INTEGRATING INDIGENOUS PLANT RESOURCES IN FUNCTIONAL DAIRY PRODUCTS: A STUDY ON YOGHURT FORTIFIED WITH ALLIGATOR WEED AND FLAVOURED WITH CARDAMOM

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ABSTRACT

Yoghurt is a nutritious dairy product, but often lacks essential micronutrients like iron and vitamin C. *Alternanthera philoxeroides*, a plant rich in bioactive compounds, offers a potential solution for fortifying dairy products. Cardamom, known for its flavour and antioxidant properties, can enhance the sensory appeal of the yoghurt. The study investigates the effects of fortifying yoghurt with *A. philoxeroides* leaf juice at concentrations of 3%, 5%, and 7%, along with cardamom powder (1.5%) on the yoghurt. Yoghurt was prepared with varying concentrations of *A. philoxeroides* leaf juice and fixed cardamom flavouring. The samples were stored at 4°C, and analyses were conducted on Day 1 and Day 7. Proximate composition, antioxidant activity, physicochemical properties, microbial stability, and sensory attributes were evaluated for consumer acceptability. Fortification with *A. philoxeroides* led to a significant ($p < 0.05$) increase in protein, carbohydrate, calcium, and vitamin C content in the fortified samples compared to the control. The 7% fortified yoghurt exhibited the highest nutritional values. Antioxidant activity, assessed by the DPPH method, increased in a dose-dependent manner, with the 7% sample demonstrating the strongest free radical scavenging activity (IC_{50} of 38.57 µg/mL). Physicochemical analysis revealed that the 7% formulation had the highest water holding capacity (94.56%), whereas viscosity peaked at 5% fortification (2437.33 ± 2.08 mPa.s). Syneresis was significantly reduced in the fortified samples, particularly at 5% and 7% fortification. Sensory evaluation showed a marked improvement in flavour attributes such as leafiness and cardamom aroma in the fortified samples, with the 5% formulation demonstrating the best overall sensory acceptability (7.15/10). Fortifying yoghurt with *A. philoxeroides* leaf juice significantly enhances its nutritional composition, antioxidant capacity, and microbiological stability. The 5% fortification provided the best balance of nutritional improvement, sensory appeal, and stability, while the 7% fortification maximized the nutritional and bio-functional properties but compromised texture. These findings suggest that *A. philoxeroides* is a promising functional ingredient for the development of nutritionally enhanced dairy products, particularly for addressing micronutrient deficiencies in regions where this plant is locally available.

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ABBREVIATION

AP: *Alternanthera philoxeriodes*

C: Control

TSS: Total Soluble Solids

ANOVA: Analysis of Variance

CFU/g: Colony Forming Unit/gram

FAO: Food and Agriculture Organization

FDA: Food and Drug Administration

FTNS: Food Technology and Nutritional Science

MBSTU: Mawlana Bhashani Science and Technology University

NA: Nutrient Agar

PDA: Potato Dextrose Agar

SD: Standard Deviation

SPC: Standard Plate Count

TFC: Total Fungal Count

TPC: Total Plate Count

TVC: Total Viable Count

USA: United States of America

USDA: United States Department of Agriculture

CHAPTER-01

INTRODUCTION

CHAPTER 01: INTRODUCTION

1.0 INTRODUCTION

1.1 Background of the study

Yoghurt is a cultured dairy product produced through the fermentation of milk by lactic acid bacteria, and it holds a significant position among the most widely consumed fermented dairy foods worldwide (Mbaeyi-Nwaoha et al., 2024). According to the regulations established by the United States Food and Drug Administration (USFDA), yoghurt is defined as a food product resulting from the fermentation of dairy ingredients—such as cream, milk, partially skimmed milk, or skim milk—used individually or in combination, with a defined bacterial culture primarily consisting of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Mbaeyi-Nwaoha et al., 2024). This fermentation process not only enhances the shelf life and sensory qualities of the product but also contributes to its notable nutritional profile. Yoghurt is regarded as a valuable source of high-quality proteins, essential minerals, and health-promoting lactic acid bacteria. Moreover, it is particularly distinguished by its enhanced digestibility compared to milk, a characteristic shared with other fermented dairy products (Jakopovic et al., 2022).

In continuation of its nutritional significance, recent advancements have focused on enhancing the health benefits of yoghurt through the incorporation of plant extracts, which serve as rich sources of functional compounds such as phenolics and bioflavonoids. This approach has attracted increasing interest, particularly because milk and conventional dairy products are inherently low in these bioactive, health-promoting components (Barukci et al., 2022). The formulation of innovative functional dairy products typically includes the enrichment of yoghurt with probiotic microorganisms, prebiotic fibers, oligosaccharides, conjugated linoleic acid, omega-3 fatty acids, phytosterols, essential minerals, vitamins, and bioactive peptides (Barukci et al., 2022). In the context of public health, fortified yoghurt is steadily gaining popularity in Bangladesh as a strategic nutritional intervention aimed at combating micronutrient deficiencies, especially among children (Pramanik et al., 2025). Furthermore, the incorporation of such additives into yoghurt remains one of the most straightforward yet impactful methods to modify and enhance its physicochemical and functional properties (Abdullah et al., 2023).

Among the potential plant-based ingredients for functional food development, *A. philoxeroides* (Mart.) Griseb., commonly known as alligator weed or locally referred to as 'Malancha shak' or 'Henchi shak' in Bangladesh, offers notable promise (Suraiya et al., 2023). Belonging to the family Amaranthaceae (Nahar et al., 2022), this leafy vegetable is an immersed aquatic plant native to South America. It is characterized as a sprawling herb that typically grows in water, with pinkish stems that may become hollow as they enlarge, reaching lengths of up to 3.3 feet. The leaves are oppositely arranged, narrowly elliptic to spatulate in shape, extending up to 9 cm, sometimes featuring a few indistinct marginal teeth. The plant produces small, bisexual flowers in round white heads on long stalks arising from the upper leaf axils, with each flower containing 4–5 papery bracts, five stamens, and a single pistil. The fruit is a tiny, one-seeded, thin-walled structure (Deb et al., 2018). Traditionally, *A. philoxeroides* has been consumed as a leafy vegetable and used in folk medicine for managing conditions such as hazy vision, night blindness, malaria, postnatal ailments, diarrhea, dysentery, and puerperal fever (Suraiya et al., 2023). It is a rich source of amino acids and contains a considerable amount of total nitrogen (Puro et al., 2025), as well as various phytochemicals, including glycosides, tannins, flavonoids, and saponins, with medicinal potential (Puro et al., 2025). Additionally, its high iron content allows it to be consumed in salads, further supporting its nutritional relevance (Suraiya et al., 2023). Considering that yoghurt is inherently rich in protein and calcium but lacks adequate levels of certain essential micronutrients such as iron and vitamin C, the incorporation of *A. philoxeroides* into yoghurt could offer a synergistic nutritional enhancement.

Spices have long been valued not only for their ability to enhance the flavour and shelf life of food but also for their diverse therapeutic properties. A wide range of spices exhibit significant biological activities, including anti-diabetic, antibacterial, analgesic, antioxidant, and anticancer effects, in addition to inhibiting microbial spoilage of food products (De et al., 1999). Among these, cardamom seed (*Elettaria cardamomum* (L.) is particularly esteemed in both the culinary and pharmaceutical industries for its distinct and rich aroma, which is primarily attributed to oxygenated mono-terpenoids such as α-terpinyl acetate and 1,8-cineole, key constituents of cardamom essential oil (Hamdy et al., 2025). Traditionally used as a condiment, cardamom is also a natural source of flavonoids like quercetin, pelargonidin, and luteolin, which contribute to its antioxidant potential. Additionally, cardamom has been shown to effectively reduce blood pressure and enhance fibrinolysis in patients with stage 1 hypertension, without impacting blood lipid or fibrinogen levels (Verma et al., 2009; Abdullah

et al., 2023). In the present study, cardamom is employed as a natural flavouring agent to enhance the sensory appeal of the yoghurt and to mask any strong herbal undertones imparted by *A. philoxeroides*.

The present study aims to develop a functional yoghurt by incorporating juice from *Malancha* (*A. philoxeroides*) and enhancing its sensory appeal with cardamom, a widely used natural flavouring agent, to increase consumer acceptability. In the context of Bangladesh, where micronutrient deficiencies remain a significant public health concern, especially among vulnerable groups, there is a growing need for affordable, nutrient-dense functional foods. Yoghurt, although nutritionally rich in protein and calcium, lacks sufficient levels of certain micronutrients and bioactive compounds. The integration of *A. philoxeroides*, a locally available but underutilized leafy vegetable with notable medicinal and nutritional properties, offers an innovative approach to enhance the functional value of yoghurt. While both *A. philoxeroides* and cardamom are traditionally known for their health-promoting effects, their combined application in functional dairy products has not yet been investigated in Bangladesh.

This study seeks to fill this research gap by formulating and assessing the functional yoghurt against a control sample, with a comprehensive evaluation of its antioxidant potential, physicochemical properties, nutritional profile, microbiological quality, and sensory characteristics during storage.

1.2 Problem Statement

Conventional yoghurt, while beneficial as a probiotic-rich dairy product, lacks sufficient levels of natural antioxidants and bioactive compounds necessary for combating oxidative stress and related health issues. Although plant-based fortification is a promising solution, there is limited research on the incorporation of underutilized local plants such as *A. philoxeroides* into yoghurt. Furthermore, the functional effects of combining *A. philoxeroides* with natural flavouring agents like cardamom have not been extensively studied. This gap in knowledge hinders the development of accessible, affordable, and nutritionally enhanced functional dairy products using locally available ingredients.

1.3 Justification of the Study

In the face of growing global challenges such as malnutrition, hidden hunger, climate change, and food insecurity, there is an urgent need for sustainable, affordable, and nutritionally enhanced food solutions. Millions of people, especially in developing countries like

Bangladesh, still suffer from micronutrient deficiencies, iron-deficiency anemia, and poor dietary diversity. The COVID-19 pandemic in the recent past has exacerbated this situation, with forecasts for 2030 suggesting that the current efforts are far from sufficient to eradicate malnutrition within the next decade (FAO et al., 2021). Furthermore, food systems encounter significant obstacles due to environmental degradation, loss of resources, influence of climate change, emissions of greenhouse gases, and population expansion (Mila et al., 2024). Addressing these issues requires innovative approaches that utilize local, nutrient-dense food sources to develop accessible and functional food products.

Yoghurt, as a widely accepted fermented dairy product, offers numerous health benefits due to its probiotic content. However, its nutritional profile can be significantly improved by fortifying it with plant-based ingredients rich in antioxidants, polyphenols, and essential micronutrients. This opens the door to functional food innovation aimed at both preventive healthcare and improved nutrition.

A. philoxeroides (Mart.) Griseb (Family Amaranthaceae), commonly known as alligator weed, is used in Chinese folk medicine for the treatment of acute brain fever, measles, and herpes zoster (Fang et al., 2009). The decoction of this plant is also used for the treatment of anemia in India (Purkayastha et al., 2005). This plant is also used for hazy vision, night blindness, malaria, post-natal complaints, diarrhea, dysentery, and puerperal fever in Rajshahi, Bangladesh (Rahman et al.,2014). Despite its abundance and therapeutic potential, it remains largely ignored in mainstream food product development and scientific research. Incorporating this locally available vegetable into yoghurt not only enhances its functional properties but also promotes dietary diversity and the utilization of indigenous resources.

This study directly aligns with several United Nations Sustainable Development Goals (SDGs):

SDG 2: Zero Hunger – by contributing to the development of affordable, nutrient-rich functional foods to combat undernutrition and micronutrient deficiencies.

SDG 3: Good Health and Well-being – by addressing health through food-based solutions that reduce the risk of non-communicable diseases.

SDG 12: Responsible Consumption and Production – through the sustainable use of local, natural plant resources in food manufacturing.

SDG 13 & 15: Climate Action & Life on Land – by promoting the use of hardy, climate-resilient, and biodiversity-supporting crops like *A. philoxeroides* that grow without requiring intensive agricultural inputs.

Additionally, the use of cardamom in the formulation enhances the product's sensory properties and offers further antioxidant benefits, contributing to the product's acceptability and health impact.

By utilizing underexplored plant species and integrating them into familiar food systems like yoghurt, this research supports sustainable nutrition, local agriculture, and food innovation. It also contributes to global food security by reducing dependence on imported or industrially processed additives and promoting resilience through local food systems.

Thus, this study is not just a step toward developing a novel functional yoghurt—it is a strategic, health-driven, and sustainability-focused initiative that bridges traditional knowledge with modern nutritional science, benefiting both consumers and local communities.

1.4 Research Objectives

1.4.1 General objective

To develop and comparative analysis of control yoghurt and functional yoghurt fortified with *A. philoxeroides* leaf juice and flavoured with cardamom, focusing on its nutritional, physicochemical, antioxidant, and sensory appeal.

1.4.2 Specific objective

To formulate yoghurt with varying concentrations of *A. philoxeroides* leaf juice while incorporating cardamom as a flavouring agent.

1. To investigate key physicochemical parameters, including pH, titratable acidity, syneresis, water holding capacity and rheological behavior of yoghurt samples.
2. To determine the proximate and micronutrient composition, with emphasis on protein, lipid, carbohydrate, ash, moisture, vitamin C, calcium and iron content of yoghurt samples and leaves of *A. philoxeriodes*.
3. To assess the antioxidant activity of the yoghurt samples and leaves of *Al. philoxeriodes.*
4. To assess the microbiological test of the yoghurt samples and juice of Malancha (*A. philoxeriodes*).

5. To conduct a comprehensive sensory evaluation to determine consumer perception and acceptability.
6. To elucidate the functional potential of *A. philoxeroides* as a sustainable and underutilized bioresource for advancing food security and nutritional resilience.

CHAPTER-02

LITERATURE REVIEW

CHAPTER 02: LITERATURE REVIEW

2.0 REVIEW OF LITERATURE

2.1 Rediscovering an Invasive Weed: The Untapped Potential of *A. philoxeroides*

A. philoxeroides exhibits strong nutritional and pharmaceutical potential, spanning antimicrobial, antioxidant, neuroprotective, and environmental clean-up roles.

Prasad (2018) emphasized its value as a source of dietary fiber, essential minerals like calcium, iron, potassium, and various sterol compounds.

Table 2.1: Based on data from Serdiati et al. (2024), the dry weight nutritional composition is presented below:

Nutrient	**Content per 100g(dry weight)**
Proximate Composition	
Crude Protein	18.5g
Crude Fat	3.2g
Crude Fibre	21.0 g
Ash	14.7g
Carbohydrates	42.6g
Minerals	
Calcium (Ca)	1200mg
Phosphorusv (P)	310mg
Potassium (K)	2350mg
Magnesium (Mg)	320mg
Sodium (Na)	45mg
Iron (Fe)	35mg
Zinc (Zn)	4.8mg
Vitamins	
Vitamin A	1450IU
Vitamin C	35mg
Vitamin E	3.1mg
Amino Acids	
Lysine	1.2g
Methionine	0.4g
Threonine	0.9g
Valine	1.0g
Fatty Acids	
Linoleic Acid (Omega-6)	1.1g
α-Linolenic Acid (Omega-3)	0.3g

In a study by Suraiya et al. (2023), three aquatic weeds—Helencha (*Enhydra fluctuans*), Malancha (*A. philoxeroides*), and kalmi (*Ipomoea aquatica*)—were used to produce edible

paper sheets. Among these, AP was the most nutritious. It showed that Crude protein was 21.66%, Lipid content was 2.72%, Carbohydrate content was 60.89%, and Calcium was 442.85 ± 4.53 mg/100g. The plant was rich in amino acids such as glutamic acid (20.86%), serine (8.28%), aspartic acid (7.82%), and arginine (6.52%). Essential amino acids like leucine (4.3%), isoleucine (4.24%), and lysine (3.88%) were also present. It contained approximately 24% total nitrogen, reinforcing its potential as a valuable dietary source (Suraiya et al., 2023). Further, Suraiya et al. (2024) developed edible packaging sheets (EPSs) from *A. philoxeroides* and *Hypophthalmichthys molitrix* (silver carp), exploring sustainable food-packaging alternatives. Farooq et al. (2021) studied whole plant fodder (excluding roots) and found nitrogen (1.8–2.3%), phosphorus (1.5–2.0%), potassium (1.6–2.1%), copper (5.9–9.2 mg/kg), iron (39.8–99.9 mg/kg), zinc (18.5–31.4 mg/kg), manganese (34.4–64.5 mg/kg), ash (15.1–29.8%), and fat (2.3–3.6%). The plant's nutritive value is comparable to alfalfa, sorghum, and maize

Recent investigations into invasive plant utilization have highlighted *A. philoxeroides* as a rich source of bioactive compounds. According to Zhang et al. (2023), this aquatic weed contains significantly higher polyphenol concentrations (32.7 mg GAE/g) than many cultivated leafy vegetables, including spinach (24.1 mg GAE/g) and kale (28.9 mg GAE/g). Chen et al. (2022) identified its dominant flavonoid, kaempferol-3-O-glucoside, which demonstrated 40% stronger free radical scavenging activity compared to common dietary flavonoids. Serdiati et al. (2024) further identified multiple phytochemicals with therapeutic potential, as given below:

Table 2.2: Phytochemical Composition (per 100g dry weight)

Phytochemical	**Content (g/100g)**
Alkaloids	0.65
Flavonoids	2.35
Tannins	1.20
Saponins	1.75
Phenolic Compounds	3.80
Terpenoids	0.95
Glycosides	0.50
Steroids	0.45
Coumarins	0.30
Anthocyanins	0.20

Puro et al. (2025) reported the total phenolic content of AP as 2.23 ± 0.32 mg GAE/g, and the flavonoid content as 1.44 ± 0.19 mg QE/g in fresh samples. Phytochemical analyses identified substantial quantities of flavonoids (2.35 g/100g) and phenolic compounds (3.80 g/100g).

Antioxidant activity was measured via the DPPH assay, with methanol extracts showing the highest inhibition (79.4% at 10 mg/mL). Despite promising findings, limitations exist. These include geographical constraints affecting generalizability and short-term duration, which does not assess long-term impacts (serdiati et al., 2024)

In a study by Zhang et al. (2020), methanolic extracts of AP exhibited strong activity in both ABTS radical cation decolourization and Ferric Reducing Antioxidant Power (FRAP) assays. These results confirmed the electron-donating ability and total antioxidant capacity of the extract. Li et al. (2022) reported that extracts of AP significantly inhibited lipid peroxidation in egg yolk and linoleic acid models, showing a comparable effect to α-tocopherol. This indicates potential for food preservation or nutraceutical applications

Serdiati et al. (2024) showed antioxidant activity Using the DPPH method, antioxidant activity was compared with Vitamin C as the standard:

Table 2.3: Antioxidant Activity (% DPPH Inhibition)

Sample	Dose (mg/mL)	Solvent	% Inhibition
Alligator Weed Extract	1	Water	30.5
	5	Water	55.2
	10	Water	68.7
	1	Methanol	40.3
	5	Methanol	63.8
	10	Methanol	79.4
	1	Ethanol	35.1
	5	Ethanol	58.4
	10	Ethanol	72.5
Vitamin C (Standard)	1	Water	85.6
	5	Water	92.3
	10	Water	95.1

Its antimicrobial properties are equally noteworthy. Gupta et al. (2021) observed inhibition zones of 18.2 mm against *Staphylococcus aureus*, surpassing green tea extracts (12.4 mm). However, Li et al. (2022) cautioned that the plant's high saponin content (12.3%)—though pharmacologically valuable—can interact with dairy proteins, potentially altering the texture of food products. Akbar et al. (2021) tested organic extracts of *A. philoxeroides* against phytopathogens (*Erwinia carotovora, Ralstonia solanacearum, Xanthomonas axonopodis*). The n-hexane leaf extract showed an inhibition zone diameter of 28.1 mm against *R. solanacearum*, while penicillin produced 48mm.

Rahim et al. (2025) explored the phytochemical and biological properties of methanol and ethanol extracts from *Digera muricata*, *Trianthema portulacastrum*, and *A. philoxeroides*. The methanol extract of AP demonstrated strong cytotoxicity (LC50: 13.89 ± 1.33μg/mL, $p < 0.05$), underscoring its potential in food chemistry. In poultry research, dietary supplementation with 1% AP significantly reduced oxidative stress and enhanced immune responses under cold stress conditions (Roy et al., 2019). This suggests its utility in functional feeds and veterinary applications.

2.2 Taxonomic Classification and Morphology

Family: Amaranthaceae (**Order:** Caryophyllales)

Growth habit: Perennial amphibious herb with hollow stems (5-10 mm diameter) and lanceolate leaves (3-10 cm length) (Sainty et al., 2018)

Reproductive biology: Predominantly clonal propagation with <2% seed viability in non-native ranges (Geng et al., 2020)

2.3 Cardamom Phytochemistry and Flavour Modulation

Elettaria cardamomum, or cardamom, exhibits a sophisticated flavour profile with various functional advantages:

Volatile Profile:

GC-MS analysis has shown that the predominant volatile compounds in cardamom are 1,8-cineole (34.7 ± 2.1%) and α-terpinyl acetate (25.3 ± 1.8%) (Nair et al., 2023). These volatile oils, along with additional bioactive compounds in cardamom seeds, are responsible for its characteristic fragrance and contribute to its role as a functional food, as well as its pharmaceutical and nutraceutical value (Hamzaa & Osman, 2012). Mani et al. (2017) analyzed the constituents of cardamom oil during distillation, finding that different fractions collected during the process contained key volatile compounds, which are essential for flavour and fragrance applications. These results are significant for industries that utilize cardamom oil to enhance product aromas. Anwar et al. (2016) discussed the properties of cardamom oils in food preservation, showing that the oil possesses antimicrobial properties and can be effectively used to improve food safety and flavour, extending the shelf life of food products. Amma et al. (2010) studied the chemical composition and antioxidant activity of various cardamom varieties, discovering that the spices contained high levels of flavonoids and phenolic compounds, which contributed significantly to their radical scavenging activity and antioxidant

potential, indicating their usefulness in combating oxidative stress. Omanakutty and Joy (2007) focused on the retention of cardamom's fresh flavour through cold grinding, finding that this method preserved the volatile compounds responsible for its aroma better than traditional grinding methods.

Bitterness Masking:

Molecular dynamics simulations indicate that hydrophobic interactions (ΔG = -8.2 kcal/mol) between α-terpinyl acetate and bitter phytochemicals play a role in masking bitterness, improving the overall flavour experience (Patel & Menon, Journal of Agricultural and Food Chemistry, 2023).

Health Benefits:

Cardamom seeds are known for their diverse biological functions, including antioxidant, antihypertensive, antidiabetic, gastroprotective, laxative, antispasmodic, antibacterial, antiplatelet aggregation, and anticancer properties (Marongiu et al., 2004; Padmakumari et al., 2010). Clinical studies (n=45) have shown that cardamom accelerates gastric emptying by 18% ($p<0.01$) through ultrasound analysis (Kumar et al., 2022). Research by Heydarian et al. (2023) revealed that cardamom significantly reduced inflammation markers such as hs-CRP (SMD: −0.60 mg/dL), IL-6 (WMD: −1.25 mg/dL), and TNF-α (WMD: −2.10 kg; $p<0.001$), as well as systolic (WMD: −0.54 mmHg) and diastolic blood pressure (WMD: −0.90 mmHg).

A 2021 clinical trial involving women with obesity and prediabetes, who took 3g of cardamom daily for two months, showed a significant reduction in total cholesterol and LDL cholesterol while maintaining HDL cholesterol. According to the United States Department of Agriculture (USDA)Trusted Source Nutritional Profile (per 1 tablespoon of ground cardamom):

- Calories: 18 kcal
- Total Fat: 0.4 g
- Carbohydrates: 4.0 g
- Fiber: 1.6 g
- Protein: 0.6 g

Rajathi et al. (2017) reviewed the processing and medicinal uses of cardamom, confirming its anti-inflammatory, antioxidant, and digestive health benefits, with numerous therapeutic

properties backed by traditional and modern scientific findings. Nagashree et al. (2017) examined the anti-hypercholesterolemic effects of cardamom in experimental rats and found that cardamom supplementation significantly reduced cholesterol levels, suggesting its potential as a natural treatment for managing cholesterol. Joshi et al. (2013) analyzed the essential oil of large cardamom grown in different agroclimatic zones and found substantial variations in the chemical composition of the oil, which could influence both its medicinal and flavour properties, indicating that the growing environment plays a crucial role in cardamom's characteristics. Lastly, Baby and Ranganathan (2016) investigated the effect of enzyme pre-treatment on cardamom oil extraction, revealing that enzyme treatment improved both the yield and quality of the oil, making this method more efficient for industrial applications. Overall, these studies highlight cardamom's versatility and health benefits, showcasing its use in food, fragrance, and medicinal applications, while also emphasizing the importance of processing techniques to optimize its properties.

2.4 Advanced Yoghurt Fortification Technologies and recent innovations in dairy fortification

Probiotic yoghurt, recognized for its health benefits, has been increasingly enriched with various functional ingredients that improve its nutritional profile, antioxidant properties, shelf life, and sensory characteristics. This literature review summarizes studies on the incorporation of different types of ingredients in probiotic yoghurt, focusing on their effects on microbiological, sensory, physicochemical, and antioxidant properties.

Farooq et al. (2023) studied the use of whey protein-pectin complexes improved the stability of polyphenols in probiotic yoghurt, increasing their shelf life from 7 to 21 days. This enhancement is beneficial in maintaining the antioxidant properties of the yoghurt over time. Pourghorban et al. (2021) produced yoghurt samples that were enriched with different concentrations of olive leaf powder (OLP) and its extract (OLE). The results showed that the incorporation of OLP or OLE increased the shelf life, antioxidant activity percentage (AA%), and total phenol content (TPC). Over 21 days, TPC, AA%, and pH decreased, while titratable acidity (TTA) increased, indicating the effects of OLP and OLE on the product's preservation and quality.Taha et al. (2024) studied nano-powders derived from fruit wastes such as apricot and peach kernels, watermelon rind, and banana peel were added to yoghurt at 0.5% concentration. The fortification process significantly increased the total solids, fat, protein, and

ash content, with apricot and peach kernels notably elevating fat and protein by 3.46% and 3.70%, respectively, compared to the control sample. These ingredients were positively impacted nutritional value and antioxidant activity of yoghurt. Jakopovi et al. (2022) enriched yoghurt with moringa extract (ME) at concentrations of 1%, 3%, and 4% showed improvements in acidity, microbiological parameters, syneresis, and water holding capacity compared to control yoghurt. ME addition also decreased syneresis while enhancing water retention, improving the rheology and sensory properties of the yoghurt. **(**Kasapidou et al. (2025) incorporated processed (hydrolyzed) and unprocessed (non-hydrolyzed) orange peels into the diets of lactating ewes were investigated to examine the influence on yoghurt's composition. The study found that yoghurt produced from ewes fed orange peels had higher protein and fat contents (5.93% and 6.79% for processed, 5.53% and 6.24% for unprocessed) compared to the control diet (5.42% and 6.06%), suggesting that orange peels positively influence yoghurt's nutritional content. Addition of green pepper extract at concentrations of 100, 200, and 300 ppm to yoghurt was evaluated for its impact on various properties. The results indicated that increasing the amount of extract improved antioxidant activity, phenolic compounds, and sensory properties, enhancing yoghurt's health benefits and sensory appeal (Kovsari et al., 2024). Incorporation of 2% wolfberry dietary fiber into yoghurt improved the product's texture by reducing syneresis and increasing viscosity and hardness compared to control yoghurt. This suggests that wolfberry fiber can enhance both the sensory attributes and stability of probiotic yoghurt (Fan et al., 2023). Studies have found that peaches and apples are the most suitable fruits for enriching probiotic yoghurt. Furthermore, dragon fruit and orange sweet potato purees were also incorporated into yoghurt, and consumer acceptance was positive, indicating that fruit-based additions can enhance the taste and nutritional profile of probiotic yoghurt (Atik et al., 2021). Mixing 5% gobdin to yoghurt containing Lactobacillus acidophilus resulted in a product that was deemed acceptable by consumers. This study shows that the addition of certain natural ingredients can improve the sensory qualities and acceptability of probiotic yoghurt (Ertem et al., 2018). Fortification of yoghurt with pomegranate juice and probiotics enhanced sensory attributes, such as colour intensity, carrot flavour, creaminess, mouth coating, and chalkiness. These changes resulted in an improved overall sensory profile, making the yoghurt more appealing to consumers (Al-Aswad et al., 2018).Walnut oil was encapsulated in nano/microcapsules and incorporated into yoghurt. This addition resulted in improved nutritional properties, such as increased polyunsaturated fatty acids (PUFAs) content, making walnut oil a valuable ingredient for fortifying yoghurt (Turek et al., 2023).Carrot juice was added to skimmed milk before fermentation to produce carrot-

enriched yoghurt. The study evaluated the effect of carrot juice on lactic acid content, chemical composition, syneresis, texture, and rheology. The addition of carrot juice contributed to the formation of a firm structure in the yoghurt, thanks to the interaction between milk proteins and the dietary fiber in carrot juice (Bo et al., 2023). The addition of seaweed to yoghurt was studied for its impact on physicochemical, functional, and sensory properties. Two species of brown macroalgae, *Saccharina latissima* (both blanched and unblanched) and *Alaria esculenta*, were added in concentrations of 0.25%, 0.50%, 0.75%, and 1%, both in flakes and powder forms. Seaweed incorporation improved yoghurt's texture, flavour, and antioxidant properties, suggesting that seaweed could be a promising functional ingredient (Rodríguez et al., 2024).Beetroot puree was added to yoghurt at concentrations of 2%, 2.03%, and 8%, and its effects on yoghurt's viscosity and colour were evaluated. The incorporation of beetroot puree slightly influenced the viscosity, but it significantly impacted the colour of the yoghurt. A consumer acceptance test showed that yoghurt with 2.03% beetroot puree had the highest acceptance (7.42), followed by the control sample (7.28). The 8% beetroot puree formulation was the least preferred (6.08) (Adjei et al., 2024).Also, Salehi et al. (2021) explored the addition of common purslane extract to yoghurt, which improved its antioxidant activity while maintaining its physicochemical properties such as pH and acidity. The sensory evaluation showed that the fortified yoghurt was well-accepted, making purslane a promising ingredient for functional yoghurt production. Ahmad et al. (2020) focused on fortifying probiotic yoghurt with apple peel polyphenol extract. The study found a significant increase in the yoghurt's antioxidant capacity and stability, without negatively affecting its probiotic content or sensory properties. The apple peel extract helped maintain higher levels of probiotics during storage, making it a beneficial addition to probiotic yoghurt. Ani et al. (2018) investigated plant-based yoghurt made from bambaranut, soybean, and Moringa oleifera seed milks. These plant-based yoghurts showed good physicochemical and microbiological stability. Sensory testing revealed that bambaranut and soybean-based yoghurts were better accepted compared to Moringa, which had a stronger flavour. All variants exhibited good storage stability. Lastly, Hamed et al. (2021) fortified buffalo yoghurt with peanut skin extract powder. This fortification increased the antioxidant activity and slightly improved the texture, while the sensory evaluation indicated good acceptability. The extract also helped maintain the yoghurt's antioxidant properties during storage. Alwazeer et al. (2020) investigated how plant extracts influenced the acidification and reducing capacities of yoghurt bacteria, finding that certain extracts enhanced the fermentation process, potentially improving yoghurt's functional benefits. Bulut et al. (2021) examined the effects of plant extracts on the physicochemical, rheological, textural, and

sensory properties of set-type yoghurt during storage. They found that fortification did not negatively impact these properties and, in some cases, improved the texture, firmness, and overall sensory acceptability of the yoghurt. Hasneen et al. (2020) compared different herbs for fortifying skimmed milk yoghurt and Kariesh cheese, highlighting their positive impact on antioxidant activity and overall nutritional value. Shokery et al. (2017) studied the effects of green tea and Moringa leaf extracts, noting significant improvements in antioxidant properties, texture, and sensory qualities, making the yoghurt more appealing and stable during storage. Dhawi et al. (2020) found that fortifying buffalo yoghurt with fenugreek and Moringa oleifera flours increased antioxidant and antibacterial activities, as well as mineral content, enhancing the yoghurt's health benefits. Finally, El-Said et al. (2014) demonstrated that fortifying stirred yoghurt with pomegranate peel extract improved both antioxidant activity and physical properties, making the yoghurt nutritionally richer.

In summary, fortifying yoghurt with natural plant extracts, such as purslane, apple peel, peanut skin, and others, resulted in enhanced antioxidant properties, maintained sensory qualities, and stable storage, offering potential for creating functional dairy products with additional health benefits.

2.5 Challenges in Yoghurt Fortification

Fortifying plant-based yoghurt with additional nutrients has proven to be technically challenging. O'Connor et al. (2023) found that adding 2% moringa powder increased the iron content by 35%, but it negatively impacted the yoghurt's viscosity and mouthfeel. Similarly, spirulina fortification at 1% improved the protein content but was rejected by consumers due to its unnatural colour (Santos et al., 2023). In another study, Kumar et al. (2022) observed that adding chia seeds to plant-based yoghurt increased omega-3 content but caused undesirable changes in texture, making the yoghurt too gritty. Moreover, Patel et al. (2021) explored the use of turmeric powder to enhance antioxidant levels, yet the resultant product was unappealing due to the strong, overpowering flavour. These examples underline the critical challenge of optimizing both nutritional enhancements and sensory attributes to ensure consumer acceptance while achieving the desired health benefits.

CHAPTER-03

MATERIALS AND METHODS

CHAPTER 03: MATERIALS AND

3 MATERIALS AND METHODOLOGY

3.1 Study Design

This research employed an experimental approach under a completely randomized design to formulate and evaluate functional yoghurt enriched with *A. philoxeroides* leaf juice. Multiple formulations, including a control, were developed and stored at 4 ± 1°C for 7 days to simulate short-term refrigerated storage.

Comprehensive assessments were conducted on Day 0 and Day 7 to monitor quality changes. The evaluation encompassed physicochemical properties, nutritional attributes, functional properties, microbiological tests, and sensory acceptability. All analyses were performed in triplicate, and data were statistically interpreted to identify significant effects of formulation and storage.

3.2 Study Area

The present analytical study was conducted at the General Laboratory of the Department of Food Technology and Nutritional Science (FTNS) of Mawlana Bhashani Science and Technology University, Tangail-1902, Bangladesh. The sample analysis was conducted at the Food Processing Laboratory of the Department of Food Technology and Nutritional Science (FTNS), Mawlana Bhashani Science and Technology University, Tangail-1902, Bangladesh.

3.3 Duration of study

The duration of the study period was 11 months from September 2024 to July 2025, which includes sample collection, laboratory analysis, sensory analysis, and paper writing.

3.4 Materials

3.4.1 Raw Materials

Primary raw materials utilized in this study that are collected from the local market of Tangail city, such as,

1. Fresh cow whole Milk.

2. *A. philoxeroides* leaf juice.

3. Starter culture (commercial yoghurt as the inoculum).

4. Sugar.

5. Cardamom powder.

3.4.2 Equipment

Equipment used for this experiment is a Digital weighing balance, Filter paper (Double Rings 102), Water bath, Blender, Hot air oven, Muffle Furnace (JSMF 45 T), refrigerator, viscometer, laminar airflow, colourimeter, vortex, spectrophotometer, centrifuge machine, incubator, autoclave, flask, test tube, and beaker.

3.4.3 Reagents and Chemicals

Various types of chemicals were used including Sulfuric acid, Sodium Hydroxide (Merck), Ethyl alcohol (Merck), Copper sulphate (K_2SO_4 and $CuSo_{4.5}H_2O$), Anhydrous sodium carbonate, Diethyl ether, Hydrochloric acid, Acetone, n-Hexane, Ammonium Thiocyanate, Aluminium chloride, Calcium chloride, 8-Hydroxyquinoline, OCPC, Ascorbic acid, Folin-ciocalteu, Bovine serum Albumin, 2,6-dichlorophenol indophenols, Anthrone reagent, Dextrose anhydrous, Gallic acid, Quercetin etc. Moreover, various culture media were used, including Nutrient Agar (Merck), Potato Dextrose Agar (PDA)(Merck), MRS, and MacConkey Agar.

3.5 Methods

3.5.1 Plant Material Collection and Preparation

Alligator weed (*A. philoxeroides*) was collected from a natural habitat located in Santosh, Tangail. The leaves of the plants were harvested and thoroughly washed with distilled water to

remove any adhering dirt and contaminants. Following the cleaning process, the leaves were air-dried in a shaded area for 5 minutes. Subsequently, leaves were first blanched at 90ºC for 30 seconds to inactivate any enzymes that may later affect the taste and flavour of the yoghurt. The blanched leaves were then blended into a fine paste. The paste was then pressed through a sieve to remove only the juice particles that were going to be used in the yoghurt production.

(a) Blanching of Alligator weed

(b) Alligator weed (Malancha)

Figure 3.1: *A. philoxeroides*leaf collection and preparation

3.5.2 Proximate analysis of *Alternanthera philoxeriodes* (Malancha) leaf

3.5.2.1 Determination of Moisture Content

The moisture content for alligator weed was determined by the procedure AOAC (2005).

3.5.2.2 Determination of Ash content

The ash content for alligator weed was determined by the procedure AOAC (2005).

3.5.2.3 Determination of Carbohydrate Content

The Carbohydrate content was determined according to the procedure given by Hedge and Hofreiter (1962).

3.5.2.4 Determination of Protein Content

Protein assay was performed according to the method presented by the Lowry method (Lowry et al., 1951). Lowry's method for protein estimation is one of the most common colourimetric methods (Hartree, 2025; Waterborg and Matthews, 2023)

3.5.2.5 Determination of Fat Content

The fat content for the sample was determined by the procedure given in AOAC (2000).

3.5.2.6 Determination of crude fiber

The AOAC technique was used to calculate the crude fiber.

3.5.2.7 TSS Determination Procedure (°Brix using Refractometer)

Total soluble solids were determined using a portable refractometer following the method of Hemalatha et al. (2018).

3.5.3 Physicochemical analysis of *Alternanthera philoxeriodes* (Malancha) leaf

3.5.3.1 Determination of pH

pH was measured using a calibrated HI2210 pH meter (Hanna Instruments) with a temperature-compensating probe.

3.5.3.2 Determination of colour

The colour of the yoghurt samples was measured using a CR-400 Chroma Meter (Konica Minolta Chroma Co., Japan) according to the method used by Ilic et al (2024)

$$\Delta E = \sqrt{(L*2 - L*1)2 + (a* 2 - a* 1)2 + (b*2 - b*1)2}$$

3.5.4 Bioactive compound analysis of *Alternanthera philoxeriodes* (Malancha) leaf

3.5.4.1 Determination of Total Phenolic Content

Total phenolic content was estimated by the Folin-Ciocalteu method (Bhalodia et al., 2011; Kalpoutzakis et al., 2023; Singleton et al., 1999)

3.5.4.2 Determination of Total Flavonoids Content

Total flavonoid content was determined by aluminium chloride colourimetric method (Suborna and M.N., 2024; Nyangena et al., 2019; Rashid et al., 2011) with some modification.

3.5.4.3 Determination of Antioxidant Activity

Antioxidant activity was estimated by the DPPH(2,2-diphenyl-2-picrylhydrazyl) method (Manzoor,2016; Akter et al., 2024; Suborna and M.N., 2024; Nyangena et al., 2019) with some modifications.

3.5.5 Nutritional analysis of *Alternanthera philoxeriodes* (Malancha)

3.5.5.1 Determination of Vitamin-C (L-Ascorbic acid)

The titrimetric determination of VC, based on the reduction of 2,6-DCPIP in a medium containing metaphosphoric acid, was performed according to previous studies (Wu et al., 2023; Arya et al., 2000) with some modifications.

3.5.5.2 Determination of beta-carotene content

β-carotene was estimated spectrophotometrically according to the previous procedure (Akter et al., 2024; Suborna and M.N., 2024)

3.5.5.3 Determination of Calcium

Calcium in samples was determined by colourimetric method (Kaur, 2007; Stern and Lewis, 1957) with some modifications.

3.5.5.4 Determination of Iron

Iron was determined in the food samples by the spectrophotometer method (Balarabe and Folashade, 2019; Jha, 2023; P.V., 2022).

3.5.6 Microbiological analysis of *Alternanthera philoxeriodes* (Malancha) leaf

Total Viable Count (TVC), Total Coliform Count (TCC), and Total Fungal Count (TFC) were conducted for the samples

3.5.6.1 Determination of Total Viable Count

The total viable count of the sample was estimated using serial dilutions and surface plating techniques (Jay et al., 2005; ICMSF,1986)

3.5.6.2 Determination of Total Coliform Count

Coliform count of the sample was done according to the method described in Introductory Microbiology Lab Skills and Techniques in Food Science (Shen and Zhang, 2022).

3.5.6.3 Determination of Total Fungal Count

The yeast and mold count of the sample was according to the method as described in the Standard Methods for Examination of Dairy Products (Robertson, 1952).

3.5.7 Production of yoghurt fortified with Malancha juice

- 500ml of milk was heated to 85ºC for 30 minutes, reducing its volume to 400ml. Then, 60 ml of sugar was added to the milk.
- Divide the milk into 2 sections (1st section contains 100ml milk, another contains 300ml). In the 2nd section, 1.5% of cardamom powder was added to the milk and stirred for a while, and another section remained without cardamom powder for the control.
- Milk was cooled down to 45ºC for optimum growth of the culture.
- For inoculation, 15 ml of previous yoghurt as culture for each 100 ml of milk was then added to the milk and stirred well to mix all the ingredients.
- Poured the milk into four plastic cups.
- Lastly, alligator weed juice was added in various proportions (0%,3%,5%,7%).
- Based on this proportion, the samples were marked as

  C (Milk+0% alligator weed extract),

  A1 (Milk+3% alligator weed extract),

A2 (Milk+5% alligator weed extract),

A3 (Milk+7% alligator weed extract).

- The samples were then placed inside an incubator and maintained at a temperature between 37-40°C for 6-8 hours, approximately until the desired degree of acidity and coagulation was achieved.
- After incubation, the samples were cooled rapidly to 5-10ºC. Finally intended yoghurt was formed.

Figure 3.2: Yoghurt samples(control, 3%, 5%, 7%)

3.5.8 Proximate analysis of Yoghurt

3.5.8.1 Determination of Moisture Content

The moisture content for yoghurt was determined by the procedure AOAC (2005).

Procedure:

- At first, the empty crucible was washed, cleaned, and dried in the hot air oven for one hour at 110ºC.
- The empty crucible was weighed (W_1) through a digital electronic balance,
- then about 5g of sample was weighed into the crucible (W_2)
- The crucible with the sample was placed in the hot air oven for 6hr at 105ºC.
- After drying, the crucible is transferred to the desiccator to cool.

- When cooled, the crucible with the sample is weighed (W_3)

Calculation:

Moisture (%) = $\frac{w2-w3}{w2-w1} \times \frac{100}{1}$

Here,

W_1 = Weight of empty dish

W_2 = Weight of crucible + sample before drying

W_3 = Weight of crucible + sample after drying

3.5.8.2 Determination of Ash content

The ash content for yoghurt was determined by the procedure AOAC (2005).

Procedure:

- The process began with the preheating of a crucible inside a hot air oven, followed by cooling and recording its weight as W_1.
- Then, 5g of each sample was carefully weighed and placed into the crucible, noted as W_2.
- The sample within the crucible was transferred into a muffle furnace that had been preheated to 600ºC and left there for 4 hours until it achieved a white or light grey appearance.
- Afterwards, the sample was allowed to cool within a desiccator and was then weighed, recorded as W_3.

Calculation:

Percentage ash content = $\frac{W3-W1}{W2-W1} \times \frac{100}{1}$

Here,

W_1 = Weight of empty crucible

W_2 = Weight of crucible + sample before ashing

W_3 = Weight of crucible + ash after ashing

3.5.8.3 Determination of Carbohydrate Content

The Carbohydrate content was determined according to the procedure given by Hedge and Hofreiter (1962).

Procedure:

Stock Glucose Solution(100µg/ml)

- 100 mg of dextrose/glucose was weighed in a flask.
- Distilled water was added to the dextrose/ glucose to make up to 100ml. It was then mixed to prepare the stock solution.

Working Standard (100µg/ml)

- 10 ml of the stock solution was taken and added to a separate conical flask.
- 10 ml of stock solution was diluted to 100 ml with distilled water. Mixed it well to prepare the working standard.
- **Anthrone Reagent Preparation**
- 200 mg of Anthrone powder was taken in a conical flask.
- 100 ml H_2SO_4 was slowly added to the flask slowly while stirring continuously in anice bath.
- After preparing it was kept aside in a dark bottle

Preparation of the standard Glucose solution:

Pipette different volumes of glucose standard into clean test tubes (e.g. 20 µg to 100 µg)

Sample Preparation

- 1 g of sample was weighed into a centrifuge tube.
- 10 ml of 80% hot ethanol was added to the sample. Mixed thoroughly and was left to sit for 5 minutes.
- The mixture was then centrifuged at 4000-5000 rpm for 30 minutes.
- After centrifugation supernatant from the top was collected.

- Again, added 10 ml of ethanol to the residue inside the tube and centrifuged for repeated extraction. After centrifugation supernatant was collected. The first and second supernatant was combined in a beaker. The beaker containing the combined supernatant was placed in a water bath at 60ºC until most of the ethanol evaporated. The beaker lid was slightly open for the ethanol to evaporate.

Reaction step:

- 1 ml of sample extract was taken into a test tube.
- Then, 4 ml of Anthrone reagent was added to each test tube.
- Using a vortex, the contents inside the tube were mixed thoroughly.
- All the test tube was then placed into a water bath for 10 minutes.
- Test tubes were cooled in an ice bath after being it from the water bath, to stop the reaction.
- Lastly, absorbance was measured at 620 nm.

Calculation:

$$\frac{Absorbance\ of\ unknown}{Conc.\ of\ unknown} = \frac{Absorbance\ of\ standard}{Conc.\ of\ standard}$$

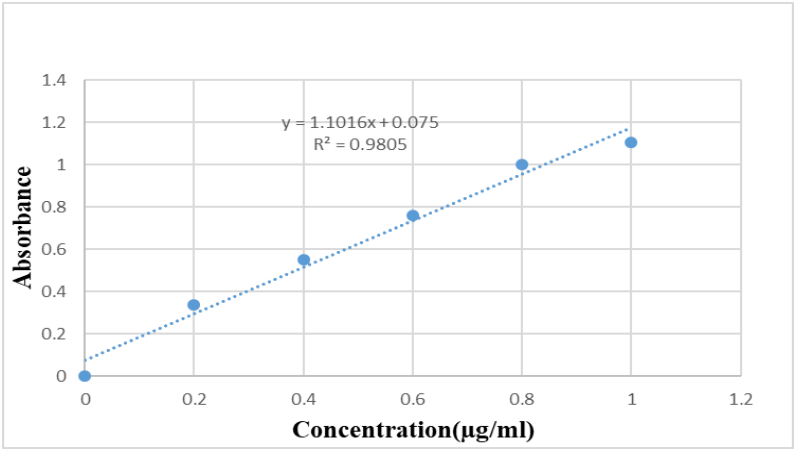

Figure 3.3: Standard curve of Carbohydrate determination (1st day of storage)

3.5.8.4 Determination of Protein Content

Protein assay was performed according to the method presented by Lowry method (Lowry et al., 1951). Lowry's method for protein estimation is one of the most common colourimetric methods (Hartree, 2025; Waterborg and Matthews, 2023).

Procedure:

Reagent preparation:

•**Reagent A (2% Na2CO3 in 0.1 N NaOH):** 2g Na2CO3 was dissolved in 100 mL of distilled water.

•**Reagent B (0.5% CuSO4):** 0.5 g CuSO4.5H20 dissolved in 100 ml of distilled water. 1g of sodium potassium tartrate was dissolved in 100 ml of distilled water. Both solutions were mixed in equal volume in a conical flask.

•**Reagent C (Alkaline copper Reagent):** 100 parts of reagent were mixed with 1 part of reagent B.

•**Folin-Ciocalteu reagent:** 10 ml of Folin-Ciocalteu was mixed with 10 ml of distilled water.

Protein Standard Preparation:

prepare the standard 1 mg/ml concentration of BSA. Pipette out into clean glass tubes 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the protein solution and make up the total volume to 1ml with addition of distilled water.

Sample Preparation: 0.1 ml of sample was mixed with 0.9 ml of water (1:10).1 ml of this mixture was taken in a test tube.

Reaction step:

- Add 5 ml of reagent C was added to both the standard and sample test tubes and mixed well. The test tubes were set aside for 10 minutes at room temperature.
- Then, 0.5 ml of Folin-Ciocalteu reagent was added to each test tube.
- Later on, the test tubes were incubated for 30 min at room temperature.

- After 30 min, measurements were taken in a spectrophotometer at an absorption of 750 nm.

Calculation:

$$\text{Protein Concentration(mg/ml)} = \frac{(Absorption\ of\ sample - intercept\ of\ the\ standard\ curve)}{Slope\ of\ the\ calibration\ curve}$$

$$\text{Protein(mg/100g)} = \frac{Protein\ conc.\left(\frac{mg}{ml}\right) \times Volume of\ sample}{Weight\ of\ sample} \times 100$$

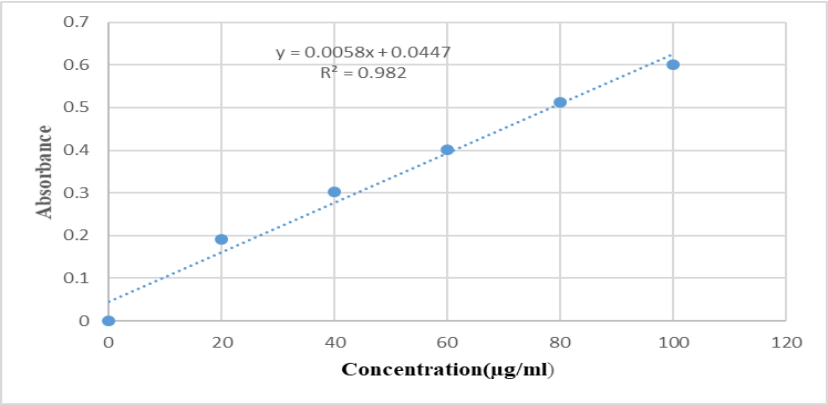

Figure 3.4: Standard curve of protein determination (1st day of storage)

3.5.8.5 Determination of Fat Content

The fat content for the sample was determined by the procedure given in AOAC (2000).

Procedure:

- The sample was dried in a hot air oven for 4-5 hours at 105ºC.
- From that dried sample, 5 g was weighed onto filter paper and wrapped.
- The sample was taken into an extraction thimble and transferred into a Soxhlet, which was then filled with petroleum ether (B.P.40-60ºC), about 150 ml, into the bottle and placed on the heating mantle.
- After connecting the Soxhlet apparatus, the water was turned on to cool it, and then switched on the heating mantle.
- The sample was heated for about 3-4 hours.
- An empty beaker of 250 ml was dried at 100ºC and weighed.

- Then the extracted sample with the solvent was poured into the beaker.
- Then the beaker was heated for about 20-30 minutes at 103ºC in an oven until the solvent completely evaporated, leaving only the sample residue.
- After drying, the beaker was transferred to the desiccators to cool for 30 minutes, and the beaker was weighed again and its dry content.
- The percentage of fat was calculated as the equation below.

Calculation:

$$\%\text{Fat (Dry basis)} = \frac{(Weight\ of\ beaker+Oil)-(Weight\ of\ beaker\ without\ sample)}{(Weight\ of\ sample)} \times 100$$

3.5.8.6 Determination of crude fiber

The AOAC technique was used to calculate the crude fiber.

Procedure:

- Two-gram (2g) sample was weighed into a 750ml Erlenmeyer flask. 200ml of 1.25% H_2SO_4 was added, and immediately flask was immediately set on a hotplate and connected to the condenser.
- The contents were boiled within 1 minute of contact with the solution.
- At the end of 30 minutes, the flask was removed and immediately filtered through a linen cloth in a funnel and washed with a large volume of water.
- Filtrate (containing sample from acid hydrolysis) was washed and returned into the flask with 200 mL 1.25% NaOH solution.
- Flask was connected to the condenser and was boiled for exactly 30 minutes.
- It was then filtered through Fischer's crucible and washed thoroughly with water, and 15ml of 96% alcohol.
- The crucible and contents were dried for 2 hours at 105 °C and cooled in a desiccator, and it was weighed.

- The crucible was ignited in a furnace for 30 minutes, and after that, it was cooled and reweighed.

Calculation:

Percentage crude fiber =W2 − W3 /W1 × 100

Here,

W1=initial weight of empty crucible; W2 =weight of crucible +weight of sample before incineration; and W3=eight of crucible +weight of sample after incineration

3.5.8.7 TSS Determination Procedure (°Brix using Refractometer)

Total soluble solids were determined using a portable refractometer following the method of Hemalatha et al. (2018).

Procedure:

- Calibrate the refractometer with distilled water → set to 0.0 °Brix.
- Prepare the liquid sample → filter if needed.
- Place 1–2 drops of the sample on the prism.
- Close the cover plate and wait a few seconds.
- Read the °Brix value on the display or eyepiece.
- Clean the prism with distilled water after each use.

3.5.9 Physicochemical analysis of Yoghurt

3.5.9.1 Determination of pH

pH was measured using a calibrated HI2210 pH meter (Hanna Instruments) with a temperature-compensating probe.

Procedure:

- The probe was first washed with distilled water, then it was immersed in pH 4.01 buffer and pressed until stable.
- This process was again repeated for 2nd buffer, pH 7.01.

- Yoghurt was then taken in a small beaker of about 50ml and stirred gently.
- The probe was then fully submerged and waited for 30-60 seconds until the probe was stabilized.
- pH was recorded once the reading was steady.

3.5.9.2 Determination of Titratable Acidity

Titratable acidity was determined using the method described by Nejad et al. (2014). Exactly 1 ml of the sample was weighed in a flask and mixed with 9 ml of distilled water. Three drops of phenolphthalein were added to the mixture and titrated against 0.1 M NaOH. The titration endpoint was indicated by the appearance of a pink colour, and titratable acids (lactic acid percentage equivalent) were calculated from the titration values.

Calculation:

Titratable acidity=$\frac{(10 \times volume\ of\ NaOH \times 0.009 \times 0.1)}{W} \times 100\%$

Where, 10 = Dilution factor; W = weight of sample for titration; V NaOH = Volume of NaOH used to neutralize the lactic acid; 0.1 = Normality of NaOH; 0.009 = equivalent of lactic acid normality.

3.5.9.3 Determination of colour

The colour of the yoghurt samples was measured using a CR-400 Chroma Meter (Konica Minolta Chroma Co., Japan) according to the method used by Ilic et al (2024)

Procedure:

- Samples were placed in a Petri dish, and their International Commission on Illumination (CIE) L*a*b* parameters were measured. Here,
- The instrument was first placed onto a white surface to set the calibration to Y=85.1, X=3171, and Y=3234.
- After setting the calibration, sample colours were measured using the instrument. The instrument was placed on top of the sample and pressed on the power button.
- A white flash came out, which marked that the reading was successful.

- All tests were carried out in triplicate.
- The total colour difference (ΔE) between the samples was calculated using the following equation:

$$\Delta E = \sqrt{(L*2 - L*1)2 + (a* 2 - a* 1)2 + (b*2 - b*1)2}$$

3.5.9.4 Water holding capacity

Water holding capacity (WHC) was determined using a modified centrifugal method according to Feng et al and method described by Cardines et al.20 g of yoghurt was weighed and taken in a centrifugal tube. The tube was then centrifuged at 5000 rpm for 10 min at 4 °C. The yoghurt, after centrifugation separated into two layers. The upper whey portion was drained and expelled. The residual precipitate was weighed, and WHC was calculated according to the Equation.

WHC (%) = [weight (drained gel)/ (weight (sample)] ×100

3.5.9.5 Syneresis

Syneresis was determined according to the modified method by Joung et al.20 g of yoghurt was taken in a 50 ml centrifuge tube. The tube was then centrifuged at 5000rpm/min for 10 min. In the tube yoghurt was divided into two layers after centrifugation. The upper layer known as whey, was drained and weighed. Syneresis index was calculated according to the Equation:

S (%) = [weight (supernatant)/ (weight (sample)] 100

3.5.9.6 Viscosity

Viscosity was determined following the method described by Fetahagi´c et al. Yoghurt viscosity was measured using a viscometer (DVS-80S). Approximately 100 ml of the yoghurt sample was measured into a 100 ml beaker, and the viscosity was determined at 20 ± 0.1ºC. Measurement was taken using motor 3, rotor velocity 30 rpm, and spindle 3. The measurement was taken for each sample after 3 min of rotation. The spindle was washed after each test with distilled water.

3.5.10 Bioactive compound analysis of yoghurt

3.5.10.1 Determination of Total Phenolic Content

Total phenolic content was estimated by the Folin-Ciocalteu method (Bhalodia et al., 2011; Kalpoutzakis et al., 2023; Singleton et al., 1999).

Procedure:

Sample preparation

- 5g of sample was weighed on a digital weighing balance and placed inside a centrifuge tube.
- 10ml,70% acidified ethanol was added to the centrifuge tube.
- The tube was vortexed or stirred vigorously for 5 minutes. The tube was then let stand for 30-60 minutes at room temperature.
- Finally, tubes were centrifuged at 5000 rpm for 15 min at 4ºC.
- After the completion of centrifugation, the supernatant was collected from the tube and concentrated to 5 ml at <30ºC in a water bath.

Preparation of Gallic acid solution(1mg/ml):

- Stock solution:10 mg of Gallic acid was dissolved in 10 ml of distilled water in a conical flask.
- Standard solution: 5µg/ml,10µg/ml,15µg/ml,20µg/ml,25µg/ml of gallic acid

Preparation of FCR solution:

2 ml FCR reagent was mixed with 18 ml distilled water to prepare the FCR solution.

Preparation of Sodium Carbonate Solution:

7.5 g Na_2CO_3 was dissolved in 100 ml of distilled water.

Reaction step:

- A blank was made containing just 0.5 ml of distilled water.

- 0.5 ml of sample extract and standard solution were taken in a test tube.
- Then added 2.5 ml of Folin-Ciocalteu reagent was added and shaken vigorously.
- The mix was allowed to stand for 5 minutes at room temperature.
- After 5 minutes, 2 mL of 7.5% Na_2CO_3 was added to each test tube.
- It was allowed to incubate at room temperature for 30 minutes in the dark. Intense blue colour was developed.
- After incubation, absorbance was measured at 750nm spectrophotometer.
- Blank was performed with blank reagent, with solvent, and measurements were taken in triplicate.

The TPC was expressed as mg of Gallic acid equivalents per g fresh weight by following the formula below:

$y=mx+ c$

Here

y = Absorbance of samples.

x = Total phenol content (TPC).

The value of c and m was found from the regression line plotted for each sample separately.

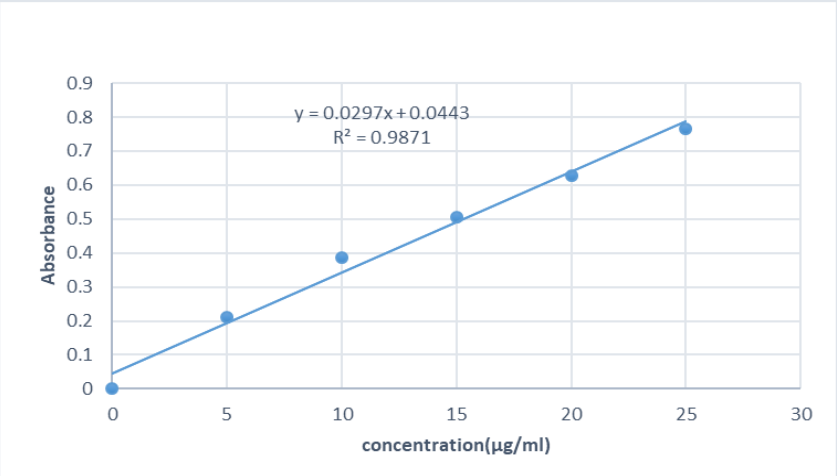

Figure 3.5: Standard curve of Total phenolic content (TPC) determination (1st day of storage)

3.5.10.2 Determination of Total Flavonoids Content

Total flavonoid content was determined by aluminum chloride colourimetric method (Suborna and M.N., 2024; Nyangena et al., 2019; Rashid et al., 2011) with some modifications.

Procedure:

Sample preparation

- 5g of sample was weighed on a digital weighing balance and placed inside a centrifuge tube.
- 10ml,70% acidified ethanol was added to the centrifuge tube.
- The tube was vortexed or stirred vigorously for 5 minutes.
- The tube was then let stand for 30-60 minutes at room temperature.
- Finally, tubes were centrifuged at 5000 rpm for 15 min at 4ºC.
- After the completion of centrifugation, the supernatant was collected from the tube and concentrated to 5 ml at <30ºC in a water bath.

Preparation of a standard solution

Quercetin solution (1mg/ml): 10 mg of Quercetin was mixed in 10 ml of Ethanol. A working standard was prepared. For standard, different concentration 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml of Quercetin were taken.

10% Aluminum Chloride solution

10 g of Aluminum chloride was dissolved in 100 ml of methanol. Stored in a dark bottle.

1 M Potassium Acetate Solution

9.8 g of potassium acetate was dissolved in 100 mL of distilled water.

Reaction step:

- A blank was prepared containing 2.8 ml distilled water,2.1 ml ethanol, and 0.1 ml Potassium acetate.
- 0.5 ml of the sample extract was taken in a test tube along with all the other standards.
- To each test tube, 0.1 ml of Aluminum chloride was added and mixed.

- 5 min later, 0.1 ml of potassium acetate was added.
- To each test tube, 1.5 mL of ethanol was added.
- Lastly, 2.8ml of distilled water was added and mixed.
- It was then incubated for 30 minutes in the dark and absorbance measurement was taken at 415 nm.

The outcome data of TFC was calculated from the quercetin standard calibration curve and expressed as mg quercetin equivalent (QE)/g fresh weight by following the formula as below,

$y=mx+ c$

Here,

y = Absorbance of samples.

x = Total flavonoid content (TFC).

The value of c and m was found from the regression line plotted for each sample

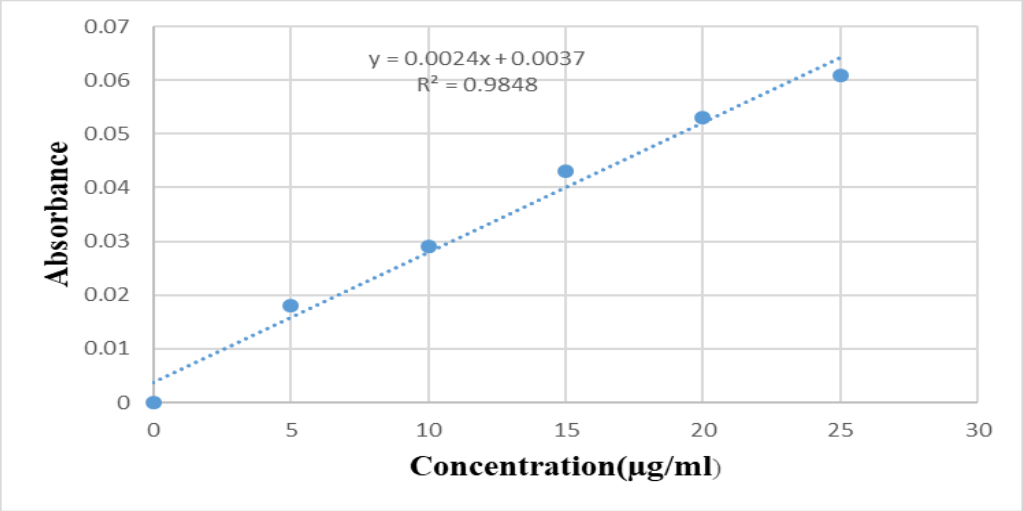

Figure 3.6: Standard curve of Total flavonoid content (TFC) determination (1st day of storage)

3.5.10.3 Determination of Antioxidant Activity

Antioxidant activity was estimated by the DPPH(2,2-diphenyl-2-picrylhydrazyl) method (Manzoor,2016; Akter et al., 2024; Suborna and M.N., 2024; Nyangena et al., 2019) with some modifications.

Procedure:

Sample preparation

- 5g of sample was weighed on a digital weighing balance and placed inside a centrifuge tube.
- 10ml,70% acidified Methanol was added to the centrifuge tube. The tube was vortexed or stirred vigorously for 5 minutes.
- The tube was then let stand for 30-60 minutes at room temperature.
- Finally, tubes were centrifuged at 5000 rpm for 15 min at 4ºC.
- After the completion of centrifugation, the supernatant was collected from the tube and concentrated to 5 ml at <30ºC in a water bath.

Preparation of standard

Ascorbic acid standard: 10 mg of Ascorbic acid was dissolved in 10 mL of distilled water. different concentrations of 5µg/ml,10µg/ml,15µg/ml,20µg/ml, and 25µg/ml of Ascorbic acid were prepared.

Preparation of DPPH solution

3.94 mg DPPH was dissolved in 100 mL of methanol. Mixed well, kept in the dark for colour formation.

Preparation of sample stock solution

1ml of sample extract was dissolved in 9 mL of ethanol. Then, this stock was used to prepare different concentrations of the sample standard. For the standard solution, different concentrations, 20µg/ml,40µg/ml,60µg/ml,80µg/ml, and 100µg/ml, were taken.

Reaction step:

- For each concentration of ascorbic acid and sample extract solution,1ml of DPPH solution was mixed.
- Blank was prepared by 2ml of methanol.
- Control was prepared by 1 ml of DPPH solution and 1 ml of ethanol.
- Then prepared solution was incubated in the dark for 30 minutes.

- Absorbance was taken at 517 nm.

The radical scavenging activity was calculating by following formula:

Radical Scavenging Activity (%) = $\frac{A0-A1}{A0}\times 100$

ere,

A_0=DPPH solution absorbance.

A_1=Sample absorbance

- The values of IC50 used in this study were generated from a regression line graph that was plotted by % radical scavenging activity of 5 concentrations against 5 concentrations of each extract sample of the standard and test sample.
- IC50 can scavenge 50% of DPPH free radicals in the DPPH free radical scavenging method. As a lower IC50 value corresponds with a higher antioxidant activity (Akter et al., 2024; Suborna and M.N., 2024; Nyangena et al., 2019)

IC50 was calculated by following the formula below:

IC50=y−b/ a × 100

Here,

Y is replaced by 50 in the above equation.

Value of a and b was found from the regression line plotted for each sample separately.

3.5.11 Nutritional analysis of Alternanthera philoxeriodes (Malancha)

3.5.11.1 Determination of Vitamin-C (L-Ascorbic acid)

The titrimetric determination of VC, based on the reduction of 2,6-DCPIP in a medium containing metaphosphoric acid, was performed according to previous studies (Wu et al., 2023; Arya et al., 2000) with some modifications.

Reagent preparation:

Dye solution:100 mg of 2,6-dichlorophenol indophenol and 105 g sodium bicarbonate were dissolved in distilled water and made up to 500 ml. The solution was then filtered.

3% Meta phosphoric acid reagent: 15 g of meta phosphoric acid was dissolved in 40 ml of acetic acid and made up to 500 ml with distilled water.

Standard vitamin C (0.1 mg/ml): 10 mg pure vitamin C dissolved in 3% metaphosphoric acid and made up to 100 ml,100% Metaphosphoric acid.

Sample Preparation

- A 10 g sample was homogenized well with 40 mL of Metaphosphoric-acetic acid solution. It was then filtered through a double layer of muslin cloth.
- The filtrate was later centrifuged at 3000 rpm for 10 minutes.
- After centrifugation, only the supernatant was collected.

Titration Procedure:

- 10 ml of standard vitamin C solution was taken in a conical flask and titrated with dye solution.
- 10 ml of sample extract was mixed with 5 ml of metaphosphoric-acetic acid solution and titrated with 2,6-dichlorophenol indophenol solution.

Calculation:

Vitamin C (mg/100 g) =volume of DCPIP used(ml)×mg ascorbic acid per ml of DCPIP

Vitamin C content (%) = $\frac{Vitamin\ C\ Obtained(mg)}{Weight\ of\ sample(gm)} \times 100$

3.5.11.2 Determination of β-Carotene content

β-Carotene was estimated spectrophotometrically according to the previous procedure (Akter et al., 2024; Suborna and M.N., 2024).

Procedure:

Sample preparation

- A 5 g sample was weighed in a test tube.10-15 ml of acetone was then added to the test tube.
- The test tube was swirled gently or vortexed to obtain a homogenous mixture.

Extraction and Collection:

- The test tube containing the sample was placed in a test tube holder.
- Added 15-20 ml n-hexane to the test tube.
- It was then vortexed vigorously for 2 minutes.
- Distilled water, about 10-15 ml, was added carefully along the tube wall. For better separation, it was centrifuged at 3000 rpm for 5 min.
- It was allowed to stand until two separate layers were obtained, and beta carotene moved to the upper hexane layer.
- The top layer was carefully collected. The extraction was repeated for the bottom layer with fresh n-hexane to pool the layers.
- Pure n-hexane was used as a blank.
- The absorbance of the extract was measured using a spectrophotometer at a wavelength of 450 nm.

Calculation:

$$\text{Concentration of pigment(mg/100g)} = \frac{Absorbance}{Extinction\ co{-}efficient \times Thickness\ of\ cuvettes}$$

3.5.11.3 Determination of Calcium

Calcium in samples was determined by colourimetric method (Kaur, 2007; Stern and Lewis., 1957) with some modifications.

Procedure:

Sample Preparation

A 5 g sample was heated at 500ºC for 4 hours in a muffle furnace. After cooling, 10 mL of 0.1 M HCL was added to the sample in a beaker. Distilled water was added to the mix to make up to 25 ml. Lastly, the mix was filtered.

OCPC Reagent preparation: 2.06 mg of OCPC was weighed in a beaker.2 mL of methanol was added to the beaker. Lastly, it was diluted with 10 ml of distilled water.

Tris-HCl preparation: 6.06 g of Tris-HCl was added to 80 mL of water. It was diluted with 0.1M NaOH drop wise to make pH approximately 10.

8-Hydroxyquinoline reagent: 1 g of 8-hydroxyquinoline was weighed in a beaker. 100 ml of 95% ethanol was added to the beaker.

0.1 M HCL preparation: 1.67 ml was added to 200 ml distilled water.

Standard Solution preparation:36.7 mg $CaCl_2$ was weighed in a beaker. It was made up to 100 ml with distilled water.

Reaction step:

- For standard, different concentration was prepared such as,1µg/ml, 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml,10µg/ml of calcium chloride.
- A blank was prepared with 1 ml of distilled water.
- 1 mL of sample was added to the test tube and1 mL of each standard solution was added to the test tubes.
- Then, to all the test tubes, 1 ml of Tris-HCl,1 ml of OCPC, and 0.5 ml of 8-hydroxyquinoline were added.
- Test tubes were swirled to mix all the solutions. It was then left to stand at room temperature for 15 minutes.
- The absorbance was measured using a spectrophotometer at a wavelength of 570-580 nm.

Calculation:

The outcome data of calcium was calculated from $CaCl_2$ the standard calibration curve and expressed as mg/g fresh weight by following the formula as below,

$y=mx+ c$

Here,

y = Absorbance of samples.

x = Total calcium content.

The value of c and m was found from the regression line plotted for each sample

3.5.11.4 Determination of Iron

Iron was determined in the food samples by the spectrophotometer method (Balarabe and Folashade, 2019; Jha, 2023; P.V., 2022) with some modifications.

Procedure:

Sample Preparation

A 5 g sample was heated at 500ºC for 4 hours in a muffle furnace. After cooling, 10 mL of 1 M HCL was added to the sample in a beaker. Distilled water was added to the mix to make up to 25 ml. Lastly, the mix was filtered.

Reagent preparation:

1 M H_2SO_4: 5.6 ml of Sulfuric acid was weighed and taken in a beaker. About 100 mL of distilled water was added to prepare the solution.

1M HCL: 1.67 g Hydrochloric acid was weighed and taken in a beaker. About 200 ml of distilled water was added to prepare the solution.

1 M Ammonium thiocyanate: 0.7629 g of ammonium thiocyanate was weighed and taken in a beaker. About 10 mL of water was added to prepare the solution.

Preparation of Standard Solution

0.001m M Ferric ammonium sulfate standard solution was prepared by dissolving 1.2059 g ferric in 10 ml of H_2SO_4. It was left to stand for proper dissolution. This solution was transferred to a 250 ml flask and filled up to the mark with distilled water.

Reaction step:

- For standard, 1µg/ml,2µg/ml, 4µg/ml,6µg/ml, and 8µg/ml of Ferric ammonium sulfate standard solution to test-tubes.
- 2 mL of sample was transferred to a test tube.
- Then, 0.5 ml of ammonium thiocyanate was added to the test tubes.
- Test tubes were left to stand for 10-15 minutes.
- The absorbance was measured using a spectrophotometer at a wavelength of 490 nm.

Calculation:

The outcome data of iron was calculated from ferric ammonium sulphate standard calibration curve and expressed as mg/g fresh weight by following the formula as below,

$y=mx+ c$

Here,

y = Absorbance of samples.

x = Total iron content.

Value of c and m was found from the regression line plotted for each sample.

3.5.12 Microbiological analysis of Yoghurt

3.5.12.1 Determination of Total Viable Count

The total viable count of the sample was estimated using serial dilutions and surface plating techniques (Jay et al., 2005; ICMSF,1986).

Procedure:

- Nutrient agar powder of about 14 g was weighed on a digital electronic balance.
- The media was prepared by rehydrating the agar powder with 500 mL of distilled water in a flask.
- The flask was heated and stirred in a magnetic stirrer for 10 minutes.
- Then it was shut closed with foil paper and placed inside the autoclave machine along with all the other apparatus such as a petri dish, test tube, measuring cylinder, and spatula.
- The machine was tightly shut and ran for 15 minutes at 121ºC.
- Before plating, the media were kept in a boiling water bath, and pouring was done at 45ºC.
- 1 g sample was weighed and transferred into a 9 ml dilution blank of sterile distilled water (10^{-1}).
- This dilution process was repeated 2 more times, resulting in dilutions 10^{-2},10^{-3}.

- About 15-20 ml of melted agar at 45 °C was poured into each plate.
- As the agar media solidified, about 0.1 ml of dilution was added in drops onto the solidified media and incubated Petri dishes for 24 hours at 37°C

Calculation:

TVC (CFU/mL or CFU/g) = Number of colonies ×Dilution factor/volume plated ml)

3.5.12.2 Determination of Total Coliform Count

Coliform count of the sample was done according to the method described in Introductory Microbiology Lab Skills and Techniques in Food Science (Shen and Zhang, 2022).

Procedure:

- MacConkey Agar of about 50 g was weighed on a digital electronic balance.
- The media was prepared by rehydrating the agar powder with 1 L of distilled water in a flask.
- The flask was heated and stirred in a magnetic stirrer for 10 minutes.
- Then it was shut closed with foil paper and placed inside the autoclave machine along with all the other apparatus such as petri dish, test tube, a measuring cylinder, spatula.
- The machine was tightly shut and ran for 15 minutes at 121ºC.
- Before plating, the media was kept in a boiling water bath, and pouring was done at 45ºC.
- For dilution blank, 8.5 g of table salt was dissolved in 1 L of distilled water.
- After mixing, the lid of the flask was shut off and placed in the incubator for 15 minutes at 121ºC.
- After heating was done, it was left to cool at room temperature.
- Dilution blanks were prepared by pouring 9 mL of sterile saline into sterilized test tubes.
- This dilution process was repeated 2 more times, resulting in dilutions 10^{-2},10^{-3}.
- About 15-20 ml of melted agar at 45 °C was poured into each plate.

- As the agar media solidified, about 0.1 ml of dilution was added in drops onto the solidified agar media.
- After some time, the petri dishes were inverted and placed inside an incubator at 35-37°C for 24 hours.

Calculation:

TVC (CFU/mL or CFU/g) = Number of colonies ×Dilution factor/volume plated ml

3.5.12.3 Determination of Total Fungal Count

The yeast and mold count of the sample was according to the method as described in the Standard Methods for Examination of Dairy Products (Robertson, 1952).

Procedure:

- Potato Dextrose Agar (PDA) of about 39 g was weighed on a digital electronic balance.
- The media was prepared by rehydrating the agar powder with 1000 mL of distilled water in a flask.
- The flask was heated and stirred in a magnetic stirrer for 10 minutes.
- Then it was shut closed with foil paper and placed inside the autoclave machine along with all the other apparatus such as petri dish, test tube, measuring cylinder, spatula.
- The machine was tightly shut and ran for 15 minutes at 121ºC.
- Before plating, the media was kept in boiling water bath, and pouring was done at 45ºC.
- 1 g sample was weighed and transferred into a 9 ml dilution blank of sterile saline (10^{-1}).
- This dilution process was repeated 2 more times, resulting in dilutions 10^{-2},10^{-3}.
- About 15-20 ml of melted agar at 45 °C was poured into each plate.
- As the agar media solidified, about 0.1 ml of dilution was added in drops onto the solidified agar media.
- After some time, the petri dishes were inverted and placed inside an incubator at 25ºC for 5 days.

- Yeast colonies were characterized by their smooth, moist, and elevated surface, whereas mold colonies were identified by the profuse growth of hyphae.

Calculation:

TVC (CFU/mL or CFU/g) = Number of colonies ×Dilution factor/volume plated ml)

3.5.12.4 Determination of Total Lactobacillus Count

The lactobacillus count of the sample was determined according to the method as described in the Standard Methods for Examination of Dairy Products (Robertson, 1952)

Procedure:

- MRS agar of about 65.13 g was weighed on a digital electronic balance.
- The media was prepared by rehydrating the agar powder with 1000 mL of distilled water in a flask.
- The flask was heated and stirred in a magnetic stirrer for 10 minutes.
- Then it was shut closed with foil paper and placed inside the autoclave machine along with all the other apparatus such as petri dish, test tube, a measuring cylinder, spatula.
- The machine was tightly shut and ran for 15 minutes at 121ºC. Before plating, the media were kept in a boiling water bath, and pouring was done at 45ºC.
- 1 g sample was weighed and transferred into a 9 ml dilution blank of sterile saline (10^{-1}).
- This dilution process was repeated 2 more times, resulting in dilutions 10^{-2},10^{-3}.
- About 15-20 ml of melted agar at 45 °C was poured into each plate.
- As the agar media solidified, about 0.1 ml of dilution was added in drops onto the solidified agar media. After some time, the petri dishes were inverted and placed inside an incubator at 37ºC for 48 hours.

Calculation:

TVC (CFU/mL or CFU/g) = Number of colonies ×Dilution factor/volume plated ml)

3.5.13 Sensory Analysis

A 9-point Hedonic scale was used. All tests were conducted by using 9-point Hedonic scale (Peryam et al., 1957) by a panel consisting of 20 sensory assessors aged between 20-45 years. Hedonic scale is a type of rating scale used to measure the degree of pleasure or liking of a product or service, typically ranging from "dislike extremely" to "like extremely". It's

commonly used in consumer acceptance testing, particularly in the food industry, to assess how much people like or dislike a product.

Table 3.1: The list of attributes used for sensory evaluation

Sensory Quality Factors	
Attribute	**Description**
Appearance	Whey presence
	Shiny
Colour	Greenish
	White
Texture	Smoothness
	Creaminess
	Mouth coating
Aroma	Milky
	Leafy
	Elachi
Taste	Sweetness
	Sourness
	Aftertaste

3.5.14 Statistical analysis

For the analysis of the functional yoghurt, analysis of variance (ANOVA) was performed. Using SPSS software. Significant differences between means were determined at $p< 0.05$. Tukey's HSD (Honestly Significant Difference) test is a post hoc analysis used to determine which specific group means are significantly different from each other after a statistically significant result from an ANOVA (Analysis of Variance) test.

CHAPTER-04

RESULT AND DISCUSSION

4 RESULT AND DISCUSSION

4.1 Proximate composition of yoghurt on Day 1

Table 05 presents the proximate composition of yoghurt samples on Day 01. A significant variation ($p < 0.05$) in moisture content was observed among the treatment groups. The 3% ($68.53^{a} \pm 0.30$) and 5% ($68.36^{a} \pm 0.25$) samples exhibited significantly higher moisture content compared to the control ($64.16^{c} \pm 0.20$) and 7% ($63.10^{b} \pm 0.10$) samples. Tukey's HSD post hoc analysis confirmed that no significant difference was found between the 3% and 5% samples, as indicated by their shared superscript. *A. philoxeroides* juice may increase moisture due to its water content, but it could also affect texture and water retention if it interacts with proteins or fats. Its antioxidants might alter the yoghurt matrix, affecting consistency. Notably, the 7% sample showed a significantly lower moisture content than all other groups, suggesting that higher inclusion levels may reduce water retention capacity.

The ash content increased significantly with the level of ingredient incorporation. The control sample demonstrated the lowest ash content ($3.06^{d} \pm 0.05$), whereas the 7% sample recorded the highest value ($4.60^{a} \pm 0.10$). The 3% ($4.03^{c} \pm 0.05$) and 5% ($4.30^{b} \pm 0.10$) samples showed intermediate values, each significantly different from the others. This trend indicates a consistent and significant elevation in ash content in response to higher inclusion levels. Studies have shown that plant-based ingredients often have higher mineral content than dairy alone, and their addition directly increases the mineral content in the final product (Dewanto et al., 2002). *A. philoxeroides* and cardamom both contribute to the mineral content of the yoghurt. These ingredients are rich in various minerals, such as calcium, magnesium, potassium, and iron, which would increase the overall ash content as their concentrations rise with higher inclusion levels.

Table 4.1: Proximate composition of yoghurt on day 1 and Day 7 storage

Day-01							
Sample	**Moisture**	**Ash**	**CHO**	**Protein**	**Fat**	**Fiber**	**TSS**
C	64.16^{c}±0.20	3.06^{d}±0.05	35.01^{d} ± 0.01	54.33^{d}±0.02	7.71^{a}±0.00	0.00±0.00	23.36^{a}±0.05
3%	68.53^{a}±0.30	4.03^{c}±0.05	36.96^{c} ± 0.01	87.64^{c}±0.01	7.19^{b}±0.01	0.00±0.00	20.03^{b}±0.05
5%	68.36^{a}±0.25	4.30^{b}±0.10	39.65^{b} ± 0.02	104.32^{b}±0.03	6.72^{c}±0.00	0.00±0.00	21.53^{c}±0.05
7%	63.10^{b}±0.10	4.60^{a}±0.10	43.26^{a} ± 0.01	115.03^{a}±0.02	5.00^{d}±0.00	0.01±0.00	20.76^{d}±0.05
Day-07 Storage							
Sample	**Moisture**	**Ash**	**CHO**	**Protein**	**Fat**	**Fiber**	**TSS**
C	62.20^{d}±0.20	3.20^{d}±0.00	22.89^{d} ± 0.01	52.18^{d} ± 0.01	7.30^{a}±0.58	0.00±0.00	23.43^{a}±0.05
3%	67.40^{a}±0.10	4.21^{c}±0.10	23.19^{c} ± 0.01	86.69^{c} ± 0.01	7.18^{a}±0.01	0.00±0.00	22.53^{b}±0.05
5%	66.60^{b}±0.10	4.81^{b}±0.10	24.07^{b}± 0.01	88.38^{b} ± 0.01	6.67^{a}±0.00	0.00±0.00	21.80^{c}±0.10
7%	62.73^{c}±0.20	5.03^{a}±0.05	24.28^{a} ± 0.01	90.77^{a} ± 0.01	4.97^{b}±0.01	0.01±0.00	21.33^{d}±0.05

Note: Values are means of triplicate tests ± standard deviation. Means in the same column with different superscript letters (a, b, c, d) differ significantly at $p < 0.05$ (Tukey HSD)

Significant differences ($p < 0.05$) were also evident in carbohydrate content across all groups. The 7% sample exhibited the highest carbohydrate concentration ($43.26^{a} \pm 0.01$), followed by the 5% ($39.65^{b} \pm 0.02$), 3% ($36.96^{c} \pm 0.01$), and control ($35.01^{d} \pm 0.01$) samples. Each group differed significantly, highlighting a progressive increase in carbohydrate content with increasing levels of ingredient incorporation. The crude protein content of *A. philoxeroides* (Alligator weed) has been reported as 42.6 g/100g dry weight, indicating that it can serve as a significant source of carbohydrate (Serdiati et al., 2024). This high carbohydrate content suggests that its inclusion in fortified yoghurt could substantially increase the overall carbohydrate content of the final product, providing a nutritional benefit by enhancing its carbohydrate profile.

A similar trend was observed in protein content, which also rose significantly ($p < 0.05$) with higher inclusion levels. The control group contained the lowest protein content ($54.33^{d} \pm 0.02$), while the 7% sample recorded the highest value ($115.03^{a} \pm 0.02$). Intermediate values were observed in the 3% ($87.64^{c} \pm 0.01$) and 5% ($104.32^{b} \pm 0.03$) groups, all of which were significantly different from one another. This pattern reflects a strong positive correlation between ingredient incorporation and protein enrichment. The crude protein content of

18.5g/100g dry weight suggests that Alligator weed can serve as a substantial protein source (Serdiati et al.,2024).

In contrast, the fat content demonstrated a significantly decreasing trend with increased ingredient addition. The control sample had the highest fat content ($7.71^{a} \pm 0.00$), whereas the 7% sample recorded the lowest ($5.00^{d} \pm 0.00$). The 3% (7.19^{b}) and 5% (6.72^{c}) samples presented intermediate values, all of which were statistically distinct, indicating a significant inverse relationship between fat content and ingredient inclusion. As more *A. philoxeroides* juice are added to the yoghurt, the overall percentage of fat in the mixture decreases. This is due to the dilution effect: the added ingredients (which are low in fat) increase the total mass of the yoghurt, but they do not contribute significant fat content. Research has shown that when non-fat ingredients are added to a food product, they dilute the fat content, leading to a lower overall fat percentage (Jovanović et al., 2013).

Fiber content was negligible across all samples and did not show any statistically significant differences. Although the 7% sample contained a minimal amount (0.01%), the variation was not significant, and fiber content can thus be considered unchanged across treatments on Day 01.

Finally, a significant reduction in total soluble solids (TSS) was noted with the addition of the ingredient. The control sample exhibited the highest TSS value ($23.36^{a} \pm 0.05$), while the 3% sample had the lowest ($20.03^{b} \pm 0.05$). The 5% and 7% samples showed intermediate TSS values of 21.53°Brix and 20.76°Brix, respectively, with all values being significantly different from one another. This indicates that increasing inclusion levels led to a consistent decline in TSS.

4.2 Proximate composition of yoghurt on Day 7

Seven days of storage (Table 05), significant differences were observed in the proximate composition of yoghurt samples over the storage period. The moisture content showed significant variation among the samples. The highest moisture retention was recorded in the 3% formulation ($67.40^{a} \pm 0.10$), followed closely by the 5% group ($66.60^{b} \pm 0.10$). Both values were significantly higher than those of the control ($62.20^{d} \pm 0.20$) and 7% ($62.73^{c} \pm 0.20$) treatments. Tukey's HSD post hoc analysis confirmed significant differences between the 3% and 5% groups ($p = 0.001$), justifying the assignment of distinct superscripts. Although the 7%

sample showed slightly higher moisture than the control, it was still significantly lower than the 3% and 5% samples ($p < 0.001$). As the concentration of *A. philoxeroides* juice increases, the fat-protein network in yoghurt becomes less effective, resulting in moisture migration. This reduction in moisture retention is consistent with findings from previous studies, which suggest that excessive fortification can interfere with the structural integrity of the product, leading to water loss during storage (Gänzle & Hertel, 2009; McSweeney & Sousa, 2000).

Significant increases in ash content ($p < 0.05$) were observed with increasing storage period. The lowest value was recorded in the control sample ($3.20^{d} \pm 0.00$), followed by the 3% ($4.21^{c} \pm 0.10$), 5% ($4.81^{b} \pm 0.10$), and 7% ($5.03^{a} \pm 0.05$) formulations. All groups differed significantly from one another, indicating a consistent dose-dependent enhancement of mineral content due to increasing levels of enrichment.

The carbohydrate content also exhibited significant variation among the groups ($p< 0.05$) on day 7. The control sample contained the lowest value ($22.89^{d} \pm 0.01$), while the 7% formulation demonstrated the highest ($24.28^{a} \pm 0.01$). Intermediate values were found in the 3% ($23.19^{c} \pm 0.01$) and 5% ($24.07^{b} \pm 0.01$) samples, with all pairwise comparisons showing statistically significant differences. This pattern suggests a clear and progressive increase in carbohydrate content with elevated treatment concentrations, even after storage. During fermentation, the lactic acid bacteria (LAB) in yoghurt typically break down lactose (the primary carbohydrate in milk) into lactic acid. However, non-dairy ingredients like *A. philoxeroides* juice and cardamom flavour may not undergo the same fermentation processes. These ingredients could introduce additional carbohydrates that remain largely unaffected by the microbial activity during fermentation, which results in increased carbohydrate levels in the final product.

Regarding protein content, significant differences were found among all treatment groups and reduction of protein content observed during storage. The highest protein level was observed in the 7% sample ($90.77^{a} \pm 0.01$), followed by the 5% ($88.38^{b} \pm 0.01$), 3% ($86.69^{c} \pm 0.01$), and control ($52.18^{d} \pm 0.01$) samples. All values were statistically distinct, as supported by Tukey's HSD test.

The fat content substantially reduced over the storage period. The fat content on Day 07 remained statistically similar among the control ($7.30^{a} \pm 0.58$), 3% (7.19^{a}), and 5% (6.67^{a}) formulations, with no significant differences ($p>0.05$) observed among these groups.

Consequently, they were assigned the same superscript. In contrast, the 7% treatment exhibited a markedly lower fat content ($4.97^{b} \pm 0.01$), which differed significantly from all other groups ($p<0.001$). This decrease suggests that higher concentrations may have induced fat migration, oxidation, or degradation during storage, whereas lower concentrations preserved fat stability over time.

Fiber content remained negligible across all groups. The control, 3%, and 5% samples contained no detectable fiber (0.00 ± 0.00), while only a trace amount was found in the 7% formulation (0.01 ± 0.00). These results indicate that the treatment had minimal effect on fiber content, even at the highest inclusion level.

Finally, during the storage period, water loss due to evaporation could lead to a higher concentration of soluble solids in the product, which increases TSS. The control group presented the highest TSS value ($23.43^{a} \pm 0.05$), followed by the 3% ($22.53^{b} \pm 0.05$), 5% ($21.80^{c} \pm 0.10$), and 7% ($21.33^{d} \pm 0.05$) samples. Each treatment group differed significantly from the others, as denoted by distinct superscripts. This consistent decline in TSS suggests that higher inclusion levels may influence solute concentration and matrix interactions, potentially altering the distribution or stability of soluble solids during storage.

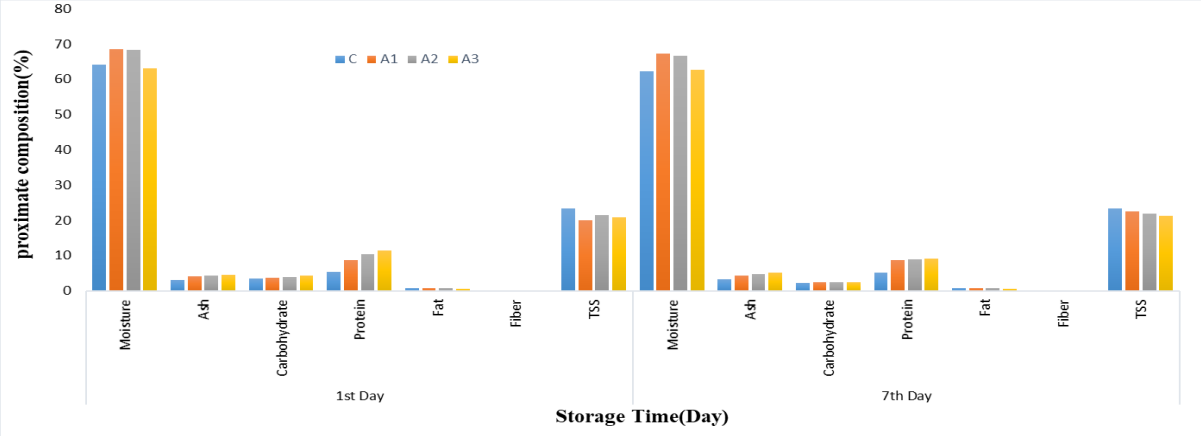

Figure 4.1: The mutual effect of storage time and amount of *A. philoxeroides* leaf juice on Proximate composition of all yoghurt samples (C= control, A1=3%, A2=5%, A3=7%)

4.3 Physicochemical test of yoghurt on Day 1

Table 06 presents the physicochemical properties of yoghurt samples on the first day of evaluation. Significant differences in pH values were observed among the treatment groups ($p < 0.05$). The control sample exhibited the highest pH ($4.45^{a} \pm 0.01$), whereas the 7% treatment

showed the lowest value ($4.31^{c} \pm 0.01$). The 3% ($4.35^{b} \pm 0.01$) and 5% ($4.34^{b} \pm 0.01$) samples demonstrated intermediate pH values that did not differ significantly from one another, indicating that moderate ingredient incorporation exerted minimal influence on acidity levels compared to the control and higher concentration treatments.

Titratable acidity increased progressively with higher treatment levels and was significantly different across all groups ($p < 0.05$). The highest acidity was observed in the 7% treatment ($0.81^{a} \pm 0.01$), while the control group had the lowest value ($0.57^{d} \pm 0.01$). Intermediate values were recorded for the 3% ($0.72^{c} \pm 0.02$) and 5% ($0.77^{b} \pm 0.01$) samples, each forming statistically distinct groups, suggesting a dose-dependent increase in acidity with higher levels of enrichment. Milk pH and acidity are widely used as indirect indicators of microbiological quality (Kasapidou et al,2021). Bacterial growth in milk typically involves lactose fermentation, leading to lactic acid production, increased acidity, and a reduction in pH (Kasapidou et al., 2024). Azad et al. (2012), "Phytochemical screening and antioxidant activity of *A. philoxeroides* leaf extracts". This study discusses the presence of organic acids and other compounds in the leaves of *A. philoxeroides*, which may affect the acidity of the juice when used as a fortifier.

Water holding capacity (WHC) also varied significantly among the treatments ($p < 0.05$). The 7% ($94.23^{a} \pm 0.15$) and 5% ($94.16^{a} \pm 0.15$) formulations exhibited the highest WHC values and did not differ significantly from one another ($p > 0.05$). Both were significantly higher than the control ($93.20^{b} \pm 0.10$) and 3% ($93.50^{b} \pm 0.10$) groups, which also did not differ significantly between themselves. These findings suggest that higher inclusion levels may enhance the structural integrity of the yoghurt matrix, improving water retention.

Table 4.2: Physicochemical test of yoghurt

Day-01					
Sample	**P^{H}**	**TA**	**WHC**	**Syneresis**	**Viscosity**
Control	$4.45^{a} \pm 0.01$	$0.57^{d} \pm 0.01$	$93.20^{b} \pm 0.10$	$7.10^{a} \pm 0.10$	$2524.67^{c} \pm 5.03$
3%	$4.35^{b} \pm 0.01$	$0.72^{c} \pm 0.02$	$93.50^{b} \pm 0.10$	$6.70^{b} \pm 0.10$	$2675.33^{b} \pm 5.03$
5%	$4.34^{b} \pm 0.01$	$0.77^{b} \pm 0.01$	$94.16^{a} \pm 0.15$	$4.03^{c} \pm 0.05$	$2709.00^{a} \pm 3.60$
7%	$4.31^{c} \pm 0.01$	$0.81^{a} \pm 0.01$	$94.23^{a} \pm 0.15$	$4.03^{c} \pm 0.05$	$2108.33^{d} \pm 7.63$
Day-07 Storage					
Sample	**P^{H}**	**TA**	**WHC**	**Syneresis**	**Viscosity**
Control	$4.31^{a} \pm 0.01$	$0.60^{d} \pm 0.00$	$93.20^{a} \pm 0.10$	$7.43^{a} \pm 0.05$	$1925.33^{a} \pm 2.51$
3%	$4.22^{b} \pm 0.01$	$0.73^{c} \pm 0.01$	$93.76^{b} \pm 0.05$	$6.16^{b} \pm 0.15$	$2273.00^{b} \pm 5.00$
5%	$4.18^{c} \pm 0.01$	$0.80^{b} \pm 0.01$	$94.20^{c} \pm 0.20$	$4.23^{c} \pm 0.20$	$2437.33^{c} \pm 2.08$
7%	$4.17^{c} \pm 0.00$	$0.83^{a} \pm 0.01$	$94.56^{d} \pm 0.11$	$3.80^{d} \pm 0.10$	$1916.00^{a} \pm 5.29$

Note: Values are means of triplicate test ± standard deviation. Means in the same column with different superscripts letters (a, b, c, d) differ significantly at $p < 0.05$ (Tukey HSD)

Syneresis showed a significant decreasing trend with increasing treatment concentration ($p < 0.05$). The control sample demonstrated the highest syneresis ($7.10^{a} \pm 0.10$), significantly exceeding all other groups. The 3% treatment presented an intermediate value, while the lowest levels were recorded in the 5% and 7% treatments ($4.03^{c} \pm 0.05$), which did not differ significantly from one another. This trend indicates that ingredient enrichment contributed to a reduction in whey separation, likely through improved gel structure. However, AP contain dietary fibers as well as proteins that can absorb and hold water (Serdiati et al., 2024). As the juice concentration increases, the breaking effect is compensated by the water-holding capacity of AP that absorbs the whey released by the gel.

Viscosity also differed significantly among the samples ($p < 0.05$). The highest viscosity was observed in the 5% treatment ($2709.00^{a} \pm 3.60$ cP), which was significantly greater than all other groups. The 3% treatment recorded the second highest value ($2675.33^{b} \pm 5.03$ cP), also significantly higher than the control ($2524.67^{c} \pm 5.03$ cP) and 7% ($2108.33^{d} \pm 7.63$ cP) samples. The lowest viscosity was found in the 7% group, suggesting that excessive addition may compromise the textural stability of the yoghurt. At the 7% concentration, the lower viscosity may result from an oversaturation of the active compounds in *A. philoxeroides* leaf juice. At higher concentrations, the molecular interactions between the bioactive compounds may become less effective at forming a thickening network, reducing the overall viscosity. These results collectively indicate that moderate enrichment levels improved the functional properties of the yoghurt, whereas higher levels may negatively impact consistency.

4.4 Physicochemical test of yoghurt on Day 7

Table 06 summarizes the physicochemical characteristics of yoghurt samples following seven days of storage. A significant decline in pH values was observed during storage days. The control sample exhibited the highest pH ($4.31^{a} \pm 0.01$), followed by the 3% treatment ($4.22^{b} \pm 0.01$). The 5% ($4.18^{c} \pm 0.01$) and 7% ($4.17^{c} \pm 0.00$) treatments recorded the lowest pH values. According to Tukey's HSD post hoc test, all groups differed significantly from each other except for the 5% and 7% treatments, which were not statistically different.The observed decrease could be as a result of production of lactic acid and other organic acids by the activities of the starter and non-starter bacteria that ferment lactose in yoghurt. It is imperative to note

that the activity of lactic acid bacteria is not completely stopped during refrigerated storage conditions. It is marked with the production of lactic acid leading to a drop of pH (Makinde et al., 2022).

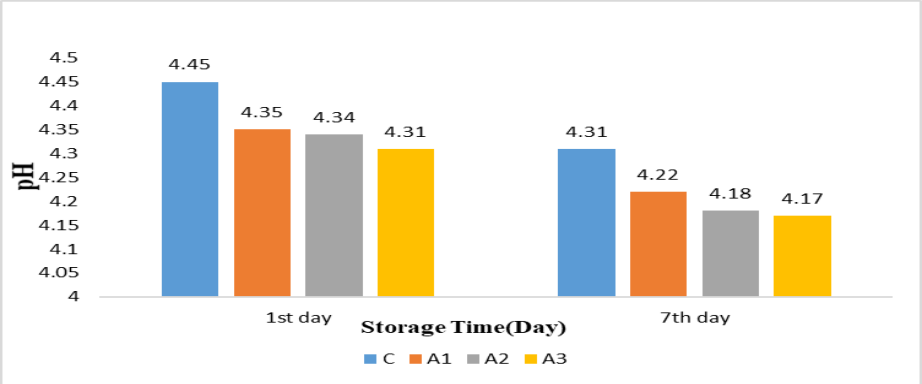

Figure 4.2: The mutual effect of storage time and amount of *A. philoxeroides*leaf juice on pH of all yoghurt samples (C= control, A1=3%, A2=5%, A3=7%)

Titratable acidity increased significantly ($p < 0.05$) over the storage period. The 7% treatment showed the highest acidity ($0.83^{a} \pm 0.01$), followed by the 5% ($0.80^{b} \pm 0.01$) and 3% ($0.73^{c} \pm 0.01$) groups, all of which were significantly higher than the control ($0.60^{d} \pm 0.00$). The difference between the 5% and 7% treatments was not statistically significant ($p = 0.016$), indicating a plateau effect in acidity development at the highest concentration. The increase in titratable acidity observed with the control sample was a result of the action of lactic acid bacteria, accompanied by the production of lactic acid at refrigerated storage conditions of 4°C. This result agrees with previous findings as reported by (Njoya et al.,2018).

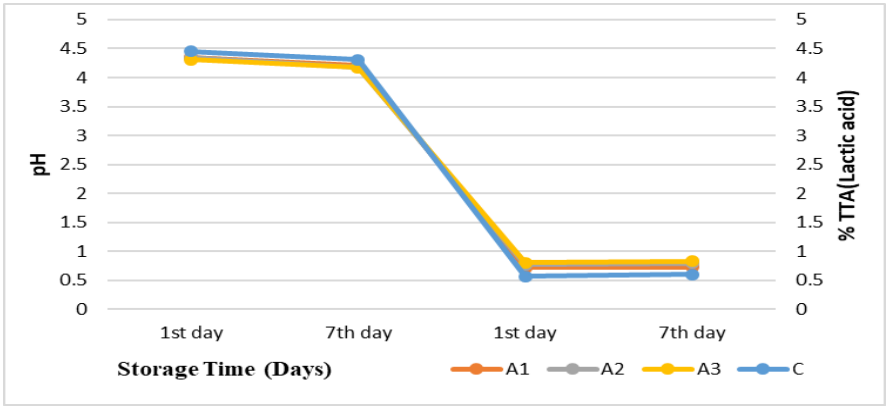

Figure 4.3: Changes in pH values and titratable acidity (% lactic acid) in yoghurt samples and with *A. philoxeroides*leaf juice during 7 days of cold storage (C= control, A1=3%, A2=5%, A3=7%)

Figure 9 illustrates the comparison between pH and titratable acidity (TTA) over storage period, expressed as % lactic acid, for different samples labeled A1, A2, A3, and C. The x-axis represents the storage time in days, specifically the 1st day and 7th day. The left y-axis indicates pH values, ranging from 0 to 5, while the right y-axis represents % TTA (lactic acid), also ranging from 0 to 5. On the 1st day of storage, all samples (A1, A2, A3, and C) show initial pH values around 4.5. Correspondingly, their TTA values are approximately 0.75% to 0.8% lactic acid. This suggests a consistent starting point for all samples in terms of acidity and pH.

As the storage time progresses to the 7th day, there is a noticeable decline in pH for all samples. The pH values for A1, A2, A3, and C decrease to approximately 4.25. Simultaneously, a significant increase in TTA is observed. On the 7th day, the TTA values for all samples rise to around 4.25% lactic acid. This inverse relationship between pH and TTA is expected, as a decrease in pH indicates an increase in acidity, which is quantified by TTA.

The lines connecting the 1st day and 7th day data points for both pH and TTA demonstrate a clear trend. The pH lines show a downward slope, indicating a decrease in pH over the 7-day storage period. Conversely, the TTA lines exhibit an upward slope, signifying an increase in acidity during the same period. This pattern suggests that during storage, lactic acid production or other acid-generating processes are occurring in all samples, leading to a more acidic environment. The similar trends observed across all samples (A1, A2, A3, and C) indicate that the general behavior regarding pH and TTA changes over storage time is consistent among them, although specific magnitudes might slightly vary depending on the individual sample characteristics.

WHC remained stable or improved slightly in all samples over 7 days. The control sample did not show any change in WHC (93.20% on both days), implying no improvement in water retention ability during storage. The treated samples (3%, 5%, 7%) showed progressive increases in WHC.The control sample recorded the lowest WHC value ($93.20^{a} \pm 0.10$), while the 7% treatment achieved the highest ($94.56^{d} \pm 0.11$). All treatment groups were significantly different from each other, as confirmed by Tukey's HSD test. This upward trend reflects a dose-dependent enhancement of WHC, likely due to the incorporation of functional

components that improve the matrix's ability to retain water. The findings suggest that higher inclusion levels contribute positively to the textural stability and perceived juiciness of the product during storage.

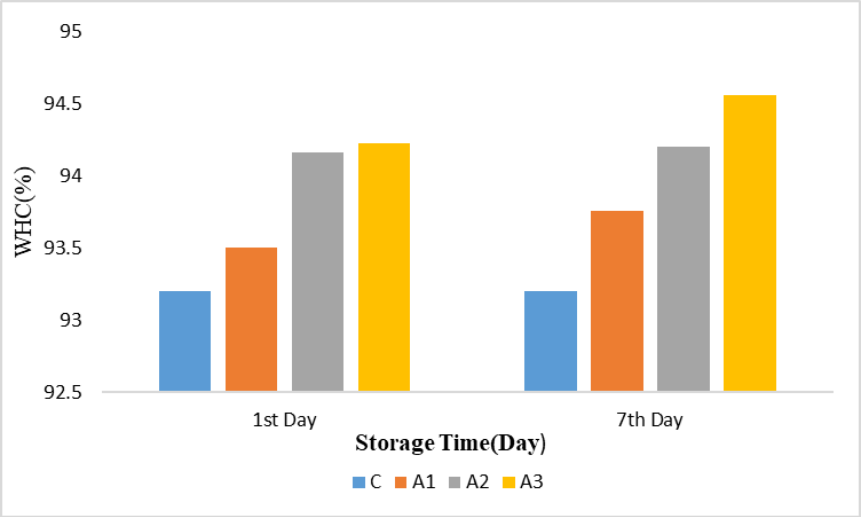

Figure 4.4: The mutual effect of storage time and amount of *A. philoxeroides*leaf juice on WHC of all yoghurt samples (C= control, A1=3%, A2=5%, A3=7%)

Syneresis decreased over the storage period. The control group demonstrated the highest syneresis ($7.43^{a} \pm 0.05$), which was significantly greater than those of all treated groups. The 7% treatment showed the lowest syneresis ($3.80^{d} \pm 0.10$), while the 3% ($6.16^{b} \pm 0.15$) and 5% ($4.23^{c} \pm 0.20$) treatments presented intermediate values. All treatment groups were significantly different from one another, with the 5% and 7% samples differing significantly from the 3% treatment ($p = 0.023$). This reduction in whey separation suggests improved gel strength and water-holding structure at higher enrichment levels.

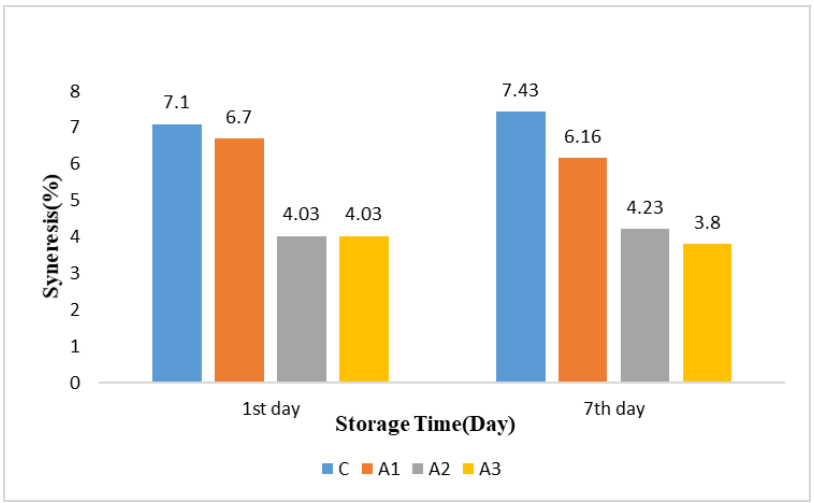

Figure 4.5: The mutual effect of storage time and amount of *A. Philoxeriodes* leaf juice on syneresis of all yoghurt samples (C= control, A1=3%, A2=5%, A3=7%)

Viscosity decreased significantly over 7 days for all treatments, indicating structural breakdown in the sample matrix during storage. The greatest drop was observed in the control (599.34 cP), showing no structural stability without additives. The 5% and 7% treatments retained more viscosity, indicating superior resistance to molecular breakdown (possibly due to stabilizers or hydrocolloids). Overall, the 5% treatment appeared most effective in improving consistency and maintaining desirable viscosity during storage.

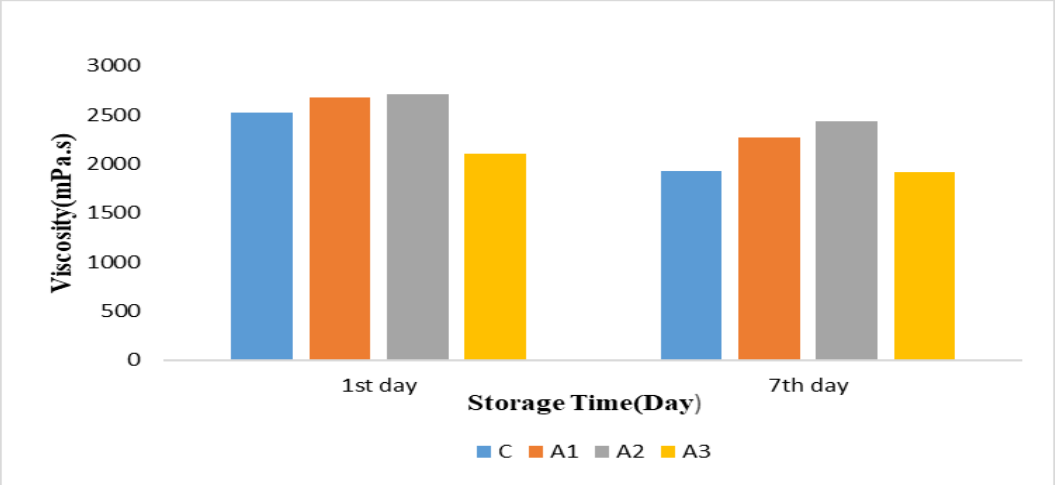

Figure 4.6: The mutual effect of storage time and amount of *A. Philoxeriodes* leaf juice on viscosity of all yoghurt samples (C= control, A1=3%, A2=5%, A3=7%)

4.5 Colour analysis table of yoghurt on Day 1

Table 07 presents the results of colour analysis for the yoghurt samples on Day 1. A significant reduction in lightness (L*) values was observed across all treatment groups compared to the control ($p < 0.05$). The control sample exhibited the highest lightness ($79.54^{a} \pm 0.02$), while the 7% treatment showed the lowest value ($78.09^{d} \pm 0.01$). Each group was significantly different from the others, forming four statistically distinct groups. This reduction in L* values with increasing treatment levels suggests that higher concentrations of the incorporated ingredient contributed to a perceptible darkening of the product.

In terms of the red-green axis (a*), the values became progressively more negative with increasing treatment levels, indicating a shift toward greenness. The control sample had the highest a* value ($-0.26^{a} \pm 0.02$), whereas the 7% treatment recorded the most negative value ($-1.28^{d} \pm 0.02$), reflecting a more intense green hue. All samples differed significantly from

one another ($p < 0.05$), demonstrating a dose-dependent colour shift along the a* axis due to the added ingredients. Similarly, a significant decreasing trend was observed in b* values ($p < 0.05$), indicating a reduction in yellowness with increasing treatment levels. The control group exhibited the highest b* value ($5.21^{a} \pm 0.01$), and the 7% sample had the lowest ($3.88^{d} \pm 0.01$). Each treatment level resulted in significantly different b* values, confirming a consistent and significant decline in the yellow tone of the samples with higher ingredient inclusion. inclusion. Our findings are in accordance (Barukci et al.,2022) with who used OLE (1.5, 3, and 5% v/v) in manufacturing of functional and healthy yoghurt, which led to a significant decline in lightness and yellowness with increased redness could be due to the darker colour of olive leaf. The total colour difference (ΔE) values increased with the level of treatment, reflecting cumulative changes in the colour attributes relative to the control. Although ΔE was not subjected to Tukey's HSD test due to its inapplicability to the control sample, the values ranged from 0.95 in the 3% treatment to 2.22 in the 7% treatment. This progression indicates a greater perceptible colour deviation from the control as the concentration of the added ingredient increased.

Table 4.3: Colour analysis table of yoghurt

Sample	**Day-01 Colour values**			
	L^{*}(D1)	a^{*}(D1)	b^{*}(D1)	ΔE(D1)
Control	79.54^{a}±0.02	-0.26^{a}±0.02	5.21^{a}±0.01	-
3%	79.02^{b}±0.02	-1.02^{b}±0.01	5.02^{b}±0.01	0.95±0.01
5%	78.79^{c}±0.01	-1.12^{c}±0.02	4.52^{c}±0.01	1.35±0.01
7%	78.09^{d}±0.01	-1.28^{d}±0.02	3.88^{d}±0.01	2.22±0.01
Sample	**Day-07 Storage Colour values**			
	L^{*}(D7)	a^{*}(D7)	b^{*}(D7)	E(D7)
Control	79.53^{a}±0.01	-0.26^{a}±0.02	5.19^{a}±0.05	-
3%	78.75^{b}±0.01	-1.09^{b}±0.01	4.98^{b}±0.01	1.16±0.01
5%	77.96^{c}±0.01	-1.18^{c}±0.01	4.44^{c}±0.01	1.95±0.01
7%	77.55^{d}±0.01	-1.37^{d}±0.01	4.34^{d}±0.01	2.91±0.01

Note: Values are means of triplicate test ± standard deviation. Means in the same column with different superscripts letters (a, b, c, d) differ significantly at $p < 0.05$ (Tukey HSD)

4.6 Colour analysis table of yoghurt on Day 7

Table 07 presents the colour attributes of yoghurt samples after seven days of storage. A significant reduction in lightness (L*) values was observed with increasing treatment concentrations ($p < 0.05$). The control group maintained the highest L* value ($79.53^{a} \pm 0.01$),

while the 7% treatment exhibited the lowest ($77.55^{d} \pm 0.01$). All treatment groups were significantly different from one another, forming four distinct statistical groups. This consistent decline in lightness suggests that higher inclusion levels of the added ingredient contributed to perceptible darkening over the storage period. The a* values demonstrated a progressive shift toward the green axis with increased treatment levels. The control sample recorded the highest a* value ($-0.26^{a} \pm 0.02$), whereas the 7% treatment showed the most negative value ($-1.37^{d} \pm 0.01$), indicating a more intense green coloure. All groups differed significantly ($p < 0.05$), forming four statistically distinct subsets. These results reflect a dose-dependent change in colour along the red-green spectrum due to the additive effect of the incorporated ingredients. A significant decreasing trend in b* values was also observed ($p < 0.05$), indicating reduced yellowness with higher treatment concentrations. The control group exhibited the highest b* value ($5.19^{a} \pm 0.05$), while the 7% treatment showed the lowest ($4.34^{d} \pm 0.01$). All treatment groups were significantly different; however, the 5% and 7% samples showed a narrower significance gap ($p = 0.016$), suggesting a potential saturation point in the reduction of yellowness at higher concentration.The Control lightness remains almost unchanged (79.53), indicating the colour is stable. The 3%, 5%, and 7% samples all show a slight decrease in lightness, with the 7% sample being the darkest on Day 7 (77.55).

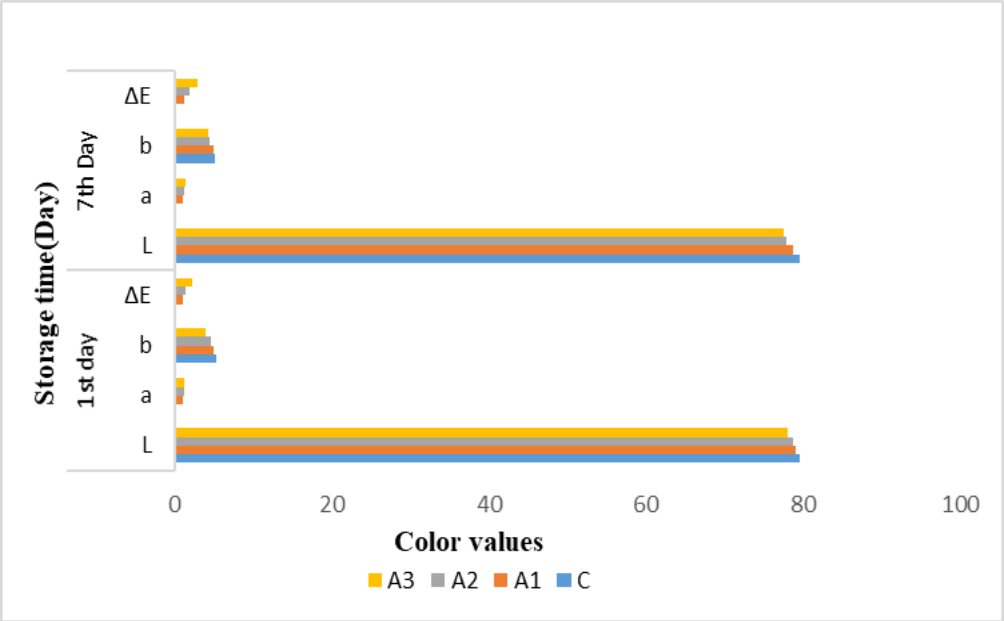

Figure 4.7: The mutual effect of storage time and amount of *A. Philoxeriodes* leaf juice on colour of all yoghurt samples (C= control, A1=3%, A2=5%, A3=7%)

The total colour difference (ΔE) increased progressively with treatment level, indicating greater visual deviation from the control as concentration increased. As ΔE was not applicable to the

control sample, it was not subjected to statistical comparison. However, values ranged from 1.16 in the 3% treatment to 2.91 in the 7% treatment, with the highest ΔE observed in the 7% group. The colour of yoghurt is pH-dependent; however, the change in colour during storage was a result of the heat treatment applied to milk, leading to the release of certain colour pigments such as carotene, as reported (by García-Pérez et al.,2005).

4.7 Bioactive compound of yoghurt on Day 1

Table 08 presents the analysis of bioactive compounds in yoghurt samples. On the first day, total phenolic content (TPC) exhibited a significant increase with the addition of treatments compared to the control. The control sample demonstrated the lowest TPC value (0.04 ± 0.00 mg GAE/g). The 3% treatment group showed a significant rise, distinctly differing from both the control and the higher concentration groups. Meanwhile, the 5% and 7% treatments recorded similar phenolic content values and did not differ significantly from each other. This trend suggests a concentration-dependent increase in phenolic compounds up to the 5% treatment level, beyond which the effect plateaued, indicating no further statistically significant enhancement.

Similarly, total flavonoid content (TFC) showed a marked and statistically significant increase ($p < 0.05$) across treatment levels. The control group had the lowest TFC ($0.03^{d} \pm 0.00$ mg QE/g), while the highest value was observed in the 7% treatment ($0.98^{a} \pm 0.00$ mg QE/g). All treatment groups were significantly different from each other, forming four distinct significance levels in the following order: Control < 3% < 5% < 7%. This pattern reflects a consistent elevation in flavonoid content corresponding to increasing treatment concentration.

Table 4.4: Bioactive compounds of yoghurt

Test Name	Sample	Day-01 (Mean ± SD)	Day-07 (Mean ± SD)
TPC	Control	$0.04^{a} \pm 0.00$	$0.04^{d} \pm 0.00$
	3%	$0.09^{b} \pm 0.00$	$0.07^{c} \pm 0.00$
	5%	$0.09^{c} \pm 0.00$	$0.07^{b} \pm 0.00$
	7%	$0.09^{c} \pm 0.00$	$0.08^{a} \pm 0.00$
TFC	Control	$0.03^{d} \pm 0.00$	$0.02^{d} \pm 0.00$
	3%	$0.07^{c} \pm 0.00$	$0.05^{c} \pm 0.00$
	5%	$0.86^{b} \pm 0.00$	$0.06^{b} \pm 0.00$
	7%	$0.98^{a} \pm 0.00$	$0.08^{a} \pm 0.00$

Note: Values are means of triplicate tests ± standard deviation. Means in the same column with different superscript letters (a, b, c, d) differ significantly at $p < 0.05$ (Tukey HSD)

Table 09 presents the comparative analysis of DPPH free radical scavenging activity of different yoghurt samples on Day 1. At the 20% inhibition level, all treated groups exhibited a significant increase in activity compared to the control. The control group showed the lowest inhibition value of $31.00^{a} \pm 1.00\%$. In contrast, the 3%, 5%, and 7% treatment groups demonstrated higher inhibition values of $35.26^{b} \pm 0.15\%$, $37.00^{b} \pm 1.00\%$, and $39.66^{b} \pm 1.52\%$, respectively. Statistical analysis indicated no significant differences among the 3%, 5%, and 7% groups, suggesting comparable efficacy in free radical inhibition within these concentrations. However, all treatment groups differed significantly from the control, highlighting the positive effect of supplementation on antioxidant activity.

At a concentration of 40 µg/ml, the radical scavenging activity (RSA%) on Day 1 showed notable variation across treatment groups. The 7% group demonstrated the highest inhibition value ($61.00^{a} \pm 1.00\%$), followed by the 5% ($54.66^{b} \pm 1.52\%$), 3% ($52.16^{b} \pm 0.05\%$), and the control group ($45.00^{c} \pm 1.00\%$). Tukey's HSD post hoc test revealed that the 7% treatment was significantly different from all other groups ($p < 0.05$). The control also showed significant differences compared to all treatments. However, there was no statistically significant difference between the 3% and 5% groups ($p = 0.072$), indicating their antioxidant activities were statistically similar.

At a concentration of 60 µg/ml on Day 1, the 7% group exhibited the highest radical scavenging activity ($80.33^{a} \pm 0.57\%$), followed by the 5% ($72.66^{b} \pm 1.52\%$) and control ($71.00^{b} \pm 1.00\%$) groups, while the 3% group showed the lowest activity ($65.53^{c} \pm 0.02\%$). Statistical analysis confirmed that the 7% group was significantly different from all other groups ($p < 0.001$). No significant difference was observed between the control and 5% groups ($p = 0.222$), placing them in the same statistical category. The 3% group, however, was significantly different from both the control and 5% treatments ($p = 0.001$ and $p = 0.000$, respectively), forming a separate statistical group.

At 80 µg/ml concentration on Day 1, the control group recorded the lowest mean value ($80.13^{a} \pm 0.03\%$), significantly lower than all treatment groups. The 3% and 7% groups displayed similar values ($86.27^{b} \pm 0.02\%$ and $86.00^{b} \pm 1.00\%$, respectively), indicating no significant difference between them. The 5% group exhibited the highest mean value ($88.00^{bc} \pm 1.00\%$),

which was significantly higher than the 7% group but not significantly different from the 3% group. These findings suggest that treatment at all concentrations improved radical scavenging activity compared to the control, with the 5% concentration showing a marginally greater effect. However, the effects of the 3% and 7% treatments were statistically comparable.

At 100 µg/ml concentration on Day 1, the 7% treatment group showed the highest scavenging activity ($95.16^{a} \pm 0.15\%$), followed by the 5% ($94.50^{b} \pm 0.10\%$), 3% ($92.33^{c} \pm 0.20\%$), and control ($87.14^{d} \pm 0.02\%$) groups. Statistical evaluation confirmed that each group was significantly different from the others.

Finally, the IC_{50} values corroborated this pattern. The 7% treatment exhibited the lowest IC_{50} value (38.57 µg/ml), indicating the highest antioxidant potency. This was followed by the 5% (41.52 µg/ml), 3% (44.13 µg/ml), and control (47.25 µg/ml) groups. These findings affirm that increasing treatment concentration enhances free radical scavenging efficiency in a concentration-dependent manner, although the difference between the 3% and 5% groups was not statistically significant. The overall order of efficacy in terms of antioxidant activity was: Control < 3% < 5% < 7%.

4.8 Bioactive compound of yoghurt on Day 7

On 07 days (Table 08) of storage, total phenolic content (TPC) continued to increase progressively with the level of treatment but decreased compared to day 1. The control group exhibited the lowest TPC value ($0.04^{d} \pm 0.00$ mg GAE/g), indicating a minimal presence of phenolic compounds in the untreated yoghurt samples. In contrast, the 7% treatment demonstrated the highest TPC ($0.08^{a} \pm 0.00$ mg GAE/g), reflecting a marked enhancement in phenolic content following fortification. All pairwise comparisons were statistically significant ($p < 0.05$), including the comparison between the 5% and 7% treatments ($p = 0.046$), suggesting that even minor increases in treatment concentration produced meaningful differences. The 3% treatment recorded a TPC value of $0.07^{c} \pm 0.00$, which was significantly lower than both the 5% ($0.07^{b} \pm 0.00$) and 7% ($0.08^{a} \pm 0.00$) groups (Pourghorban et al.,2022) observed a decrease in TPC during storage of yoghurt supplemented with olive leaf and its extract (a rich source of polyphenols). This might be the result of the decomposition of polymeric phenolics in the presence of lactic acid bacteria during cold storage. Furthermore, the production of lactic acid during fermentation may cause degradation of unstable phenolic components at an acidic p^{H}. Although the 3% and 5% treatments shared the same numerical value, they differed

statistically, confirming the presence of four distinct significance levels: Control < 3% < 5% < 7%.

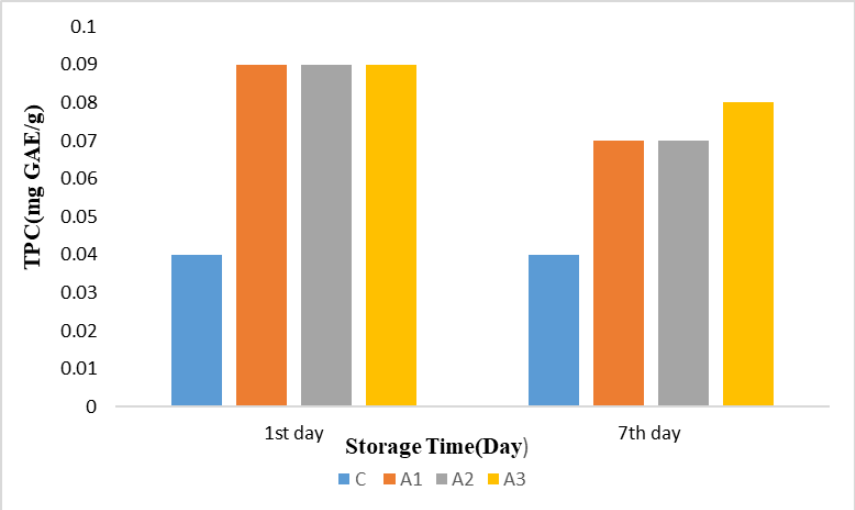

Figure 4.8: The mutual effect of storage time and amount of *A. Philoxeriodes* leaf juice on TPC of all yoghurt samples (C= control, A1=3%, A2=5%, A3=7%)

A similar trend was observed in total flavonoid content (TFC). The control group showed the lowest TFC ($0.02^{d} \pm 0.00$ mg QE/g), indicating low baseline levels of flavonoids. The highest TFC was recorded in the 7% treatment ($0.08^{a} \pm 0.00$ mg QE/g), suggesting that higher treatment concentrations effectively enhanced flavonoid retention after storage. All treatment groups differed significantly from each other ($p < 0.001$ for all pairwise comparisons), with the 3%, 5%, and 7% treatments yielding TFC values of 0.05^{c}, 0.06^{b}, and 0.08^{a} mg QE/g, respectively.

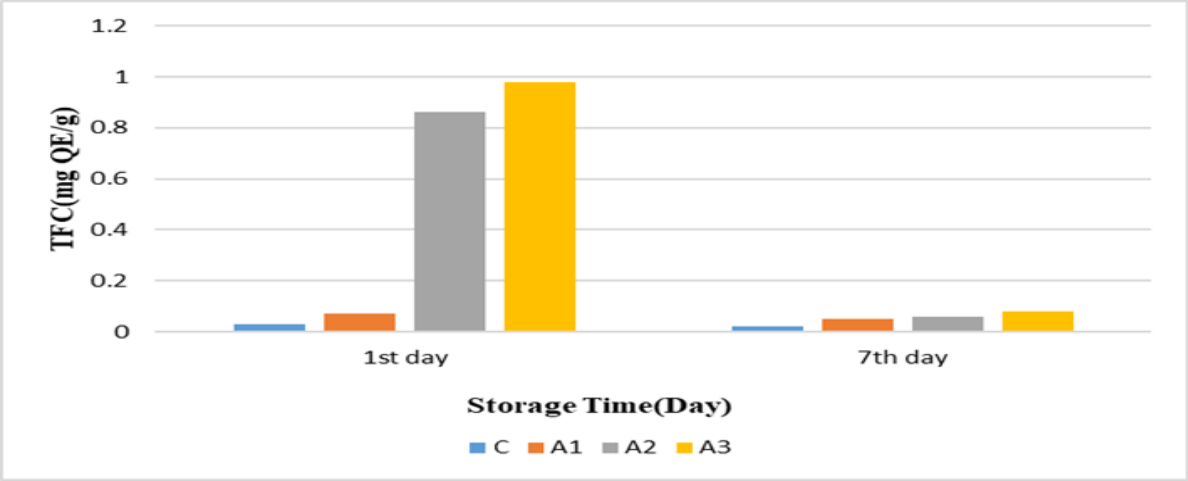

Figure 4.9: The mutual effect of storage time and amount of *A. Philoxeriodes* leaf juice (C= control, A1=3%, A2=5%, A3=7%)

At 7 days (Table 09) of storage, the radical scavenging activity of yoghurt samples at various concentrations (20–100 µg/ml) showed a decreasing pattern of antioxidant activity compared to the 1st day. At a concentration of 20 µg/ml on Day 7, the 5% treatment exhibited the highest radical scavenging activity ($50.55^{a} \pm 0.01\%$), followed by the 7% ($45.23^{b} \pm 0.15\%$), 3% ($36.73^{c} \pm 0.01\%$), and control ($29.81^{d} \pm 0.01\%$) groups. Statistical analysis confirmed that all treatment groups differed significantly from each other as well as from the control

Table 4.5: Comparative analysis of DPPH free radicals scavenging activity of different yoghurt samples (Antioxidant)

Sample	**Percentage of inhibition at different concentration(ug/ml) Day-01**					**IC_{50}**
	20	**40**	**60**	**80**	**100**	
Control	31.00^{a}±1.00	45.00^{c} ±1.00	71.00^{b}±1.00	80.13^{a}±0.03	87.14^{d} ± 0.02	47.25
3%	35.26^{b} ±0.15	52.16^{b} ±0.05	65.53^{c} ±0.02	86.27^{b}±0.02	92.33^{c} ± 0.20	44.13
5%	37.00^{b}±1.00	54.66^{b}±1.52	72.66^{b}±1.52	88.00^{bc}±1.00	94.50^{b} ± 0.10	41.52
7%	39.66^{b}±1.52	61.00^{a}±1.00	80.33^{a}±0.57	86.00^{c} ±1.00	95.16^{a} ± 0.15	38.57
Sample	**Percentage of inhibition at different concentration(ug/ml) Day-07**					**IC_{50}**
	20	**40**	**60**	**80**	**100**	
Control	29.81^{d} ±0.01	46.95^{d}±0.01	62.97^{c} ±0.02	66.16^{d}±0.11	81.48^{d} ± 0.02	52.74
3%	36.73^{c} ± 0.01	54.96^{b}±0.01	59.38^{d}±0.01	70.42^{c} ±0.01	87.55^{c} ± 0.01	48.04
5%	50.55^{a} ±0.01	50.51^{c}±0.01	65.81^{b}±0.13	78.22^{b}±0.01	89.22^{b} ± 0.01	41.09
7%	45.23^{b} ±0.15	62.97^{a}±0.02	68.73^{a}±0.06	80.18^{a}±0.01	91.14^{a} ± 0.05	40.07

Note: Values are means of triplicate tests ± standard deviation. Means in the same column with different superscript letters (a, b, c, d) differ significantly at $p < 0.05$ (Tukey HSD)

Table 4.6: Ascorbic acid standard radical scavenging activity (%)

Sample	**Percentage of inhibition at different concentration(ug/ml) Day-01 & 07 (Same)**					**IC_{50}**
Ascorbic	20	40	60	80	100	28.57
	58.70±0.50	77.00±0.40	85.45±0.30	89.34±0.20	97.20±0.10	

At a concentration of 40 (Table 09), µg/ml on Day 7, the 7% treatment demonstrated the highest radical scavenging activity ($62.97^{a} \pm 0.02\%$), followed by the 3% ($54.96^{b} \pm 0.01\%$), 5% ($50.51^{c} \pm 0.01\%$), and control ($46.95^{d} \pm 0.01\%$) groups. Each group was statistically distinct, as indicated by the unique superscripts, reflecting significant pairwise differences in antioxidant potential.

At a concentration of 60 µg/ml on Day 7, the 7% treatment again exhibited the highest radical scavenging activity ($68.73^{a} \pm 0.06\%$), followed by 5% ($65.81^{b} \pm 0.13\%$), control ($62.97^{c} \pm 0.02\%$), and the lowest in the 3% treatment ($59.38^{d} \pm 0.01\%$). Tukey's HSD test confirmed statistically significant differences across all groups. The notably lower antioxidant activity in the 3% group compared to the control, despite both being lower than 7%, suggests variability in dose response. Treatments with 5% and 7% concentrations demonstrated superior antioxidant activity compared to both control and 3%, with the 7% concentration being the most effective.

At a concentration of 80 µg/ml on Day 7, the highest radical scavenging activity was observed in the 7% treatment group ($80.18^{a} \pm 0.01\%$), followed by 5% ($78.22^{b} \pm 0.01\%$), 3% ($70.42^{c} \pm 0.01\%$), and control ($66.16^{d} \pm 0.11\%$). Statistical analysis confirmed that each group was significantly different from the others, as denoted by the distinct superscripts, reaffirming a clear trend of increasing antioxidant activity with higher extract concentrations.

At a concentration of 100 µg/ml on Day 7, the 7% treatment again demonstrated the highest radical scavenging activity ($91.14^{a} \pm 0.05\%$), followed by the 5% ($89.22^{b} \pm 0.01\%$), 3% ($87.55^{c} \pm 0.01\%$), and control ($81.48^{d} \pm 0.02\%$) groups. The Tukey HSD test indicated that all treatments differed significantly from one another, with each group representing a unique level of activity. This clear separation suggests a strong concentration-dependent enhancement of antioxidant properties with increased levels of extract.

Finally, the IC_{50} values corroborated this pattern, with the 7% treatment showing the lowest IC_{50} value (40.07 µg/ml), indicating the highest antioxidant potency. In contrast, the control exhibited the highest IC_{50} (52.74 µg/ml), reflecting the lowest radical scavenging activity. Overall, the results demonstrated a consistent dose-dependent increase in antioxidant activity across treatments, following the order: Control < 3% < 5% < 7%. During cold storage, the antioxidant activity values of control or all enriched products were declined till the end of storage which may be due to the degradation or oxidation of the phenolic compounds during storage time. (Kabir et al.,2021) added BP extract with levels of 0,100, 200, 400, 600, 800, and 1,000 µl per 100 mL of yoghurt milk which elevated the total phenolic content and exhibited higher antioxidant activities against DPPH and ABTS+ radicals of the yoghurt with the increase of BP extract addition. (El-Batawy et al.,2014) supplemented yoghurt with 2% of both mango and pomegranate peels powders that increased the total phenolic content and antioxidant

activity of product which remarkedly dropped during storage periods especially after 14 and 21 days.

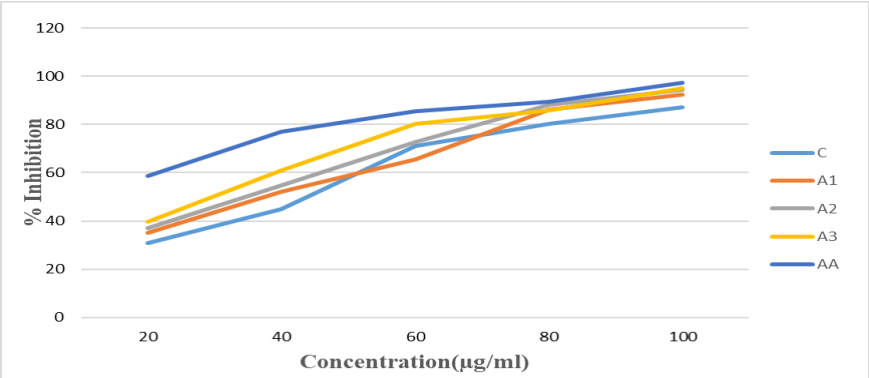

Figure 4.10: DPPH free radical scavenging activity (% inhibition vs concentration graph for standard and test sample)

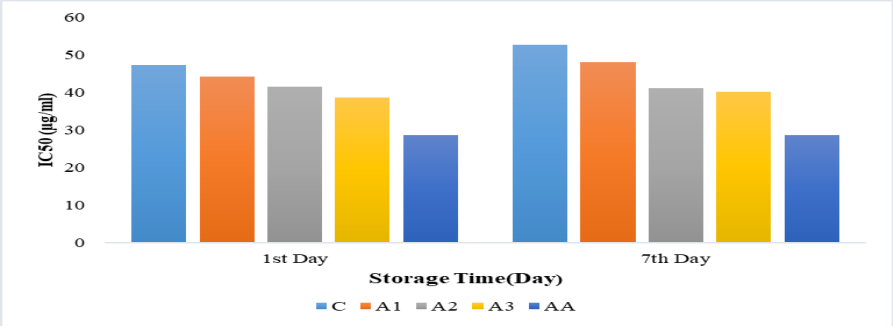

Figure 4.11: The mutual effect time of storage and amount of *A. Philoxeriodes* leaf juice on the IC50 value of standard and test samples (C= control, A1=3%, A2=5%, A3=7%).

Figure 17 compares the IC50 values of samples C, A1, A2, and A3 against a standard (AA) on Day 01 and Day 07 of storage. Lower IC50 values indicate higher antioxidant activity. On Day 01, the standard AA had the lowest IC50 (28.57 µg/ml), signifying the highest antioxidant efficacy. Samples C (47.25 µg/ml), A1 (44.13 µg/ml), A2 (41.52 µg/ml), and A3 (38.57 µg/ml) all showed higher IC50 values than AA, with A3 having the highest activity among the samples. By Day 07, the standard AA maintained its stable IC50 (28.57 µg/ml). However, the IC50 values for samples C (52.74 µg/ml) and A1 (48.04 µg/ml) increased, indicating a decrease in their antioxidant activity. Sample A2's IC50 remained stable at around 41 µg/ml, and A3's IC50 slightly increased to approximately 40.07 µg/ml. In conclusion, the standard AA consistently demonstrated superior and stable antioxidant activity, while samples C and A1

experienced a notable decline in activity during storage. Samples A2 and A3 showed more stable, albeit lower, antioxidant activity compared to the standard.

4.9 Nutritional compound of yoghurt on Day 1

Table 11 presents the nutritional composition of yoghurt samples on the first day of observation. Regarding Vitamin C content, a progressive increase was observed with increasing treatment concentration. The highest value was recorded in the 7% treatment group ($0.20^{a} \pm 0.00$ mg/g), followed by 5% ($0.14^{b} \pm 0.00$ mg/g), 3% ($0.10^{c} \pm 0.00$ mg/g), and the control group ($0.05^{d} \pm 0.00$ mg/g). Statistical analysis using Tukey's HSD indicated that all treatment groups differed significantly from each other, as denoted by distinct superscripts. The observed rise in ascorbic acid reflects the contributory role of the added ingredients in enhancing the antioxidant profile from the initial stage of storage. The observed rise in ascorbic acid reflects the vitamin C of AP juice.

The β-Carotene content also increased in a treatment-dependent manner. The control group exhibited the lowest β-Carotene value ($0.18^{a} \pm 0.02$ mg/g), while the 3%, 5%, and 7% treatments showed increasing levels, indicating a steady enhancement with concentration. The highest content was found in the 7% sample ($1.93^{d} \pm 0.07$ mg/g), representing nearly a tenfold increase compared to the control. The pattern highlights a strong positive correlation between treatment concentration and β-Carotene accumulation in the yoghurt matrix.

A similar concentration-dependent increase was observed for Calcium content across all treatment groups. The highest level was recorded in the 7% sample ($2.35^{a} \pm 0.01$ mg/g), followed by 5% ($2.28^{b} \pm 0.01$ mg/g),3%($1.82^{c} \pm 0.01$ mg/g), and the control($1.40^{d} \pm 0.03$ mg/g). Tukey's HSD analysis confirmed that each group differed significantly from the others, as indicated by distinct superscripts. Although the difference between 5% and 7% groups was relatively small, it remained statistically significant ($p = 0.020$), emphasizing that even marginal increases in treatment concentration effectively contributed to calcium enhancement. These findings reinforce the efficacy of the fortification strategy in improving the mineral profile of yoghurt from the earliest point of analysis.

In the case of Iron content, a substantial increase was observed only at the highest treatment level. The 7% treatment group exhibited the highest value ($0.43^{a} \pm 0.00$ mg/g), while both the 3% and 5% groups recorded significantly lower and statistically similar values

($0.03^{b} \pm 0.00$ mg/g). No detectable iron content was found in the control group (0.00 ± 0.00 mg/g). Tukey's HSD test confirmed that the 7% treatment was statistically distinct from all other groups, whereas the 3% and 5% samples, despite numerical variation, did not differ significantly and shared the same superscript. The control differed significantly from all treatments. These results suggest a threshold effect for iron enrichment, whereby a substantial increase in iron content was achieved only at the 7% supplementation level.

Table 4.7: Nutritional compounds of yoghurt

Day-01				
Sample	**Vitamin-C**	**Beta-Carotene**	**Calcium**	**Iron**
Control	$0.05^{d} \pm 0.00$	$0.18^{a} \pm 0.02$	$1.40^{d} \pm 0.03$	$0.00^{c} \pm 0.00$
3%	$0.10^{c} \pm 0.00$	$0.92^{b} \pm 0.04$	$1.82^{c} \pm 0.01$	$0.03^{b} \pm 0.00$
5%	$0.14^{b} \pm 0.00$	$1.45^{c} \pm 0.06$	$2.28^{b} \pm 0.01$	$0.03^{b} \pm 0.00$
7%	$0.20^{a} \pm 0.00$	$1.93^{d} \pm 0.07$	$2.35^{a} \pm 0.01$	$0.43^{a} \pm 0.00$
Day-07 Storage				
Sample	**Vitamin-C**	**Beta-Carotene**	**Calcium**	**Iron**
Control	$0.03^{d} \pm 0.00$	$0.16^{a} \pm 0.01$	$1.38^{d} \pm 0.00$	$0.00^{d} \pm 0.00$
3%	$0.08^{c} \pm 0.00$	$0.71^{b} \pm 0.03$	$1.85^{c} \pm 0.01$	$0.02^{c} \pm 0.00$
5%	$0.08^{b} \pm 0.00$	$1.09^{c} \pm 0.05$	$2.31^{b} \pm 0.02$	$0.02^{b} \pm 0.00$
7%	$0.16^{a} \pm 0.00$	$1.41^{d} \pm 0.06$	$2.38^{a} \pm 0.01$	$0.03^{a} \pm 0.00$

Note: Values are means of triplicate test ± standard deviation. Means in the same column with different superscripts letters (a, b, c, d) differ significantly at $p < 0.05$ (Tukey HSD)

4.10 Nutritional compound of yoghurt on Day 7

On Day 7 (Table 11), vitamin C content demonstrated a distinct upward trend with increasing treatment concentration but decreasing compare to day 1.

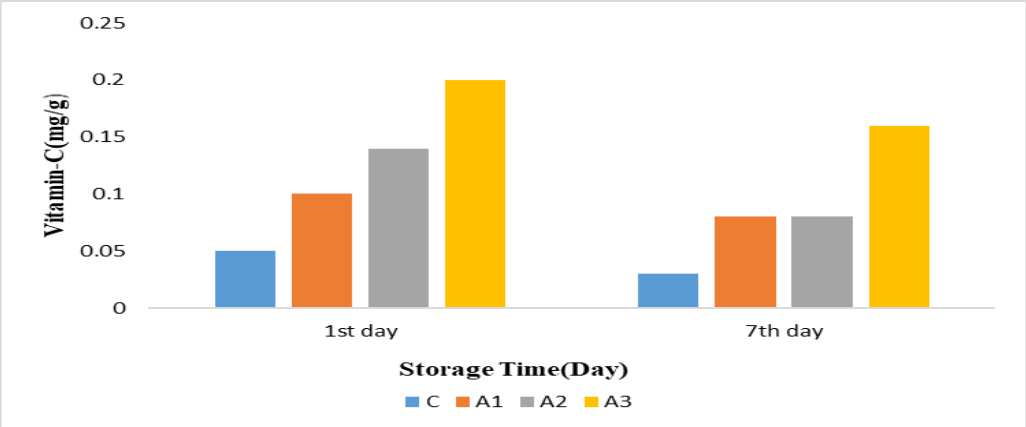

Figure 4.12: The mutual effect of storage time and amount of *A. Philoxeriodes* leaf juice on the vitamin C content (C= control, A1=3%, A2=5%, A3=7%)

Beta-carotene content showed a slight reduction over the 7-day storage period when compared to Day 1; however, a consistent dose-dependent increase with treatment concentration was maintained. The control group exhibited the lowest beta-carotene content ($0.16^{a} \pm 0.01$), while the highest was recorded in the 7% treatment group ($1.41^{d} \pm 0.06$). Intermediate values were observed for 3% and 5% treatments, with each concentration level resulting in a statistically higher beta-carotene content than the preceding one. These findings indicate that although some degradation occurred over time, the treatment remained effective in preserving higher beta-carotene levels, emphasizing the contribution of treatment concentration to carotenoid retention.

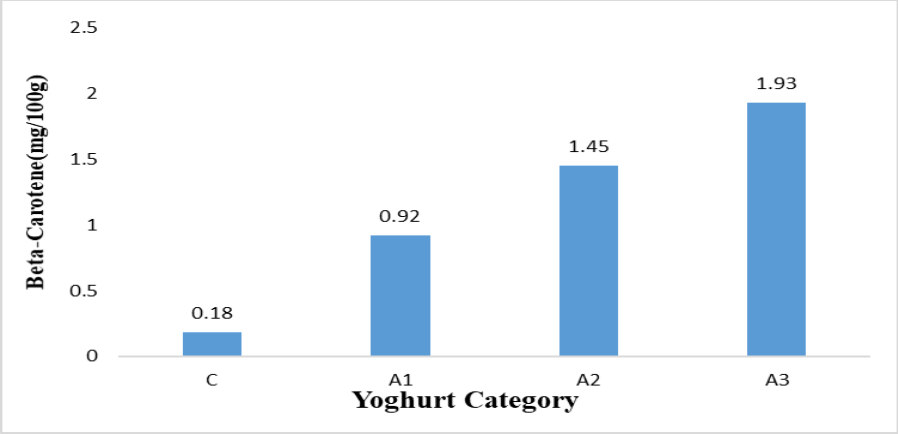

Figure 4.13: Comparison of Beta-Carotene among yoghurt samples (C= control, A1=3%, A2=5%, A3=7%)

Calcium content also followed a clear and consistent dose-dependent pattern. The highest calcium level was recorded in the 7% treatment ($2.38^{a} \pm 0.01$ mg/g), followed by 5% ($2.31^{b} \pm 0.02$ mg/g), 3% ($1.85^{c} \pm 0.01$ mg/g), and the lowest in the control group ($1.38^{d} \pm 0.00$ mg/g). Tukey HSD analysis confirmed that all pairwise differences were statistically significant ($p < 0.05$), including the relatively small difference between the 5% and 7% treatments. These results demonstrate that increasing the concentration of the treatment effectively improved calcium fortification in the product during storage. Calcium increases in yoghurt fortified with *A. philoxeroides* after 7 days because enzymes and acidic conditions during storage help release bound calcium from the plant material, increasing the soluble calcium content in the yoghurt matrix. Previous studies (Harikrishnan et al., 2020; Singh & Wani, 2017) have shown that lactic acid fermentation enhances mineral bioavailability,

including calcium, by degrading anti-nutritional factors such as phytates and fibers that usually bind minerals in the food matrix.

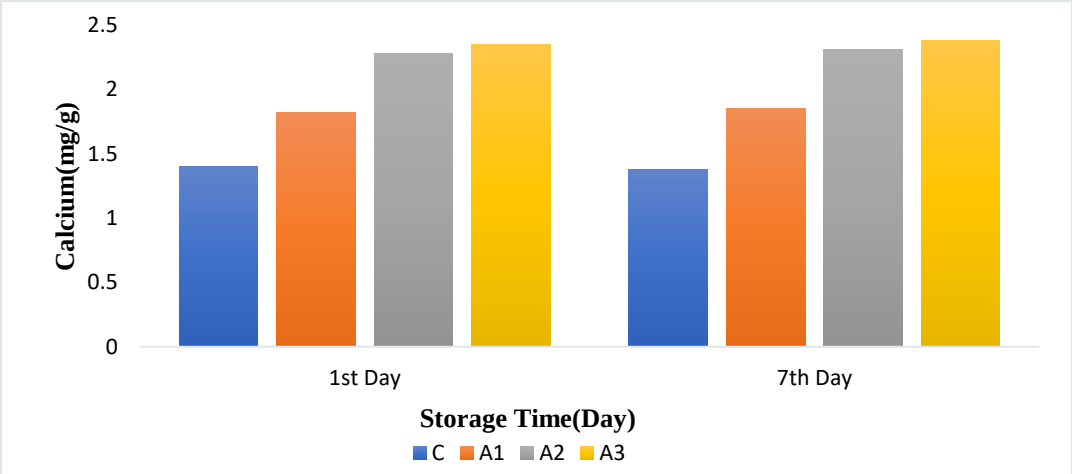

Figure 4.14: The mutual effect of storage time and amount of *A. Philoxeriodes* leaf juice on the Calcium content (C= control, A1=3%, A2=5%, A3=7%)

Iron content showed a gradual decrease during storage. The 7% treatment yielded the highest iron concentration ($0.03^{a} \pm 0.00$ mg/g), followed by 5% ($0.02^{b} \pm 0.00$ mg/g), 3% ($0.02^{c} \pm 0.00$ mg/g), while the control group exhibited no detectable iron ($0.00^{d} \pm 0.00$ mg/g). Although the mean values for the 3% and 5% treatments appeared visually similar, statistical analysis via Tukey HSD confirmed significant differences among all group comparisons ($p = .000$), as indicated by distinct superscripts. These findings suggest that even minor treatment increments contributed significantly to iron enhancement, with the 7% concentration resulting in the greatest improvement in iron content during the observed storage period.

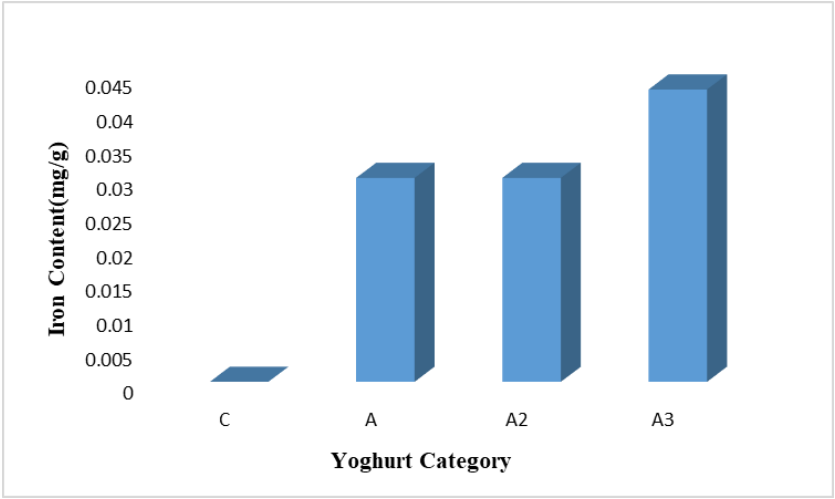

Figure 4.15: Comparison of iron content among yoghurt samples (C= control, A1=3%, A2=5%, A3=7%)

4.11 Microbiological test of yoghurt on Day 1

Table 12 presents the microbiological analysis of yoghurt samples. On Day 1, the total viable count (TVC) showed a narrow range across all samples, varying from 6.06^{a} log CFU/g in the control to 6.11^{ab} log CFU/g in the 5% and 7% treatment groups. Although the numerical differences were minor, certain pairwise comparisons exhibited statistical significance. Notably, both the 5% and 7% treatments showed significantly higher TVC values compared to the control ($p = .013$ and $p = .028$, respectively). In contrast, comparisons among the 3%, 5%, and 7% treatments revealed no statistically significant differences, indicating these groups were microbiologically similar in terms of viable counts. The highest microbial load was recorded in the 5% and 7% treatments, while the control group had the lowest. Despite statistical differences, the overall increase in microbial load was minimal, suggesting only a modest elevation in total viable count at Day 1 due to treatment.

Regarding *Lactobacillus* counts, a notable increase was observed in response to treatment, indicating a concentration-dependent effect. The 7% treatment group recorded the highest count ($5.66^{a} \pm 0.01$ log CFU/g), followed closely by the 5% ($5.65^{a} \pm 0.02$) and 3% ($5.59^{b} \pm 0.01$) groups, whereas the control exhibited a markedly lower count ($4.35^{c} \pm 0.02$). According to Tukey's HSD post hoc analysis, all groups differed significantly from one another ($p < 0.05$), except between the 5% and 7% groups ($p = .996$), which were statistically similar and, therefore, share the same superscript. The difference between 3% and both 5% and 7% treatments were statistically significant, despite their close numerical values, justifying distinct grouping. These findings demonstrate a clear dose-responsive enhancement in *Lactobacillus* proliferation, with the 5% and 7% treatments showing the most substantial effects. The control group remained significantly lower, reinforcing the effectiveness of the treatment in promoting beneficial microbial growth from the initial day of storage. This suggests that the concentration of *A. philoxeroides* leaf juice at 5% creates an environment that is favorable for the growth of Lactobacillus. This could be due to the presence of nutrients (such as sugars, amino acids, or other growth-promoting factors) in the juice that support probiotic growth. Additionally, cardamom might have synergistic effects in promoting the growth of Lactobacillus, as spices

like cardamom are known for their antimicrobial properties and could create a selective environment that favors the growth of beneficial microbes over pathogenic ones

Table 4.8: Microbiological test of yoghurt

Test Name	Sample Name	Day-01 (Mean ± SD)	Day-07 (Mean ± SD)
TVC	Control	$6.06^{a} \pm 0.01$	$6.05^{ab} \pm 0.00$
	3%	$6.08^{a} \pm 0.01$	$6.07^{a} \pm 0.00$
	5%	$6.11^{ab} \pm 0.01$	$6.11^{b} \pm 0.01$
	7%	$6.11^{b} \pm 0.01$	$6.05^{ab} \pm 0.00$
Lactobacillus count (CFU/g)	Control	$4.35^{c} \pm 0.02$	$4.31^{d} \pm 0.01$
	3%	$5.59^{b} \pm 0.01$	$5.56^{c} \pm 0.01$
	5%	$5.65^{a} \pm 0.02$	$5.67^{a} \pm 0.01$
	7%	$5.66^{a} \pm 0.01$	$5.62^{b} \pm 0.00$
Coliform count (CFU/g)	Control	ND	ND
	3%	ND	ND
	5%	ND	ND
	7%	ND	ND
Total Fungal Count (CFU/g)	Control	ND	ND
	3%	ND	ND
	5%	ND	ND
	7%	ND	ND

Note: Values are means of triplicate test ± standard deviation. Means in the same column with different superscripts letters (a, b, c, d) differ significantly at $p < 0.05$ (Tukey HSD)

4.12 Microbiological test of yoghurt on Day 7

On Day 7 (Table 12), TVC remained generally stable during storage. The 7% sample showed a significant decrease, possibly due to antimicrobial activity from a higher concentration of additive. In other samples, fluctuations were minor and not statistically meaningful.

The total viable count (TVC) was found to be highest in the 5% sample ($6.11^{b} \pm 0.01$ log CFU/g), followed by the 3% ($6.07^{a} \pm 0.00$), and both the control and 7% ($6.05^{ab} \pm 0.00$) samples. According to Tukey's post hoc analysis, a statistically significant difference ($p < 0.05$) was observed between the 5% treatment and all other groups, indicating enhanced microbial proliferation at this concentration. No significant difference was noted between the control and 7% samples ($p = .936$), suggesting similar microbial loads. Similarly, the comparison between the 3% and 7% groups did not reach statistical significance ($p = .078$); however, the 3%

treatment differed significantly from both the control and 5% groups, thereby justifying a distinct superscript assignment. These results suggest that the 5% treatment supported the highest level of microbial growth, while the 7% sample demonstrated microbial counts comparable to the untreated control group.

Lactobacillus counts decreased slightly in all but the 5% treatment, where they slightly increased (not significantly). This suggests that 5% treatment provided optimal protection for probiotic viability during storage. In control and 3%/7%, slight declines indicate moderate environmental stress (e.g., pH shifts, competition, moisture loss). The Tukey HSD analysis confirmed statistically significant differences among all groups ($p < 0.05$), including between the closely related 5% and 7% samples ($p = .002$). The increase in Lactobacillus count after 7 days in the 5% sample is likely due to a combination of the optimal concentration of nutrients, the fermentation environment, and the selective inhibition of harmful microbes, creating an ideal setting for the proliferation of Lactobacillus.

Regarding the microbiological quality (Table 12), coliform bacteria were not detected in any of the samples throughout the storage period. This absence indicates that the stirred yoghurt was produced and stored under hygienic conditions, effectively preventing coliform contamination. Similarly, no yeast or mold growth was observed in either the control or the fortified yoghurt samples till the 7th day of storage. This lack of fungal contamination suggests that proper aseptic techniques and strict hygiene measures were maintained during the production process, contributing to the microbial safety and extended shelf life of the products.

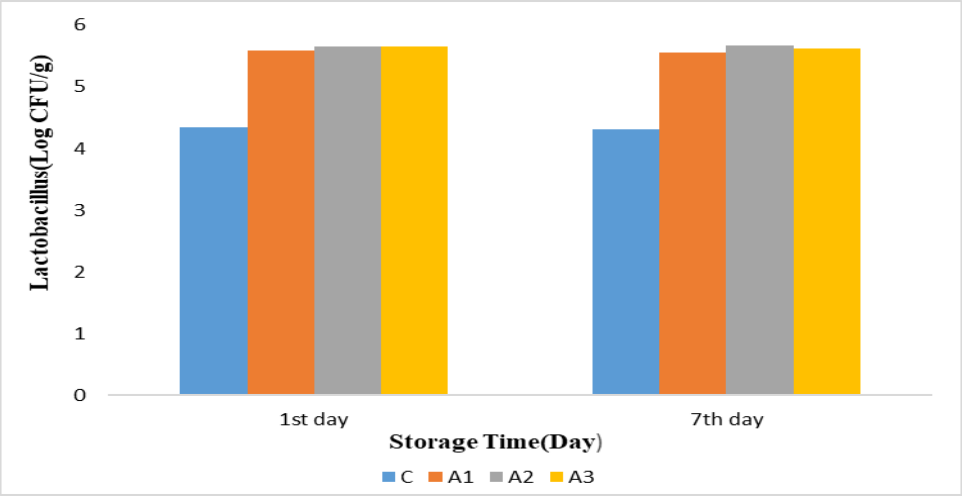

Figure 4.16: The mutual effect of storage time and amount of *A. Philoxeriodes* leaf juice (C= control, A1=3%, A2=5%, A3=7%)

4.13 Sensory evaluation result of the yoghurt sample

Table 13 presents the sensory evaluation results of yoghurt samples, highlighting the influence of different treatment concentrations on various sensory attributes. The whey presence score was highest in the control sample (7.90 ± 0.30) and lowest in the 5% treatment group (7.25 ± 0.44), with intermediate values observed in the 3% and 7% samples. According to Tukey's post hoc analysis, a significant difference was detected only between the control and 5% groups ($p = .001$), indicating a perceptible reduction in whey presence at the 5% treatment level. No significant differences were noted among the control, 3%, and 7% groups ($p > 0.05$), implying that these samples shared comparable whey characteristics. The shared superscripts among the control, 3%, and 7% groups further support the conclusion that the whey presence remained within a similar sensory range, while the 5% treatment distinctly reduced this attribute.

Table 4.9: Sensory evaluation result of the yoghurt sample

Sample	Appearance		Colour		Texture		
	Whey	Shiny	Greenish	White	Smoothness	Creaminess	Mouth coating
C	$7.90^{a}\pm0.30$	$7.55^{a}\pm0.60$	-	$7.40^{a}\pm0.59$	$7.90^{a}\pm0.30$	$8.15^{a}\pm0.58$	$7.70^{a}\pm0.47$
3%	$7.65^{ab}\pm0.48$	$7.40^{a}\pm0.75$	$6.85^{a}\pm0.98$	$7.35^{a}\pm0.67$	$7.85^{a}\pm0.87$	$7.75^{ab}\pm0.91$	$7.35^{ab}\pm0.67$
5%	$7.25^{b}\pm0.44$	$7.10^{a}\pm0.44$	$7.60^{b}\pm0.50$	$6.70^{b}\pm0.80$	$7.25^{b}\pm0.71$	$7.20^{bc}\pm0.69$	$6.90^{bc}\pm0.44$
7%	$7.55^{ab}\pm0.68$	$7.20^{a}\pm0.52$	$7.62^{b}\pm0.60$	6.65b±0.67	$7.25^{b}\pm0.85$	$6.90^{c}\pm0.64$	$6.85^{bc}\pm0.58$
Sample	**Aroma**			**Taste**			**Overall**
	Milky	Leafy	Cardamom	Sweetness	Sourness	Aftertaste	
C	$7.50^{a}\pm0.51$	-	-	$7.50^{a}\pm0.76$	$6.65^{b}\pm0.74$	$6.75^{a}\pm0.71$	$7.30^{a}\pm0.57$
3%	$7.70^{a}\pm0.65$	$7.05^{a}\pm0.82$	$6.90^{a}\pm0.85$	$6.85^{b}\pm1.04$	$6.75^{b}\pm0.85$	$6.60^{a}\pm1.04$	$7.10^{a}\pm0.55$
5%	$7.40^{a}\pm0.50$	$6.95^{a}\pm0.75$	$6.95^{a}\pm0.39$	$6.80^{b}\pm0.61$	$7.70^{a}\pm0.47$	$7.00^{a}\pm0.56$	$6.90^{b}\pm0.30$
7%	$7.45^{a}\pm0.60$	$7.50^{ab}\pm0.76$	$7.55^{b}\pm0.51$	$7.40^{a}\pm0.59$	$7.30^{ab}\pm0.97$	$7.15^{a}\pm0.58$	$7.15^{a}\pm0.36$

Note: Values are means of triplicate tests ± standard deviation. Means in the same column with different superscript letters (a, b, c, d) differ significantly at $p < 0.05$ (Tukey HSD)

In terms of shiny appearance, the scores across all treatment groups ranged from 7.10 ± 0.44 (5% sample) to 7.55 ± 0.60 (control). Despite these numerical differences, Tukey's post hoc test confirmed no statistically significant variation among the samples ($p > 0.05$). This suggests that the inclusion of dried herbs, even at varying concentrations, did not significantly affect the

perceived shininess of the yoghurt, and all samples may be considered part of a homogeneous group for this attribute.

For the greenish attribute, a significant increase was observed from the 3% treatment to both the 5% and 7% groups, which did not significantly differ from each other. The 5% and 7% treatments exhibited the highest mean values (7.60 and 7.62, respectively), indicating a more pronounced greenish appearance. In contrast, the 3% group recorded the lowest score (6.85). These results suggest that higher concentrations of dried herbs intensify the greenish hue, with the 5% and 7% groups yielding similar sensory outcomes, both significantly greener than the 3% group.

Regarding the white attribute, no significant difference was found between the Control and 3% groups, indicating consistency in whiteness at these levels. However, both the 5% (6.70) and 7% (6.65) treatments showed significantly lower whiteness scores compared to Control and 3%, reflecting a reduction in white intensity at higher concentrations. The similarity in scores between the 5% and 7% treatments further implies that the decrease in whiteness plateaus at these higher treatment levels.

The smoothness attribute followed a similar trend. The Control and 3% groups achieved the highest scores and did not differ significantly from each other, suggesting that low treatment levels do not adversely affect smoothness. Conversely, the 5% and 7% treatments resulted in significantly lower smoothness, with no meaningful difference between them. This pattern indicates that higher concentrations negatively impact smoothness, while control and lower-level treatments maintain a favorable texture.

In evaluating creaminess, the Control group again yielded the highest score, followed by the 3% treatment, which was not significantly different from Control. A further decrease was observed in the 5% group, which differed significantly from Control but not from 3%. The 7% treatment showed the lowest creaminess, significantly different from both Control and 3%, but comparable to 5%. These results suggest a gradual but consistent reduction in creaminess with increasing treatment concentration, especially evident beyond the 3% level.

Mouth coating was most prominent in the Control group, with a slight reduction in the 3% group, which remained statistically similar to Control. The 5% and 7% treatments showed significantly reduced scores, indicating a notable decline in mouth coating at higher

concentrations. While 5% and 7% were similar to each other, the 3% treatment occupied an intermediate position showing marginal difference with 5% but significantly higher than 7%. This trend reflects a dose-dependent decrease in mouth coating, with optimal sensory performance maintained in the Control and 3% groups.

The milky attribute exhibited no statistically significant variation among the Control and treated samples at 3%, 5%, and 7%. All groups recorded comparable mean scores ranging from 7.40 to 7.70, indicating that varying treatment concentrations did not impact the milky perception. This uniformity implies that the inherent milky flavour of the yoghurt was retained consistently across all formulations, preserving the core sensory quality regardless of the added ingredient levels. For the leafy sensory attribute, no significant difference was found between the 3% and 5% samples, both of which had similar mean scores around 7.0. The 7% formulation showed a slightly elevated mean value of 7.50, suggesting a potential enhancement of leafy perception at higher concentration. However, this increase was not statistically significant when compared to either 3% or 5%, indicating that all three treated samples were largely comparable in this sensory aspect. This trend points toward a possible, though inconclusive, intensification of leafy notes with increased concentration.

In the case of the cardamom attribute, the 3% and 5% samples exhibited closely aligned mean values, showing no significant difference between them. Notably, the 7% formulation demonstrated a significantly higher mean score than both 3% and 5%, signifying a pronounced enhancement of the cardamom flavour at this higher concentration. This result suggests that increasing the treatment concentration to 7% significantly elevates the perception of cardamom, in contrast to the lower concentrations that remain statistically similar.

Regarding sweetness perception, both the Control and 7% samples attained significantly higher mean ratings compared to the 3% and 5% formulations. These two groups Control and 7% were categorized within the same homogeneous subset, reflecting equivalent sweetness intensity. Conversely, the 3% and 5% treatments also formed a separate statistical group characterized by significantly reduced sweetness scores. While no statistical difference was observed between the 3% and 5%, or between these and the 7% sample, the pattern suggests a temporary decline in sweetness at intermediate concentrations, with a restoration at the highest treatment level. This may be indicative of a threshold masking effect, where sweetness is subdued at mid-levels but becomes prominent again at higher intensities, aligning with the control. The sourness attribute demonstrated the highest mean rating in the 5% formulation,

followed by the 7% sample. Both the Control and 3% samples recorded relatively lower sourness scores. A statistically significant increase in sourness was observed for the 5% sample when compared to both the Control and 3% samples, indicating enhanced sourness perception at this concentration. The 7% treatment did not significantly differ from any group, placing it in an intermediate category, overlapping with both the higher and lower intensity groups. These findings imply that sourness increased at the 5% inclusion level and remained perceptible at 7%, though not sharply distinct. The Control and 3% formulations shared the lowest sourness intensity, forming a homogeneous group. With respect to aftertaste**,** there was no statistically significant variation across all tested samples. Although the 7% treatment yielded the highest numerical mean and the 3% the lowest, these differences were not statistically meaningful. Consequently, all samples belonged to a single homogeneous group for this attribute, indicating that the inclusion of the functional ingredient did not significantly affect the aftertaste perception. This consistency supports the notion of a stable aftertaste profile regardless of treatment concentration.

Lastly, Overall acceptability scores ranged from 6.90 to 7.30 across the samples, with the control sample receiving the highest mean score and the 5% formulation the lowest. The comparison between the control and the 5% sample showed a statistically significant difference, indicating that the 5% sample was less preferred in terms of overall acceptability. However, no significant differences were observed among the control, 3%, and 7% samples, which all fell into the same significance group. This suggests that while a moderate concentration (5%) led to a reduction in overall liking, both lower (3%) and higher (7%) concentration remained acceptability levels comparable to the control.

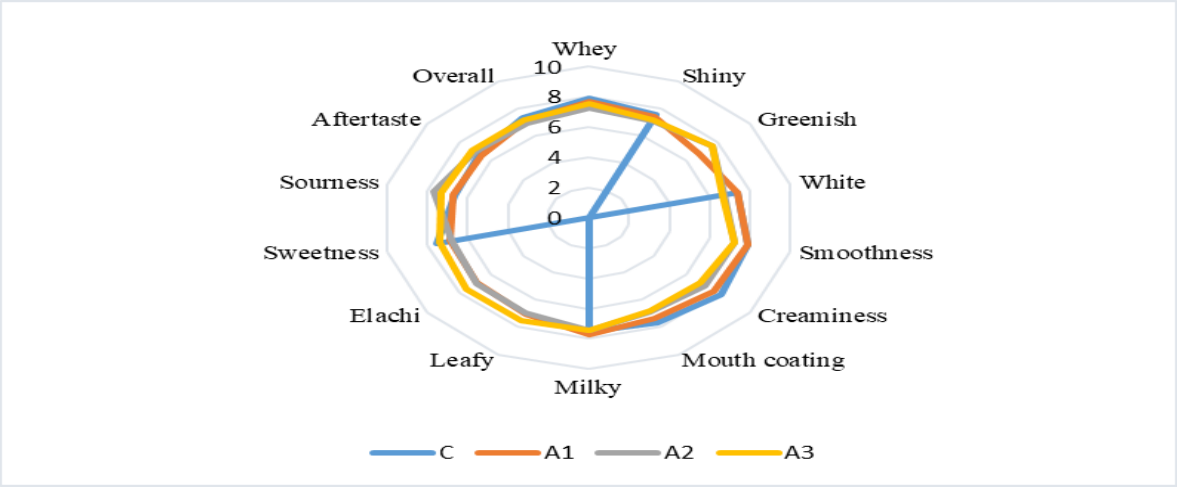

Figure 4.17: Radar chart representing the sensory evaluation of yoghurt samples: Control (without fortification), and A1–A3 (fortified with increasing levels of *A. philoxeroides* juice). Here, (C= control, A1=3%, A2=5%, A3=7%)

Figure 23, The radar chart presents a comparative analysis of the sensory profiles of four yoghurt samples Control (C), A1, A2, and A3 evaluated across thirteen sensory attributes encompassing appearance, texture, flavour, and overall acceptability.

The Control sample (C), which did not contain any fortification with *A. philoxeroides* juice or cardamom, achieved the highest ratings for creaminess and smoothness. This indicates that the unfortified base yoghurt possessed an inherently desirable textural quality, which may have been slightly diminished by the addition of plant extract and spice in the fortified formulations.

In contrast, the fortified samples (A1, A2, and A3), containing varying concentrations of *A. philoxeroides* juice and cardamom as a flavouring agent, demonstrated notable improvements in several other sensory dimensions. These included attributes such as mouth coating, leafy and *elachi* flavour, whey presence, aftertaste, sweetness, and overall acceptability, suggesting that the fortification enhanced the complexity and appeal of the product's flavour profile.

Among all samples, A3 exhibited the most favorable and well-balanced sensory characteristics, occupying the largest area on the radar chart. It particularly excelled in greenish tint, mouth coating, creaminess, *elachi* flavour, aftertaste, and overall acceptability. These results indicate that A3 was perceived as the most organoleptically pleasing formulation.

Additionally, visual changes were observed in terms of colour: The control sample maintained a whiter appearance, while the fortified samples, especially A3, developed a more pronounced greenish hue, attributable to the presence of the plant extract.Overall, the findings suggest that while the incorporation of *A. philoxeroides* and cardamom slightly compromised certain textural attributes such as creaminess and smoothness, it significantly enhanced the flavour, aftertaste, and overall sensory quality of the yoghurt. Among the samples, A3 emerged as the most preferred, highlighting the potential of these ingredients in developing a functional dairy product with improved sensory appeal.

4.14 Properties of *A. philoxeroides* (Malancha) Leaf

4.14.1 Proximate composition

Table 14 presents the proximate composition of the analyzed leaf sample, including its moisture, ash, carbohydrate, protein, fat, fiber, and total soluble solids (TSS) content. The moisture content was found to be 88.4%**,** indicating a higher water content, which is typical for fresh leafy vegetables and suggests that the leaf is highly perishable and may require proper preservation techniques to maintain its quality during storage and processing.

The ash content was recorded at 2.4%, which reflects the total mineral content of the sample. This value suggests a moderate presence of inorganic nutrients such as calcium, magnesium, and potassium that contribute to the nutritional value of the leaf.

The carbohydrate content was measured at 0.496 mg/g**,** which is relatively low, consistent with the general composition of leafy vegetables that are not primary sources of carbohydrates. Similarly, the protein content was 0.162 mg/g, indicating that while the leaf does contain protein, it may not serve as a significant source of dietary protein on its own.

Table 4.10: Proximate composition

Sample	Composition	Value
Leaf	Moisture (%)	88.4
	Ash (%)	2.4
	Carbohydrate (mg/g)	0.496
	Protein (mg/g)	0.162
	Fat (%)	2
	Fiber (%)	18.75
	TSS (ºBrix)	1.3

The fat content of the leaf was 2%**,** which is relatively higher compared to many leafy greens that typically have negligible fat levels. This might suggest the presence of essential fatty acids or oil-soluble compounds contributing to the nutritional profile of the sample.

The crude fiber content was found to be 18.75%, indicating that the leaf is a rich source of dietary fiber. This is nutritionally beneficial as fiber aids in digestion, helps regulate blood sugar levels, and supports overall gut health.

Lastly, the total soluble solids (TSS) value was 1.3 ºBrix, which represents the concentration of soluble substances, primarily sugars, in the sample. This low TSS is typical for non-sweet leafy vegetables and reflects limited simple sugar content.Overall, the proximate composition suggests that the leaf is high in moisture and dietary fiber, with moderate levels of minerals and fats, and low levels of carbohydrates and proteins. These characteristics make it potentially suitable as a functional ingredient in health-oriented food formulations, particularly those targeting dietary fiber enrichment.

4.14.2 Physicochemical properties

Table 15, presents the physicochemical property of *A. philoxeroides* (commonly known as Malancha) leaf, focusing specifically on its pH value. The recorded pH of the leaf sample was 5.5, indicating that it is mildly acidic in nature.This pH level falls within the typical range for many edible leafy vegetables, which often exhibit slightly acidic to neutral pH values. The mildly acidic nature of the leaf may influence its shelf life, microbial stability, and potential applications in food formulations. From a preservation standpoint, a pH of 5.5 may not provide sufficient inhibitory effects against microbial growth on its own; therefore, additional preservation methods, such as refrigeration or drying, may be required to enhance its stability during storage.

Table 4.11: Physicochemical properties

Sample	Physicochemical properties	Value
Leaf	pH	5.5

Furthermore, the acidity level may impact the sensory characteristics and the interaction of the leaf with other ingredients in food product development. Overall, the pH value of 5.5 suggests that *A. philoxeroides* leaf is suitable for various culinary and nutritional applications, especially where slightly acidic plant materials are desirable.

4.14.3 Colour analysis

Table 16 illustrates the colour characteristics of the *A. philoxeroides* leaf based on the CIELAB colour space parameters in the colourimeter**.** The lightness (L) value was measured at 40.23, indicating a moderately dark leaf colour. In the CIELAB scale, an L value of 0 represents black, while 100 represents white. A value of 40.23 suggests the leaf has a relatively low brightness level, typical of green leafy vegetables rich in chlorophyll.

The a* value, which represents the red-green axis, was found to be -5.75. Negative values along this axis indicate greenness, confirming the leaf's characteristic green colour. This greenness is largely attributed to the presence of chlorophyll pigments.

The b* value, representing the yellow-blue axis, was 16.67. Positive values along this axis indicate yellowness. The presence of a notable b* value along with a negative a* value suggests the leaf has a greenish-yellow tint, which is consistent with the natural appearance of many edible leafy greens.

Chroma, which was recorded at 17.65, reflects the colour intensity or saturation. A higher chroma value indicates a more vivid colour. This result suggests that the leaf exhibits a moderate to high colour saturation, making it visually appealing for use in fresh or processed food products.The hue angle was 110.23 degrees, which falls within the yellow-green region of the colour wheel. This further supports the visual observation that the leaf displays a green colour with slight yellow undertones.

Table 4.12: Colour analysis

Sample	Colour	Value
Leaf	Lightness (L)	40.23
	Red-green axis (a)	-5.75
	Yellow-blue axis (b)	16.67
	Chroma	17.65
	Hue	110.23

Collectively, the colourimetric analysis confirms that *A. philoxeroides* leaf possesses typical green pigmentation with moderate brightness and chromatic intensity, which may influence its sensory appeal and acceptability in functional food applications

4.14.4 Bioactive compound

Table 17, presents the bioactive compound profile of *A. philoxeroides* leaf, including total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity. These parameters are essential indicators of the functional and therapeutic potential of plant-based foods.

The total phenolic content was determined to be 0.60 mg GAE/g (milligrams of gallic acid equivalents per gram of sample). Phenolic compounds are well known for their antioxidant properties, contributing to the neutralization of free radicals and reduction of oxidative stress in biological systems. The presence of phenolics in the leaf suggests potential health benefits, including anti-inflammatory and antimicrobial activities.

The total flavonoid content was found to be 0.42 mg QE/g (milligrams of quercetin equivalents per gram of sample). Flavonoids are a subclass of polyphenols with strong antioxidant capacity, often associated with cardiovascular protection, anti-cancer properties, and immune system support. The moderate level of flavonoids indicates that the leaf possesses valuable phytochemicals that may enhance its nutraceutical properties.

Table 4.13: Bioactive compound

Sample	Bioactive compound	Value
Leaf	TPC (mg GAE/g)	0.60
	TFC (mg QE/g)	0.42
	Antioxidant Activity IC50 (µg/ml)	36.52

The antioxidant activity of the leaf extract was measured at 36.52 µg/ml, reflecting its capacity to scavenge free radicals. This value was likely obtained using a DPPH assay or a similar radical scavenging method. A lower IC50 value in such assays generally indicates stronger antioxidant activity. The recorded value demonstrates that *A. philoxeroides* exhibits a notable level of antioxidant potential, which can be advantageous in developing functional foods or supplements aimed at preventing oxidative damage.

4.14.5 Nutritional compound

Table 18, presents the concentrations of key nutritional compounds identified in *A. philoxeroides* (Malancha) leaf, including vitamin C, beta-carotene, calcium, and iron. These

nutrients play vital roles in supporting various physiological functions and contribute to the overall health benefits of the plant.

The vitamin C content was found to be 0.293 mg/g. Vitamin C (ascorbic acid) is a water-soluble antioxidant essential for collagen synthesis, immune function, and enhancement of iron absorption. Its presence in the leaf suggests a contribution to antioxidant defense mechanisms and potential use in promoting immunity and skin health.

The beta-carotene content was measured at 0.00118 mg/g. Beta-carotene is a precursor to vitamin A and is important for vision, skin health, and immune function. Although the value is relatively low compared to some other leafy vegetables, the presence of beta-carotene adds to the functional quality of the leaf, especially when consumed as part of a varied diet.

The calcium content was recorded at 7.48 mg/g, indicating that the leaf is a good source of this essential mineral. Calcium is vital for bone health, muscle contraction, nerve signaling, and enzymatic activity. The relatively high calcium level suggests that *A. philoxeroides* leaf could contribute significantly to daily calcium intake, particularly in populations at risk of calcium deficiency.

Table 4.14: Nutritional Compound

Sample	Nutritional Compound	Value
Leaf	Vitamin-C (mg/g)	0.293
	Beta-carotene (mg/g)	0.00118
	Calcium (mg/g)	7.48
	Iron (mg/g)	0.3

Iron content was found to be 0.3 mg/g. Iron is a key component of hemoglobin and is necessary for oxygen transport in the blood. It also plays a role in energy metabolism and immune function. The presence of iron in the leaf sample indicates its potential as a plant-based source of dietary iron, which is important in preventing anemia, especially in populations with limited access to animal-based foods. Overall, the nutritional composition of *A. philoxeroides* leaf demonstrates its potential as a valuable source of micronutrients, particularly calcium, vitamin C, and iron, which support various health functions. These findings highlight the possible use of the leaf in food formulations aimed at improving nutritional status and preventing micronutrient deficiencies.

4.14.6 Microbiological test

Table 19 presents the results of the microbiological assessment of *A. philoxeroides* (Malancha) leaf, including total viable count (TVC), coliform count, and yeast and mold count, expressed in logarithmic colony-forming units per gram (Log CFU/g). These parameters are crucial for evaluating the microbial safety and hygienic quality of the leaf sample. The total viable count was recorded at 4.30 Log CFU/g. This value reflects the total population of aerobic mesophilic microorganisms present in the sample. A TVC in this range is generally considered acceptable for fresh leafy vegetables and indicates that the sample was handled under reasonably hygienic conditions. However, it also suggests the need for proper washing or processing prior to consumption to reduce microbial load.

The coliform count was not detected in the sample. Coliform bacteria are commonly used as indicator organisms to assess possible fecal contamination and overall sanitary quality. The absence of detectable coliforms suggests that the sample was free from fecal contamination and handled under satisfactory sanitary conditions, which is a positive indicator for food safety

Table 4.15: Microbiological test

Sample	Microbiological Test	Value
Leaf	Total viable count (Log CFU/g)	4.30
	coliform count (Log CFU/g)	Not detected
	Yeast and mold count (Log CFU/g)	3.56

The yeast and mold count were found to be 3.56 Log CFU/g. Yeasts and molds are common spoilage organisms in fresh plant materials. While this count is within acceptable limits for fresh produce, it highlights the importance of appropriate post-harvest handling and storage to prevent potential spoilage, especially under high humidity or warm conditions that may promote fungal growth.

Overall, the microbiological profile of *A. philoxeroides* leaf indicates that the sample is microbiologically safe for consumption, with an acceptable total viable count, no detectable coliforms, and a moderate presence of yeasts and molds. These findings support the potential for safe consumption and processing of the leaf when standard hygienic practices are maintained.

CHAPTER-05

CONCLUSION AND RECOMMENDATION

CHAPTER 05: CONCLUSION AND RECOMMENDATION

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study not only provides a scientific basis for the utilization of *A. philoxeroides*—an underexploited leafy plant—as a functional food ingredient but also demonstrates its feasibility in dairy-based applications. The synergistic integration of plant bio-actives and traditional dairy matrices can serve as a sustainable model for developing affordable, nutrient-rich, and health-promoting functional foods, particularly in regions where micronutrient deficiencies persist. Moreover, the incorporation of cardamom as a natural flavouring agent enhanced the palatability of the product without the use of artificial additives, aligning the product with clean-label trends. This research thus bridges the gap between nutritional enhancement and consumer acceptability—an essential step in functional food innovation. In a broader context, the findings of this study may contribute to diversifying the functional dairy market while encouraging the valorization of indigenous plant resources for nutritional security and public health promotion.

5.2 Recommendations:

Based on the outcomes of this study and statistical interpretations, the following recommendations are proposed for formulation development, future research, and potential applications of yoghurt fortified with *A. philoxeroides* juice and cardamom:

- Among all tested formulations, the 5% *A. philoxeroides* juice with fixed cardamom level is recommended for general consumption, offering a balanced combination of nutritional improvement, antioxidant activity, and sensory acceptability.
- The 3% formulation may be suitable for consumers who prefer minimal changes in colour and texture while still receiving moderate nutritional benefits.
- The 7% formulation, despite providing the highest levels of micronutrients and antioxidant capacity, showed reduced viscosity and creaminess, and may therefore be

considered only with texture-enhancing techniques such as encapsulation or natural stabilizers.

- Further research is advised to explore the storage stability beyond 7 days, especially focusing on vitamin C retention, phenolic degradation, and microbial viability.
- Since cardamom was kept constant in all fortified samples, future studies should examine its individual and interactive effects by varying its concentration alongside *A. philoxeroides.*
- Only one variety of *A. philoxeroides* was used. Nutritional composition may vary by geographical source or plant maturity, which can be explored.
- Although the yoghurt showed enhanced nutritional and antioxidant properties in vitro, in vivo trials or bioavailability studies can be conducted to confirm actual health benefits.

CHAPTER-06

REFERENCES

CHAPTER 06: REFERENCES

6.0 REFERENCES:

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CHAPTER-07

APPENDIX

CHAPTER 07: APPENDIX

7.1 Sample Photograph

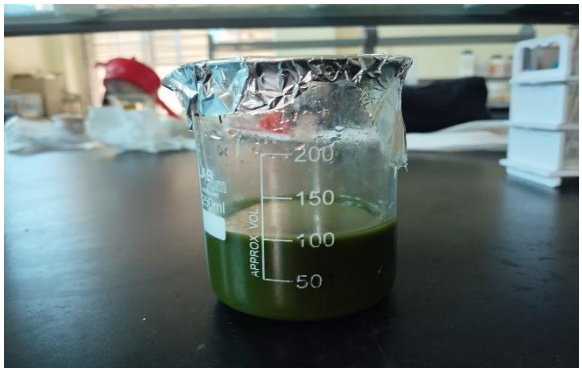

Figure: Alligator leaf juice

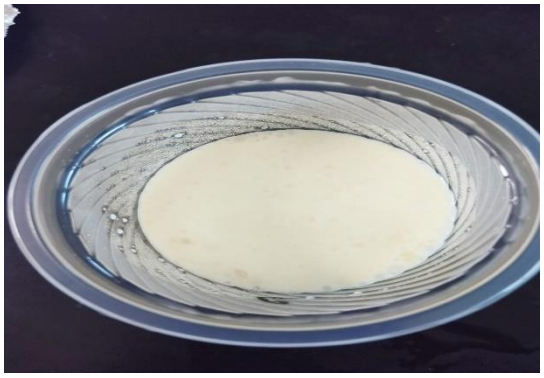

Figure: Yoghurt (Control)

Figure: Yoghurt (5% leaf juice)

Figure: Yoghurt (3% leaf juice)

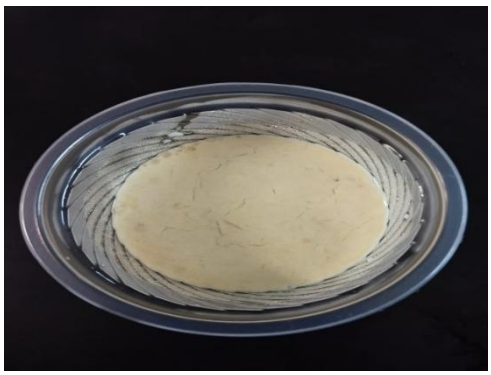

Figure: Yoghurt (7% leaf juice)

7.2 Processing of Yoghurt

Figure: Pasteurization of milk

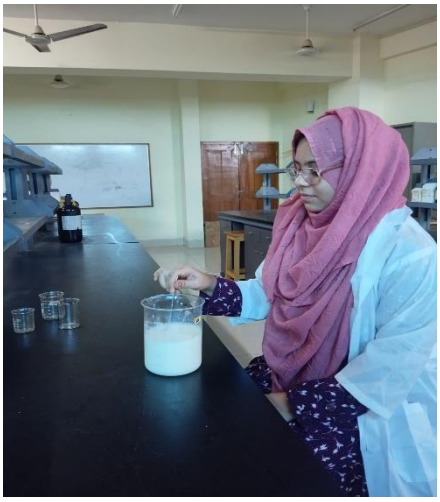

Figure: Inoculation of milk

Figure: Mixing of juice

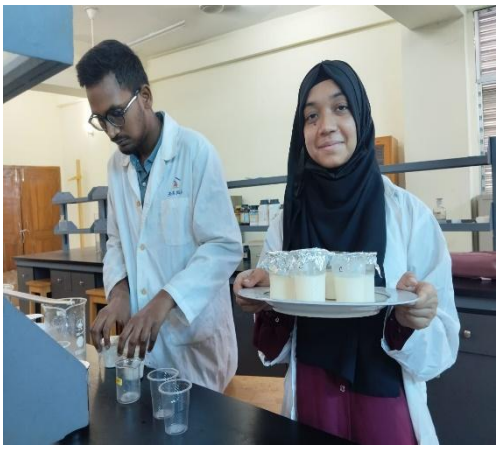

Figure: covering by foil paper

Figure: Incubation for fermentation

Figure: Cardamom powder

7.3 Photographs of procedure of some test and reagent

Figure: Bovine serum albumin

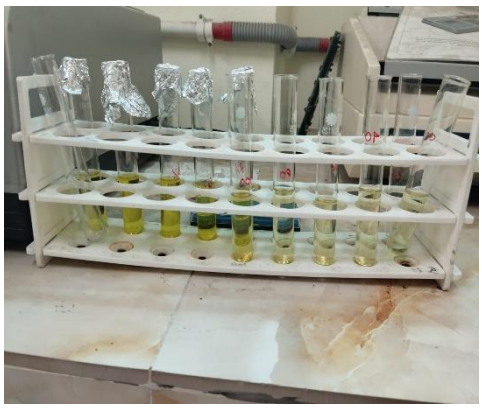

Figure: Quercetin solution

Figure: MRS agar media

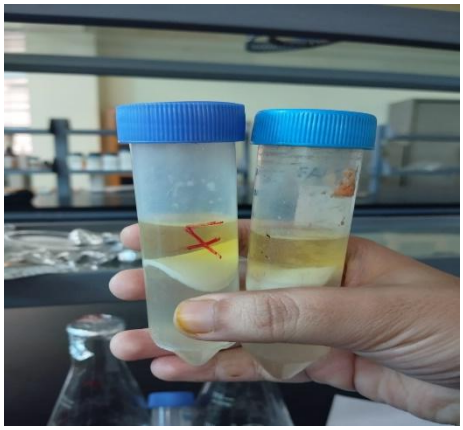

Figure: Beta-carotene extraction

Figure: Viscometer

Figure: Ferrous ammonium

7.4 Sensory Analysis Form

Scorecard for the sensory quality evaluation using 9-point hedonic scale

Product information:

Product Name: _____

Sample Code: _____

Date: _____

Evaluator Information:

Name: _____

Age: _____

Gender: _____

Note:

Taste the sample and tick ✓ how much you like or dislike each of the characteristics.

Please drink water to clean the flavour left in your mouth in between sample

Sensory Quality Factors		**Sensory Scale Factors**								
Attribute	**Description**	**Like extremely (9)**	**Like very much (8)**	**Like moderately (7)**	**Like slightly (6)**	**Neither like or dislike (5)**	**Dislike slightly (4)**	**Dislike moderately (3)**	**Dislike very much (2)**	**Dislike extremely (1)**
Appearance	Whey presence									
	Shiny									
Colour	Greenish									
	White									
Texture	Smoothness									
	Creaminess									
	Mouth coating									
Aroma	Milky									
	Leafy									
	Elachi									
Taste	Sweetness									
	Sourness									
	Aftertaste									
Overall acceptability										