

Effects of probiotic cultures on bioactive peptides, composition, and sensory traits of yogurt

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Abstract

Milk proteins include many bioactive peptides (α -, β -, γ -, and κ -casein) with different therapeutic effects. This study aimed to evaluate the effects of different probiotic cultures on proximate composition, organic acids, quantitative analysis, proteolysis, bioactivities, and sensory attributes. Probiotic yogurt was prepared using a starter and probiotic bacteria (CHR-Hansen, Denmark) in combination with a standard protocol with slight modifications. It was stored at 6–8°C for 21 days. Results indicated that probiotic culture and storage significantly impacted the moisture, ash, protein, citric acid, and lactic acid composition of yogurt during storage. Regarding proximate parameters, maximum moisture, fat, protein, acetic acid, citric acid (treatments-wise), and sensory attributes were found in the following treatments (T): T₅ (84.78±1.44%), T₇ (4.69±0.05%), T₇ (4.99±0.04%), T₆ (109.26±0.73 ppm), T₆ (377.81±6.57 ppm), and T₆ (4.58±0.33%) by sequence during storage of 21 days. Probiotic cultures examined in this research possessed the capacity to enhance technological aspects and flavor profile of probiotic yogurt.

Keywords: HPLC; organic acid; bioactive component; antioxidant; antihypertensive; proteolysis

Introduction

A lacteal secretion produced by the mammary glands of mammals is milk (Kalita *et al.*, 2025). In Pakistan, the main milk-producing animals include sheep, goats, camels, cows, and buffalo. In terms of global milk production, Pakistan is placed at fourth place. In the fiscal year 2016–2017, the gross annual production of milk was projected

around 56.08 million tons (Government of Pakistan (GOP), 2021).

The highest produced milk was the buffalo milk, with a production of about 34.12 million tons. Cows produce about 20.14 million tons of milk annually, which is still a small amount when compared to buffalo milk (GOP, 2021).

The nutritional value of milk is correlated with its chemical constitution. Regarding milk composition, 3.5% of milk comprises proteins, 80% of which are casein and 20% are whey. There are three types of α -, β -, and κ -caseins. Whey contains b-lactoglobulin, α -lactalbumin, and countless other small proteins with a variety of biological properties, such as the ability to bind enzymatically minerals and to have an immunological effect (Ren *et al.*, 2024).

With a proline residue at the carboxyl-terminal end, the angiotensin-converting enzyme (ACE)-inhibitory peptides are known to withstand digestion by digestive enzymes, enter the bloodstream as tiny sequence peptides, and travel straight to the small intestine. ACE is recognized for its ability to regulate peripheral blood pressure and is a crucial component of the renin-angiotensin system (RAS) (Minj and Vij, 2025).

In vitro chemical, *in vitro* cellular, and *in vivo* animal approaches are the three primary categories to assess antioxidant activity. Xu *et al.* (2024) characterized the biological impacts of these three techniques by including the scavenging of free radicals, chelation of metal ions, inhibition of lipid peroxidation, inhibition of oxidative enzyme activities, and activation of antioxidant enzymes and non-enzymatic systems. Exchange in lifestyle, nutritional modification, and physical workout are the key components to deal with diabetes without impacting any pharmacological drug. High fiber weight-reduction plan, food regimen low in saturated fat, intake of lactobacilli, and the overall low carbohydrate diet therapies are beneficial to attenuate blood glucose degree (Tahir *et al.*, 2023).

Mammals are evolved to produce milk to serve as the source of nutrients needed for the growth and development of newborns. Milk contains a range of physiologically active compounds, including bioactive proteins, in addition to necessary nutrients, for newborn's health. Peptides with a range of applications, including antioxidant qualities, are released when these milk proteins are degraded by enzymes during digestion, processing, or fermentation (such as the production of cheese) (Amigo *et al.*, 2020).

According to Robinson *et al.* (2021), human milk contains hundreds to thousands of peptides produced by milk proteins in the mammary gland, which are also present in cheese and yogurt, among other dairy products. After consumption, the human milk's proteins are further broken down into smaller bits and peptides in the stomach and intestines. Some peptides present in the human milk are excreted through newborn's feces (Beverly *et al.*, 2020). We should increase the utilization of Ca in our white flour through organic source like egg shell, while our dairy product intake is less than

required values. In this way we can overcome Ca deficiency diseases. (Quddoos *et al.*, 2022).

The biological significance of peptides produced from milk proteins in different products and at different sites of digestion should be understood by comparing peptidomics data of a library of known bioactive peptides (BPs). Nielsen *et al.* (2024) point out that the creation of the Milk Bioactive Peptide Database (MBPDB) established this kind of research possible. The first published MBPDB contained 737 unique peptide sequence-function pairs. It included 606 unique peptide sequences from the milk of 10 different animals. Using the MBPDB, researchers discovered hundred more peptides in the human milk and infant formula. These peptides were very similar to the known bioactive milk peptides. They also discovered these peptides in the stomach and intestinal contents of babies as well as in their poop after they consumed human milk (Nielsen *et al.* 2020).

Dormant in their parent protein chains, bioactive peptides become active when enzymes and microbes break down these sequences. However, partial hydrolysis and the availability of raw materials restrict the utilization of enzymatic hydrolysis. Bioactive peptides are inactive within the parent protein's sequence and can be released in three ways: (i) by digestive enzymes hydrolyzing the protein, (ii) by proteolytic microorganisms hydrolyzing the protein, and (iii) by proteolytic enzymes derived from plants or microorganisms (Hong *et al.*, 2025). Among the various types of peptides are those that are antihypertensive, antioxidant, antithrombotic, hypocholesterolemic, opioid, anti-appetizing, antimicrobial, immunomodulatory, cytomodulatory, mineral binding, cholesterol-reducing effects, essential for human health. Whey proteins are used to make a variety of bioactive peptides as they contain various discerned bioactive peptides, such as lactoferricin, albutensin A, serorphin, β -lactotensin, β -lactorphin, and α -lactorphin (César *et al.*, 2024).

Streptococcus thermophilus and *Lactobacillus delbrueckii*, subsp. *bulgaricus*, the two specialized lactic acid-producing bacteria, are cultured in milk to produce yogurt under controlled environmental conditions (Abedin *et al.*, 2024). People use yogurt because it has health benefits. The immune system, metabolism, mental health, and digestive health are benefited from functional yogurt. As per the recommended daily allowance (RDA), yogurt contains between 30% and 45% phosphorus and 40% calcium per serving. Yogurt contains various amino acids, but glycine and proline are particularly significant. Because milk has a high protein content, yogurt is thought to have a positive biological value. Both gastrointestinal (GI) motility and performance of the immune system are enhanced by yogurt. Yogurt contains peptides and proteins that improve physiological activity of the

human body, along with minerals (calcium and phosphorus), proteins, carbohydrates, vitamins (cobalamin, folate, niacin, riboflavin, vitamin A, and thiamine), and other nutrients (Pannerchelvan *et al.*, 2024). The microbial communities that inhabit the vagina of wholesome reproductive age women typically incorporate high proportions of *Lactobacillus* spp., which are ruled by *Lactobacillus iners*, *Lactobacillus crispatus*, *Lactobacillus gasseri*, and *Lactobacillus jensenii* (Altaf *et al.*, 2022). The body's cholesterol level and lipid profile are reduced by lactic acid bacteria (LAB; Saeed *et al.*, 2023). The pH of yogurt varies between 4.4 and 4.6. Numerous factors contribute to the sourness of yogurt. For instance, if the pH is low (4.4), it indicates that the milk used to make yogurt is of inferior quality and the starter culture is likewise tainted. The culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* is also responsible for the bitter flavor of yogurt. Because it lowers the pH of milk, lactic acid is advantageous for producing yogurt (Ikram *et al.*, 2021). Yogurt's thermophilic culture, which grows at 40–45°C, causes acidity and fermentation of milk. The required ingredients to prepare yogurt are 8.25% milk solids, 3.25% milk fat, and a titrable acidity of at least 0.9% (Sionek *et al.*, 2024).

Functional foods offer several fitness benefits, together with the prevention and remedy to illnesses, and a way to the purposeful food bioactive additives together with flavonoids, polyphenols, saponins, prebiotics, and natural sulfides (Zerlasht *et al.*, 2024). Probiotic-containing dairy products are known to be beneficial for human health because they treat a wide range of physical abnormalities and illnesses. Probiotic dairy products enhance immune system performance and vaginal microbiota. Additionally, it decreases lactose intolerance and elevated blood cholesterol levels (Pannerchelvan *et al.*, 2024). More than 500 distinct types of bacteria have positive functions in the human digestive system. As we get older (aged), fewer bacteria are present, making other microorganisms more potent immune system attackers (Keresztény *et al.*, 2024).

The aim of this study was to evaluate the effects of different probiotic cultures on the physicochemical composition, organic acids, and sensory attributes of proteolytic peptide during the production and preservation of yogurt.

Materials and Methods

Raw milk collection

Raw buffalo and cow milk were procured from local dairy farms in the Sargodha region of Pakistan for preparing probiotic yogurt.

Starter cultures

Yogurt starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) and probiotic cultures (*Lactobacillus acidophilus* and *Bifidobacterium bifidum animalis* subsp. *lactis*) of Chr. Hansen, Denmark, were purchased from scientific stores.

The starter culture should be kept in a freezer at –18°C (0°F) as soon as it is received. To prepare a starting culture for making yogurt, starter culture was defrosted for a few hours at room temperature (20–25°C). Then it was rehydrated for 30 min⁻¹ h at room temperature by combining it with a small amount of sterile milk, as directed by the manufacturer. In order to prepare milk, it was heated to an appropriate temperature (often between 85°C and 90°C) and then cooled to inoculation temperature (about 40–45°C). To inoculate milk, the activated starting culture was added to the chilled milk and stirred gently. Then the infected milk was stored at a warm and draft-free area (around 40–45°C) and let it incubate for 4–8 h.

Probiotic yogurt preparation and storage

Probiotic yogurt was prepared using starter and probiotic bacteria by following the standard protocol with slight modifications as described by Cai *et al.* (2024); Dan *et al.* (2023); and Yang and Yoon (2022). It was stored at 6–8°C for 21 days. A detailed treatment plan for preparing yogurt is provided in Table 1, and was prepared according to the steps shown in Figure 1.

Proximate and physicochemical analyses

All yogurt treatments were analyzed for compositional analyses (moisture, fat, protein, and ash content), pH, and acidity at intervals of 7, 14, and 21 days during storage following the standard procedures of American Association of Cereal Chemists (AACC, 2000) and Association of Official Analytical Chemists (AOAC, 2006).

A drying oven with natural convection (Model: ED–115; Binder, Germany) was used to determine the moisture content of all yogurt treatments by the method described in AACC (2000). Crude protein content in all treatments of yogurt was determined by following Method No. 46–30 of AACC (2000) by using Kjeldhal's apparatus (Model No: LBS-KDDC-3, Labsol enterprises, unit no 105, tower 2, DLF Corporate, Gurugram - 122004, Haryana, India). Crude fat content in all treatments of yogurt was determined by following the procedure described in Method No. 30–25 of AACC (2000) by using Soxhlet's apparatus (Model No: Z556203. Sigma-Aldrich,

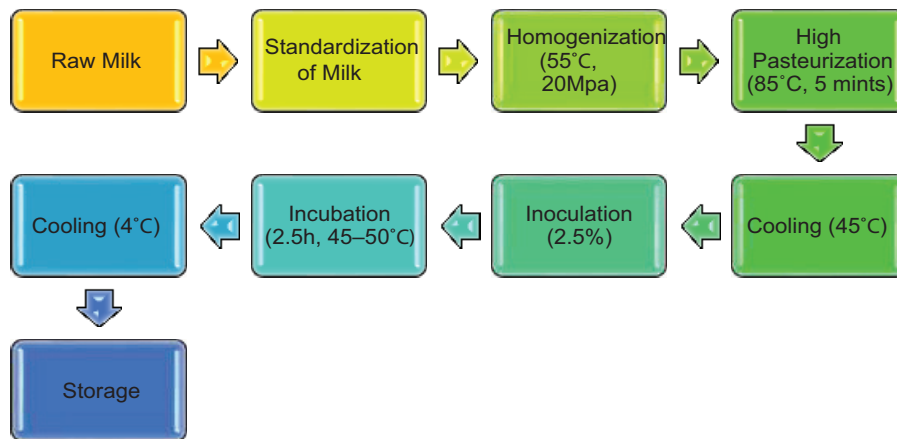


Figure 1. Steps for preparing yoghurt with specifications.

Table 1. Detailed treatment plan for yogurt manufacturing.

Cow milk	T ₁	C (Control)	<i>Streptococcus thermophilus</i> + <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>
	T ₂	C + BF	<i>Streptococcus thermophilus</i> + <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> + <i>Bifidobacterium bifidum</i>
	T ₃	C + LA	<i>Streptococcus thermophilus</i> + <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> + <i>Lactobacillus acidophilus</i>
	T ₄	C + BF + LA	<i>Streptococcus thermophilus</i> + <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> + <i>Bifidobacterium bifidum</i> + <i>Lactobacillus acidophilus</i>
Buffalo milk	T ₅	C (control)	<i>Streptococcus thermophilus</i> + <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>
	T ₆	C + BF	<i>Streptococcus thermophilus</i> + <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> + <i>Bifidobacterium bifidum</i>
	T ₇	C + LA	<i>Streptococcus thermophilus</i> + <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> + <i>Lactobacillus acidophilus</i>
	T ₈	C + BF + LA	<i>Streptococcus thermophilus</i> + <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> + <i>Bifidobacterium bifidum</i> + <i>Lactobacillus acidophilus</i>

LA: lactic acid fermentation; BF: bacterial cultures; C: control sample.

Merck SA, an affiliate of Merck KGaA, Darmstadt, Germany). A pH meter (CPH-102; Contech, India) was used to measure pH values of each yogurt sample. All measurements of the samples were conducted in duplicate (AOAC, 2006).

Determination of organic acids

For 21 days, probiotic yogurt's organic acids (acetic, lactic, and citric acids) were measured at different intervals (day 7, 14, and 21). With some modifications, the approach outlined by Akalin *et al.* (2002) was used to measure organic acids. This was accomplished by the use of high-performance liquid chromatography (HPLC). Aqueous 0.5% (wt/vol) (NH₄)₂HPO₄ (0.038 M) – 0.2% (vol/vol) acetonitrile (0.049 M) adjusted to pH 2.24 with H₃PO₄ was combined with 7 g of grated probiotic yogurt sample. About 1 h was needed to remove the material. The extraction process was agitated

using a shaker (T18 Basic, JK Ultra Turrax IKA-Werke GmbH & Co. KG, Janke & Kunkel-Str. 1079219,Staufen, Germany). The sample was centrifuged at 6,000 rpm for 5 min. The supernatant was filtered using Whatman filter No. 01. A 0.45-µm was used for second filtration. The filtrate was prepared for HPLC analysis. A Shimadzu liquid chromatograph (LC-10 AT VP Series, Shimadzu Corporation, Japan) was equipped with a SCL-10AVP framework processor. Both 20-µL test circle and SPD-10AVP UV-VIS indicator were added to chromatograph injector. At 214 nm, the detector was set up to detect organic acids.

Finding bioactive peptides

Relevant peptides were extracted from probiotic yogurt samples after 7, 14, and 21 days, and their corresponding bioactivities were examined. Kuchroo and Fox's (1982) acid coagulation process was used to identify bioactive peptides.

Purification of peptides

The extracted peptides were purified using RP-HPLC by using the procedure described by Ong and Shah (2008).

In vitro bioactivity determination

The corresponding bioactivities of purified peptides were examined.

Antihypertensive activity of peptides

The antihypertensive action of peptides was assessed using the spectrophotometric method described by Borges *et al.* (1971). The spectrophotometric technique was applied based on the liberation of hippuric acid from hippuryl-L-histidyl-L-leucine (HHL), which was catalyzed by ACE. To assess *in vitro* ACE-inhibitory activity, samples of a predetermined volume (20 μ L) were combined with 100 μ L of buffered substrate solution at an operating temperature of 37°C.

Then the reaction mixture was added to 5-mM hippuryl-L-histidyl-L-leucine (Sigma-Aldrich, MO, USA) in 50-mM HEPES-HCl buffer containing 300-mM NaCl, exhibiting a pH of 8.3. About 40 μ L of ACE (0.1 U/mL) (obtained from rabbit lung and supplied by Sigma-Aldrich) was added for carrying out the reaction, followed by incubation at 37°C for 30 min. After completion of the reaction, about 150 μ L of 1-M HCl was added. The reaction led to the release of heptylic acid, which was further extracted with ethyl acetate (1 mL), followed by transference of organic phase to a glass tube and subjected to pyrolysis. The dissolution of the residue was performed in distilled water (800 μ L) and was further subjected to spectrophotometric determination of absorbance wavelength at 228 nm; the results were reported in terms of percentage ACE-inhibitory activity (ACEI) using the following formula:

where A is the absorbance without water-soluble extract (WSE), and B is the represented absorbance without ACE. Conversely, C is indicative of absorbance in the presence of both ACE and WSE. The WSE activity from yogurt was also expressed in terms of the concentration of protein needed for inhibition (IC 50 value) of 50% of ACE activity. Captopril (1 mM; Sigma-Aldrich) was employed as a positive ACE-inhibitor control in this spectrophotometric measurement.

Antioxidant activity of peptides

The antioxidant activity of peptides was determined by the method reported by Apostolidis *et al.* (2007) using the 2,2-diphenyl-1-picrylhydrazyl (DPPH)-radical scavenging spectrophotometric assay at 517 nm with some modifications. In brief, 1-mL aliquots at varying concentrations of WSP extract (250, 500, 750 μ g/mL) were

taken in test tubes and subjected to mixing with freshly prepared DPPH (1 mL: 60 μ M) methanol solution, followed by homogenization. After completion of 45-min incubation period under darkness, the absorbance was measured by employing a spectrophotometer (Elx 800 TM Absorbance Microplate Reader; BioTek Instrument, VT, USA) at a detection wavelength of 517 nm. Similarly, about 1 mL of distilled water was employed as a blank, and results were calculated using Equation (4) given below:

$$\text{DPPH scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where

A_s = sample's absorbance,

A_c = absorbance of the blank.

Proteolysis assessment

Using HPLC, as described by Rulikowska *et al.* (2013), the proteolysis process in probiotic yogurt was assessed at different storage intervals. To separate fat, a 5-g sample was homogenized in 20 mL of 0.1-M citrate buffer (pH 3.6) at 40°C for 1 h. The suspension was centrifuged for 30 min at 3,000 \times g at 4°C. A disassociating solution (5 mL) was used to dissociate about 100 mg of the resulting pellet, which was gently and periodically mixed in a water bath at 40°C for 1 h. A 0.45-mm nylon syringe filter was utilized for the filtration process after the mixture was centrifuged once again for 10 min at 20,000 \times g at room temperature. With an ultraviolet (UV) visible detector (Shimadzu Corporation, Kyoto, Japan) set at 214 nm, a 20-mL aliquot was placed into a Shimadzu liquid chromatograph (LC-VP Series 10 AT; Shimadzu Corporation, Kyoto, Japan). For complete separation, two gradients of mobile phases were prepared: (a) 10% acetonitrile + 0.1% trifluoroacetic acid and 90% water; and (b) 10% water + 0.1% trifluoroacetic acid and 90% acetonitrile at a flow rate of 0.8 mL/min. The gradient was sustained at 20% A for 3 min after being lowered from 75% A (12 min) to 51% A (31 min), then to 20% A (13 min). After increasing the gradient to 75% A for 3 min, the entire running duration (54 min) was maintained at this concentration for 2 min. We determined the amount of water-soluble nitrogen (WSN) and total free amino acids (FAA).

Sensory evaluation

The present study used a descriptive sensory evaluation technique to evaluate probiotic yogurt's sensorial parameters, such as color, taste, odor, sourness, consistency, appearance, and general acceptability. All treatments of

yogurt were examined for sensory parameters by a panel of evaluators during storage by a method carried out by Murtaza *et al.* (2013). Among students and employees of the University of Sargodha, Pakistan, 25 evaluators, aged 18–60 years, were selected. A combined acceptance preference test by using a 9-point Hedonic scale, comprising 1 ('I disliked very much') to 9 ('I liked very much'), was applied. Each evaluator was provided with samples packed in coded packets distributed in a balanced and randomized manner along with a glass of water.

Statistical analysis

Analysis of variance and completely randomized design (CRD) were used to collect data, and One-Way Analysis of Variance (ANOVA) was used to analyze the results. The paired *t*-test was used to measure differences between mean values, and significance was noted at $P < 0.05$. Mean \pm standard deviation (SD) for three replicates ($n = 3$) was used to express all of the acquired results (Montgomery, 2008).

Results and Discussions

Proximate and physicochemical analyses

All samples of yogurt were analyzed for their moisture content at different intervals of storage. Statistical findings showed that probiotic cultures and storage significantly impacted the moisture content of yogurt (Table 2). The highest moisture content was observed in T_5 ($84.78 \pm 1.44\%$), and the lowest was in T_1 ($84.23 \pm 1.0\%$) on 0 day. During storage, the treatments showed minor alterations in moisture content, the moisture

content of probiotic yogurt reduced from $84.78 \pm 1.44\%$ to $84.12 \pm 1.46\%$, and moisture values for probiotic yogurt decreased as water evaporated for several days of storage. All interventions yielded statistically significant outcomes ($P < 0.05$), as there was a consistent reduction in moisture content. The trend of decreasing moisture content during storage was also identified in a study conducted by Terpilowski *et al.* (2023), which examined how storage conditions affected the physicochemical characteristics of yogurt. The moisture levels and other physical and chemical properties of the final yogurt didn't change much when researchers kept starter cultures at 5°C for up to 8 weeks. This finding matched what we already know: both type of probiotic culture and proper cold storage helped to keep yogurt's moisture stable over time. In a different study, Ranok *et al.* (2021) looked at how whey protein concentrate (WPC) affected probiotic yogurt. The authors kept the moisture at the same level, but found that adding WPC boosted probiotics' survival and yogurt's ability to fight off oxidation during storage. So, as WPC, it can make yogurt more nutritious without messing up how well it keeps its moisture when stored in a fridge.

Protein

Table 3 shows the protein content of yogurts made with different probiotic cultures and storage periods. On day 0, each treatment had a different protein content. Treatments T_6 – T_8 had higher protein content (4.98–4.9%) than treatments T_1 – T_4 (3.9–4.12%). Protein content decreased as storage time increased for all treatments. The decrease was more pronounced in cow milk-based yogurts, while buffalo milk-based yogurts had higher protein content throughout the storage period.

Younas *et al.* (2024) added *Lactobacillus acidophilus* to buffalo milk yogurt. After 21 days of refrigeration, its

Table 2. Effect of probiotic cultures and days on moisture content (%).

Treatments	Storage days			
	0	7	14	21
T_1	84.23 ± 1.00^a	84.21 ± 1.43^a	84.15 ± 1.44^a	84.12 ± 1.46^a
T_2	84.45 ± 1.47^a	84.43 ± 1.43^a	84.39 ± 1.43^a	84.32 ± 1.44^a
T_3	84.46 ± 1.44^a	84.44 ± 1.45^a	84.4 ± 1.45^a	84.33 ± 1.45^a
T_4	84.46 ± 1.45^a	84.44 ± 1.44^a	84.4 ± 1.44^a	84.33 ± 1.45^a
T_5	84.78 ± 1.44^a	84.75 ± 1.43^a	84.69 ± 1.44^a	84.66 ± 1.46^a
T_6	83.98 ± 1.47^a	83.96 ± 1.71^a	83.9 ± 1.71^a	83.83 ± 1.72^a
T_7	83.98 ± 1.72^a	83.95 ± 1.73^a	83.92 ± 1.73^a	83.85 ± 1.74^a
T_8	83.98 ± 1.73^a	83.95 ± 1.72^a	83.91 ± 1.72^a	83.84 ± 1.73^a

Mean values are displayed as mean \pm standard deviation (SD) and reflect the outcome of three replications ($n = 3$). This indicates that there are substantial differences ($P < 0.05$) between the same superscript letters (a–d) in the column.

Table 3. Effect of probiotic cultures and days on protein content (%).

Treatments	Storage days			
	0	7	14	21
T ₁	3.9±0.01 ^{jk}	3.86±0.05 ^k	3.82±0.05 ^k	3.78±0.03 ^k
T ₂	4.12±0.03 ^g	4.1±0.06 ^{g-i}	4.05±0.06 ^{g-i}	3.98±0.04 ^{ij}
T ₃	4.12±0.03 ^g	4.1±0.03 ^{g,h}	4.07±0.04 ^{g-i}	4±0.04 ^{h-j}
T ₄	4.12±0.02 ^g	4.1±0.04 ^{g,h}	4.06±0.05 ^{g-i}	3.99±0.04 ^{h-j}
T ₅	4.45±0.02 ^a	4.41±0.06 ^{a,f}	4.36±0.05 ^{a,f}	4.33±0.04 ^f
T ₆	4.98±0.04 ^a	4.96±0.05 ^{a,b}	4.9±0.06 ^{a-d}	4.83±0.05 ^d
T ₇	4.99±0.04 ^a	4.95±0.03 ^{a-c}	4.92±0.03 ^{a-d}	4.85±0.03 ^{b-d}
T ₈	4.98±0.01 ^a	4.95±0.04 ^{a-c}	4.91±0.04 ^{a-d}	4.84±0.04 ^{c,d}

Mean values are the result of three replications (n = 3) and are represented as mean ± standard deviation (SD). Accordingly, identical superscript letters ^(a-d) in the column differ significantly from one another (P < 0.05).

protein content decreased slightly. This was due to probiotic cultures' metabolic process, which caused protein hydrolysis. Buffalo milk is suitable for making protein-rich probiotic yogurts because despite this decrease, the protein content remained high. Younas *et al.* (2022) also examined the impact of different probiotic strains, such as *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, and *Bifidobacterium animalis* subsp. *lactis*, on goat milk yogurt. After 28 days of refrigeration, the researchers found that probiotics affected the texture, and organoleptic and biological activities of yogurt. The result of the study showed that adding specific probiotic strains to goat milk yogurt could affect its physicochemical characteristics, such as protein content, over time. Proteolysis increased in all samples during storage, which showed the proteolytic activity of LAB.

Fat

Table 4 shows how the fat content of yogurt samples is affected by different probiotic cultures and storage

periods. All treatments showed different fat proportions at the beginning (day 0), although the initial fat content of the buffalo milk-based yogurts (T₅ through T₈) was greater (4.46–4.69%) than those of the cow milk-based yogurts (T₁ through T₄) (3.91–4.13%). Throughout the 21-day storage, all treatments showed a small decrease in fat content. The decrease was more noticeable in cow milk-based yogurts, while buffalo milk-based yogurts kept their fat content comparatively higher over the course of storage.

Results of Younas *et al.*'s (2024) study on addition of *Lactobacillus acidophilus* to buffalo milk yogurt was in line with this. The study found that after 21 days of refrigeration, the fat content of buffalo milk yogurt decreased slightly. This was due to the probiotic culture metabolic process which caused lipid hydrolysis. Buffalo milk is suitable for making rich probiotic yogurts as evidenced by the relatively high fat content that remained after this reduction. The same result was demonstrated by

Table 4. Effect of probiotic cultures and days on fat content (%).

Treatments	Storage days			
	0	7	14	21
T ₁	3.91±0.02 ^{hi}	3.87±0.04 ^{ij}	3.83±0.03 ^{ij}	3.79±0.02 ^j
T ₂	4.13±0.03 ^f	4.11±0.04 ^f	4.06±0.05 ^{fg}	3.99±0.03 ^{g,h}
T ₃	4.13±0.02 ^f	4.11±0.02 ^f	4.08±0.03 ^{fg}	4.01±0.03 ^g
T ₄	4.13±0.01 ^f	4.11±0.03 ^f	4.07±0.04 ^{fg}	4±0.03 ^g
T ₅	4.46±0.01 ^{cd}	4.42±0.05 ^{d,e}	4.37±0.04 ^{d,e}	4.34±0.03 ^e
T ₆	4.69±0.03 ^a	4.67±0.04 ^a	4.61±0.05 ^{a,b}	4.54±0.05 ^{b,c}
T ₇	4.69±0.05 ^a	4.66±0.02 ^a	4.63±0.02 ^{a,b}	4.56±0.02 ^b
T ₈	4.69±0.01 ^a	4.67±0.03 ^a	4.62±0.03 ^{a,b}	4.55±0.03 ^b

Mean values are the result of three replications (n = 3) and are represented as mean ± standard deviation (SD). Accordingly, the identical superscript letters ^(a-d) in the column differ significantly from one another (P < 0.05).

Tavakoli *et al.* (2019), who studied how the physicochemical characteristics of probiotic yogurt during storage were affected by the fat content of milk and starter culture. According to the study, regardless of the starter culture used, yogurt samples with higher initial fat content decreased slightly in fat percentage. This finding implied that although probiotic activity might be involved in slight lipid deterioration, fat content at the beginning had a major impact on the total stability of fat throughout the storage.

pH is used in the culinary industry to determine the quality of food. Statistical findings showed that probiotic cultures and storage significantly impacted the pH of yogurt (Table 5). The highest pH was observed in T₂ (4.56±0.03), and the lowest was in T₅ (4.17±0.02) on day 0. During storage duration, the treatments showed minor alterations in pH content. During storage, the pH of probiotic yogurt reduced from 4.56±0.03 to 4.06±0.06. Notably, decrease in pH was more pronounced in buffalo milk-based yogurts whereas cow milk-based yogurts maintained relatively higher pH levels throughout the storage.

After fermentation with various starting cultures, Taha *et al.* (2017) evaluated the antibacterial and antioxidant properties of bioactive peptides in buffalo yogurt. Titratable acidity showed an upward trend throughout the 15-day storage period; a range of 0.65±0.005–1.3±0.028 was recorded. The effects of storage conditions on the physicochemical properties of the final product were also investigated by Terpiłowski and colleagues (2023) in relation to the dry ingredients used in yogurt. Variations in temperature and storage period of dry components were shown to have an impact on the incubation time for yogurt to reach a pH of 4.6. Keeping starter cultures at 5°C for up to 8 weeks did not significantly affect the incubation period or the vitality of cultures,

demonstrating that proper storage conditions are crucial for maintaining the consistency and quality of yogurt products.

A study conducted by Kim *et al.* (2022) evaluated the effects of several probiotic LAB on the quality and preservation qualities of Greek yogurt, which further supported these conclusions. The researchers observed that pH of each yogurt sample decreased after being stored at 4°C for 21 days. pH of Greek yogurt, including *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus gasseri* BNR17, were notably stable, compared to other formulations. Accordingly, the rate at which pH decreased during storage could be influenced by specific probiotic strains, thereby affecting yogurt's flavor and shelf life.

Quantitative analysis of organic acids

Acetic acid

The effects of different probiotic cultures and storage period on the quantity of acetic acid in yogurt samples are shown in Table 6. Acetic acid range was numerous throughout all remedies at the start (day 0), with buffalo milk-based yogurts (T₅–T₈) frequently displaying better initial concentrations than cow milk-primarily based remedies (T₁–T₄). The quantity of acetic acid in every remedy increased gradually during 21-day storage. Considerably, buffalo milk-based yogurts showed a greater boom in acetic acid, especially in treatments that blanketed extra probiotic traces together with *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (T₆–T₈). The consequences of Younas *et al.*'s (2024) research on the addition of *Lactobacillus acidophilus* to buffalo milk yogurt were steady with this study. Over 21 days of refrigeration, the authors discovered that

Table 5. Effect of probiotic cultures and days on pH.

Treatments	Storage days			
	0	7	14	21
T ₁	4.51±0.01 ^{a-d}	4.47±0.03 ^{a-d}	4.43±0.03 ^{b-d}	4.39±0.03 ^{d,e}
T ₂	4.56±0.03 ^a	4.51±0.07 ^{a-d}	4.47±0.06 ^{a-d}	4.4±0.04 ^d
T ₃	4.54±0.03 ^{a-c}	4.52±0.03 ^{a-d}	4.48±0.03 ^{a-d}	4.41±0.03 ^{c,d}
T ₄	4.55±0.01 ^{a,b}	4.52±0.05 ^{a-d}	4.48±0.04 ^{a-d}	4.41±0.03 ^d
T ₅	4.17±0.02 ^{f-j}	4.14±0.07 ^{f-j}	4.09±0.06 ^{i,j}	4.06±0.06 ^j
T ₆	4.26±0.06 ^{a,f}	4.24±0.07 ^{f-h}	4.2±0.07 ^{f-i}	4.13±0.05 ^{g-i}
T ₇	4.25±0.04 ^{f,h}	4.23±0.04 ^{f-h}	4.19±0.04 ^{f-i}	4.12±0.04 ^{h-j}
T ₈	4.26±0.02 ^{f,g}	4.24±0.05 ^{f-h}	4.2±0.06 ^{f-i}	4.13±0.05 ^{h-j}

Mean values are the result of three replications (n = 3) and are represented as mean ± standard deviation (SD). Accordingly, identical superscript letters (^{a-d}) in the column differ significantly from one another ($P < 0.05$).

Table 6. Effect of probiotic cultures and days on acetic acid (ppm).

Treatments	Storage days			
	0	7	14	21
T ₁	25.34±0.01 ^s	38.69±1.01 ^r	52.04±0.84 ^{n,o}	67.4±1.88 ^j
T ₂	49.2±1.31 ^{o,p}	62.56±2.22 ^{h,i}	75.91±1.08 ^{e-g}	91.26±0.73 ^b
T ₃	46.7±1.6 ^{p,q}	60.06±3.17 ^{l,m}	73.41±2.11 ^{g,h}	88.76±1.41 ^{b,c}
T ₄	47.95±2.49 ^{o,p}	61.31±2.69 ^{k,l}	74.66±1.59 ^{f,g}	90.01±1 ^b
T ₅	42.17±2.03 ^{q,r}	55.52±1.41 ^{m,n}	68.88±0.76 ^{h,i}	84.23±1.53 ^{c,d}
T ₆	67.2±0.87 ^{i,j}	80.56±2.22 ^{d,e}	93.91±1.08 ^b	109.26±0.73 ^a
T ₇	64.7±1.6 ^{i,l}	78.06±3.17 ^{e-g}	91.41±2.11 ^b	106.76±1.41 ^a
T ₈	65.95±2.49 ^{i-k}	79.31±2.69 ^{d-f}	92.66±1.59 ^b	108.01±1 ^a

Mean values are the result of three replications (n = 3), and are represented as mean ± standard deviation (SD). Accordingly, identical superscript letters (a–j) in the column differ significantly from one another (P < 0.05).

pH decreased notably, with the biggest drop occurring in yogurt that protected 100% probiotic way of life. *L. acidophilus*'s metabolic strategies, which generated lactic acid and increased yogurt's acidity, were attributed for pH decrease. These effects highlighted the effect of probiotic cultures on acidification that took place in yogurt.

Similar to this, Heydari *et al.* (2021) investigated the biochemical traits of probiotic yogurt produced by the use of *Lactobacillus casei* and other local Iranian strains. Yogurts with *L. casei* had better levels of acetic acid than probiotic-unfastened control samples.

Lactic acid

Lactic acid's acidity influences the rheological and sensory properties of milk, enhancing its flavor and maintaining its shelf life (Cheng, 2010). Table 7 shows how the lactic acid content of yogurt samples is affected by

different probiotic cultures and storage periods. All treatments had variable amounts of lactic acid at the beginning (day 0); however, yogurts made from buffalo milk (T₅–T₈) typically had higher starting concentrations than those made from cow milk (T₁–T₄). The amount of lactic acid in each treatment increased gradually throughout the 21-day storage. Strangely, remedies that blanketed extra probiotic strains, such as *Bifidobacterium bifidum* and *Lactobacillus acidophilus* (T₂–T₄ and T₆–T₈) confirmed an extra noticeable increase in lactic acid.

In addition, Kim *et al.* (2022) assessed how diverse probiotic lactic acid microorganisms affected the yogurt which is stored at home. According to researchers' findings, level of lactic acid in each yogurt sample increased over 21 days of storage at 4°C. Interestingly, Greek yogurt fermented with *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus gasseri* BNR17 confirmed exceptional

Table 7. Effect of probiotic cultures and days on lactic acid (ppm).

Treatments	Storage days			
	0	7	14	21
T ₁	768.77±2.08 ^r	858.12±8.02 ^{p,q}	901.48±5.75 ⁿ	951.83±2.72 ^m
T ₂	892.64±8.46 ^{n,o}	981.99±9.35 ^{i,j}	1025.34±5.98 ^{f,g}	1075.7±1.18 ^{a,b}
T ₃	855.47±9.51 ^{p,q}	934.82±6.1 ^m	978.18±8.01 ^{j,k}	1028.53±3.22 ^{e-g}
T ₄	874.05±7.85 ^{o,p}	958.41±7.66 ^{k,l}	1001.76±6.99 ^{h,i}	1052.11±2.18 ^{c,d}
T ₅	848.6±8.65 ^q	937.96±5.05 ^{l,m}	981.31±4.36 ^{i,j}	1031.66±9.64 ^{d-g}
T ₆	910.64±17.3 ⁿ	999.99±9.35 ^{h,i}	1043.34±5.98 ^{d-f}	1093.7±1.18 ^a
T ₇	873.47±9.51 ^{o,p}	952.82±6.1 ^m	996.18±8.01 ^{i,j}	1046.53±3.22 ^{d,e}
T ₈	892.05±7.85 ^{n,o}	976.41±7.66 ^{j,k}	1019.76±6.99 ^{g,h}	1070.11±2.18 ^{b,c}

Mean ± standard deviation (SD) represent mean values, which are the outcome of three replications (n = 3). This indicates that identical superscript letters (a–d) in the column differ considerably (P < 0.05) from one another.

increase in lactic acid concentration, which improved its physicochemical traits and sensory acceptability.

Similarly, after 12 h of fermentation, the medium with 2% probiotic, 1.5% galactooligosaccharides (GOS), and 1.5% maltodextrin had maximum acidity, with a median value of 5, 91% consistent with the findings of Quddos *et al.* (2018). The medium that contained 1.5% GOS, 1.5% maltodextrin, and 1% probiotic (T17) had a mean value of 5, 79% consistent. In order to be away from cardiovascular ailments and electrolytic imbalance, the protein and calorie consumption of patients is reduced considerably, but consumption of a suitable diet may additionally restrict the loss of various critical nutrients and additional use the banifical bacteria, such as lactic acid microorganisms (*Lactobacillus bulgaricus* and *Lactobacillus acidophilus*) (Hameed *et al.*, 2024).

Citric acid

Table 8 shows the effect of probiotic cultures and storage period on citric acid content (ppm) in yogurt. Across all treatments, citric acid levels increased gradually throughout the course of 21-day storage, suggesting the ongoing metabolic activity during storage. Interestingly, treatments with more probiotic strains (such as T₂, T₆, and T₈) showed noticeably higher levels of citric acid than the control (T₁), indicating that the increased fermentation activity of these probiotic cultures increased the synthesis of organic acids. For example, treatment T₆ achieved 377.81 ppm on day 21, but the control T₁ only managed to reach 270.61 ppm. When many strains of probiotic cultures are utilized, the metabolic processes they stimulate (such the conversion of citrate into other metabolites) are more active, which improves yogurt's acid profile and possibly its sensory qualities.

The results of Akgun *et al.* (2018), who looked into the combined impact of probiotic cultures and final

incubation pH on the quality of buffalo milk yogurt during cold storage, were consistent with these results. According to the study, generation of organic acids, particularly citric acid, during storage was greatly impacted by the type of starter culture. In particular, citric acid concentrations of yogurts fermented with probiotic cultures were higher than those of yogurts fermented with conventional starter cultures. This suggested that the metabolic activities of the probiotics helped in the gradual increase in citric acid generation.

Evaluation of proteolysis

Water-soluble nitrogen

All yogurt samples were analyzed for their WSN content at different storage periods. The results are provided in Table 9, which shows an analysis of variance for the WSN content of different yogurt treatments kept at different storage periods. We observed significant results between storage days and various yogurt treatments, but nonsignificant results were observed between their interactions.

Table 9 displays the impact of days and treatments on the WSN content of various yogurt treatments. It is evident that treatment T₆ had the highest overall WSN content (0.16±0A) among the treatments, and that 21 days of storage had the highest overall WSN content (0.16±0). Treatment T₆ had the highest WSN level during interaction on day 21 of storage (0.18±0), while Treatment T₁ had the lowest WSN level on day 0 of storage (0.11±0).

Similarly, Niamah (2017) examined the effect of incorporating *Saccharomyces boulardii* to yogurt and determined that WSN increased over 21-day storage. The elevated level of WSN was related to *S. boulardii*'s proteolytic interest, which simplified the breakdown of milk proteins into smaller peptides and amino acids,

Table 8. Effect of probiotic cultures and days on citric acid content (ppm).

Treatments	Storage days			
	0	7	14	21
T ₁	175.45±3.06 ⁿ	200.5±1.01 ^m	235.55±0.82 ^{k,l}	270.61±2.33 ^{a,h}
T ₂	264.65±3.91 ^{h,i}	289.7±4.67 ^f	324.75±5.47 ^d	359.81±6.57 ^b
T ₃	228.48±7.84 ^l	253.53±2.99 ^j	288.59±3.94 ^f	323.64±5.22 ^d
T ₄	246.56±6.64 ^k	271.62±3.83 ^{g,h}	306.67±4.69 ^e	341.72±5.87 ^c
T ₅	222.28±7.22 ^l	247.33±1.41 ^k	282.39±0.92 ^g	317.44±2.19 ^{d,e}
T ₆	282.65±3.73 ^g	307.7±4.67 ^e	342.75±5.47 ^c	377.81±6.57 ^a
T ₇	246.48±7.84 ^k	271.53±2.99 ^{g,h}	306.59±3.94 ^e	341.64±5.22 ^c
T ₈	264.56±6.64 ^{h,i}	289.62±3.83 ^f	324.67±4.69 ^d	359.72±5.87 ^b

Mean ± standard deviation (SD) represent mean values, which are the outcome of three replications (n = 3). This indicates that identical superscript letters (a-d) in the column differ considerably (P < 0.05) from one another.

Table 9. Effect of treatments and days on WSN content.

Treatments	Storage days				Total
	0	7	14	21	
T ₁	0.11±0 ^l	0.12±0 ^{-l}	0.13±0 ^{n-r}	0.14±0 ^{-o}	0.13±0 ^f
T ₂	0.13±0 ^{q-s}	0.14±0 ^{m-q}	0.15±0 ^{g-m}	0.16±0 ^{d-g}	0.14±0 ^{c,d}
T ₃	0.12±0 ^{s,t}	0.13±0 ^{p-s}	0.14±0 ^{k-p}	0.15±0 ^{f-k}	0.14±0 ^e
T ₄	0.13±0 ^{-l}	0.13±0 ^{n-r}	0.14±0 ⁻ⁿ	0.15±0 ⁻ⁱ	0.14±0 ^{d,e}
T ₅	0.13±0 ^{q-s}	0.14±0 ^{-o}	0.15±0 ^{e-j}	0.16±0 ^{b-e}	0.15±0 ^c
T ₆	0.15±0 ^{h-m}	0.16±0 ^{d-h}	0.17±0 ^{a-d}	0.18±0 ^a	0.16±0 ^a
T ₇	0.14±0 ^{m-p}	0.15±0 ^{f-i}	0.16±0 ^{c-f}	0.17±0 ^{a-c}	0.15±0 ^b
T ₈	0.14±0 ^{-o}	0.15±0 ^{e-j}	0.16±0 ^{b-e}	0.17±0 ^{a,b}	0.16±0 ^{a,b}
Total	0.13±0 ^d	0.14±0 ^c	0.15±0 ^b	0.16±0 ^a	

Values not sharing superscript letters in a column or row are significant statistically.

enhancing the yogurt's nutritional value and taste. Owing to the breakdown of casein into low-molecular weight water-soluble amino acids and peptides via non-starter lactic acid, milk, and LAB, WSN—also referred to as total nitrogen—is a distinguished proteolysis product. Owing to the continuous breakdown of casein into smaller peptides and amino acid fractions and large peptides by using starter and non-starter lactic acid microorganism (NSLAB), sure enzymes, and remaining rennet, the range of nitrogen increased with maturing period (Adouard *et al.*, 2016).

Free amino acids

Table 10 illustrates the impact of various treatments (T₁–T₈) and storage periods (0, 7, 14, and 21 days) on the FAA content of a product. As storage time increased and other applications were implemented, the quantity of FAA inside the mixture increased. As storage days increased, the FAA content material also increased throughout all

treatments. The FAA content material in treatment 1 (T₁), for instance, increased from 31.77 mg/100 g on day 0 to 41.17 mg/100 g on day 21. This showed that proteins spoiled down into FAA throughout storage because of ongoing proteolytic pursuit. Through day 21, the overall FAA content, when averaged over all treatments, increased to 47.15 mg/100 g on day 0. The idea that storage time had a vital role in the development of loose amino acids—probably because of nonstop enzymatic hobby—was supported through this standard growth.

Similar styles have been referred to in research on plant-based totally beverages, where higher quantities of FAA were determined after storage, suggesting proteolysis. Likewise, Akan (2022) tested the impact of yogurt microorganisms and fermentation period on FAA content of probiotic goat yogurt. The authors observed that, depending on particular probiotic strains applied, The starter culture's proteolytic pursuit, which hydrolyzes

Table 10. Effect of treatments and days on FAA content.

Treatments	Storage days				Total
	0	7	14	21	
T ₁	31.77±0 ^p	34.9±1 ^{o,p}	38.04±1.02 ^{m-o}	41.17±1.04 ^{l-n}	36.47±1.14 ^q
T ₂	42.77±1.07 ^{l,m}	45.9±2.08 ^{k,l}	49.04±2.08 ^{j,k}	52.17±2.08 ^{h-j}	47.47±1.38 ^d
T ₃	36.94±2.08 ^{n-p}	40.04±2.24 ^{m-o}	43.18±2.26 ^{l,m}	46.31±2.26 ^{k,l}	41.62±1.43 ^f
T ₄	39.85±2.26 ^{m-o}	42.97±2.16 ^{l,m}	46.11±2.17 ^{k,l}	49.24±2.17 ^{-k}	44.54±1.4 ^e
T ₅	52.27±2.17 ^{h-j}	55.4±1.8 ^{f-h}	58.54±1.83 ^{d-g}	61.67±1.86 ^{b-e}	56.97±1.32 ^c
T ₆	60.77±1.89 ^{-f}	63.9±2.08 ^{b-d}	67.04±2.08 ^{a,b}	70.17±2.08 ^a	65.47±1.38 ^a
T ₇	54.94±2.08 ^{g-i}	58.04±2.24 ^{e-g}	61.18±2.26 ^{c-e}	64.31±2.26 ^{b,c}	59.62±1.43 ^c
T ₈	57.85±2.26 ^{e-h}	60.97±2.16 ^{c-f}	64.11±2.17 ^{b-d}	67.24±2.17 ^{a,b}	62.54±1.4 ^b
Total	47.15±2.17 ^d	50.27±2.17 ^c	53.4±2.17 ^b	56.54±2.17 ^a	

Values not sharing a superscript letter in a column or row are significant statistically.

milk proteins into peptides and FAA, improves yogurt's dietary value and sensory attraction.

Evaluation of bioactivities

Antihypertensive activity

At various stored periods, the antihypertensive property of every yogurt pattern was examined. Table 11 shows how distinctly bacterial remedies and storage periods affect yogurt samples' antihypertensive effectiveness, which is expressed as percentage suppression of ACE. With values of 22.45–46.14%, all therapies confirmed the baseline antihypertensive action at the beginning (day 0). Antihypertensive activity gradually improved for all treatments in the course of 21-day storage. Interestingly, treatments with additional probiotic strains, such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (T_2 , T_4 , T_6 , and T_8), showed more antihypertensive action than the control (T_1). On day 21, for example, T_6 had 62.48% antihypertensive effect, and T_1 had 38.78% effect. The outcomes of Kong *et al.*'s (2021) research about the effect of probiotic yogurt on blood strains in mice with spontaneous hypertension are constant with this discovery. The authors found that consuming probiotic yogurt dramatically decreased coronary heart fee and blood stress. This changed through reworking of intestinal microbiota and the improved synthesis of quick-chain fatty acids having antihypertensive properties. Altering intestinal microbiota and producing bioactive compounds highlight the potential of probiotic-fermented dairy products in the treatment of high blood pressure. Similarly, Juandini *et al.* (2024) investigated the antihypertensive outcomes of Greek yogurt combined with roselle extract and probiotics. Probiotic Greek yogurt's antihypertensive effect increased from 35.68% in the control probiotic yogurt to 81.36% in the supplemented

version, consistent with the study. This was due to the mixed action of probiotics and the bioactive substances in roselle extract that increased ACE-inhibitory pursuit.

Antioxidant activity

All the samples of yogurt were analyzed for their antioxidant activity at different intervals of storage. The results of analyses are shown in Table 12.

Table 12 shows the analysis of variance for antioxidant activity of different treatments of yogurt stored at different storage intervals. We observed that highly significant results were obtained between storage days and various treatments of yogurt; however, nonsignificant results were observed for their interaction. It was observed that the highest overall antioxidant activity in the case of treatments was found for treatment T_6 (63.41 ± 2.32), while the highest overall antioxidant activity during storage was found on day 21 of storage (51.75 ± 9.23). For interaction, the highest antioxidant activity was observed for treatment T_6 (66.21 ± 1.05) on day 21 of storage, while the lowest antioxidant activity was found for treatment T_1 on day 0 of storage (31.73 ± 1.53). However, in the fourth sample of yogurt, significant antioxidant values were recorded when yoghurt and probiotic bacteria were used in conjunction after day 0 assessment. We found results of the present study in compliance with results of the research conducted by Gupta and Garg (2009).

This trend aligned with the findings of Akan *et al.* (2022), who investigated the combined effect of probiotic cultures and incubation final pH on the quality of buffalo milk yogurt during cold storage. The study reported that the type of starter culture significantly influenced the production of organic acids, including citric acid, during storage. Specifically, yogurts fermented with probiotic cultures exhibited higher citric acid concentrations

Table 11. Effect of treatments and days on antihypertensive activity.

Treatments	Storage days				Total
	0	7	14	21	
T_1	22.45±0.99 ^w	28.12±0.45 ^v	33.45±0.73 ^t	38.78±1.24 ^{q,r}	30.7±6.39 ^h
T_2	37.78±1.56 ^{q-s}	43.45±0.99 ^{m-o}	48.78±0.99 ^{h,i}	54.12±1.28 ^{e,f}	46.03±6.43 ^d
T_3	33.73±1.52 ^l	39.4±0.97 ^{p,q}	44.73±1.05 ^{k-m}	50.06±1.39 ^{g,h}	41.98±6.44 ^f
T_4	35.76±1.54 ^{s,t}	41.42±0.98 ^{o,p}	46.76±1.02 ^{i-k}	52.09±1.34 ^{f,g}	44.01±6.43 ^e
T_5	30.81±1.53 ^u	36.48±1.19 ^s	41.81±1.58 ^{n-p}	47.14±2.06 ^{j-k}	39.06±6.49 ^g
T_6	46.14±1.99 ^l	51.81±1.53 ^{f,g}	57.14±1.76 ^{c,d}	62.48±2.13 ^a	54.39±6.54 ^a
T_7	42.09±2.02 ^{n,o}	47.76±1.59 ^{h-j}	53.09±1.85 ^{e,f}	58.42±2.24 ^{b,c}	50.34±6.56 ^c
T_8	44.12±2 ^{l-n}	49.78±1.56 ^{g,h}	55.12±1.81 ^{d,e}	60.45±2.18 ^{a,b}	52.37±6.55 ^b
Total	36.61±7.55 ^d	42.28±7.49 ^c	47.61±7.51 ^b	52.94±7.57 ^a	

Values not sharing superscript letter in a column or row are significant statistically.

Table 12. Effect of treatments and days on antioxidant activity.

Treatments	Storage days				Total
	0	7	14	21	
T ₁	31.73±1.53 ^x	33.73±1.49 ^w	35.55±1.66 ^v	37.4±1.88 ^u	34.6±2.61 ^h
T ₂	42.37±0.79 ^s	44.37±0.73 ^{p,q}	46.2±0.9 ^p	48.05±1.11 ⁿ	45.25±2.33 ^e
T ₃	38.49±1.01 ^u	40.49±1.01 ^t	42.32±1.2 ^s	44.17±1.42 ^{q,r}	41.37±2.42 ^g
T ₄	40.43±0.87 ^t	42.43±0.85 ^{r,s}	44.26±1.03 ^q	46.11±1.25 ^{o,p}	43.31±2.37 ^f
T ₅	48±0.89 ⁿ	50±0.84 ^m	51.82±1.01 ^{k,l}	53.67±1.22 ^{l,j}	50.87±2.36 ^d
T ₆	60.54±0.77 ^{d,e}	62.54±0.7 ^c	64.36±0.84 ^b	66.21±1.05 ^a	63.41±2.32 ^a
T ₇	51.23±0.98 ^{l,m}	53.19±0.9 ^k	55.02±1.09 ^{h,i}	56.87±1.3 ^g	54.08±2.37 ^c
T ₈	55.89±0.86 ^{g,h}	57.87±0.78 ^f	59.69±0.95 ^e	61.54±1.16 ^{c,d}	58.75±2.34 ^b
Total	46.08±9.21 ^d	48.08±9.2 ^c	49.9±9.21 ^b	51.75±9.23 ^a	

Values not sharing superscripted letter in a column or row are significant statistically.

compared to those with traditional starter cultures, suggesting that the metabolic activities of added probiotics contribute to increased production of citric acid over time.

Nishat *et al.* (2024) revealed the antioxidant potential of muskmelon seed oil that there is a significant interaction between all the treatments. The interaction effect of days and treatments is also significant.

Descriptive sensory analysis of yogurt

Significant variations in descriptive sensory attributes were noted while investigating the impact of probiotic culture on yogurt. Statistical findings showed that probiotic cultures and storage significantly impacted the color of yogurt (Table 13). The highest color was observed in T₆ (4.76±0.13) and the lowest was in T₁ (3.97±0.15) on

day 0. All treatments exhibited slight changes in colors over the course of storage. Over time, probiotic yogurt's hue increased from 3.97±0.15 to 4.94±0.17.

The rheological traits and sensory profile of yogurt were made by using a unique aggregate of probiotic cultures comprising *Lactobacillus plantarum* HA119 and *Bifidobacterium animalis* subsp. *lactis* B94 (Ilić *et al.*, 2024), and their findings were found to be steady with the present commentary. In the study, adding probiotic cultures produced yogurt with decreased fats content, similar to the sensory features of excessive-fat yogurts, inclusive of enhanced color capabilities. Probiotic culture's metabolic functions were attributed to producing improved sensory color, which could additionally have an effect on pigment balance. Similarly, Kim *et al.* (2022) investigated how probiotic LAB affected Greek yogurt's exceptional and storage acts, including the bacteria, such *Lactobacillus gasseri* BNR17 and *Lactobacillus*

Table 13. Effect of probiotic cultures and days on sensory color.

Treatments	Storage days			
	0	7	14	21
T ₁	3.97±0.15 ^f	4.03±0.12 ^q	4.06±0.26 ^q	4.13±0.29 ^p
T ₂	4.18±0.14 ^o	4.24±0.02 ^{m,n}	4.3±0.06 ^{k,l}	4.38±0.13 ^j
T ₃	4.21±0.1 ^{n,o}	4.26±0.17 ^{l,m}	4.31±0.21 ^k	4.35±0.12 ^j
T ₄	4.2±0.27 ^o	4.25±0.22 ^{m,n}	4.31±0.18 ^k	4.37±0.12 ^j
T ₅	4.51±0.23 ⁱ	4.56±0.08 ^h	4.62±0.04 ^g	4.69±0.33 ^f
T ₆	4.76±0.13 ^d	4.81±0.32 ^c	4.87±0.24 ^b	4.91±0.17 ^a
T ₇	4.72±0.35 ^{e,f}	4.8±0.18 ^c	4.86±0.09 ^b	4.94±0.17 ^a
T ₈	4.75±0.28 ^{d,e}	4.8±0.26 ^c	4.86±0.02 ^b	4.92±0.31 ^a

Mean ± standard deviation (SD) represent mean values, which are the outcome of three replications (n = 3). This indicates that identical superscripted letters (a-d) in the column differ considerably (P < 0.05) from one another.

plantarum HY7714, which improved yogurt's physicochemical characteristics and sensory characteristics along with coloration. Probiotic microorganism's synthesis of exopolysaccharides (EPS) could modify yogurt's texture and appearance.

Appearance

Table 14 shows how probiotic microorganisms and storage time affect the appearance of yogurt samples. Across all treatments, the results showed a general increase in appearance scores throughout 21-day storage, indicating that probiotic cultures gradually improved yogurt's visual appeal. Treatments containing particular probiotic strains, such as T_6 – T_8 , demonstrated noticeably superior appearance ratings during storage period, whereas the control sample (T_1) had the lowest score. T_6 had the highest score on day 21 (4.54), suggesting that this formulation produced yogurt with maximum visual appeal.

Probiotic bacteria, according to previous studies, can improve the physical and sensory qualities of yogurt, especially its appearance. Adding *Lactobacillus casei* and *Bifidobacterium animalis* to yogurt recipes enhanced their overall sensory appeal, especially in terms of texture, consistency, and appearance (Jaman *et al.*, 2022). EPS-producing probiotic strains would contribute to a more consistent and glossy appearance, which would account for higher scores in probiotic culture treatments. Smooth, natural, and vibrant hues are essential qualities for yoghurt. Yogurt that is white to off-white on color may be flavorless. The surface should exhibit a uniform appearance, devoid of any signs of whey growth, separation, or discoloration. It is critical to ensure consistency in color, size, and distribution of ingredients.

Consistency of yogurt

Table 15 illustrates the effect of various probiotic cultures and storage periods on the consistency of yogurt

samples as evaluated by sensory panels. Consistency scores increased progressively over 21-day storage across all treatments, indicating an improvement in the perceived texture of yogurt over time. Remarkably, treatments with greater probiotic bacteria, such as *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* (T_2 , T_4 , T_6 , and T_8), confirmed better consistency than the control (T_1). On day 21, treatment T_6 had a consistency of 4.27, while treatment T_1 had a consistency of 3.47. This indicated that the addition of specific probiotic culture improved the consistency of yogurt.

Similarly, Fan *et al.* (2022) checked how adding *Lactocaseibacillus casei* and *Levilactobacillus brevis* affected yogurt's textural traits. The authors confirmed that in comparison to manipulated samples, yogurt containing both probiotic lines had better hardness and adhesiveness in addition to decreased syneresis. Probiotics' metabolic activities, which boosted yogurt's mouthfeel and structural integrity, were associated with their improved textural qualities. These findings were consistent with those of Turgut and Çakmakçi (2018), who found that yogurt consistency varied appreciably. During storage, no discernible alternative to consistency was discovered in any of the seven formulations.

It was determined that for all seven yogurt formulations, maximum sensory qualities did not adjust through 10-day chilling period. Yogurt's texture and body must be firm, like a custard, free of lumps. A spoonful of yogurt should retain its shape with no jagged edges. It must be made sure that bulk flavoring components are dispersed equally throughout the finished product.

Sourness

The yogurt should possess a pleasant, refreshing, and sour acidic taste. Undesirable flavors, such as bitterness, rancidity, oxidation, staleness, sourness, and dirtiness,

Table 14. Effect of probiotic cultures and days on appearance.

Treatments	Storage days			
	0	7	14	21
T_1	3.51±0.07 ^q	3.63±0.13 ^p	3.59±0.21 ^{p,q}	3.73±0.4 ^o
T_2	3.78±0.31 ^{m-o}	3.77±0.07 ^{n,o}	3.9±0.29 ^{j-k}	3.98±0.16 ⁱ
T_3	3.78±0.2 ^{n,o}	3.83±0.06 ^{k-n}	3.85±0.14 ⁻ⁿ	3.89±0.37 ^{-l}
T_4	3.8±0.31 ^{l-o}	3.78±0.08 ^{m-o}	3.87±0.33 ^{-m}	3.93±0.12 ^{ij}
T_5	4.11±0.17 ^h	4.1±0.26 ^h	4.15±0.15 ^{g,h}	4.23±0.02 ^{fg}
T_6	4.32±0.18 ^{c-f}	4.37±0.1 ^{b-e}	4.42±0.27 ^b	4.54±0.21 ^a
T_7	4.29±0.11 ^{d-f}	4.38±0.11 ^{b-d}	4.43±0.39 ^b	4.44±0.29 ^b
T_8	4.28±0.23 ^{e,f}	4.37±0.13 ^{b-d}	4.39±0.37 ^{b,c}	4.45±0.14 ^{a,b}

Mean values are the result of three replications (n = 3) and are shown as mean ± standard deviation (SD). Values with the same superscript letters (a–d) in the column are significantly ($P < 0.05$) different from one another.

Table 15. Effect of probiotic cultures and days on consistency.

Treatments	Storage days			
	0	7	14	21
T ₁	3.24±0.13 ^l	3.36±0.23 ^{ij}	3.33±0.25 ^{ij}	3.47±0.09 ^{h-j}
T ₂	3.52±0.13 ^{g-j}	3.51±0.25 ^{h-j}	3.63±0.28 ^{d-j}	3.71±0.13 ^{b-i}
T ₃	3.51±0.26 ^{g-j}	3.56±0.31 ^{ij}	3.58±0.04 ^{e-j}	3.62±0.16 ^{d-j}
T ₄	3.53±0.03 ^{g-j}	3.52±0.29 ^{g-j}	3.61±0.33 ^{d-j}	3.67±0.16 ^{c-j}
T ₅	3.84±0.13 ^{a-h}	3.83±0.14 ^{a-h}	3.89±0.12 ^{a-h}	3.96±0.09 ^{a-g}
T ₆	4.05±0.3 ^{a-d}	4.1±0.28 ^{a-c}	4.16±0.1 ^{a,b}	4.27±0.33 ^a
T ₇	4.03±0.05 ^{a-e}	4.11±0.05 ^{a-c}	4.17±0.19 ^a	4.17±0.07 ^a
T ₈	4.01±0.07 ^{a-f}	4.1±0.31 ^{a-c}	4.13±0.24 ^{a,b}	4.19±0.33 ^a

Mean ± standard deviation (S.D.) represent mean values, which are the outcome of three replications (n = 3). This indicates that identical superscripted letters (a-d) in the column differ considerably ($P < 0.05$) from one another.

must be eliminated. Statistical findings showed that probiotic cultures and storage significantly impacted sourness of yogurt (Table 16). The highest sourness was noted in T₆ (4.58±0.04) and the lowest was in T₁ (3.77±0.08) on day 0. During storage, the treatments showed minor alterations in sourness, which increased slightly from 3.77±0.08 to 4.80±0.03. These results were consistent with those of Soni *et al.* (2020), who revealed substantial variation in the sourness of yogurt. The used probiotic strain greatly impacted the flavor, texture, aroma, sourness, and sweetness of yogurt.

Soni *et al.* (2020) reported no significant change in total soluble solids (TSS), total viable counts, flavor, aroma, appearance, and mouthfeel in all seven formulations during storage ($P < 0.05$). However, in yogurt with *Lactobacillus acidophilus* and *Bifidobacterium bifidum animalis*, water-holding capacity, sourness, and sweetness changed significantly ($P < 0.05$).

Odor intensity

Statistical findings indicated that probiotic cultures and storage nonsignificant impacted the odor intensity of yogurt (Table 17). The highest odor intensity was noted in T₆ (4.68±0.26) and the lowest was in T₁ (3.87±0.17) on day 0. During storage, the treatments showed minor alterations in odor intensity, which nonsignificant changed from 3.87±0.17 to 4.78±0.37.

The findings of Ilić *et al.* (2024), who examined the rheological characteristics and sensory profile of yogurt and made a unique combination of probiotic cultures, were consistent with this observation. According to the study, yogurts with a more noticeable consistency—which is a result of probiotic bacteria's metabolic activity—had an overall milder odor. Accordingly, probiotic cultures may improve some textural qualities, but their effect on the strength of odor may differ according on the strains utilized and their metabolic byproducts.

Table 16. Effect of probiotic cultures and days on sourness.

Treatments	Storage days			
	0	7	14	21
T ₁	3.77±0.08 ^l	3.9±0.06 ^{ij}	3.86±0.37 ^{ij}	4±0.04 ^{h-j}
T ₂	4.05±0.32 ^{g-j}	4.04±0.17 ^{g-j}	4.16±0.11 ^{e-i}	4.24±0.26 ^{c-i}
T ₃	4.04±0.11 ^{g-j}	4.09±0.26 ^{g-j}	4.11±0.24 ^{f-i}	4.15±0.05 ^{f-j}
T ₄	4.07±0.17 ^{g-j}	4.05±0.28 ^{g-j}	4.14±0.06 ^{f-j}	4.2±0.06 ^{d-i}
T ₅	4.37±0.34 ^{b-h}	4.36±0.32 ^{b-h}	4.42±0.09 ^{a-g}	4.49±0.13 ^{a-f}
T ₆	4.58±0.04 ^{a-c}	4.63±0.05 ^{a,b}	4.69±0.2 ^{a,b}	4.8±0.03 ^a
T ₇	4.56±0.09 ^{a-d}	4.65±0.35 ^{a,b}	4.7±0.37 ^{a,b}	4.71±0.36 ^{a,b}
T ₈	4.55±0.17 ^{a-e}	4.64±0.39 ^{a,b}	4.66±0.28 ^{a,b}	4.72±0.04 ^{a,b}

Mean ± standard deviation (S.D.) represents mean values, which are the outcome of three replications (n = 3). This indicates that identical superscripted letters (a-d) in the column differ considerably ($P < 0.05$) from one another.

Table 17. Effect of probiotic cultures and days on odor intensity.

Treatments	Storage days			
	0	7	14	21
T ₁	3.87±0.17 ⁿ	4±0.38 ^{l-n}	3.96±0.12 ^{m,n}	4.1±0.16 ^{k-n}
T ₂	4.15±0.39 ^{h-n}	4.14±0.25 ^{f-n}	4.26±0.35 ^{c-n}	4.34±0.2 ^{a-n}
T ₃	4.14±0.05 ⁱ⁻ⁿ	4.19±0.33 ^{f-n}	4.21±0.11 ^{e-n}	4.25±0.16 ^{d-n}
T ₄	4.17±0.08 ^{g-n}	4.15±0.29 ^{h-n}	4.24±0.13 ^{d-n}	4.3±0.21 ^{b-n}
T ₅	4.47±0.16 ^{a-l}	4.46±0.31 ^{a-m}	4.52±0.07 ^{a-k}	4.59±0.11 ^{a-k}
T ₆	4.68±0.26 ^{a-f}	4.64±0.02 ^{a-j}	4.71±0.15 ^{a-e}	4.78±0.37 ^{a,b}
T ₇	4.66±0.23 ^{a-g}	4.65±0.21 ^{a-h}	4.72±0.23 ^{a-e}	4.81±0.22 ^a
T ₈	4.57±0.38 ^{a-k}	4.65±0.05 ^{a-i}	4.76±0.39 ^{a-c}	4.72±0.22 ^{a-d}

Mean values are the result of three replications (n = 3) and are represented as mean ± standard deviation (SD). Identical superscript letters (a-d) in the column differ significantly from one another (P < 0.05).

Likewise, a study done by Akan (2022) looked at how probiotic viability, antioxidant activity, and free amino acid content were affected by fermentation duration and yogurt bacteria in probiotic goat yogurts. The addition of yogurt culture was shown to boost lactic acid concentrations and show a shorter fermentation period, which impacted the final product's sensory qualities, particularly the strength of its odor. According to the study, the sensory qualities of yogurt are greatly influenced by the probiotic strains used and the conditions in which they fermented.

Taste

Statistical findings indicated that probiotic cultures and storage significantly impacted the taste of yogurt (Table 18). Maximum taste was noticed in T₇ (4.76±0.39) and minimum taste was in T₁ (3.97±0.08) on day 0. During storage, the treatments showed minor alterations in taste, which changed significantly from 3.87±0.17 to 4.81±0.19.

These results were consistent with those of Yilmaz-Ersan and Topcuoglu (2022), who revealed significant variation in the taste of yogurt during storage. Based on panelists' descriptions, substituting almond milk resulted in nutty flavor, sweetness, and a slightly bitter taste.

General acceptability

The data in Table 19 illustrate the effect of various probiotic cultures and storage periods on the general acceptability of yogurt samples. General acceptability scores increased slightly over 21-day storage across all treatments, suggesting that the perceived overall quality of the yogurt was maintained or marginally enhanced with time. Notably, treatments incorporating additional probiotic strains, such as *Bifidobacterium bifidum* and *Lactobacillus acidophilus* (T₂, T₄, T₆, and T₈), demonstrated higher acceptability scores compared to the control (T₁). For instance, on day 21, treatment T₆ reached an acceptability rating of 4.58 whereas the control T₁

Table 18. Effect of probiotic cultures and days on taste.

Treatments	Storage days			
	0	7	14	21
T ₁	3.97±0.08 ^f	4.1±0.21 ^{d-f}	4.06±0.03 ^{e,f}	4.2±0.16 ^{e-f}
T ₂	4.25±0.19 ^{b-f}	4.27±0.09 ^{b-f}	4.36±0.37 ^{a-f}	4.44±0.29 ^{a-f}
T ₃	4.24±0.18 ^{b-f}	4.29±0.32 ^{a-f}	4.31±0.17 ^{a-f}	4.39±0.3 ^{a-f}
T ₄	4.27±0.11 ^{b-f}	4.25±0.23 ^{b-f}	4.34±0.08 ^{a-f}	4.4±0.09 ^{a-f}
T ₅	4.51±0.04 ^{a-f}	4.56±0.38 ^{a-e}	4.62±0.35 ^{a-d}	4.66±0.07 ^{a-c}
T ₆	4.65±0.08 ^{a-c}	4.77±0.23 ^{ab}	4.81±0.19 ^a	4.76±0.25 ^{ab}
T ₇	4.76±0.39 ^{a,b}	4.64±0.36 ^{a-c}	4.82±0.24 ^a	4.71±0.11 ^{a-c}
T ₈	4.67±0.28 ^{a-c}	4.75±0.24 ^{a,b}	4.68±0.32 ^{a-c}	4.72±0.07 ^{a-c}

Mean ± standard deviation (SD) represents mean values, which are the outcome of three replications (n = 3). This indicates that identical superscript letters (a-d) in the column differ considerably (P < 0.05) from one another.

Table 19. Effect of probiotic cultures and days on general acceptability.

Treatments	Storage days			
	0	7	14	21
T ₁	3.61±0.21 ^o	3.73±0.07 ⁿ	3.7±0.18 ⁿ	3.83±0.14 ^m
T ₂	3.88±0.18 ^{l,m}	3.89±0.32 ^{l,m}	3.99±0.06 ^{h,j}	4.07±0.28 ^h
T ₃	3.88±0.29 ^{l,m}	3.93±0.37 ^l	3.95±0.33 ^l	4±0.4 ^{h,j}
T ₄	3.9±0.36 ^{k-m}	3.89±0.19 ^{l,m}	3.98±0.04 ^{i-k}	4.04±0.35 ^{h,i}
T ₅	4.2±0.05 ^g	4.2±0.26 ^g	4.26±0.16 ^{f,g}	4.33±0.18 ^{e,f}
T ₆	4.4±0.13 ^{c-e}	4.45±0.12 ^{b-d}	4.5±0.24 ^{a,b}	4.58±0.33 ^a
T ₇	4.4±0.22 ^{c-e}	4.44±0.24 ^{b-d}	4.52±0.16 ^{a,b}	4.52±0.26 ^{a,b}
T ₈	4.37±0.29 ^{d,e}	4.45±0.26 ^{b-d}	4.48±0.14 ^{b,c}	4.52±0.14 ^{a,b}

Mean ± standard deviation (SD) represents mean values, which are the outcome of three replications (n = 3). This indicates that identical superscript letters (a-d) in the column differ considerably (P < 0.05) from one another.

exhibited a rating of 3.83. This implies that the overall sensory appeal of yogurt for the duration of storage is undoubtedly impacted by the addition of unique probiotic cultures.

Lestari *et al.* (2022) investigated the microbiological and sensory evaluation of probiotic yogurt produced using numerous starter cultures. The authors showed that during a 4-week storage period, probiotic yogurts retained higher sensory rankings, including universal acceptability, than manipulated samples. Thus, the use of particular probiotic lines can enhance yogurt's sensory traits and customer acceptability even as it is stored in a fridge.

Conclusion

The effects of different probiotic cultures on the sensory attributes of proteolytic peptides were evaluated during the production and preservation, proximate composition, quantitative analyses (organic acids, acetic acid, lactic acid, citric acid), proteolysis (WSN and FAA), and bioactivities of yogurt. Results indicated that probiotic cultures and storage significantly impacted the moisture content (decreased), ash content, protein content (decreased), citric acid (increased), lactic acid (increased), WSN (increased), FAA (increased), antihypertensive activities (increased), and antioxidants of yogurt during storage. In summary, the production of probiotic yogurt incorporating different probiotic cultures was accomplished effectively, and sensory assessments revealed that it possessed qualities similar to those of conventional yogurt, including appearance, flavor, texture, and the overall character. The dairy sector may find the current study useful in creating new probiotic products and in justifying the choice

of probiotic strain combinations. Even though the idea of probiotics has existed since the time of the Bible, we are still in the early stages of creating consistently beneficial products for human use. The market for probiotic products is expanding quickly with growing health concerns of consumers.

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Data Availability Statement

All data are available in this manuscript.

Author Contributions

Conceptualization, Data curation, Formal analysis and Methodology (Azhar Iqbal); Project administration (Mian Anjum Murtaza Aysha Sameen); Journal format setting and uploading to journal (Muhammad Yousaf Quddoos); Resources, Funding acquisition (Amanullah Sabir, Tawfiq Alsulami); software, data curation: (Iram Hafiz), Supervision (Mian Anjum Murtaza Aysha Sameen); Writing original draft (Azhar Iqbal); Review & editing (Mian Anjum Murtaza, Muhammad Yousaf Quddoos).

Conflict of Interest

The authors declared that they had no conflict of interest.

Ethical Approval

Because no human or animal study was involved, no ethical approval was required.

Declaration

All authors declare that this manuscript was exclusively contributed to the 'Dairy and Food Sciences Research'.

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