± 0.02^{aA}	9.53 ± 0.01^{aA}	8.06 ± 0.02^{aB}	9.01 ± 0.01^{aA}	8.07 ± 0.03^{aB}
24	24	8.73 ± 0.02^{cC}	8.81 ± 0.02^{cB}	8.93 ± 0.01^{cA}	7.97 ± 0.03^{bB}	8.43 ± 0.01^{cA}	7.41 ± 0.10^{cC}
	48	9.08 ± 0.02^{bC}	9.28 ± 0.01^{bA}	9.21 ± 0.01^{bB}	8.01 ± 0.02^{aB}	8.71 ± 0.01^{bA}	7.82 ± 0.04^{bC}
	72	9.01 ± 0.03^{aB}	9.54 ± 0.01^{aA}	9.53 ± 0.01^{aA}	8.03 ± 0.01^{aC}	9.00 ± 0.03^{aA}	8.07 ± 0.03^{aB}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a-c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-C}$Mean within rows with different superscript are significantly ($p<0.05$) different

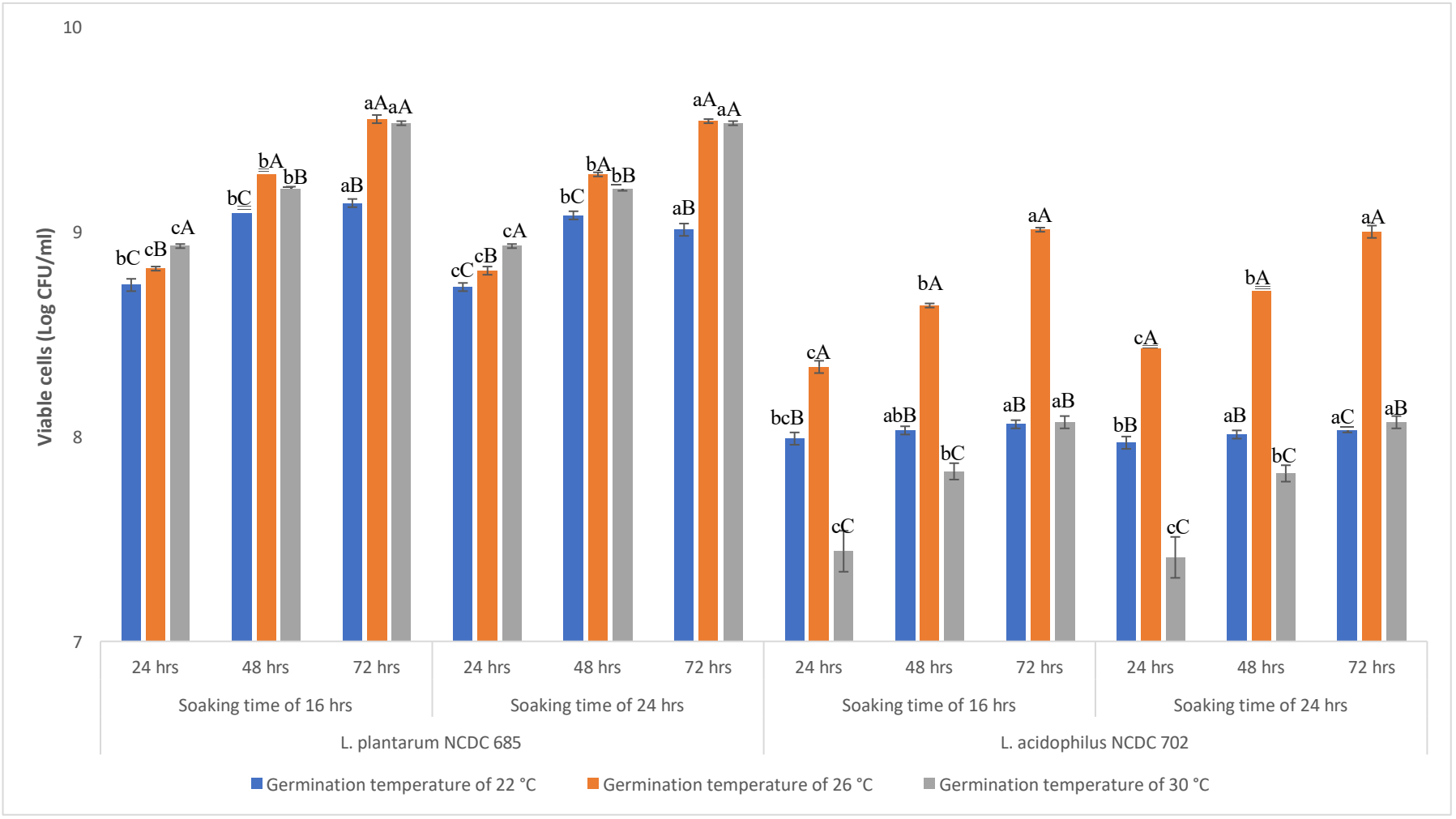


Figure. 6.22. Prebiotic effect of the germinated finger millet dried at 60 ˚C (Log CFU/g)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different

Table 6.17 and Figure 6.23., represents the prebiotic effect of pearl millet that significantly ($p<0.05$) increased with increase in germination time with both *L. acidophilus* NCDC 600 and *L. plantarum* NCDC 685. With the 16 hrs soaking time and germination at 30˚C, *L. plantarum* NCDC 685 had significantly ($p<0.05$) increased the prebiotic effect from 8.96 Log CFU/g at 24 hrs to 9.111 Log CFU/g at 72 hrs of germination. Similarly, *L. acidophilus* NCDC 600 had significant ($p<0.05$) increase in prebiotic effect from 7.44 Log CFU/g at 24 hrs to 8.07 Log CFU/g at 72 hrs under the same conditions. After 72 hrs of germination, the prebiotic effect of *L. plantarum* NCDC 685 significantly ($p<0.05$) increased to 9.26 Log CFU/g at 26˚C and 9.11 Log CFU/g at 30˚C, compared to 9.80 Log CFU/g at 22˚C.The *L. plantarum* NCDC 685 presented viable counts of 9.23 Log CFU/g for pearl millet germinated at 26˚C for 72 hrs that was soaked for 24 hrs to 9.26 Log CFU/g for pearl millet soaked for 16 hrs. Similar trend was observed with *L. acidophilus* NCDC 600 where, pearl millet soaked for 16 and 24 hrs were 9.021 Log CFU/g and 8.88 Log CFU/g.

Table. 6.17. Prebiotic effect of the germinated pearl millet dried at 60 ˚C (Log CFU/g)

Soaking time (hrs)	Germination temperature	*L. plantarum* NCDC 685			*L. acidophilus* NCDC 600		
		22 ˚C	26 ˚C	30 ˚C	22 ˚C	26 ˚C	30 ˚C
	Germination time (hrs)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)
16	24	8.86 ± 0.02^{bC}	9.05 ± 0.01^{cB}	8.96 ± 0.03^{cA}	7.77 ± 0.03^{cC}	7.95 ± 0.03^{cA}	7.88 ± 0.02^{cB}
	48	9.06 ± 0.01^{aC}	9.15 ± 0.02^{bA}	9.09 ± 0.01^{bB}	8.32 ± 0.01^{bC}	8.70 ± 0.04^{bA}	8.60 ± 0.02^{bB}
	72	9.80 ± 0.02^{aB}	9.26 ± 0.03^{aA}	9.11 ± 0.01^{aB}	8.40 ± 0.02^{aC}	9.02 ± 0.02^{aA}	8.95 ± 0.01^{aB}
24	24	8.77 ± 0.02^{cC}	9.03 ± 0.03^{cA}	8.93 ± 0.01^{cB}	7.67 ± 0.03^{cC}	7.86 ± 0.02^{cA}	7.75 ± 0.03^{cB}
	48	9.04 ± 0.03^{bB}	9.13 ± 0.02^{bA}	9.04 ± 0.03^{bB}	8.30 ± 0.01^{bC}	8.61 ± 0.03^{bA}	8.41 ± 0.01^{bB}
	72	9.06 ± 0.01^{aC}	9.23 ± 0.01^{aA}	9.10 ± 0.02^{aB}	8.39 ± 0.02^{aC}	8.88 ± 0.01^{aA}	8.71 ± 0.03^{aB}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a-c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-C}$Mean within rows with different superscript are significantly different ($p<0.05$) different

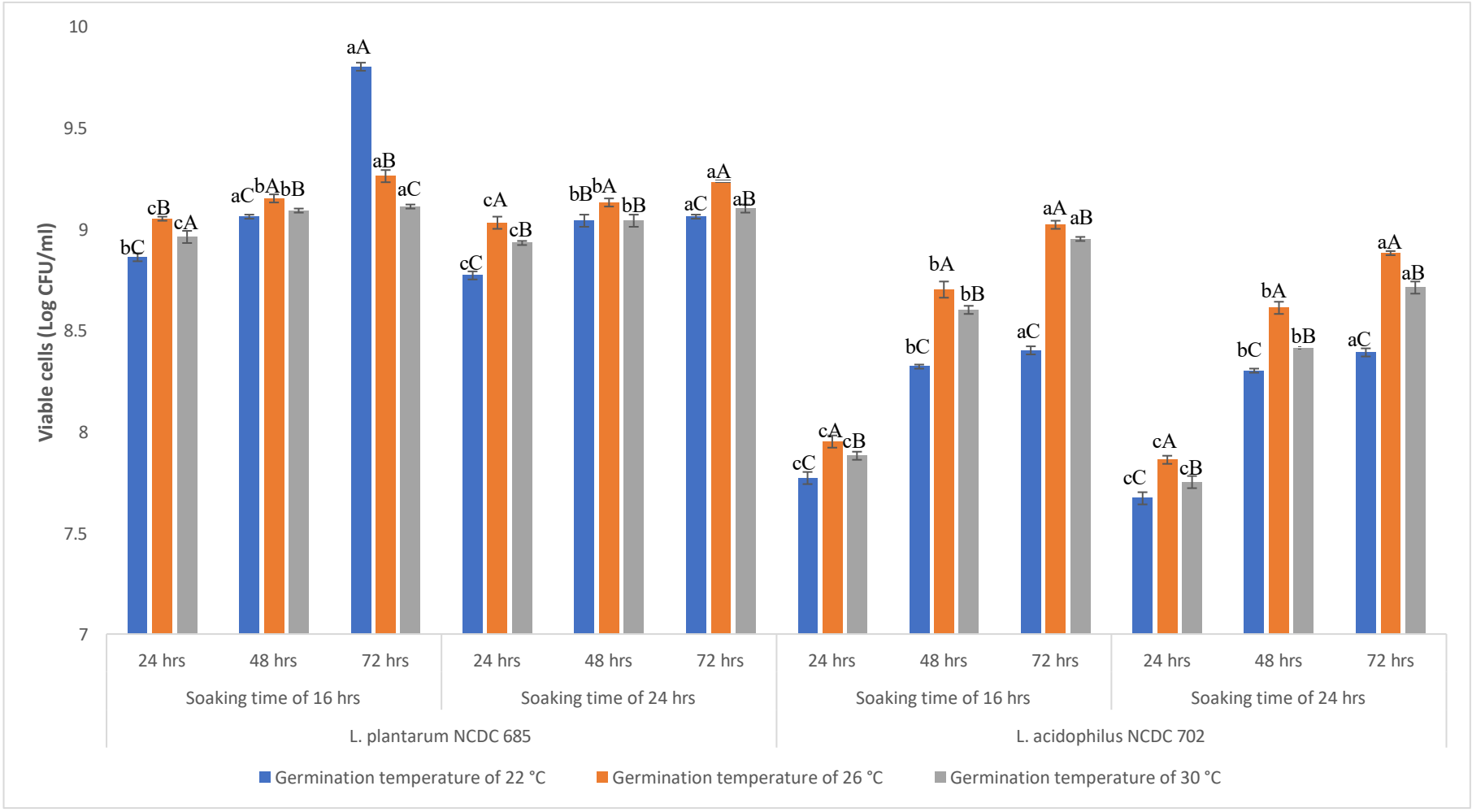


Figure. 6.23. Prebiotic effect of the germinated pearl millet dried at 60 ˚C (Log CFU/g)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different

The prebiotic effects of germinated buckwheat as shown in Table 6.18. Figure 6.24., revealed that increasing germination time consistently led to significant ($p<0.05$) increase in prebiotic effect. *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 both exhibited maximum growth at 72 hrs of germination after 16 hrs of soaking, at 22˚C (9.86±0.02 Log CFU/g and 9.26±0.01 Log CFU/g, respectively), and 26˚C (9.79 Log CFU/g and 9.25 Log CFU/g, respectively). The prebiotic effect for *L. plantarum* NCDC 685 were highest at 22˚C across all germination times and soaking durations. After 72 hrs of germination and 16 hrs of soaking, the count was 9.86 Log CFU/g at 22˚C, while it significantly ($p<0.05$) decreased to 8.89 Log CFU/g at 30˚C. In contrast, *L. acidophilus* NCDC 600 also exhibited the highest growth at 22˚C, with counts reaching 9.26 Log CFU/g at 72 hrs of germination and 16 hrs of soaking. At germination temperature of 30˚C, the count was 8.419 Log CFU/g after 72 hrs germination and 24 hrs soaking. The soaking time also plays a significant ($p<0.05$) role in the prebiotic effect of germinated buckwheat. After 16 hrs of soaking, *L. plantarum* NCDC 685 showed significantly ($p<0.05$) higher cell counts, after 72 hrs of germination and germination temperature of 22˚C (9.86 Log CFU/g), compared to 24 hrs of soaking (9.84 Log CFU/g). *L. acidophilus* NCDC 600 exhibited a similar trend, with the highest growth also observed after 72 hrs of germination at 22˚C, but with slightly lower sensitivity to soaking time changes (9.26 Log CFU/g at 16 hrs soaking to 9.24 Log CFU/g at 24 hrs soaking).

The prebiotic effect of germinated cereals has been found to be significantly higher ($p<0.05$) with increased bacterial cell count than the un-germinated cereal due to the presence of components that are suitable for optimal growth of bacteria that are synthesised by enzymatic reaction during germination (Budhwar et al., 2020).

Table. 6.18. Prebiotic effect of the germinated buckwheat and dried at 60 ˚C (Log CFU/g)

Soaking time (hrs)	Germination temperature	*L. plantarum* NCDC 685			*L. acidophilus* NCDC 600		
		22 ˚C	26 ˚C	30 ˚C	22 ˚C	26 ˚C	30 ˚C
	Germination time (hrs)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)
16	24	8.90 ± 0.02^{cA}	8.81 ± 0.02^{cB}	8.83 ± 0.00^{cB}	8.06 ± 0.03^{cB}	8.04 ± 0.02^{cB}	8.43 ± 0.02^{aA}
	48	9.75 ± 0.02^{bA}	9.68 ± 0.01^{bB}	8.90 ± 0.00^{bC}	8.88 ± 0.01^{bA}	8.46 ± 0.03^{bB}	8.44 ± 0.01^{aB}
	72	9.86 ± 0.02^{aA}	9.79 ± 0.02^{aB}	8.89 ± 0.00^{aC}	9.26 ± 0.01^{aA}	9.25 ± 0.02^{aA}	8.42 ± 0.03^{aB}
24	24	8.87 ± 0.03^{cA}	8.77 ± 0.01^{cA}	8.39 ± 0.13^{aB}	8.05 ± 0.02^{cB}	8.04 ± 0.01^{cB}	8.43 ± 0.02^{aA}
	48	9.73 ± 0.01^{bA}	9.66 ± 0.02^{bB}	8.46 ± 0.01^{aC}	8.84 ± 0.03^{bA}	8.45 ± 0.02^{bB}	8.44 ± 0.01^{aB}
	72	9.84 ± 0.01^{aA}	9.77 ± 0.02^{aB}	8.47 ± 0.03^{aC}	9.24 ± 0.01^{aA}	9.23 ± 0.03^{aA}	8.41 ± 0.03^{aB}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. $^{a\text{-}c}$Mean within column with different superscript are significantly ($p<0.05$) different; $^{A\text{-}C}$Mean within rows with different superscript are significantly different ($p<0.05$) different

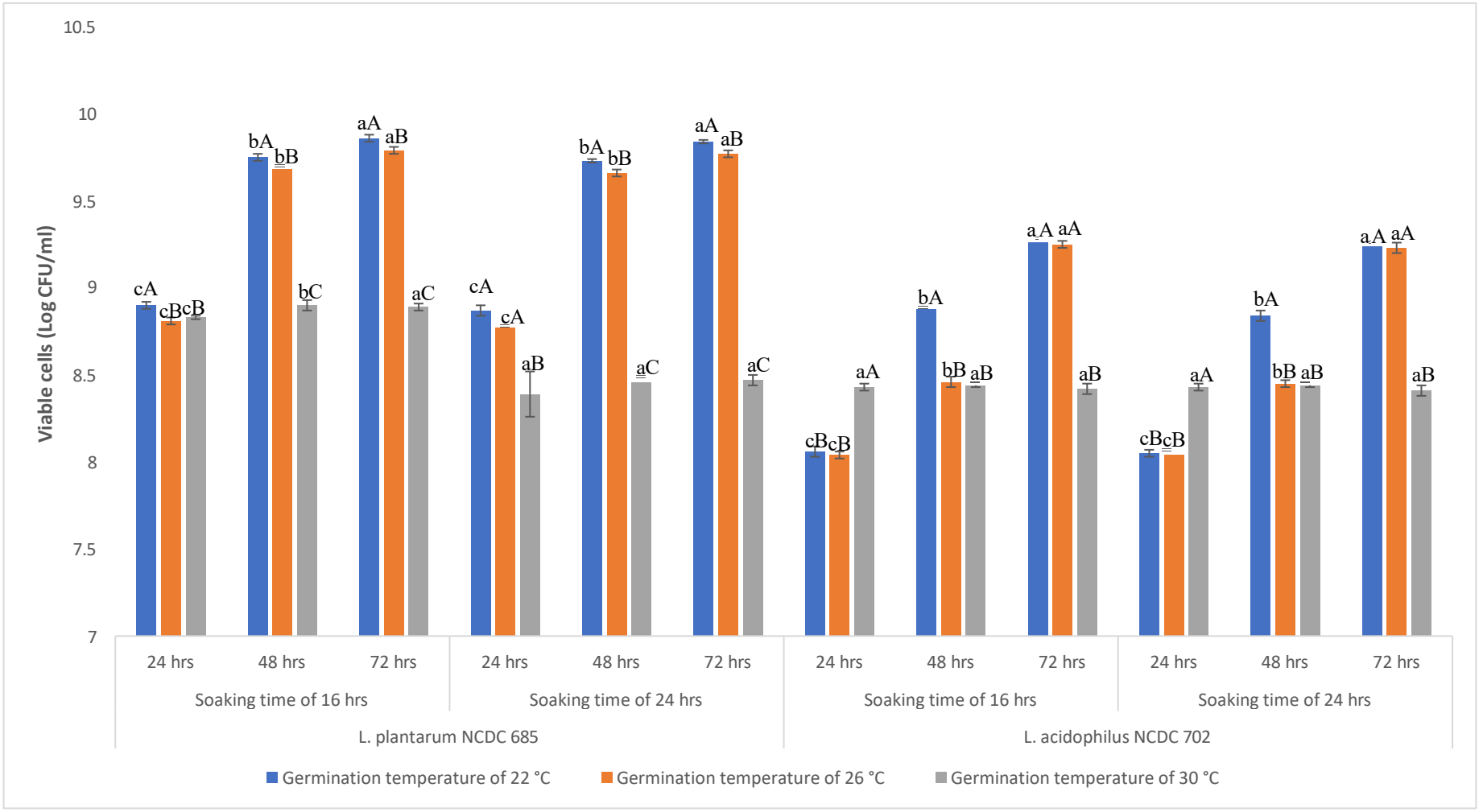


Figure. 6.24. Prebiotic effect of the germinated buckwheat and dried at 60 ˚C (Log CFU/g)

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-C]Mean within rows with different superscript are significantly ($p<0.05$) different

6.7. Optimization of the process for the development of nutricereals based beverage

6.7.1. Preparation of nutricereal malt extract

The optimal germination condition chosen for finger millet and pearl millet were soaking time of 16 hrs and germination temperature of 26 ˚C for 72 hrs and buckwheat was 22 ˚C after 16 hrs of soaking and germinating for 72 hrs, while the drying temperature was 60 ˚C. The sensory attributes of different concentration of the nutricereal malt extract are given in Table 6.19. Five different ratios of pearl millet, finger millet, and buckwheat were prepared, as C1 (50:30:20), C2 (60:25:15), C3 (70:20:10), C4 (80:15:5) and C5 (90:10:0). C1 had the highest bitterness score (6.85), which might have been influenced by its highest buckwheat content among the combination. C4 improved appearance (7.38) and consistency (7.08) but has similar overall acceptability (6.85) compared to C1 (6.84) and C3 (6.84). The minimal buckwheat content seems to positively impact the sensory attributes, especially in reducing bitterness (6.35) in C4. C5 had the highest appearance score (7.23) but shows a decline in flavour (5.73) and overall acceptability (6.66). The absence of buckwheat eliminates its bitterness but might also reduce the complexity of flavour. Though C1 and C3 also have good overall acceptability scores, considering all attributes, C4 still stands out slightly better. Therefore, C4 was used for the development of the beverage (Figure 6.7.). The prepared nutricereal malt extract had a pH of 5.84.

Figure. 6.25. Nutricereal malt extract chosen for further study (C4).

Table. 6.19. Sensory profile of different combinations of pearl millet, finger millet and buckwheat in the nutricereal malt extract.

Malt	Pearl millet (g)	Finger millet (g)	Buck-wheat (g)	Appearance	Consistency	Flavour	Bitterness	Overall Acceptability
C1	50	30	20	6.92 ± 0.86^{ab}	6.77 ± 1.54^{a}	6.31 ± 1.55^{ab}	6.85 ± 1.14^{abcd}	6.84 ± 0.91^{abc}
C2	60	25	15	6.77 ± 1.30^{a}	7.23 ± 1.01^{abc}	6.35 ± 1.46^{abc}	6.61 ± 1.56^{ab}	6.82 ± 0.87^{ab}
C3	70	20	10	7.15 ± 0.80^{abc}	6.92 ± 1.19^{ab}	6.54 ± 1.45^{bcd}	6.69 ± 1.65^{abc}	6.84 ± 1.03^{abcd}
C4	80	15	5	7.38 ± 0.65^{abcd}	7.08 ± 1.19^{abc}	6.69 ± 0.85^{bcd}	6.35 ± 1.41^{a}	6.85 ± 0.71^{abcd}
C5	90	10	0	7.23 ± 0.83^{abcd}	6.92 ± 1.61^{ab}	5.73 ± 1.54^{a}	6.69 ± 1.18^{abcd}	6.66 ± 0.92^{a}

Data are expressed as mean ±S.D. (n=13). One-way ANOVA used for statically analysis.
[a-d] Mean within column with different superscript are significantly ($p>0.05$) different

6.7.2. Formulation of nurticereal based beverage

The nutricereal beverage was formulated with nutricereal malt extract and skimmed milk with sugar as constant, using Design expert RSM software with CCD design. The formulated beverages were analysed for total solids, TPC, tannin content, prebiotic effect and overall acceptability as shown in Table 6.20.

Table. 6.20. Effect of treatment combinations of nutricereal malt extract to skimmed milk on the dependent factors for optimizing a functional drink.

		Factor 1	Factor 2	Response 1	Response 2	Response 3	Response 4	Response 5
Standard	Run	A: nutricereal malt extract (ml)	B: Skimmed milk (ml)	Total Solids (%)	TPC (mg GAE/100 ml)	Tannin (mg /TA100 ml)	Prebiotic effect (Log CFU/ ml)	Overall acceptability
8	1	50	64.1421	18.73	20.51	11.23	9.12	7.2
2	2	60	40	21.27	25.99	17.48	11.43	7.0
6	3	64.1421	50	21.67	25.52	17.15	11.56	7.0
9	4	50	50	19.77	22.91	14.19	10.58	7.1
13	5	50	50	19.87	22.31	13.44	10.56	6.9
4	6	60	60	19.90	22.41	13.32	10.42	7.0
12	7	50	50	19.68	22.39	13.55	10.50	6.9
3	8	40	60	19.3	18.91	10.03	9.56	7.5
11	9	50	50	19.67	22.17	13.27	10.54	7.0
1	10	40	40	18.77	22.64	13.70	10.99	6.9
5	11	35.8579	50	19.67	19.87	10.43	10.64	7.3
7	12	50	35.8579	19.85	25.73	17.41	11.22	7.0
10	13	50	50	19.80	22.02	13.09	10.67	7.1

6.7.3. Analysis of Variance (ANOVA) of different formulations

The significant model terms of total solids (TS) are A, B, AB, A^2, B^2. The F-values, which ranged from 22.63 to 186.98, and p-values of less than 0.0001, indicates that the model was significant as given in table 7. The R^2, adjusted R^2, and predicted R^2 values were 0.9798, 0.9654, and 0.8758, respectively, demonstrating reasonable agreement between adjusted R^2, and predicted R^2 as they have a difference of less than 0.2. The adequate precision score was 27.4301, confirming that the values fit well within the quadratic model. Descriptive statistics, including the mean, standard deviation, and coefficient of variation (CV), were 19.84, 0.1533, and 0.7725, respectively. The lack of fit was found to be non-significant, with an F-value of 6.42 and a p-value of 0.0521. The coded equation for TS is provided below.

$$\text{Total Solids (\%)} = 19.76 + 0.7410A - 0.3029B - 0.4749AB + 0.4135A^2 - 0.2765B^2$$

The significant model terms of total phenolic content (TPC) are A, B. The F-values, which ranged from 3.78 to 197.93, and p-values of less than 0.0001, indicates that the model was

significant. The R^2, adjusted R^2, and predicted R^2 values were 0.9826, 0.9702, and 0.9217, respectively, demonstrating reasonable agreement between adjusted R^2, and predicted R^2 as they have a difference of less than 0.2. The adequate precision score was 29.1405, confirming that the values fit well within the quadratic model. Descriptive statistics, including the mean, standard deviation, and CV, were 22.57, 0.3729, and 1.65, respectively. The lack of fit was found to be non-significant, with an F-value of 1.50 and a p-value of 0.3422. The coded equation for TPC is provided below.

$$\text{TPC (mg/100 ml)} = 22.36 + 1.8550A - 1.8365B + 0.0375AB + 0.0625A^2 + 0.2750B^2$$

The significant model terms of tannin content are A, B. The F-values, which ranged from 2.31 to 126.26, and p-values of less than 0.0001, indicates that the model was significant. The R^2, adjusted R^2, and predicted R^2 values were 0.9722, 0.9542, and 0.8647, respectively, demonstrating reasonable agreement between adjusted R^2, and predicted R^2 as they have a difference of less than 0.2. The adequate precision score was 23.3879, confirming that the values fit well within the quadratic model. Descriptive statistics, including the mean, standard deviation, and CV, were 13.71, 0.5215, and 3.80, respectively. The lack of fit was found to be non-significant, with an F-value of 2.28 and a p-value of 0.2216. The coded equation for tannin content is provided below.

$$\text{Tannin (mg/100 ml)} = 13.508 + 2.0717A - 2.0712B - 0.1225AB + 0.0354A^2 + 0.3004B^2$$

The significant model terms of prebiotic effect are A, B, AB, A^2, B^2. The F-values, which ranged from 5.77 to 478.28, and p-values of less than 0.0001, indicates that the model was significant. The R^2, adjusted R^2, and predicted R^2 values were 0.9902, 0.9832, and 0.9467, respectively, demonstrating reasonable agreement between adjusted R^2, and predicted R^2 as they have a difference of less than 0.2. The adequate precision score was 39.4866, confirming that the values fit well within the quadratic model. Descriptive statistics, including the mean, standard deviation, and CV, were 10.60, 0.0875, and 0.8251, respectively. The lack of fit was found to be non-significant, with an F-value of 3.13 and a p-value of 0.1497. The coded equation for prebiotic effect is provided below.

$$\text{Prebiotic effect (Log CFU/ml)} = 10.57 + 0.3251A - 0.6762B + 0.1050AB + 0.2560A^2 - 0.20875B^2$$

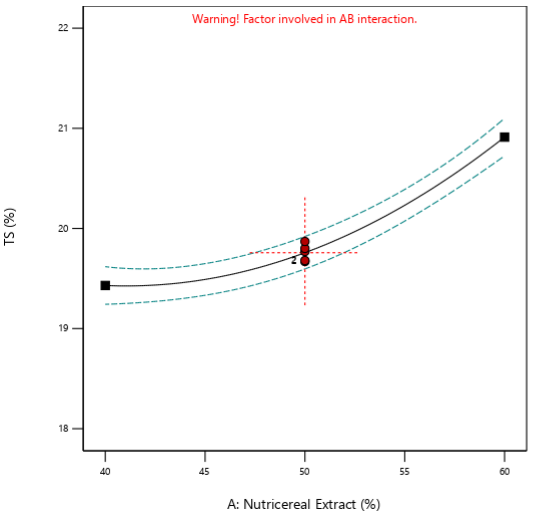
The significant model terms of overall acceptability are A, B, AB. The F-values, which ranged from 1.73 to 12.65, and p-values of 0.0078, indicates that the model was significant.

The R^2, adjusted R^2, and predicted R^2 values were 0.8534, 0.7487, and 0.5613, respectively, demonstrating reasonable agreement between adjusted R^2, and predicted R^2 as they have a difference of less than 0.2. The adequate precision score was 9.1332, confirming that the values fit well within the quadratic model. Descriptive statistics, including the mean, standard deviation, and CV, were 22.57, 0.3729, and 1.65, respectively. The lack of fit was found to be non-significant, with an F-value of 0.4632 and a p-value of 0.7233. The coded equation for overall acceptability is provided below.

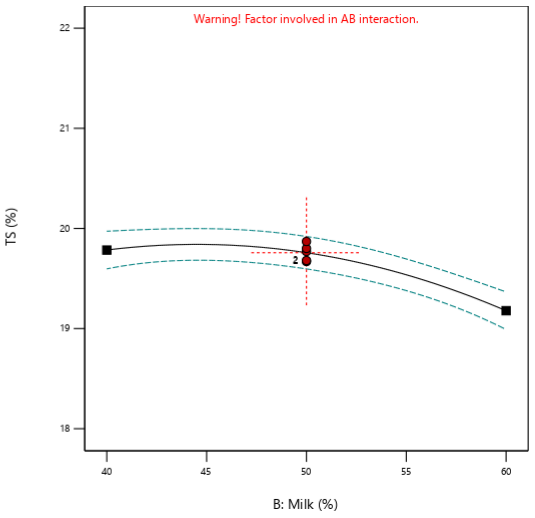
$$\text{Overall acceptability} = 7.0 - 0.1030A + 0.1104B - 0.1500AB + 0.06875A^2 + 0.04375B^2$$

Table. 6.21. Analysis of variance (ANOVA) for TS, TPC, tannin, prebiotic effect, and overall acceptability

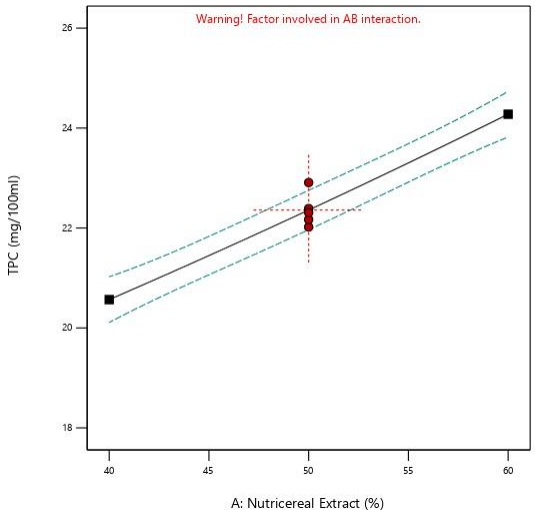
	Total Solids					TPC					Tannin					Prebiotic effect					Overall acceptability					
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	7.99	5	1.60	68.03	< 0.0001	55.05	5	11.01	79.16	< 0.0001	69.34	5	13.87	51.00	< 0.0001	5.42	5	1.08	141.72	< 0.0001	0.3138	5	0.0628	8.15	0.0078	**Significant**
A: Nutricereal malt extract	4.39	1	4.39	186.98	< 0.0001	27.53	1	27.53	197.93	< 0.0001	34.34	1	34.34	126.26	< 0.0001	0.8457	1	0.8457	110.56	< 0.0001	0.0849	1	0.0849	11.03	0.0127	
B: Skimmed milk	0.7344	1	0.7344	31.26	0.0008	26.98	1	26.98	194.00	< 0.0001	34.32	1	34.32	d126.20	< 0.0001	3.66	1	3.66	478.28	< 0.0001	0.0974	1	0.0974	12.65	0.0093	
AB	0.9025	1	0.9025	38.41	0.0004	0.0056	1	0.0056	0.0404	0.8463	0.0600	1	0.0600	0.2207	0.6528	0.0441	1	0.0441	5.77	0.0474	0.0900	1	0.0900	11.69	0.0112	
A^2	1.19	1	1.19	50.62	0.0002	0.0272	1	0.0272	0.1954	0.6718	0.0087	1	0.0087	0.0320	0.8631	0.4568	1	0.4568	59.72	0.0001	0.0329	1	0.0329	4.27	0.0776	
B^2	0.5318	1	0.5318	22.63	0.0021	0.5261	1	0.5261	3.78	0.0929	0.6277	1	0.6277	2.31	0.1725	0.3031	1	0.3031	39.63	0.0004	0.0133	1	0.0133	1.73	0.2299	
Residual	0.1645	7	0.0235			0.9736	7	0.1391			1.90	7	0.2719			0.0535	7	0.0076			0.0539	7	0.0077			
Lack of fit	0.1362	3	0.0454	6.42	0.0521	0.5160	3	0.1720	1.50	0.3422	1.20	3	0.4002	2.28	0.2216	0.0375	3	0.0125	3.13	0.1497	0.0139	3	0.0046	0.4632	0.7233	**Not Significant**
Pure Error	0.0283	4	0.0071			0.4576	4	0.1144			0.7029	4	0.1757			0.0160	4	0.0040			0.0400	4	0.0100			
Cor Total	8.16	12				56.02	12				71.25	12				5.47	12				0.3677	12				


Warning! Factor involved in AB interaction.
TS (%)
A: Nutricereal Extract (%)

(a)


Warning! Factor involved in AB interaction.
TS (%)
B: Milk (%)

(b)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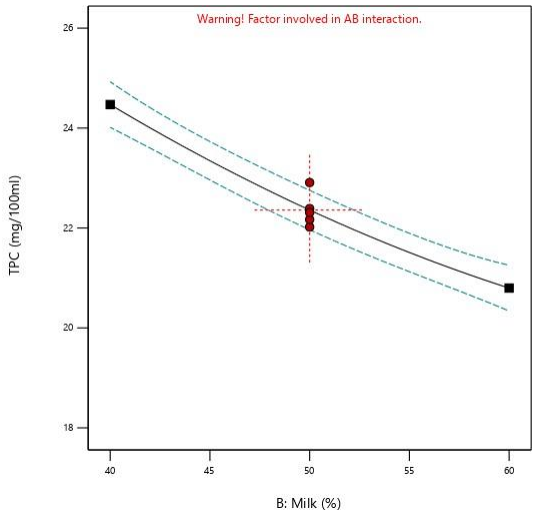
TPC (mg/100ml)
A: Nutricereal Extract (%)

(c)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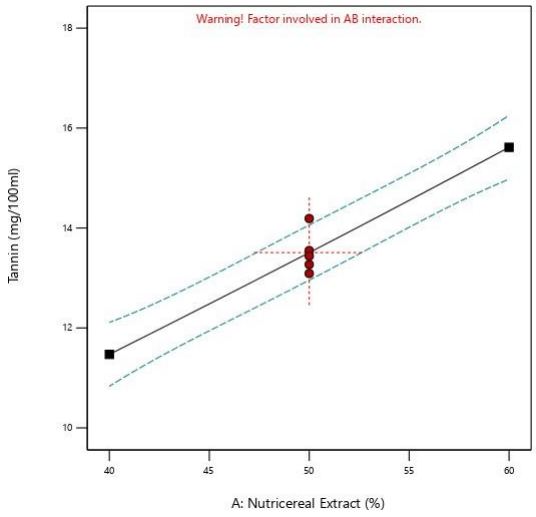
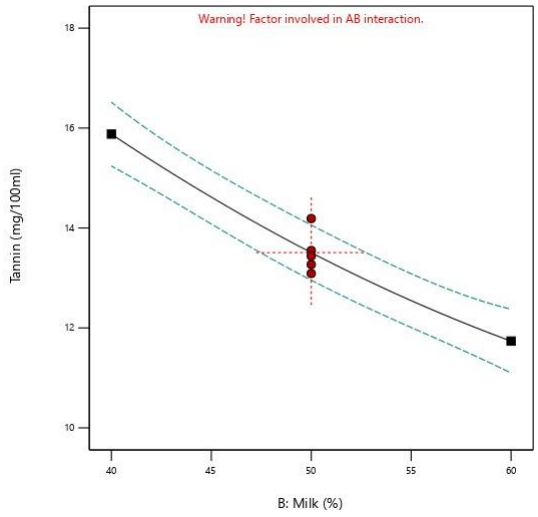
TPC (mg/100ml)
B: Milk (%)

(d)


Warning! Factor involved in AB interaction.
Tannin (mg/100ml)
A: Nutricereal Extract (%)

(e)


Warning! Factor involved in AB interaction.
Tannin (mg/100ml)
B: Milk (%)

(f)

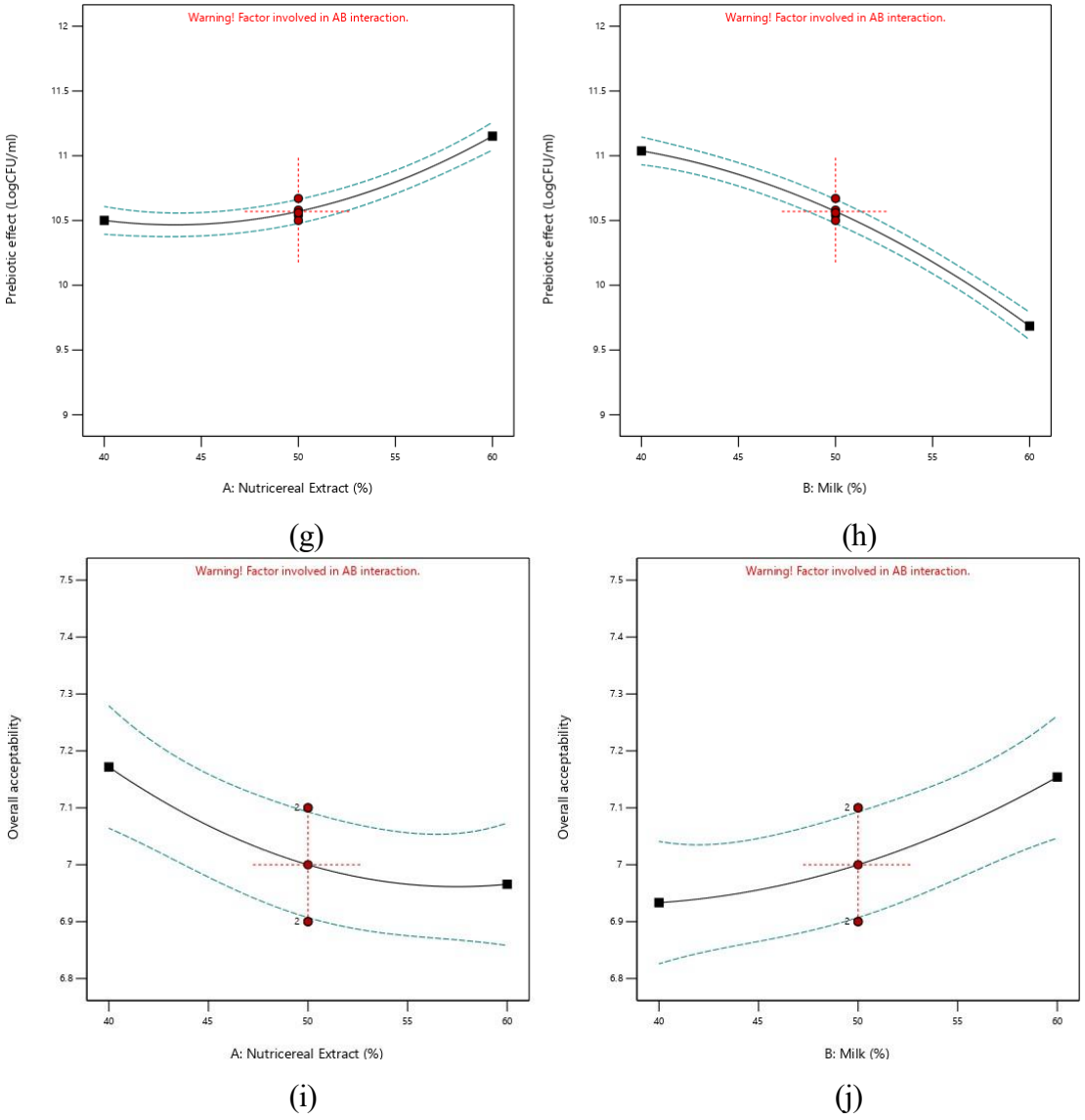


Figure. 6.26. Interaction between factors and responses, (a) nutricereal malt extract and total solids, (b) skimmed milk and total solids, (c) nutricereal malt extract and total phenolic content, (d) skimmed milk and total phenolic content, (e) nutricereal malt extract and tannin content, (f) skimmed milk and tannin content, (g) nutricereal malt extract and prebiotic effect, (h) skimmed milk and prebiotic effect, and (i) nutricereal malt extract and overall acceptability, (j) skimmed milk and overall acceptability

6.7.4. Response surface plots

The interaction between the independent variables (nutricereal malt extract and skimmed milk) and the responses was analysed using response surface plots. These plots are three-dimensional curves that depict the relationship between two independent variables.

Table. 6.22. Coefficient of regression for TS, TPC, tannin, prebiotic effect, and overall acceptability

	TS	TPC	Tannin	Prebiotic effect	Overall acceptability
Intercept	19.76	22.36	13.51	10.57	7.00
A	0.7411	1.86	2.07	0.3251	-0.1030
B	-0.3030	-1.84	-2.07	-0.6762	0.1104
AB	-0.4750	0.0375	-0.1225	0.1050	-0.1500
A^2	0.4135	0.0625	0.0354	0.2562	0.0688
B^2	-0.2765	0.2750	0.3004	-0.2087	0.0438

6.7.4.1. Effect of variables on the total solids content of the nutricereal beverage

Table 6.20 shows Total Solids (TS) across the sample runs (1-13) ranges from 18.73 to 21.67 %, with the highest value observed at a combination of 64.1421 ml nutricereal malt extract and 50 ml skimmed milk, indicating a significant ($p<0.05$) influence of higher levels of nutricereal malt extract on total solids. Figure 6.27., depicts that the increase in TS were fund with the increase in the nutricereal malt extract. The skimmed milk has a significant ($p<0.05$) negative effect on the TS content while nutricereal malt extract had a significant ($p<0.05$) positive increase. Similar trend was observed in finger millet malt-based beverage. The increase in TS due to high solid content in the nutricereal malt extract (Kumar et al., 2020a).

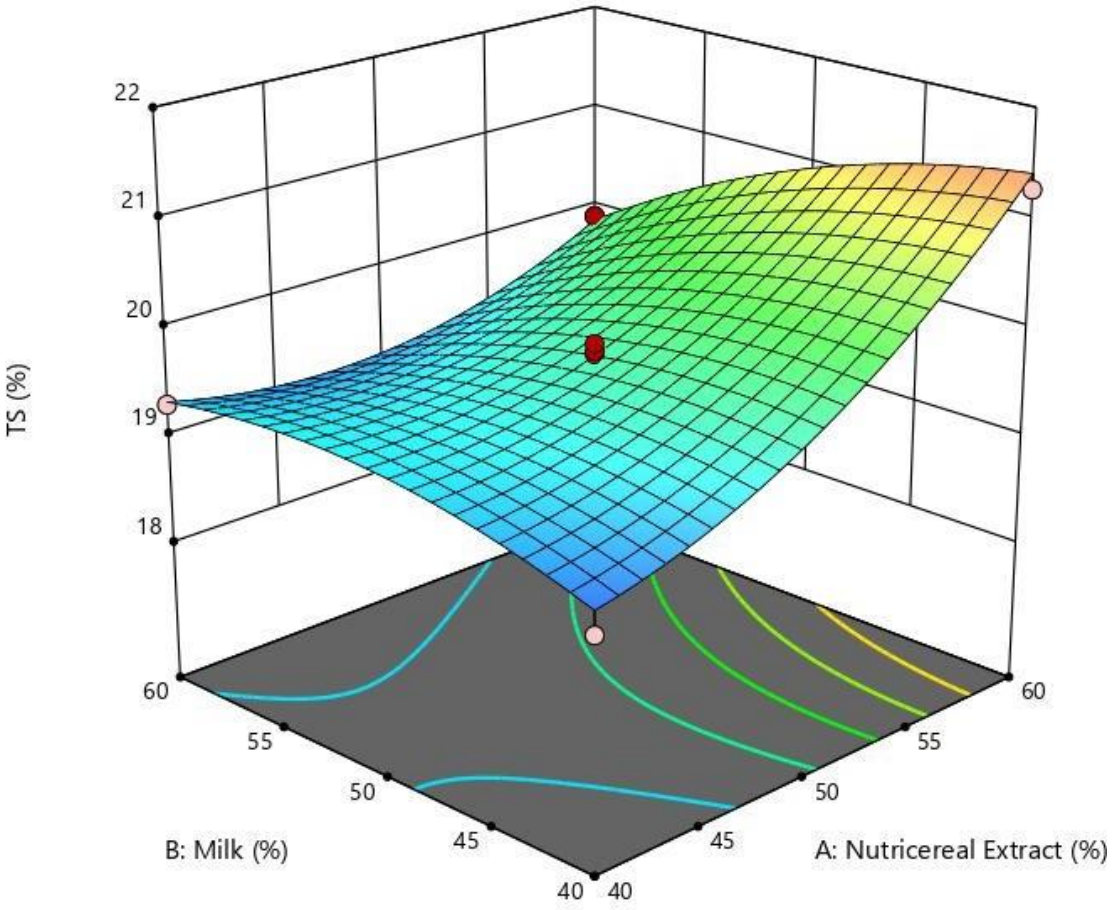


Figure 6.27. Effect of nutricereal malt extract and skimmed milk on the total solids content of the nutricereal beverage

6.7.4.2. Effect of variables on the total phenols content of the functional beverage

TPC values fluctuated between 18.91 and 25.99 mg/100ml as shown in Table 6.20., with the highest content observed at 60 ml nutricereal malt extract and 40 ml skimmed milk, suggesting that higher levels of nutricereal malt extract enhance phenolic content. The skimmed milk had a significant ($p<0.05$) negative effect on the TPC and nutricereal malt extract showed significant ($p<0.05$) increase in TPC showed in Figure 6.28. Similar trend was observed in finger millet malt-based beverage due to the higher TPC content of the nutricereals used with pearl millet, finger millet and buckwheat of 269–420 mg GAE/100g, 161.64–314.24 mg GAE/100g, 298.96–365.67 mg GAE/100g, respectively (Paschapur et al., 2021; Yousaf et al., 2021; Sowdhanya et al., 2023). However, the milk has low TPC of

3.2 to 4.9 mg/100 ml (Kumar et al., 2020a). The increase in TPC with the increase in the nutricereal malt extract is depicted in the Figure 6.10.

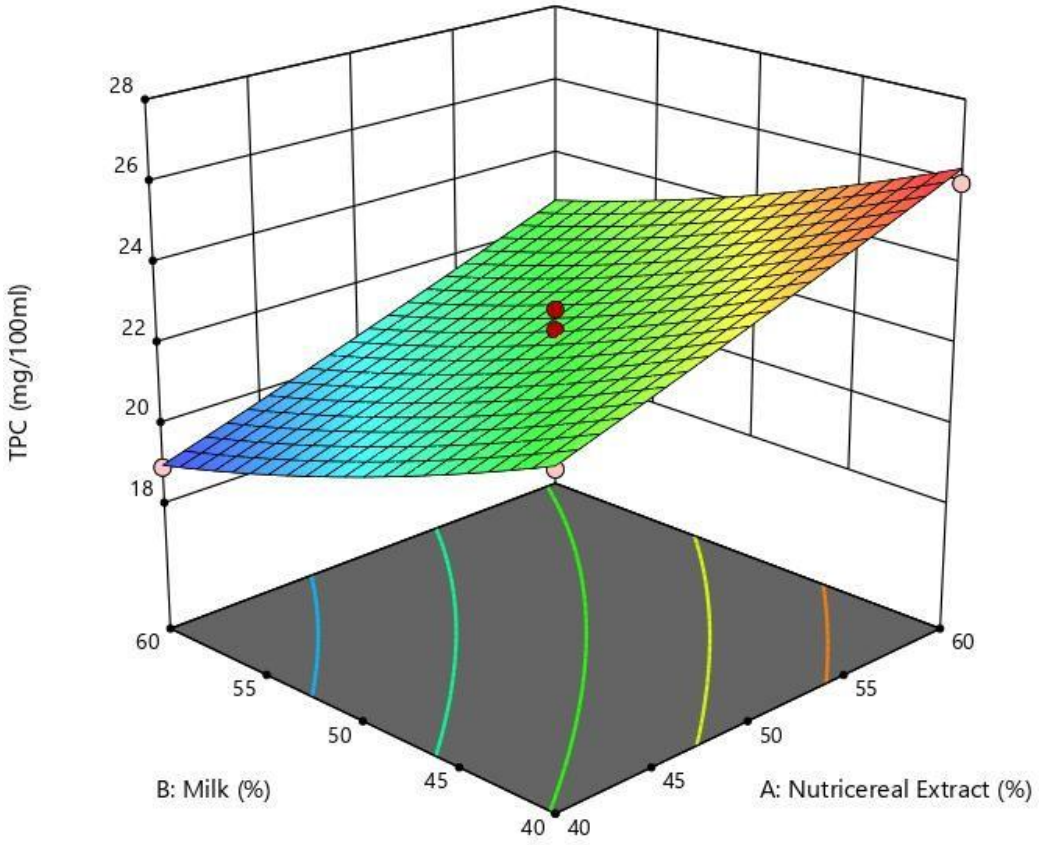


Figure. 6.28. Effect of nutricereal malt extract and skimmed milk on the total phenolic content of the nutricereal beverage

6.7.4.3. Effect of variables on the tannin content of the functional beverage

Tannin content ranges from 10.03 to 17.48 mg/100ml as depicted in table 6.20., with the maximum tannin content also occurring at 60 ml nutricereal malt extract and 40 ml skimmed milk, indicating that specific combinations of factors significantly affect ($p<0.05$) tannin levels. Tannic acid has been granted GRAS (Generally Recognized as Safe) status by the FDA (Food and Drug Administration) for direct use as a food additive. However, the FDA has set a limit of 0.005% (5 mg/100ml) in non-alcoholic beverages (Sharma et al., 2021). As there is no standard dosage for natural tannin, the tannin content of the optimised beverage can be considered safe. The Figure 6.29., depicts that the increase in tannin content were found with the increase in the nutricereal malt extract. The skimmed milk has a significant ($p<0.05$) negative effect on the tannin content while nutricereal malt extract

has a significant ($p<0.05$) increase. This increase is similar to the increase in the TPC as shown in Section. 6.8.4.2.

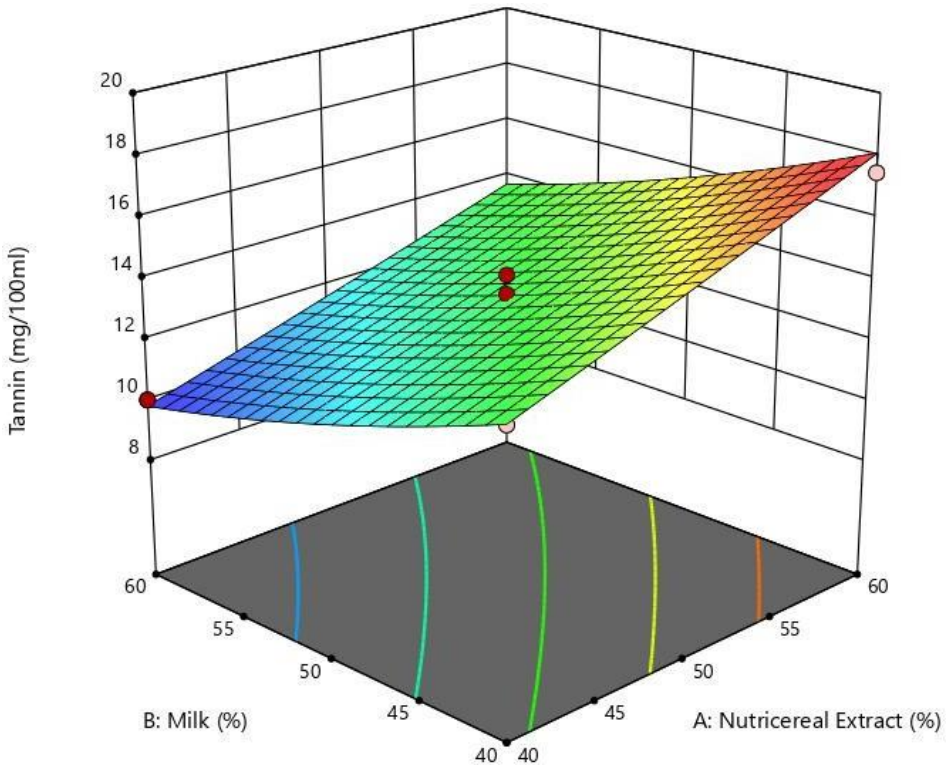


Figure. 6.29. Effect of nutricereal malt extract and skimmed milk on the tannin content of the nutricereal beverage

6.7.4.4. Effect of variables on the prebiotic effect of the functional beverage

The prebiotic effect, varies from 9.12 to 11.56 Log CFU/ml, with the highest value was observed at 64.1421 ml nutricereal malt extract and 50 ml skimmed milk. This implies that higher concentrations of nutricereal malt extract in combination with moderate levels of skimmed milk may enhance prebiotic activity. The skimmed milk had a significant($p<0.05$) negative effect on the prebiotic effect and nutricereal malt extract had a significant ($p<0.05$) positive increase. The increase in the prebiotic effect with the increase in the nutricereal malt extract is depicted in the Figure 6.30. The beverages are rich in dietary fibre, from the addition of the nutricereal extract, which is otherwise absent in dairy milk, and serve as an excellent prebiotic (Bhudwar et al., 2020).

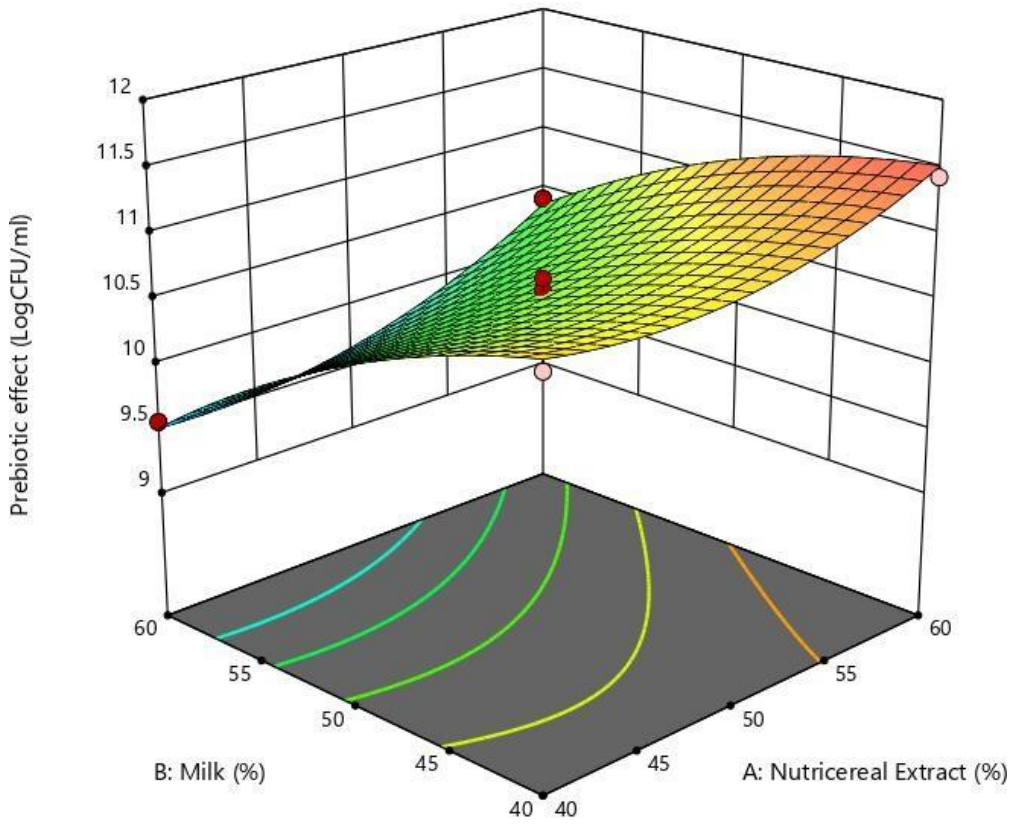


Figure. 6.30. Effect of nutricereal malt extract and skimmed milk on the prebiotic of the nutricereal beverage

6.7.4.5. Effect of variables on the overall acceptability of the functional beverage

The skimmed milk had a significant ($p<0.05$) positive effect on the overall acceptability and nutricereal malt extract had a significant ($p<0.05$) negative effect. Similar trend was observed in finger millet malt-based beverage (Kumar et al., 2020a). The increase in the prebiotic effect with the increase in the nutricereal malt extract is depicted in the Figure 6.31.

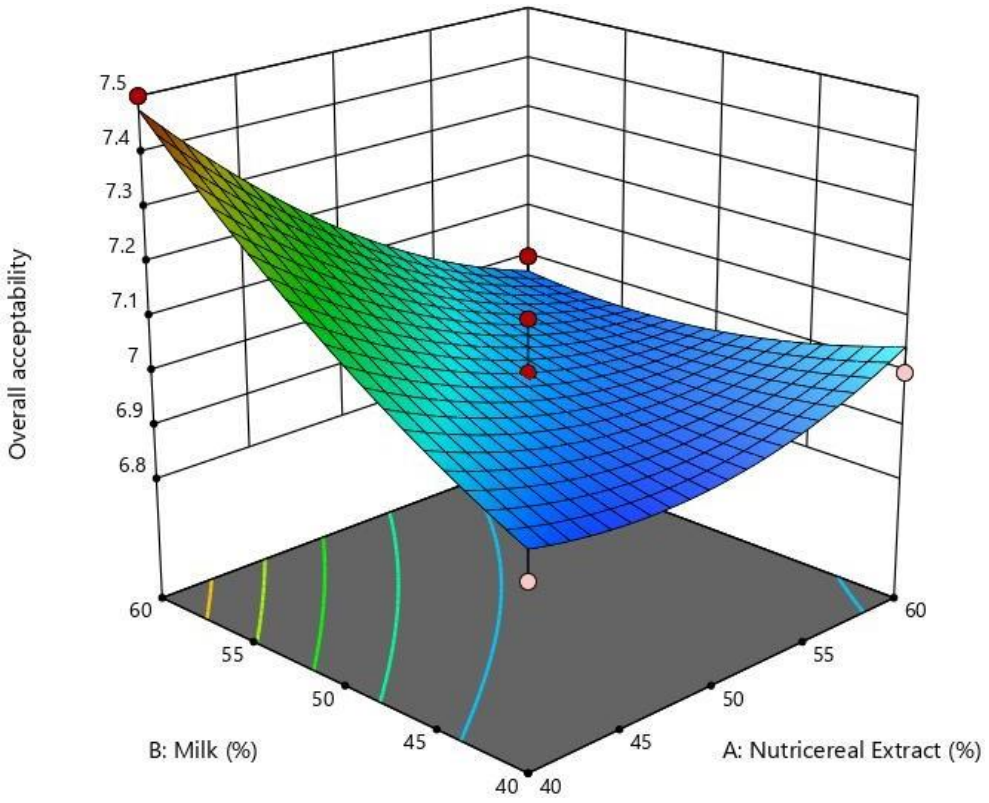


Figure. 6.31. Effect of nutricereal malt extract and skimmed milk on the prebiotic of the nutricereal beverage

6.7.5. Optimization of nutricereal beverage

The effects of the variables, nutricereal malt extract and skimmed milk, on the responses of total solids, total phenolic content, tannin content, prebiotic effect and overall acceptability are shown in Table 6.21. The optimized ratio of nutricereal malt extract to skimmed milk was obtained as 60:40 with a desirability of 0.906. A comparison between the predicted and observed values of the parameters are given in Table 6.23. The prepared beverage was pasteurized at 90˚C for 20 min, capped and cooled to refrigeration as shown in Figure 6.33).

Table 6.23. Comparison of the predicted values and observed values for the optimized nurticereal beverage

Analysis	Unit	Predicted Mean	Observed
TS	%	21.41±0.15	21.27±0.21
TPC	mg/100ml	26.35±0.37	25.99±0.13
Tannin	mg/100ml	18.11±0.52	17.48±0.25
Prebiotic effect	Log CFU/ml	11.51±0.09	11.43±0.03
Overall acceptability	-	7.04±0.09	6.90±0.40

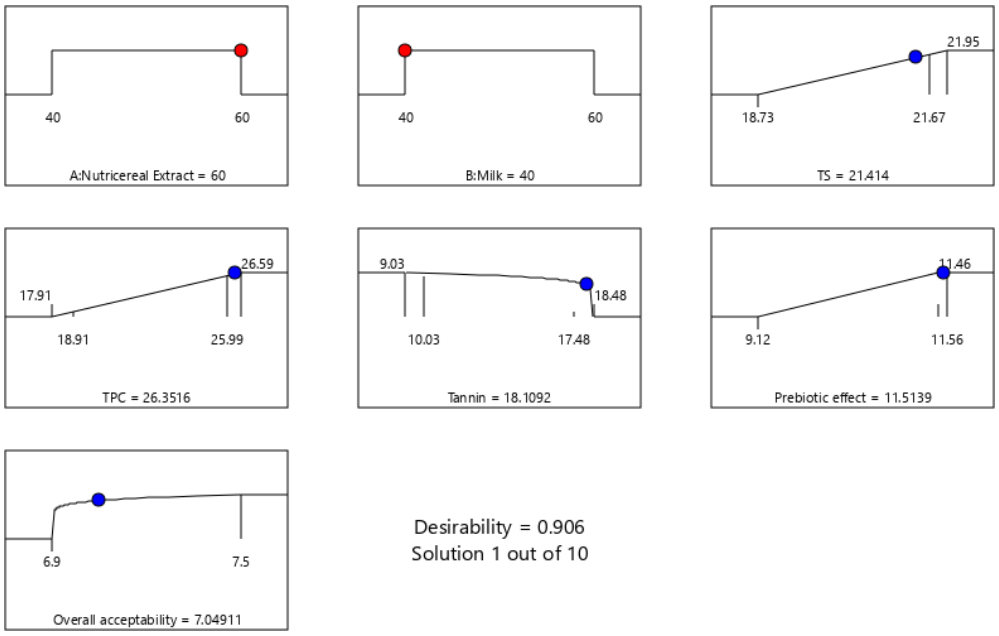


Figure. 6.32. Optimization of nutricereal based beverage.

Figure. 6.33. Prepared beverage being pasteurized at 90˚C for 20 min.

6.8. Enumeration of Probiotic Bacteria of the beverage inoculated with probiotic nanocapsules in the beverage

All the beverages with nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 had adequate viable cells present that is viability as shown in Table 6.24. The beverage with 2% nanoencapsulates, showed highest viable count of 9.72 Log CFU/ml and 10.57 Log CFU/ml, respectively (as depicted in Figure 6.16), the beverages turned thick after the nanoencapsulates absorbing the water and forming into a slurry shown in Figure 6.35. The beverage with 1% nanoencapsulates were also thick with adequate viable cells of 8.609 and 9.93 Log CFU/ ml of beverage for *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600, respectively. The beverages with 0.5 and 1% nanoencapsulated *L. plantarum* NCDC 685 had viable cell count of 7.92 Log CFU/ml and 8.73 Log CFU/ml beverage, respectively. Similarly, the beverage with nanoencapsulated *L. acidophilus* NCDC 600 had 8.40 Log CFU/ml and 9.55 Log CFU/ml of beverage with 0.5 and 1% of the nanoencapsulates. As per the standards set by FAO/WHO, these products must contain at least 10^6 to 10^7 CFU/ml (6 to 7 Log CFU/ml) of microorganisms throughout their shelf life to qualify as probiotic (FAO/WHO, 2002). According to regulatory guidelines of FSSAI, for probiotic foods, fermented foods are considered to have probiotic potential if contains LAB counts in the viable range of $\geq 10^8$ CFU/g ($\geq$8 Log CFU/g), which is believed to confer health benefits (FSSAI, 2021). As the beverage became noticeably thicker when nanoencapsulates were incorporated at concentrations above 1%. Conversely, a concentration of 0.5 and 1% nanoencapsulates resulted in a more desirable consistency. Hence, 1% of nanoencapsulated cells were taken for further storage studies.

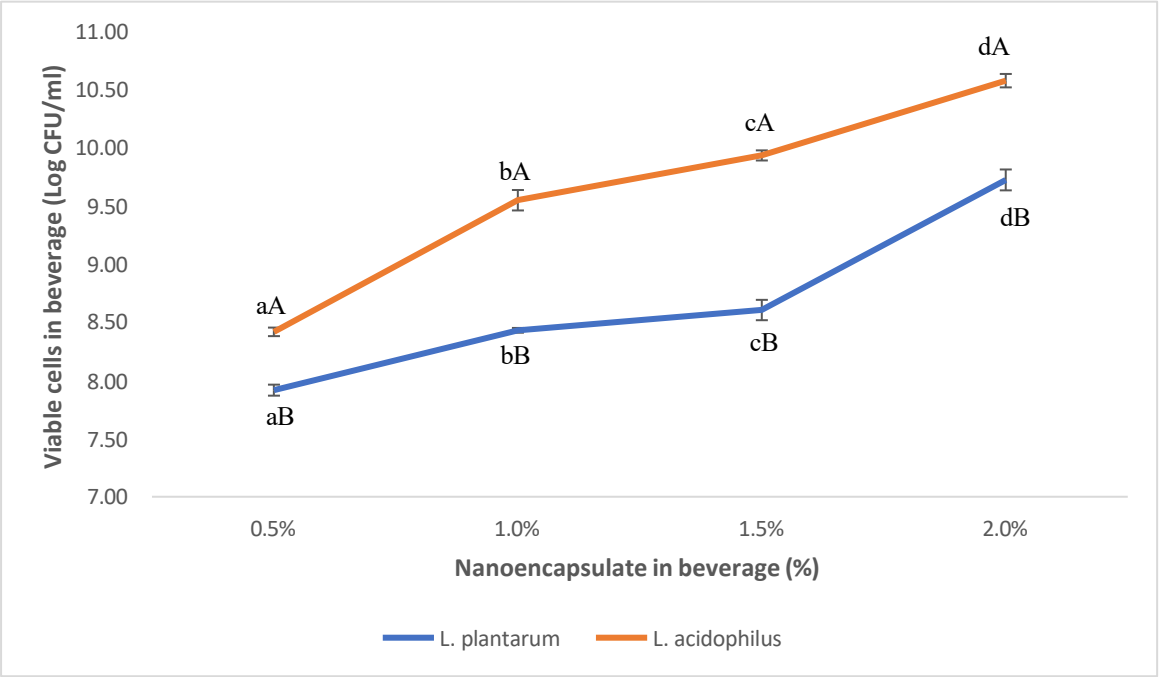


Figure. 6.34. Viability of the nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 in the beverage at different concentrations.

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-B]Mean within rows with different superscript are significantly ($p<0.05$) different

Table. 6.24. Viability of the nanoencapsulated *L. plantarum* NCDC 685 in the beverage at different concentrations.

Nanoencapsulate (%w/v)	*L. plantarum* NCDC 685 (Log CFU/ml)	*L. acidophilus* NCDC 600 (Log CFU/ml)
0.5	7.92 ± 0.05^{aB}	8.42 ± 0.04^{aA}
1.0	8.43 ± 0.02^{bB}	9.55 ± 0.09^{bA}
1.5	8.60 ± 0.09^{cB}	9.93 ± 0.04^{cA}
2.0	9.72 ± 0.09^{dB}	10.57 ± 0.06^{dA}

Data are expressed as mean ±S.D. (n=3). Two-way ANOVA used for statically analysis. [a-c]Mean within column with different superscript are significantly ($p<0.05$) different; [A-B]Mean within rows with different superscript are significantly ($p<0.05$) different

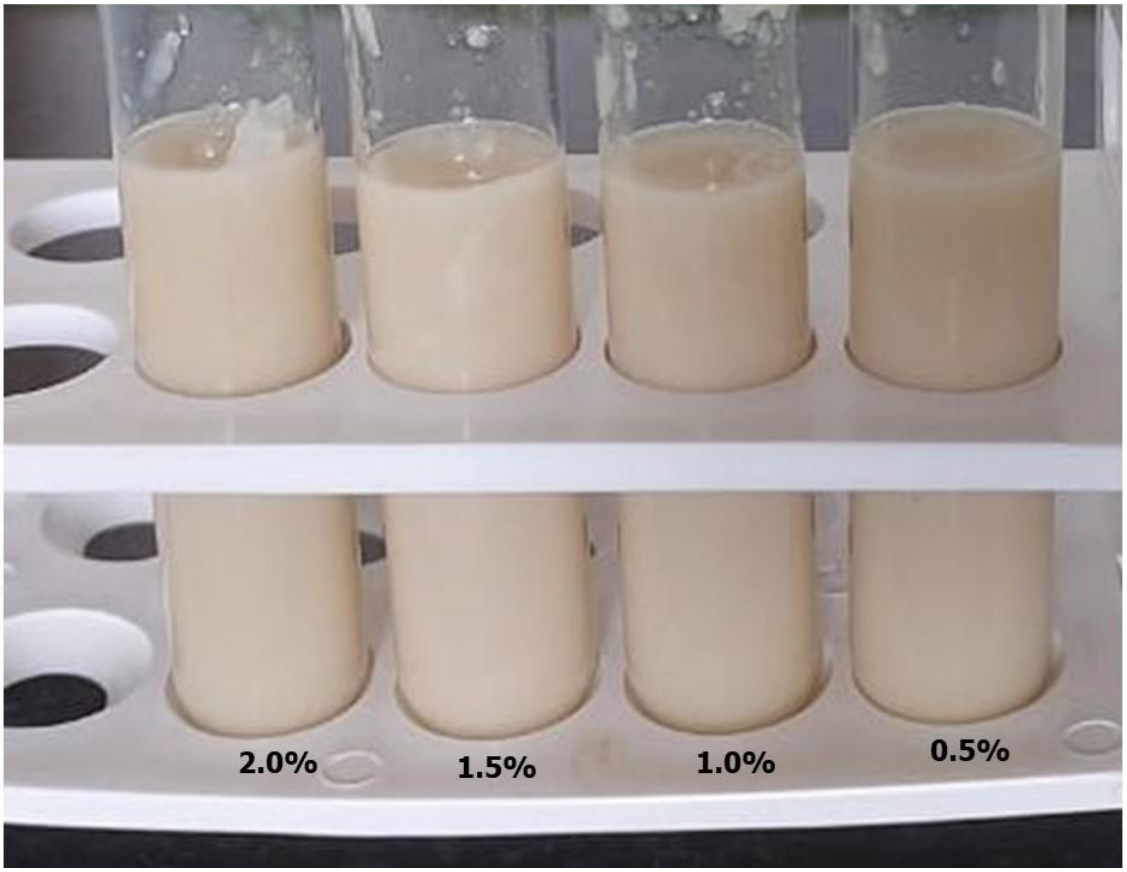


Figure. 6.35. Beverage with 0.5%, 1.0%, 1.5%, and 2.0% of nanoencapsulated *L. plantarum* NCDC 685.

6.9. Nutritional composition and physical properties

The nutritional composition and physical properties of beverages containing nanoencapsulates of *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 were analysed, and revealing comparable values across various parameters (Table 6.25). The protein content was 3.16% for beverage with *L. plantarum* NCDC 685 and 3.19% for beverage with *L. acidophilus* NCDC 600 ($p>0.05$), indicating no significant difference. Similarly, carbohydrate content was 7.15 and 7.21% ($p>0.05$) respectively, and fat content was 3.22 and 3.26% ($p>0.05$), both showing no significant differences. The fibre content and ash content also did not differ significantly ($p>0.05$) between the two beverages. Similarly, the moisture content non-significant ($p>0.05$) difference of 84.38% for B1 and 84.41% for B2. TPC was 17.61 mg GAE/100g for beverage with *L. plantarum* NCDC 685 nanoencapsulates and 17.66 mg GAE/100g for beverage with *L. acidophilus* NCDC 600 nanoencapsulates, and antioxidant activity measured was 16.17 % inhibition of DPPH and 16.39 % inhibition of DPPH, respectively. These values indicate TPC and antioxidant

activity showed non-significant ($p>0.05$) differences. Though the higher % DPPH inhibition in the *L. acidophilus* NCDC 600 beverage corresponds with its slightly higher TPC and tannin content, indicating a marginally better antioxidant activity. The tannin content was also similar at 25.69 mg TA/100g and 25.73 mg TA/100g showing no significant ($p>0.05$) differences. Calcium levels in the beverages were 321.00 mg/100g for *L. plantarum* NCDC 685 nanoencapsulates and 323.00 mg/100g for *L. acidophilus* NCDC 600 nanoencapsulates, that had no significant difference ($p>0.05$), similarly, the iron content was 0.67±0.06 mg/100g and 0.63 mg/100g, respectively, also showing no significant ($p>0.05$) difference. Phosphorus contentwas 258.62 mg/100g for beverage with *L. plantarum* NCDC 685 nanoencapsulates and 259.21 mg/100g for beverage *L. acidophilus* NCDC 600 nanoencapsulates, indicating no significant ($p>0.05$) difference. Potassium levels were 151.62 mg/100g and 152.93 mg/100g, magnesium content was 11.15 mg/100g and 11.16 mg/100g, and zinc content was 0.85±0.04 mg/100g and 0.87 mg/100g, for beverage with *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 nanoencapsulates respectively, all showing non-significant ($p>0.05$) differences ($p>0.05$). However, the sodium levels were slightly different at 51.00 mg/100g for *L. plantarum* NCDC 685 and 49.00 mg/100g for *L. acidophilus* NCDC 600, but showed non-significant ($p>0.05$) differences.

The reducing sugar content was 2.01% for the beverage with *L. plantarum* NCDC 685 nanoencapsulates and 2.0% for the beverage with *L. acidophilus* NCDC 600 nanoencapsulates, while the water activity was 0.991 for both beverages with no significant ($p>0.05$) difference. Viscosity was 10.16 and 10.18 cP, respectively, for the beverage with *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 nanoencapsulates showing no significant ($p>0.05$) differences. The pH was shown as 6.76 and 6.75 for beverage with *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 nanoencapsulates, respectively, while titaratable acidity was 0.108 % LA and 0.109 % LA, respectively, that showed non-significant ($p>0.05$) differences. These results indicate that both beverages have similar nutritional and physical profiles, and do not significantly differ in the overall composition and consistency of the beverages.

Table. 6.25. The nutritional composition and physical properties of beverages containing nanoencapsulates of *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600.

Test Parameters	Unit	Beverage with *L. plantarum* NCDC 685 (B1)	Beverage with *L. acidophilus* NCDC 600 (B2)
Protein	%	3.16±0.04[a]	3.19±0.04[a]
Carbohydrate	%	5.67±0.02[a]	5.72±0.26[a]
Fat	%	3.22±0.03[a]	3.26±0.04[a]
Fiber	%	1.47±0.05[a]	1.49±0.06[a]
Ash	%	1.87±0.21[a]	1.93±0.21[a]
Moisture	%	84.38±0.21[a]	84. 41±0.23[a]
TPC	mg GAE/100 ml	17.61±0.27[a]	17.66±0.28[a]
Antioxidant activity	% inhibition of DPPH	16.17±0.32[a]	16.39±0.32[a]
Tannin	mg TA/100 ml	25.69±0.22[a]	25.73±0.22[a]
Calcium	mg/100 ml	321.00±3.00[a]	323.00±2.00[a]
Iron	mg/100 ml	0.67±0.06[a]	0.63±0.04[a]
Phosphorus	mg/100 ml	258.62±1.88[a]	259.21±1.76[a]
Potassium	mg/100 ml	151.62±2.58[a]	152.93±2.28[a]
Magnesium	mg/100 ml	11.15±0.03[a]	11.16±0.03[a]
Zinc	mg/100 ml	0.85±0.04[a]	0.87±0.04[a]
Sodium	mg/100 ml	51.00±3.00[a]	49.00±2.00[a]
Reducing Sugar	%	2.01±0.02[a]	2.0±0.02[a]
Water activity	a_w	0.994±0.002[a]	0.994±0.001[a]
Viscosity	cP	10.16±0.02[a]	10.18±0.03[a]
pH	-	6.76±0.02[a]	6.75±0.03[a]
Titratable acidity	% LA	0.108±0.001[a]	0.109±0.001[a]

Data are expressed as mean ±S.D. (n=3). One-way ANOVA used for statically analysis.
[a] Mean within row with different superscript are significantly ($p>0.05$) different

6.10. Sensory Analysis

The sensory evaluation data compares the control sample with two variations, beverage with *L. plantarum* NCDC 685 encapsulates (B1) and *L. acidophilus* NCDC 600 encapsulates (B2), across characteristics such as Appearance, consistency, flavour, stickiness, bitterness, and overall acceptability. The results as given in Table 6.26, depicts that there were no significant differences between the control and the two variations (B1 and B2) across all sensory attributes, as indicated by the similar mean scores and overlapping standard deviations. The control scored 7.20±0.66 for control, while B1 and B2 scored 7.17 and 7.17, respectively. Consistency scores were also similar, with the control at 7.13, B1 at 7.21, and B2 at 7.14. Flavour scores were 7.37 for the control,

7.13±0.82 for B1, and 7.27 for B2. Bitterness was slightly lower for B1 (6.60) compared to the control (6.90) and B2 (6.67), but these are non-significant ($p>0.05$) differences. Overall acceptability was nearly identical for all samples, with the control at 7.34, B1 at 7.23, and B2 at 7.24 which is also non-significant ($p>0.05$) difference. These results suggest that the variations B1 and B2 are similar to the control in terms of sensory characteristics, indicating that the modifications made in B1 and B2 do not negatively affect the sensory qualities of the product.

Figure. 6.36. Prepared beverage (a) control, (b) beverage with nanoencapsulated *L. plantarum* NCDC 685 (B1) and (c) beverage with nanoencapsulated *L. acidophilus* NCDC 600 (B2).

Table 6.26. Sensory evaluation of the prepared beverages with nanoencapsulates

	Appearance	**Consistency**	**Flavour**	**Bitterness**	**Overall acceptability**
Control	7.20±0.66^{a}	7.13±0.78^{a}	7.37±0.72^{ab}	6.90±1.06^{ab}	7.34±0.47^{ab}
B1	7.17±0.75^{a}	7.21±1.03^{ab}	7.13±0.82^{a}	6.60±1.00^{a}	7.23±0.42^{a}
B2	7.17±0.79^{a}	7.14±0.86^{ab}	7.27±0.64^{ab}	6.67±1.03^{ab}	7.24±0.47^{ab}

Data are expressed as mean ±S.D. (n=30). One-way ANOVA used for statically analysis.
$^{a-b}$ Mean within column with different superscript are significantly ($p>0.05$) different

6.11. Shelf life

6.11.1. Viability

The viability of *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 in beverages, both in free cell and nanoencapsulated forms, was assessed over the storage period shown in Table 6.27. Initially, the viable counts for *L. plantarum* NCDC 685 were 8.69±0.05 Log CFU/ml for free cells and 8.55±0.06 Log CFU/ml for nanoencapsulated forms. For *L. acidophilus* NCDC 600, the initial viable counts were 9.67±0.03 log CFU/ml for free cells and 9.61±0.09 Log CFU/ml for nanoencapsulated forms. Throughout the storage period, the viability of both bacterial strains in free cell form significantly ($p<0.05$) decreased more rapidly compared to their nanoencapsulated counterparts. At 24th day, the viable counts for free cell *L. plantarum* NCDC 685 significantly ($p<0.05$) decreased to 5.87 Log CFU/ml, whereas the nanoencapsulated *L. plantarum* NCDC 685 maintained higher viability at 6.87 Log CFU/ml. Similarly, *L. acidophilus* NCDC 600 in free cell form significantly ($p<0.05$) decreased to 5.83 Log CFU/ml, while the nanoencapsulated form exhibited a slower significant ($p<0.05$) decline, maintaining a count of 7.66 Log CFU/ml by day 24. Similarly, the viability significantly ($p<0$.05) decreased to 6.16 Log CFU/ml by 36th day. These results indicate that nanoencapsulation significantly enhances ($p<0.05$) the viability of both *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 during storage, likely due to the protective effect of the encapsulation matrix, which mitigates the adverse conditions encountered during storage, thereby prolonging the functional lifespan of the probiotics in the beverage. FAO/WHO states that probiotic beverage needs to contain a minimum of 6 to 7 Log CFU/ml of live cells throughout their shelf life to qualify as probiotic (FAO/WHO, 2002). Thereby, the storage study was ceased when the viability was less than 6 Log CFU/ml. The viable counts of free *L. acidophilus* NCDC 600 in the dairy beverage stored at 4 ˚C was showed significantl ($p<0.05$) decrease by 2.6 Log units in a study, dropping from 8.8 to 6.2 Log CFU/ml over 21 days. In comparison, the viable counts in dairy beverages containing single-layer and double-layer *L. acidophilus* microcapsules declined by 1.8 and 1.4 Log decrease, respectively (Shu et al., 2018). In another study on microencapsulated *L. acidophilus* and *L. rhamnosus* in Doogh beverage, over the storage period, the bacterial count of *L. acidophilus* and *L. rhamnosus* free form significantly ($p<0.05$) decreased from 5.5×10^8 and 3.8×10^8 CFU/g to less than 10^6 CFU/g after 28 days of storage, respectively. For the microencapsulated form, the significant ($p<0.05$) decrease was observed 5.0×10^9 and 7.8×10^9 to 7.4×10^7 and 9.0×10^5 CFU/g, after 42nd day of storage for *L. acidophilus* and *L. rhamnosus*, respectively. The study demonstrated that the

microencapsulated form of the bacteria had higher survivability compared to the free form over the storage period (Pourjafar et al., 2020).

Table. 6.27. Viability of the *L. plantarum* NCDC 685 and the *L. acidophilus* NCDC 600 in beverages, both in free cell and nanoencapsulated forms over the storage period (Log CFU/ml)

Day	*L. plantarum* NCDC 685		*L. acidophilus* NCDC 600	
	Free Cell (B1C)	Nanoencapsulated (B1)	Free Cell (B2C)	Nanoencapsulated (B2)
	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)	(Log CFU/ml)
0	8.69 ± 0.05^{aB}	8.55 ± 0.06^{aC}	9.67 ± 0.03^{aA}	9.61 ± 0.09^{aA}
3	8.09 ± 0.03^{bD}	8.38 ± 0.03^{bC}	8.14 ± 0.02^{bB}	9.35 ± 0.02^{bA}
6	7.86 ± 0.07^{cA}	8.29 ± 0.02^{cB}	8.07 ± 0.05^{cC}	9.24 ± 0.03^{cA}
9	7.63 ± 0.10^{dD}	8.14 ± 0.03^{dB}	7.88 ± 0.05^{dC}	9.16 ± 0.03^{dA}
12	7.14 ± 0.04^{eD}	7.98 ± 0.06^{eB}	7.57 ± 0.05^{eC}	8.97 ± 0.05^{eA}
15	6.63 ± 0.05^{fD}	7.66 ± 0.09^{fB}	6.97 ± 0.05^{fC}	8.63 ± 0.05^{fA}
18	6.37 ± 0.04^{gD}	7.37 ± 0.04^{gB}	6.61 ± 0.09^{gC}	8.39 ± 0.03^{gA}
21	6.19 ± 0.03^{hC}	7.19 ± 0.03^{hB}	5.83 ± 0.03^{hD}	7.97 ± 0.05^{hA}
24	5.87 ± 0.04^{iC}	6.87 ± 0.04^{iB}	-	7.66 ± 0.06^{iA}
27	-	6.55 ± 0.03^{jB}	-	7.13 ± 0.04^{jA}
30	-	6.39 ± 0.04^{kB}	-	6.88 ± 0.05^{kA}
33	-	6.24 ± 0.03^{lB}	-	6.56 ± 0.05^{lA}
36	-	5.97 ± 0.02^{mB}	-	6.16 ± 0.04^{mA}

Data are expressed as mean ±S.D. (n=30). Two-way ANOVA used for statically analysis. $^{a-h}$ Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-D}$ Mean within rows with different superscript are significantly different ($p<0.05$) different

6.11.2. Titratable acidity

The titratable acidity of beverage with free cell *L. plantarum* NCDC 685 showed a steady significant ($p<0.05$) increase from 0.110 to 0.228 % Lactic Acid by 24th day (Table 6.28). The beverage with nanoencapsulated *L. plantarum* NCDC 685 had initial titratable acidity of 0.109 % LA which significantly ($p<0.05$) increased more gradually, reaching 0.196 % LA by 24th day, and continues to significantly ($p<0.05$) increase to 0.288 by day 36th day. The titratable acidity of the beverage containing free cell *L. acidophilus* NCDC 600 exhibited a gradual significant ($p<0.05$) increase from 0.107 to 0.215 % LA over a span of 24 days. In contrast, the beverage with nanoencapsulated *L. acidophilus* NCDC 600 had

initial titratable acidity of 0.108±0.004 and significantly ($p<0.05$) increased to 0.227 % LA by 36th day. Throughout the storage study, it was observed, both *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 demonstrated an increase in titratable acidity, indicative of ongoing metabolic activity and acid production. In a study, a non-dairy synbiotic beverage from riceberry malt extract was incorporated with free and encapsulated *L. plantatrum*. titratable acidity significantly ($p<0.05$) increased from 0.8% on 0th day to 1.5% by 5th day, which further increased 1.6% by 17th day whereas no further increase was observed for free cells. While the beverage with encapsulated *L. plantatrum* showed lower increase to 1.0 % by 10th day and a minimal increase by 26th day to 1.2% (Nakkarach and Withayagiat, 2018) The free cell forms of both species showed a more rapid significant ($p<0.05$) increase in titratable acidity compared to their nanoencapsulated counterparts, suggesting that nanoencapsulation moderates the rate of acid production. This effect was particularly evident as the free cell forms reached higher acidity levels by 24th day, while the nanoencapsulated forms continued to exhibit a gradual significant ($p<0.05$) increase, reaching their peak titratable acidity values by 36th day. The findings underscore the potential of nanoencapsulation to provide a controlled and sustained release of acidity, which could be advantageous for applications in food preservation and probiotic formulations, where stability and prolonged activity of bacteria are desired.

Table. 6.28. Titratable acidity of the beverages containing *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600, in both free cell and nanoencapsulated forms assessed over the storage period (% LA)

Day	*L. plantarum* NCDC 685		*L. acidophilus* NCDC 600	
	Free Cell (B1C)	Nanoencapsulated (B1)	Free Cell (B2C)	Nanoencapsulated (B2)
	(% LA)	(% LA)	(% LA)	(% LA)
0	0.110 ± 0.005^{fA}	0.109 ± 0.001^{gA}	0.107 ± 0.004^{gA}	0.108 ± 0.004^{jA}
3	0.132 ± 0.006^{eA}	0.112 ± 0.013^{gA}	0.136 ± 0.005^{fA}	0.113 ± 0.001^{jB}
6	0.154 ± 0.007^{dA}	0.113 ± 0.006^{gB}	0.153 ± 0.009^{eA}	0.115 ± 0.001^{jB}
9	$0.164\pm0.006b^{cA}$	0.118 ± 0.006^{gB}	0.163 ± 0.002^{dA}	0.124 ± 0.001^{iB}
12	0.168 ± 0.005^{cA}	0.139 ± 0.013^{fB}	0.175 ± 0.004^{cA}	0.146 ± 0.004^{hB}
15	0.174 ± 0.006^{cB}	0.163 ± 0.002^{eC}	0.184 ± 0.006^{bcA}	0.153 ± 0.001^{gD}
18	0.190 ± 0.005^{bA}	0.170 ± 0.004^{eB}	0.192 ± 0.002^{abA}	0.171 ± 0.001^{fB}
21	0.193 ± 0.005^{bAB}	0.189 ± 0.005^{dB}	0.202 ± 0.007^{aA}	0.180 ± 0.002^{eD}
24	0.228 ± 0.003^{aA}	0.196 ± 0.006^{dB}	-	0.194 ± 0.007^{dB}
27	-	0.221 ± 0.004^{cA}	-	0.203 ± 0.005^{cB}
30	-	0.229 ± 0.004^{cA}	-	0.215 ± 0.006^{bB}
33	-	0.257 ± 0.007^{bA}	-	0.224 ± 0.007^{aB}
36	-	0.288 ± 0.008^{aA}	-	0.227 ± 0.004^{aB}

Data are expressed as mean ±S.D. (n=30). Two-way ANOVA used for statically analysis. $^{a-j}$ Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-D}$ Mean within rows with different superscript are significantly different ($p<0.05$) different

6.11.3. pH

The pH of beverages containing free cell and nanoencapsulated forms of *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 was monitored over a 36-day period (Table 6.29). Initially, the pH of all samples was similar, with *L. plantarum* NCDC 685 free cells at 6.77 and nanoencapsulated at 6.76, and *L. acidophilus* NCDC 600 free cells at 6.72 and nanoencapsulated at 6.75. The pH of the beverage with free cell *L. plantarum* NCDC 685 steadily significantly ($p<0.05$) decreased, reaching 5.67 over the storage period on 24th day. In contrast, the pH of the beverage with nanoencapsulated *L. plantarum* NCDC 685 showed a more gradual decline, from 6.76to 5.63 by 24th day, continuing to significantly ($p<0.05$) decrease to 5.16 by 36th day. Similarly, the beverage with free cell *L. acidophilus* NCDC 600 experienced a significant ($p<0.05$) pH drop to 5.13 by 24th day, while the

nanoencapsulated form showed a slower significant decrease ($p<0.05$) in pH, reaching 5.74 by 24th day and 5.16 by 36th day. These findings suggest that nanoencapsulation moderates the rate of pH reduction, indicating a more controlled acidification process compared to free cells. This controlled acid production could be advantageous for applications requiring prolonged stability and significantly ($p<0.05$) slower acidification, such as in probiotic beverages and other fermented products. The pH decreases in the beverage prepared from riceberry malt extract incorporated with free *L. plantatrum* was observed to be from 5 on 0th day to <3.5 by 21st day. While the beverage with encapsulated *L. plantatrum* had a minimal decrease to <4.5 by 21st day (Nakkarach and Withayagiat, 2018).

Table. 6.29. pH of the beverages containing *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600, in both free cell and nanoencapsulated forms assessed over the storage period.

Day	*L. plantarum* NCDC 685		*L. acidophilus* NCDC 600	
	Free Cell (B1C)	**Nanoencapsulated (B1)**	**Free Cell (B2C)**	**Nanoencapsulated (B2)**
0	6.77 ± 0.02^{aA}	6.76 ± 0.02^{aA}	6.72 ± 0.03^{aA}	6.75 ± 0.03^{aA}
3	6.55 ± 0.02^{bC}	6.74 ± 0.02^{abA}	6.65 ± 0.02^{bB}	6.73 ± 0.02^{aA}
6	6.47 ± 0.02^{cB}	6.71 ± 0.02^{bA}	6.27 ± 0.02^{cC}	6.68 ± 0.02^{bA}
9	6.33 ± 0.02^{dC}	6.62 ± 0.03^{cB}	5.91 ± 0.03^{dD}	6.67 ± 0.02^{bA}
12	6.19 ± 0.01^{eC}	6.36 ± 0.02^{dB}	5.77 ± 0.04^{eD}	6.45 ± 0.02^{cA}
15	5.99 ± 0.02^{fC}	6.08 ± 0.02^{eB}	5.61 ± 0.06^{fD}	6.23 ± 0.02^{dA}
18	5.85 ± 0.02^{gB}	5.86 ± 0.01^{fB}	5.52 ± 0.03^{gC}	6.14 ± 0.02^{eA}
21	5.73 ± 0.01^{hB}	5.74 ± 0.02^{gB}	5.33 ± 0.03^{hC}	5.91 ± 0.02^{fA}
24	5.67 ± 0.02^{iB}	5.63 ± 0.03^{hB}	-	5.74 ± 0.03^{gA}
27	-	5.44 ± 0.02^{iB}	-	5.55 ± 0.02^{hA}
30	-	5.32 ± 0.02^{jB}	-	5.40 ± 0.03^{iA}
33	-	5.25 ± 0.02^{kB}	-	5.34 ± 0.03^{jA}
36	-	5.16 ± 0.05^{lB}	-	5.23 ± 0.04^{kA}

Data are expressed as mean ±S.D. (n=30). Two-way ANOVA used for statically analysis.
$^{a-l}$ Mean within column with different superscript are significantly ($p<0.05$) different;
$^{A-D}$ Mean within rows with different superscript are significantly ($p<0.05$) different

6.11.4. Sensory Analysis

6.11.4.1. Appearance

The data on the sensory evaluation parameter of colour over a 36-day storage period as shown in Table 6.30, reveals that both free cell and nanoencapsulated forms of *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 maintained stable colour characteristics with minimal variations. *L. plantarum* NCDC 685 in the beverage in free cell form (B1C) had initial a colour score of 7.20 and remained relatively consistent, recording 7.13 on 24th day before the evaluation ended. The beverage with nanoencapsulated form (B1) showed a similar trend, beginning at 7.13 and gradually non-significant decreasing to 6.73 by 36th day. In the case of beverage with *L. acidophilus* NCDC 600, the free cell form (B2C) also displayed stable colour scores initially, starting at 7.25 on day 0 and remaining consistent up to 18th day (7.13). The nanoencapsulated form (B2) had initial a colour score of 7.13 and experienced a gradual decline in colour score, reaching 6.57 by day 36. Despite these slight reductions in colour over time, the differences between the free cell and nanoencapsulated forms were not statistically significant, indicating that nanoencapsulation did not adversely affect the colour stability of the product. The results suggest that both *L. plantarum* NCD.C 685 and *L. acidophilus* NCDC 600 maintained acceptable appearance throughout the storage period, with nanoencapsulation providing a slight but not significant advantage in colour retention over time. This stability is important for ensuring the visual appeal of the product during storage, indicating that the use of nanoencapsulation could be a viable method for extending shelf life without compromising sensory quality.

Table. 6.30. Appearance of *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 in beverages, both in free cell and nanoencapsulated forms assessed over the storage period.

Day	*L. plantarum* NCDC 685		*L. acidophilus* NCDC 600	
	Free Cell (B1C)	Nanoencapsulated (B1)	Free Cell (B2C)	Nanoencapsulated (B2)
0	7.20±0.71^{aA}	7.13±0.73^{aA}	7.25±0.70^{aA}	7.13±0.78^{aA}
3	7.21±0.60^{aA}	7.12±0.72^{aA}	7.22±0.74^{aA}	7.11±0.74^{aA}
6	7.20±0.55^{aA}	7.13±0.68^{aA}	7.21±0.69^{aA}	7.10±0.71^{aA}
9	7.18±0.56^{aA}	7.12±0.84^{aA}	7.20±0.66^{aA}	7.10±0.75^{aA}
12	7.17±0.46^{aA}	7.12±0.81^{aA}	7.20±0.61^{aA}	7.08±0.81^{aA}
15	7.13±0.51^{aA}	7.10±0.81^{aA}	7.17±0.59^{aA}	7.07±0.78^{aA}
18	7.10±0.61^{aA}	7.08±0.74^{aA}	7.13±0.62^{aA}	7.03±0.76^{aA}
21	7.07±0.64^{aA}	7.01±0.73^{aA}	-	6.97±0.72^{abA}
24	7.13±0.62^{aA}	6.97±0.76^{aA}	-	6.90±0.66^{abA}
27	-	6.93±0.78^{aA}	-	6.88±0.67^{abA}
30	-	6.90±0.76^{aA}	-	6.83±0.79^{abA}
33	-	6.87±0.78^{aA}	-	6.70±0.67^{abA}
36	-	6.73±0.74^{aA}	-	6.57±0.68^{bA}

Data are expressed as mean ±S.D. (n=30). Two-way ANOVA used for statically analysis. $^{a-b}$ Mean within column with different superscript are significantly ($p<0.05$) different; ^{A} Mean within rows with different superscript are significantly ($p<0.05$) different

6.11.4.2. Consistency

The sensory evaluation of consistency in beverages containing free and nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 shows a gradual decline in consistency scores over the storage period as shown in Table 6.31. *L. plantarum* NCDC 685 in the free cell form (B1C) began with a score of 7.21 on day 0th day, remaining relatively stable until 18th day (6.90), after which a more noticeable decrease was observed, reaching 6.67 by 24th day. The nanoencapsulated form (B1) maintained slightly higher consistency scores throughout the storage period, starting at 7.27t on 0th day and gradually decreasing to 6.43 by 36th day. In the case of *L. acidophilus* NCDC 600, the free cell form (B2C) started at 7.20 on 0th day, with a consistent decline over time, reaching 6.60 by 21st day. The nanoencapsulated form (B2) also showed a gradual decrease, beginning at 7.13 on 0th day and dropping to 6.70 by 36th day. Throughout the storage period, the nanoencapsulated forms of both bacteria exhibited better retention of consistency compared

to their free cell counterparts. This suggests that nanoencapsulation may help preserve the textural qualities of the beverage over time, making it a potentially beneficial technique for maintaining product quality during extended storage. The gradual decline in consistency is typical of probiotic beverages over time, but the use of nanoencapsulation appears to mitigate this effect to some extent, ensuring a more stable product for a longer period.

Table. 6.31. Consistency of the beverages containing *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600, in both free cell and nanoencapsulated forms assessed over the storage period.

Day	*L. plantarum* NCDC 685		*L. acidophilus* NCDC 600	
	Free Cell (B1C)	**Nanoencapsulated (B1)**	**Free Cell (B2C)**	**Nanoencapsulated (B2)**
0	7.21 ± 0.71^{aA}	7.27 ± 0.64^{aA}	7.20 ± 0.71^{aA}	7.13 ± 0.57^{aA}
3	7.22 ± 0.81^{aA}	7.20 ± 0.71^{abA}	7.12 ± 0.76^{abA}	7.12 ± 0.58^{abA}
6	7.20 ± 0.75^{aA}	7.23 ± 0.67^{abA}	7.11 ± 0.70^{abA}	7.08 ± 0.56^{abA}
9	7.18 ± 0.70^{aA}	7.22 ± 0.69^{abA}	7.10 ± 0.66^{abA}	7.08 ± 0.61^{abA}
12	7.15 ± 0.71^{aA}	7.17 ± 0.69^{abA}	7.10 ± 0.71^{abA}	7.07 ± 0.64^{abA}
15	7.07 ± 0.69^{aA}	7.10 ± 0.76^{abA}	7.03 ± 0.72^{abA}	7.05 ± 0.70^{abA}
18	6.90 ± 0.71^{abA}	7.07 ± 0.74^{abcA}	6.87 ± 0.68^{bA}	7.03 ± 0.76^{abA}
21	6.73 ± 0.64^{bAB}	7.03 ± 0.76^{abcA}	6.60 ± 0.56^{bcB}	6.93 ± 0.74^{abAB}
24	6.67 ± 0.66^{bA}	7.00 ± 0.79^{abcA}	-	6.90 ± 0.71^{abA}
27	-	6.90 ± 0.76^{abcA}	-	6.88 ± 0.72^{abA}
30	-	6.83 ± 0.75^{bcA}	-	6.85 ± 0.73^{abA}
33	-	6.67 ± 0.71^{cdA}	-	6.66 ± 0.77^{abA}
36	-	6.43 ± 0.50^{dB}	-	6.70 ± 0.75^{bA}

Data are expressed as mean ±S.D. (n=30). Two-way ANOVA used for statically analysis. $^{a-d}$ Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-B}$ Mean within rows with different superscript are significantly ($p<0.05$) different

6.11.4.3. Flavour

The sensory evaluation of flavour in beverages containing free and nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 demonstrates a gradual decline in flavour scores over the 36-day storage period as shown in Table 6.32, with nanoencapsulated forms generally maintaining higher scores compared to free cells. Initially, both the free cell and nanoencapsulated forms of *L. plantarum* NCDC 685 (B1C and B1) started with similar flavour scores of 7.33 and 7.23, respectively, on 0^{th} day. The

scores remained relatively stable until day 18, after which the beverage with nanoencapsulates saw a more pronounced decline, dropping to 6.83 by 21st day and further to 6.50 by 36th day. The beverage with free cells also experienced a decline, at a higher rate, reaching 6.77 by 24th day. Similarly, *L. acidophilus* NCDC 600 in the free cell form (B2C) began with a flavour score of 7.33 on 0th day and gradually decreased to lowest score of 6.73 by 21st day. The nanoencapsulated form (B2) maintained a more consistent flavour, starting at 7.30 on 0th day and declining to 6.90 by 36th day. These results suggest that nanoencapsulation helps preserve the flavour profile of probiotic beverages over extended storage periods, making it a beneficial approach for maintaining sensory quality. The slower decline in flavour scores for nanoencapsulated samples indicates better flavour retention, which could enhance consumer acceptance over time. The more rapid decrease in flavour for free cell forms highlights the potential for flavour degradation in probiotic beverages during storage, further underscoring the advantages of nanoencapsulation.

Table. 6.32. Flavour of the beverages containing *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600, in both free cell and nanoencapsulated forms assessed over the storage period.

Day	*L. plantarum* NCDC 685		*L. acidophilus* NCDC 600	
	Free Cell (B1C)	Nanoencapsulated (B1)	Free Cell (B2C)	Nanoencapsulated (B2)
0	7.33 ± 0.71^{aA}	7.23 ± 0.82^{aA}	7.33 ± 0.71^{aA}	7.30 ± 0.60^{aA}
3	7.32 ± 0.75^{aA}	7.22 ± 0.78^{aA}	7.30 ± 0.75^{aA}	7.38 ± 0.58^{aA}
6	7.32 ± 0.70^{aA}	7.20 ± 0.76^{aA}	7.23 ± 0.77^{aA}	7.26 ± 0.74^{abA}
9	7.30 ± 0.75^{aA}	7.18 ± 0.75^{aA}	7.20 ± 0.71^{aA}	7.25 ± 0.65^{abA}
12	7.23 ± 0.68^{aA}	7.15 ± 0.73^{aA}	7.17 ± 0.79^{aA}	7.23 ± 0.63^{abA}
15	7.17 ± 0.65^{aA}	7.10 ± 0.71^{abA}	7.13 ± 0.78^{aA}	7.20 ± 0.61^{abA}
18	7.13 ± 0.68^{aA}	7.07 ± 0.69^{abA}	7.07 ± 0.69^{aA}	7.17 ± 0.59^{abA}
21	6.83 ± 0.70^{bAB}	7.03 ± 0.72^{abAB}	6.73 ± 0.58^{bB}	7.13 ± 0.57^{abA}
24	6.77 ± 0.67^{bA}	6.93 ± 0.74^{abcA}	-	7.07 ± 0.63^{abcA}
27	-	6.83 ± 0.70^{bcA}	-	7.03 ± 0.67^{abcA}
30	-	6.70 ± 0.65^{cA}	-	6.90 ± 0.71^{bcA}
33	-	6.53 ± 0.51^{cB}	-	6.73 ± 0.52^{cdA}
36	-	6.50 ± 0.57^{cA}	-	6.57 ± 0.50^{dA}

Data are expressed as mean ±S.D. (n=30). Two-way ANOVA used for statically analysis.
$^{a-d}$ Mean within column with different superscript are significantly ($p<0.05$) different;
$^{A-B}$ Mean within rows with different superscript are significantly ($p<0.05$) different

6.11.4.4. Bitterness

The sensory evaluation data for bitterness in beverages containing free and nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 indicate distinct trends over the 36-day storage period as given in Table 6.33. Initially, the beverages with both free and nanoencapsulated forms of *L. plantarum* NCDC 685 (B1C and B1) exhibited similar bitterness levels, with scores ranging from 6.80 to 6.93. Over time, bitterness score in the beverage with free cell form (B1C) showed a significant ($p<0.05$) increase, peaking at 7.20 by 18th day, before stabilizing at around 7.13 by 24th day. The beverage with nanoencapsulated *L. plantarum* NCDC 685 (B1) displayed a more consistent bitterness profile, with only a modest increase over time, reaching 7.13 by 27th day, after which a sharp decline to 6.63 was observed on 36th day. In the case of beverage with *L. acidophilus* NCDC 600, the free cell form (B2C) started with slightly lower bitterness scores compared to *L. plantarum*, ranging from 6.77 to 7.16 during the initial 18 days. A significant ($p<0.05$)

increase in bitterness score was observed by 24th day with a score of 6.77. The beverage with nanoencapsulated form (B2) of *L. acidophilus* NCDC 600 showed a gradual increase in bitterness score, similar to *L. plantarum*, reaching 7.07 by 24th day. However, a more pronounced decrease in bitterness was observed in the later days, with scores declining to 6.43 by 36th day. These findings suggest that while nanoencapsulation tends to stabilize bitterness in beverages over time, free cell forms might exhibit more variability in bitterness levels. The increase in bitterness score indicating decrease in bitterness was observed in free cells might be associated with decrease in tannin content due to metabolic activity of the probiotic during storage. Nanoencapsulation appears to mitigate this effect, resulting in a more consistent sensory experience, particularly in the latter part of the storage period.

Table. 6.33. Bitterness of the beverages containing *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600, in both free cell and nanoencapsulated forms assessed over the storage period.

Day	*L. plantarum* NCDC 685		*L. acidophilus* NCDC 600	
	Free Cell (B1C)	**Nanoencapsulated (B1)**	**Free Cell (B2C)**	**Nanoencapsulated (B2)**
0	6.93 ± 0.94^{aA}	6.80 ± 1.03^{aA}	6.77 ± 0.90^{bA}	6.73 ± 0.87^{abcA}
3	6.90 ± 0.95^{aA}	6.83 ± 0.98^{aA}	6.83 ± 0.83^{abA}	6.75 ± 0.90^{abcA}
6	6.96 ± 0.81^{aA}	6.80 ± 0.85^{aA}	6.90 ± 0.84^{abA}	6.77 ± 0.68^{abcA}
9	7.02 ± 0.70^{aA}	6.82 ± 0.75^{aA}	6.95 ± 0.77^{abA}	6.75 ± 0.64^{abcA}
12	7.10 ± 0.66^{aA}	6.88 ± 0.81^{aA}	7.03 ± 0.76^{abA}	6.80 ± 0.66^{abcA}
15	7.13 ± 0.62^{aA}	6.90 ± 0.80^{aA}	7.13 ± 0.78^{aA}	6.83 ± 0.70^{abcA}
18	7.20 ± 0.61^{aA}	6.91 ± 0.81^{aA}	7.16 ± 0.70^{aA}	6.90 ± 0.61^{abA}
21	7.17 ± 0.59^{aA}	7.07 ± 0.83^{aAB}	6.77 ± 0.63^{bB}	6.97 ± 0.72^{abAB}
24	7.13 ± 0.63^{aA}	7.10 ± 0.61^{aA}	-	7.07 ± 0.63^{abA}
27	-	7.13 ± 0.57^{aA}	-	6.91 ± 0.67^{abA}
30	-	7.07 ± 0.69^{aA}	-	6.80 ± 0.66^{abcB}
33	-	6.83 ± 0.59^{aA}	-	6.63 ± 0.55^{bcA}
36	-	6.67 ± 0.54^{aA}	-	6.43 ± 0.50^{cB}

Data are expressed as mean ±S.D. (n=30). Two-way ANOVA used for statically analysis. $^{a-d}$ Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-B}$ Mean within rows with different superscript are significantly different ($p<0.05$) different

6.11.4.5. Overall acceptability

The sensory evaluation data for overall acceptability of beverages containing free and nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 reveal significant trends over the 36-day storage period as given in Table 6.34. The beverages with both *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 initially received high acceptability scores, with minimal differences between free and nanoencapsulated forms, ranging from 7.34 to 7.38 at 0^{th} day. Throughout the first 15 days, the acceptability of both free and nanoencapsulated forms remained relatively stable, with only slight decrease. By 18^{th} day, a notable decline in acceptability was evident, in the free cell forms, where scores dropped to 7.15 for *L. plantarum* NCDC 685 and 7.15 for *L. acidophilus* NCDC 600. The decline became more pronounced beyond 21^{st} day, especially in the beverage with free cell, where, the beverages with *L. plantarum* NCDC 685 in free cell form showed overall acceptability score significantly ($p<0.05$) decreased to 6.97, while beverage with *L. acidophilus* NCDC 600 score significantly ($p<0.05$) decreased to 6.74. Beverages with nanoencapsulated forms maintained better stability, with *L. plantarum* NCDC 685 showing a score of 7.21 and *L. acidophilus* NCDC 600 maintaining a score of 7.15 on 21^{st} day. By the end of the storage period, nanoencapsulated beverages demonstrated significantly ($p<0.05$) higher overall acceptability compared to their free cell counterparts. The scores for beverages with nanoencapsulated *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 remained above 6.50, whereas the beverages with free cell forms of *L. plantarum* NCDC 685 dropped to 6.59 and *L. acidophilus* NCDC 600 to 6.55. These results suggest that nanoencapsulation plays a crucial role in preserving the overall acceptability of probiotic beverages over time. The free cell forms are more susceptible to declines in sensory quality, leading to reduced consumer acceptance as storage time increases. Nanoencapsulation appears to mitigate these effects, maintaining a higher level of overall acceptability, thus highlighting its potential for improving the shelf-life and consumer satisfaction of probiotic beverages.

Table. 6.34. Overall acceptability of the beverages containing *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600, in both free cell and nanoencapsulated forms assessed over the storage period.

Day	*L. plantarum* NCDC 685		*L. acidophilus* NCDC 600	
	Free Cell (B1C)	Nanoencapsulated (B1)	Free Cell (B2C)	Nanoencapsulated (B2)
0	7.38 ± 0.40^{aA}	7.34 ± 0.41^{aA}	7.34 ± 0.36^{aA}	7.30 ± 0.42^{aA}
3	7.37 ± 0.42^{aA}	7.32 ± 0.39^{aA}	7.33 ± 0.41^{aA}	7.29 ± 0.38^{aA}
6	7.37 ± 0.42^{aA}	7.31 ± 0.38^{aA}	7.32 ± 0.33^{aA}	7.27 ± 0.41^{aA}
9	7.37 ± 0.38^{aA}	7.30 ± 0.38^{abA}	7.31 ± 0.35^{aA}	7.25 ± 0.33^{aA}
12	7.34 ± 0.35^{aA}	7.29 ± 0.34^{abA}	7.30 ± 0.33^{aA}	7.24 ± 0.32^{aA}
15	7.31 ± 0.37^{aA}	7.25 ± 0.32^{abA}	7.29 ± 0.33^{aA}	7.22 ± 0.37^{abA}
18	7.15 ± 0.37^{bA}	7.23 ± 0.35^{abA}	7.15 ± 0.28^{bA}	7.20 ± 0.34^{abA}
21	6.97 ± 0.30^{cB}	7.21 ± 0.35^{abA}	6.74 ± 0.24^{cC}	7.15 ± 0.35^{abcA}
24	6.03 ± 0.30^{cB}	7.19 ± 0.31^{abA}	-	7.12 ± 0.35^{abcA}
27	-	7.10 ± 0.30^{bcA}	-	7.04 ± 0.34^{bcA}
30	-	6.99 ± 0.33^{cdA}	-	6.96 ± 0.29^{cdA}
33	-	6.82 ± 0.30^{dA}	-	6.80 ± 0.30^{dA}
36	-	6.59 ± 0.25^{eA}	-	6.55 ± 0.29^{eA}

Data are expressed as mean ±S.D. (n=30). Two-way ANOVA used for statically analysis. $^{a-d}$ Mean within column with different superscript are significantly ($p<0.05$) different; $^{A-B}$ Mean within rows with different superscript are significantly ($p<0.05$) different

6.12. Cost analysis

The estimated cost for a 200 ml bottle of the optimized nanoencapsulated probiotic beverage is Rs. 35.72 as shown in Table 6.36. A significant portion of this cost, Rs. 8.74, was attributed to the nanoencapsulation of the probiotic strain for 2 g of nanoencapsulate as shown in Table 6.35. This includes the cost of κ -carrageenan (Rs. 445/100g), oil (130/1L), Tween 80 (150/500ml), production cost (Rs. 6.00) and strain costs (Rs. 2.00). The cost of the optimized beverage without the nanoencapsulate is Rs. 30.27, where the expenses for refrigerated storage are not factored into the production cost calculation. This includes, pearl millet (Rs. 50/Kg), finger millet (Rs. 90/Kg), buckwheat (Rs. 180/Kg), skimmed milk (Rs. 100/L), Sugar (Rs. 50/Kg), production cost (Rs. 6.00) and packaging

cost (Rs. 5.00). The commercially available millet-based beverages are priced between Rs. 40 to 60 per serving of 200 ml which is significantly higher. The most prominent commercial probiotic, Yakult, is priced at Rs. 20 for the serving size of 60 ml. Despite the cost advantage of Yakult compared to the developed beverage, the latter offers distinct advantages due to its inclusion of nutricereals. These nutricereals enhance the nutritional profile of the beverage, thereby providing additional health benefits. Consequently, the higher price of the developed product is acceptable due to its superior nutritional value and functional attributes.

Table. 6.35. Cost analysis of the developed probiotic nanoencapsulate

Ingredient	Quantity used	Total cost (Rs.)	Cost for quantity used (Rs.)
κ -carrageenan	1g	445/100g	4.45
Oil	1ml	130/1000ml	0.03
Tween 80	100µl	150/500ml	0.03
Production	-	-	6.00
Strain	-	-	2.00
Total	2.86 g (yield)	4.37/g	12.51

Table. 6.36. Cost analysis of the developed probiotic nanoencapsulated nutricereal based probiotic beverage

Ingredient	Quantity used	Total cost (Rs.)	Cost per serving of 200 ml (Rs.)
Nanoencapsulate	2g	4.37/g	8.74
Pearl millet	24g	50/Kg	1.2
Finger millet	4.5g	90/Kg	0.41
Buckwheat	1.5g	180/Kg	0.27
Skimmed milk	80 ml	100/1L	8.00
Sugar	13g	50/Kg	0.65
Production	-	-	6.00
Packaging	-	-	5.00
Total before GST	-	-	30.27
GST	18%	-	5.45
Total beverage cost	-	-	35.72

This study provides a significant potential to influence the development of functional beverages that combine health-promoting properties with recent technological performance. It addresses a critical challenge in probiotic delivery through nanoencapsulation techniques, maintaining cell viability during processing, storage, and gastrointestinal transit. The successful application of nanoencapsulation in beverages formulated from underutilised nutricereals such as finger millet, pearl millet, and buckwheat not only adds nutritional value but also supports the diversification of functional food matrices beyond conventional probiotic products. The advantage of using nutricereals as a base provides include, the rich profile of dietary fiber, essential amino acids, and bioactive compounds, which also supports the growth and protection of probiotics. This aligns with current consumer demand for plant-based, and health-promoting beverages. Furthermore, it contributes to regional food security by utilizing grains that are drought-resistant and locally available. The findings also support the development of more stable probiotic formulations with extended shelf life. This has direct applications in the food industry, where maintaining microbial viability in functional products remains a significant obstacle. The methods and results presented in this study could serve as a guide for optimizing encapsulation processes and scaling them for commercial production. It also establishes the foundation for future research that may examine encapsulation efficiency and release kinetics highlighting the opportunities as well as the factors required for safe and effective application. Through bringing new technology into traditional food substrates, the study provides substantial knowledge in the development of functional foods.

6.13. Conclusion

Probiotics are beneficial microorganisms that promote gut health and overall wellness, but their effectiveness can be compromised by harsh gastrointestinal conditions, such as acidity and bile salts. The present study were done to prepare a formulation and optimization the nutricereal-based beverage made with finger millet, pearl millet, and buckwheat malt. The prepared nanoemulsion with 2% κ -carrageenan and 1 % oil (10% Tween 80 in oil), had the smallest droplet size, lowest PDI, and zeta potential measurement. The FE-SEM image revealed droplets predominantly exhibit a smooth sphere-like shape, which is typical for nanoemulsions. The nanoencapsulated probiotics had adequate encapsulation efficiency for *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600. The viability of nanoencapsulated

L. plantarum NCDC 685 and *L. acidophilus* NCDC 600 had significant decrease, at the bile concentration of 0.3% after 2 hrs of incubation and which were significantly higher than the free cells. The germination conditions were optimised by total phenolic content (TPC), antioxidant, tannin content and prebiotic effect at different temperatures and time. The optimal germination conditions of the nutricereals selected for the study were 26˚C for 72 hrs for finger millet and pearl millet with soaking time of 16 hrs and germination temperature of and buckwheat was 22˚C after 16hrs of soaking and germinating for 72 hrs, while the drying temperature was 60˚C for all three. The optimized ratio of Nutricereal malt extract to skimmed milk was 60:40 with 6.5 g sugar as constant. The optimized beverage showed higher total solids, TPC, adequately lower tannin content, higher prebiotic effect and overall acceptability. The beverages with 1% of nanoencapsulated cells had adequate viable without adversely affecting the texture of the beverages. The results of the storage study demonstrate that nanoencapsulation significantly enhances the viability of *L. plantarum* NCDC 685 and *L. acidophilus* NCDC 600 for up to 36th day, while the beverages with free cells had decrease viability by 24 and 21 days respectively. Therefore, incorporation of germinated cereals enhanced the beverage nutritional profile, making it a valuable addition to the diet for those seeking to improve the gut health through functional foods.

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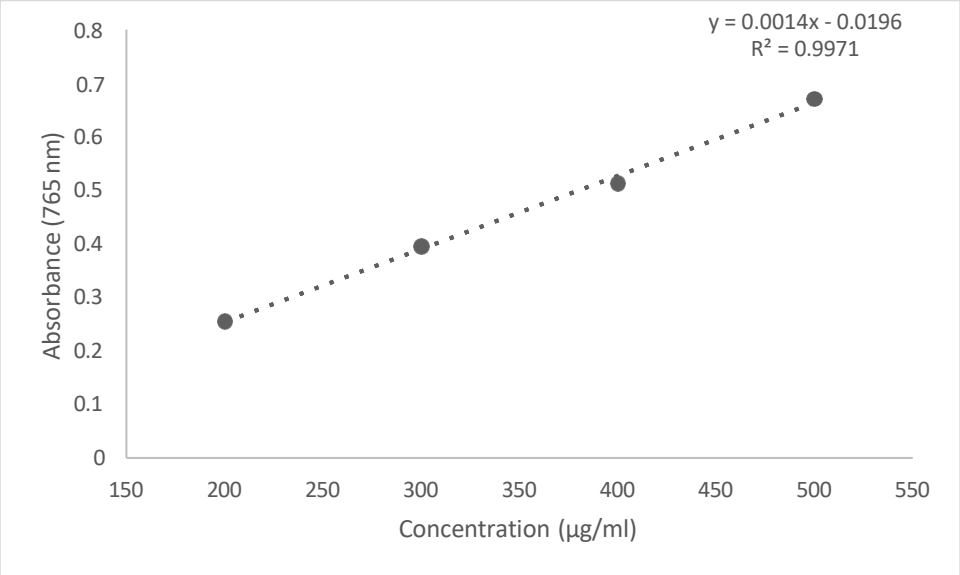
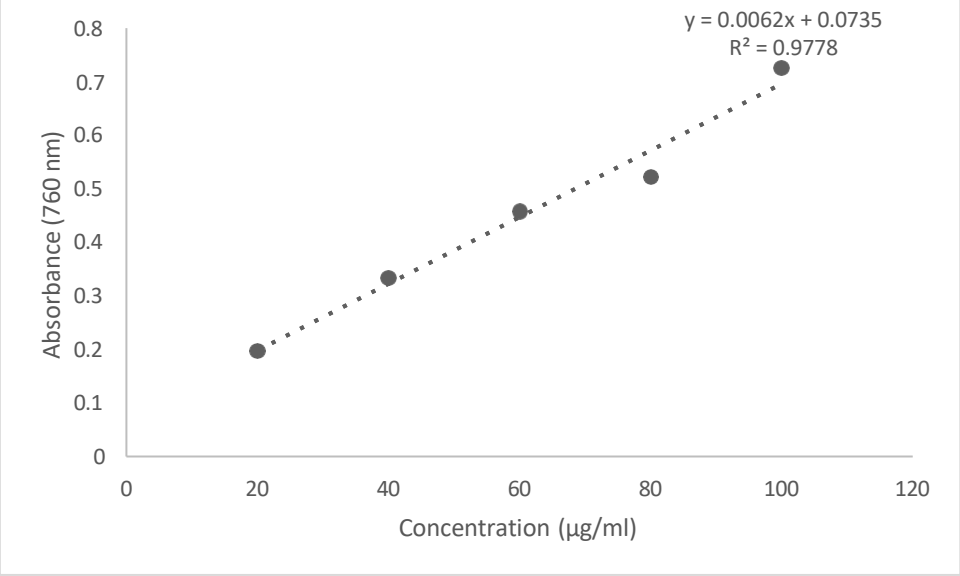
Appendix – I

Standard curve for total phenolic content



Standard curve for tannin content



Appendix – II

t Table

cum. prob	$t_{.50}$	$t_{.75}$	$t_{.80}$	$t_{.85}$	$t_{.90}$	$t_{.95}$	$t_{.975}$	$t_{.99}$	$t_{.995}$	$t_{.999}$	$t_{.9995}$
one-tail	0.50	0.25	0.20	0.15	0.10	0.05	0.025	0.01	0.005	0.001	0.0005
two-tails	1.00	0.50	0.40	0.30	0.20	0.10	0.05	0.02	0.01	0.002	0.001
df											
1	0.000	1.000	1.376	1.963	3.078	6.314	12.71	31.82	63.66	318.31	636.62
2	0.000	0.816	1.061	1.386	1.886	2.920	4.303	6.965	9.925	22.327	31.599
3	0.000	0.765	0.978	1.250	1.638	2.353	3.182	4.541	5.841	10.215	12.924
4	0.000	0.741	0.941	1.190	1.533	2.132	2.776	3.747	4.604	7.173	8.610
5	0.000	0.727	0.920	1.156	1.476	2.015	2.571	3.365	4.032	5.893	6.869
6	0.000	0.718	0.906	1.134	1.440	1.943	2.447	3.143	3.707	5.208	5.959
7	0.000	0.711	0.896	1.119	1.415	1.895	2.365	2.998	3.499	4.785	5.408
8	0.000	0.706	0.889	1.108	1.397	1.860	2.306	2.896	3.355	4.501	5.041
9	0.000	0.703	0.883	1.100	1.383	1.833	2.262	2.821	3.250	4.297	4.781
10	0.000	0.700	0.879	1.093	1.372	1.812	2.228	2.764	3.169	4.144	4.587
11	0.000	0.697	0.876	1.088	1.363	1.796	2.201	2.718	3.106	4.025	4.437
12	0.000	0.695	0.873	1.083	1.356	1.782	2.179	2.681	3.055	3.930	4.318
13	0.000	0.694	0.870	1.079	1.350	1.771	2.160	2.650	3.012	3.852	4.221
14	0.000	0.692	0.868	1.076	1.345	1.761	2.145	2.624	2.977	3.787	4.140
15	0.000	0.691	0.866	1.074	1.341	1.753	2.131	2.602	2.947	3.733	4.073
16	0.000	0.690	0.865	1.071	1.337	1.746	2.120	2.583	2.921	3.686	4.015
17	0.000	0.689	0.863	1.069	1.333	1.740	2.110	2.567	2.898	3.646	3.965
18	0.000	0.688	0.862	1.067	1.330	1.734	2.101	2.552	2.878	3.610	3.922
19	0.000	0.688	0.861	1.066	1.328	1.729	2.093	2.539	2.861	3.579	3.883
20	0.000	0.687	0.860	1.064	1.325	1.725	2.086	2.528	2.845	3.552	3.850
21	0.000	0.686	0.859	1.063	1.323	1.721	2.080	2.518	2.831	3.527	3.819
22	0.000	0.686	0.858	1.061	1.321	1.717	2.074	2.508	2.819	3.505	3.792
23	0.000	0.685	0.858	1.060	1.319	1.714	2.069	2.500	2.807	3.485	3.768
24	0.000	0.685	0.857	1.059	1.318	1.711	2.064	2.492	2.797	3.467	3.745
25	0.000	0.684	0.856	1.058	1.316	1.708	2.060	2.485	2.787	3.450	3.725
26	0.000	0.684	0.856	1.058	1.315	1.706	2.056	2.479	2.779	3.435	3.707
27	0.000	0.684	0.855	1.057	1.314	1.703	2.052	2.473	2.771	3.421	3.690
28	0.000	0.683	0.855	1.056	1.313	1.701	2.048	2.467	2.763	3.408	3.674
29	0.000	0.683	0.854	1.055	1.311	1.699	2.045	2.462	2.756	3.396	3.659
30	0.000	0.683	0.854	1.055	1.310	1.697	2.042	2.457	2.750	3.385	3.646
40	0.000	0.681	0.851	1.050	1.303	1.684	2.021	2.423	2.704	3.307	3.551
60	0.000	0.679	0.848	1.045	1.296	1.671	2.000	2.390	2.660	3.232	3.460
80	0.000	0.678	0.846	1.043	1.292	1.664	1.990	2.374	2.639	3.195	3.416
100	0.000	0.677	0.845	1.042	1.290	1.660	1.984	2.364	2.626	3.174	3.390
1000	0.000	0.675	0.842	1.037	1.282	1.646	1.962	2.330	2.581	3.098	3.300
z	0.000	0.674	0.842	1.036	1.282	1.645	1.960	2.326	2.576	3.090	3.291
	0%	50%	60%	70%	80%	90%	95%	98%	99%	99.8%	99.9%
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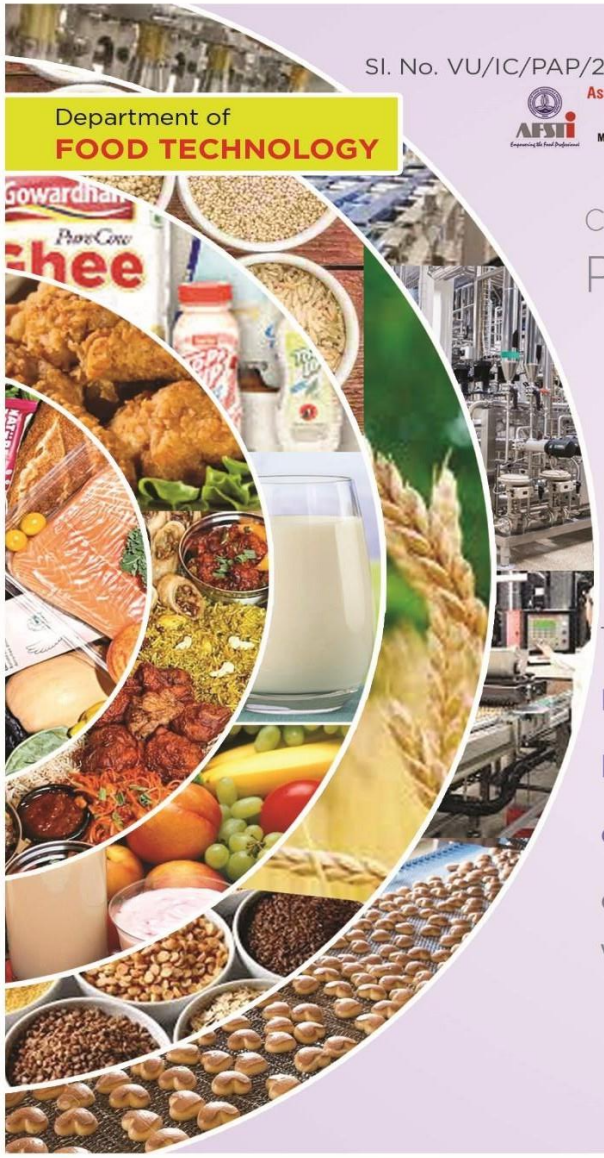

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This is to certify that Mr. / Ms. / Dr. / Prof. **Sneha K** of **Lovely Professional University, Punjab** presented (Paper presentation) on topic **Effect on germination of whole buckwheat grain (Fagopyrum esculentum) at different temperatures and on the antioxidant, total phenolic and tannin content** in International Conference, organized by Department of Food Technology, VFSTR (Deemed to be University) Vadlamudi, Guntur, Andhra Pradesh.

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Title of Abstract. To study the effect of germination of pearl grains (Pennisetum glaucum)

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Appendix – IV

Journal of
Food Chemistry & Nanotechnology

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Research Article

Open Access

To Study the Germination Effect on Finger Millet, Pearl Millet and Buckwheat: It's Impact on Phytochemical Properties and Their Prebiotic Effect

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Published by United Scientific Group

Abstract

Effect of germination temperature on the radicle length, phytochemical properties and prebiotic effect of finger millet, pearl millet and buckwheat were investigated. Germination of grain occurred at T1, T2, and T3 with different time range 24 h to 72 h. Results indicated that finger millet and pearl millet showed significantly ($p < 0.05$) better growth at T3 as compare with T1 and T2, while buckwheat had better growth at T1. Total phenolic content significantly ($p < 0.05$) decreased in finger and pearl millet at T3 (28.62 to 13.73 mg GAE/g and 26.88 to 17.85 mg GAE/g) with increase in time from 24 h to 72 h as compared with T1 and T2 (24.17 to 16.72 mg GAE/g, 30.71 to 26.95 mg GAE/g, and 27.17 to 14.46 mg GAE/g, 26.88 to 17.85 mg GAE/g). At 72 h of germination, it increased with germination time for buckwheat. The antioxidant activity of finger and pearl millet significantly ($p < 0.05$) decreased at T1, T2, T3 with an increase in germination time (24 h to 72 h) for finger millet. Therefore, buckwheat shows the higher trend in DPPH (2,2-Diphenyl-1-picrylhydrazyl) value at different germination temperature T1 and time 24 h, 48 h, 72 h. Significantly, ($p < 0.05$) decreased in tannin content of finger millet and buckwheat but increased for pearl millet with increased germination time and temperature. The prebiotic effect increased with germination time for all grains but decreased with increased germination temperature in buckwheat. These findings suggest that germination temperature directly influences the nutritional properties of cereals.

Keywords

Buckwheat, Finger millet, Grain, Germination, Pearl millet

Introduction

Millets are small seeds grasses from the Poaceae grass family which help in diabetes, celiac diseases, digestive health, and blood pressure whereas, *Eleusine coracana* (finger millet) and *Pennisetum glaucum* (pearl millet) are commonly used [1]. Finger millet is an allotetraploid belonging to the Poaceae family and subfamily of chloridoideae which contains potassium, zinc, iron, phosphorus, calcium, and fiber [2]. Pearl millet is a critical small-grain diploid of the Poaceae and Panicoideae subfamily millet contains carbohydrates, minerals, resistant starch, dietary fiber, energy, fat, crude and quality protein, vitamins A and B, and antioxidants [3, 4].

However, finger and pearl millets are excellent sources of phenolic compo-

Appendix – V

sneha thesis

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This is to certify that **Ms. Sneha K** D/o Sh. S. Kaliyaperumal participated in **Workshop on Food Analysis** organized by Lovely Professional University w.e.f. **March 04, 2024** to **March 08, 2024** and obtained **"O"** Grade.

Date of Issue : 09-05-2024
Place : Phagwara (Punjab), India

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has successfully completed training on the course
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