Journal of Halal Science, Industry, and Business, Vol. 2 No. 2 (2024) https://doi.org/10.31098/jhasib.v2i2.2756

Research Paper

Halal Yoghurt Production Aspects Related to Halal and Kosher Food Production

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Received : November 20, 2023	Revised : November 8, 2024	Accepted : November 12, 2024	Online : November 29, 2024

Abstract

Gelatin is widely used across various industries, including food, pharmaceuticals, and cosmetics. However, in the context of Halal and Kosher food production, it is considered one of the most contentious components. The acceptability of gelatin-containing products depends on the animal source, but once gelatin is integrated into food or pharmaceutical products, it becomes impossible to trace its origin. In yoghurt production, milk is incorporated with gelatin to counter the syneresis problem during storage, but the gelatin source is unknown, which led to Halal or Haram ethical issues. In response to this issue, the current study will focus on yoghurt production from transglutaminase enzymes extracted from plant sources as gelatin replacers and examine the rheological properties of yoghurt. The effects of varying transglutaminase concentrations (0.02%, 0.03%, and 0.04%) were tested at different setting temperatures (35°C, 45°C, and 55°C) and treatment times (60, 90, and 120 minutes). The findings demonstrate that the enzymatic treatment of milk helps mitigate the syneresis phenomenon during yoghurt storage at 4°C, improving water-holding capacity during centrifugation. Additionally, the post-acidification procedure and stability of yoghurt samples were both affected by cross-linking of transglutaminase with milk protein, which proved an effective tool for improving the functional properties of yoghurt. However, despite reducing syneresis, higher concentrations of transglutaminase resulted in weaker gelling properties, which negatively impacted the sensory qualities of the yoghurt. Given the increasing consumer concern regarding the authenticity of Halal and Kosher products, it is crucial to identify and quantify the origin of gelatin to ensure its compliance with Halal and Kosher standards.

Keywords *Gelatin; Kosher; Yoghurt; Tranglutaminase enzyme; Halal source*

INTRODUCTION

Pakistan's dairy industry's yoghurt processing is at a promising point. Given that Pakistan is a Muslim nation, the usage of gelatin in the making of yoghurt is controversial. Dead animal bones and occasionally pig skin are used to make gelatin, neither of which is regarded as Halal. In addition, protein gel in conventionally produced set yoghurt is held together by delicate non-covalent bonds (hydrogen, electrostatic, and hydrophobic interactions). Opportunities may arise from the addition of the transglutaminase enzyme (TGase), which eventually improves the gel consistency while decreasing syneresis. Therefore, in order to improve the yoghurt's physico-chemical qualities and nutritional value, the enzyme transglutaminase will be utilized to give it firmness and texture. Yoghurt is a popular fermented dairy product widely consumed worldwide (Shiby & Mishra, 2013).

Lactic acid bacteria, such as *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophillus* work together to ferment milk; the concentration of these bacteria is crucial in determining the fermentation rate and the yoghurt's quality (Settachaimongkon et al., 2014).

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Differences in the fat content, rituals, values, and traditions of the local population influence the choice of dairy products (Inayat, 2012). People in some countries prefer liquid milk products, while others prefer cheese and other fermented milk beverages.

Yoghurt is produced by fermenting milk and adding the microbes Lactobacillus bulgaricus and Streptococcus thermophilus. Yoghurt tastes delicious and has a smooth, custard-like consistency. 12–14% of the total milk solids are found in it. The word "yoghurt" comes from the Turkish word "*jogurt*" and the Middle East and subcontinental region utilize it as a traditional beverage. Yoghurt consumption has surged globally in recent years. Yoghurt is quickly rising to the top of the dairy product market worldwide, and its consumption is growing daily. Numerous medical professionals advised individuals with stomach and intestinal disorders to increase their intake of fermented dairy products. Lactose in yoghurt produces lactic acid, which lowers the milk's pH during fermentation (Intansari et al., 2023). Milk proteins will coagulate when the pH is lowered; casein is a key protein that will form a gel-like yoghurt structure at its isoelectric point (pH 4.6). Whey proteins link to casein micelles (mostly κ-casein) when the milk is heated to 32°C during inoculation, increasing the gel hardness of the yoghurt (Bönisch et al., 2007).

Traditionally, skim milk is added to milk to increase its total solids, but stabilizers are also used for stirred yoghurt. Nowadays, various techniques are employed to enhance the texture of dairy products produced by culture. Using high hydrostatic pressure (e.g. >200 MPa) is one of these. This is done to keep products, especially yoghurt, from becoming acidic during processing. Additionally, it aids in the breakdown of proteins like whey protein. Additional techniques include using certain cultures, cross-linking caused by enzymatic action, milk-based raw products, and innovative stabilizers (Miwa et al., 2014). Products made from goat milk, particularly cheese and yoghurt, have become increasingly popular worldwide. Because some people are allergic to bovine milk, it has a distinct flavour and taste. It's challenging to make a reliable yoghurt from goat milk. The low amount of αs1-casein in goat milk is a result of its natural characteristics. Additionally, seasonal variations might impact milk's characteristics, which negatively impact milk clotting. As1-casein plays a major part in milk coagulation and is crucial for preserving the micelle's overall structure. Goat milk has a low proportion of αs1-casein. Moreover, syneresis and gel formation time will be decreased by cross-linking of casein micelles and related whey protein molecules brought on by whey protein denaturation (Loveday et al., 2013).

Numerous food-grade ingredients are employed to improve the yoghurt's thickness and gelling qualities. One of the most creative ways to stabilize the physico-chemical characteristics of yoghurt is to use the transglutaminase enzyme in buffalo milk yoghurt. The texture of yoghurt largely depends on the balance of milk ingredients, with gel stability improving when protein content is increased (Ozer et al., 2007). Yoghurt stability is also significantly impacted by hydrophobic contact and electrostatic repulsion (Lee & Lucey, 2010). At the commercial level, a variety of conventional techniques are employed to enhance the quality of yoghurt, such as increasing the amount of total solids (TS), heating it to a high temperature, homogenizing it under high pressure, and using stabilizers like pectin and gelatin (Lucey, 2004). Because they hinder the development of big crystals, prevent component separation, and improve viscosity, pectin, tragacanth gum, and gum Arabic agar are employed as thickeners and stabilizers (Awan, 1995).

A major protein component of animal connective tissues, such as tendon, bone, and skin, gelatin is a high molecular weight polypeptide generated from collagen (Ramachandran & Ramakrishnan, 1976). Originating from the Latin word "*Gelatus*" which means frozen or fixed, the word "gelatin" was first used in 1700 (Rose, 1987). Gelatin has long been utilized in the food sector. Animal connective tissues and their byproducts have been used for their gelling properties in the food business for more than two millennia (Poppe, 1997). Fish, insects, pig skins, cow bones, hides, and the bones of killed animals can all be used to make commercial gelatin (Mariod & Adam, 2013).

Gelatin production has always remained a concern of great debate worldwide. The collagen found in animal bones, skins, and connective tissues is partially hydrolyzed to produce gelatin, a water-soluble protein. Approximately 326,000 tons of gelatin are produced worldwide (Mahamud et al., 2023). Pig skin, bovine skins, and bones accounted for 46% of production in 2007 (Sultana et al., 2018), with respective percentages of 29.5%, 23.1%, and 1.5%, and the ratio from other parts was 1.5%. Pig skin is not permitted for gelation in Islam or Judaism. Cattle gelatin is permissible as long as it is produced in accordance with religious guidelines (Riaz & Chaudry, 2003). As a result, the food mill needs to provide gelatin alternatives because the global halal market is expanding so quickly (Karim & Bhat, 2008). Furthermore, since the 1980s, when the condition known as "mad cow disease" first appeared in Europe, this issue has received increased attention (Morrison et al., 1999).

Various gelatin substitutes were tested commercially, such as transglutaminase (Yi et al., 2006), improved wheat or starch fiber gel (Wang, 2000), carrageenan (Cash, 2000), high acyl gellan gum (Morrison et al., 2002), and the mixture of low or high methoxyl pectin gels (May, 1990). Out of all these options, adding the enzyme transglutaminase (TGase) can offer chances to strengthen covalent bonds, which will ultimately improve gel consistency while lowering syneresis (Farnsworth et al., 2006).

Protein-glutamine gamma-glutamyl transferase is another name for transglutaminase. The acyl transfer process between the amine group of lysine residue (acyl receptor) and the carboxamide group of glutamine (acyl donor) can be catalyzed by this enzyme, which is a member of the transferase class. Peptide cross-linking creates a covalent bond. Protein polymers are covalently cross-linked as a result of the enzyme's reaction. In addition to pasteurization, the addition of glutathione permits TGase to cross-link milk products without the need for previous heat treatment of the milk (Bönisch et al., 2007). The current study aimed to produce high-quality yoghurt by evaluating the effects of different transglutaminase enzyme concentrations (0.02%, 0.03%, and 0.04%) at different setting temperatures (35, 45, and 55 degrees Celsius) and treatment times (60, 90, and 120 minutes). Hence, in light of the current issue, this study is designed to address the following objectives:

1. Development of yoghurt by using a halal source
2. Evaluate yoghurt's physicochemical and sensory characteristics prepared from different milk blends

LITERATURE REVIEW

Yogurt's Importance

The production of fermented foods requires careful consideration of the balance and choice of starting culture to achieve the desired flavour and texture. The microbiological value of milk and milk products can be impacted by the primary flora of raw milk (Ritcher & Vadamuthu, 2001). Probiotic cultures are frequently transported via various dairy products, such as yoghurt and fermented milk. The antiviral properties of probiotics and lactic bacteria were demonstrated in a cell culture model. One potential mechanism of antiviral activity involves preventing the virus from adhering and internalizing into cells due to the bacteria's direct capture of the virus. This includes direct antiviral effects like "crosstalk" with the cells to produce metabolites and create antiviral protection (Botić et al., 2007). Because lactic acid bacteria can ferment various foods, they are one of the oldest methods of bio-preservation used by humans. Bacterial antagonism has been known for more than a century due to the use of several lactic acid bacteria strains, but in recent years, this phenomenon has drawn more scientific attention. Because of their increased shelf life, potential effectiveness, and health benefits, these chemicals are referred to as natural substances for food preservation. The connection between nutrition and health is raising consumer awareness.

Gelatin

Gelatin is a unique hydrocolloid with a wide range of applications and numerous purposes. Cattle bones, hide, and pig skin were the main sources of gelatin. The rich vegetarian and growing halal and kosher sectors have made replacing gelatin a major issue for many years. Given its wide range of applications across numerous industries, gelatin is regarded as one of the most distinctive and unusual hydrocolloids utilized in the food industry. In the biomedical area (three-dimensional tissue regeneration and wound dressing), in the pharmaceutical industry (microspheres, hard and soft capsules), as a food ingredient (e.g., foaming and gelling agent), and in numerous other fields outside of food (photography), gelatin is utilized. Collagen was hydrolyzed to form gelatin. Collagen is a component of bones, connective tissues, and animal skin (Morrison et al., 1999). For commercial gelatin manufacture, animal skins, bones, and pig skins are the primary feedstock due to their low cost. The primary issue of replacing gelatin has existed for a long time because of the halal market, vegetarians, and kosher, but it has gained more attention recently, particularly in Europe in the 1980s when "mad cow disease" first appeared (Morrison et al., 1999). Therefore, utilizing gelatin derived from diseased animal parts raises many concerns. Pig or cowhides are used to produce the most gelatin on a commercial scale.

Use of Transglutaminase Enzyme from Figs in Yoghurt

Transglutaminase enzyme up to 0.5% was more successful in enhancing the functional qualities of goat milk-based yoghurt. Whey separation was greatly decreased, and gel consistency was improved as a result of enzymatic cross-linking. However, Farnsworth et al. (2006) found no appreciable differences between the control and enzyme-treated yoghurt samples. There were no negative consequences on the yogurt's fermentation process when the microbial transglutaminase in its inactivated condition coupled with glutathione created stirred yoghurt by covalent cross bonding. Compared to yoghurt, which was produced only by TGase, there was a significant increase in protein polymerization and perceived viscosity (Bönisch et al., 2007). Higher concentrations of the TGase enzyme decreased syneresis and increased the viscosity of the yoghurt. However, some small issues with LAB development led to less acetaldehyde and acid generation than control. The optimal concentration, however, was found to be 0.3 g L-1, which was identified as a good substitute for stabilizers for making non-fat yoghurt (Ozer et al., 2007). When yoghurt's composition was changed by adding liquid milk whey, transglutaminase seemed to be a more effective source for the development of physical qualities. It was also shown that the rate of syneresis was directly impacted by the cross-linking that TGase causes (Gauche et al., 2009).

Such enzyme effects on yoghurt's physiochemical and sensory properties were examined at various stages of manufacture and incubation. The results indicated that the addition of enzymes did not significantly alter the yoghurt's chemical makeup. However, adding an enzyme after pasteurization improved gel stability while decreasing syneresis (Şanlı et al., 2011). Transglutaminase (TGase) forms cross-links in proteins (Motoki & Seguro, 1998). Milk's protein is cross-linked by TGase, which results in improved functional qualities like hydration ability, rheological qualities, and emulsifying qualities. (O'Sullivan et al., 2001; Motoki & Seguro, 1998; Lorenzen, 2000).

For this reason, there is a lot of interest in identifying substitute sources for gelatin manufacture. Hence, our research institutions are striving to develop alternative sources with careful consideration of this context. Specifically, the objective is to advance the production of yogurt using halal-compliant sources and to evaluate the physicochemical and sensory properties of yogurt prepared from various milk blends, as outlined in the preceding introduction.

RESEARCH METHOD

Procurement of Milk

Buffalo and cow milk were purchased and stored at 4°C until used. Whey protein and whole milk powder were used to standardize raw milk for the preparation of the yoghurt.

Starter Cultures

Three commercial starter cultures, imported from Clerici Sacco, Italy, were utilized in yogurt production:

1. *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* (Y350B)
2. *Lactobacillus acidophilus* (LA 3)
3. *Bifidobacterium bifidum* (SP 9)

Table 1. Details of treatments and different milk blends for yoghurt preparation

Treatment	Cow Milk	Buffalo Milk	Transglutaminase enzyme
To	100%	0%	0%
T1	75%	25%	1%
T2	50%	50%	2%
T3	25%	75%	3%
T4	0%	100%	4%

Different milk blends were used to stabilize the yoghurt production system. In Pakistan, we usually use a mixture of milk, so it was better to use the blend instead of using only one milk type (See Table 1). The yoghurt production was followed in a sanitized system with strict hygiene practices to overcome microbiological problems. The standardization of milk was followed by the homogenization of milk by formula calculation using whey protein and milk powder in proper portions till they have standardized 3.0 % protein and 3.5% fat with total solids of 12 % as per the standardized milk.

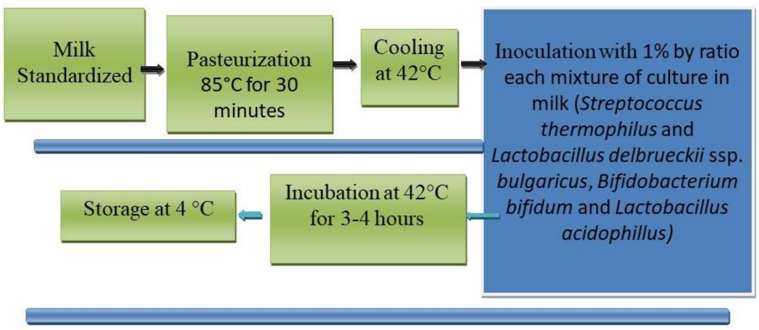

Figure 1. Development of yoghurt made with different milk blends and transglutaminase extracted from fig extract

Buffalo milk, both raw and fresh, was used to make yoghurt (Figure 1). The harmful bacteria were killed by pasteurization (at 72°C for 15 seconds) and cooling to 42°C. This milk was combined with transglutaminase enzyme that had been treated with glutathione at 42°C. In contrast, 1.5g/300 ml of gelatin was added to the control sample. Standard cultures of yoghurt, *Lactobacillus delbrueckii, subsp Lactobacillus bulgaricus,* and *Streptococcus thermophilus* were then added to milk at a rate of 2%. The culture was then incubated for three to four hours at 42°C. Following that, yoghurt was kept in a refrigerator for 28 days at 4°C for storage research (Robinson et al., 2006).

FINDINGS AND DISCUSSION

Physico-Chemical Examination

Transglutaminase enzyme treated with glutathione was used to make yoghurt. The resulting treatments were then examined for fat percentage, pH, SNF percentage, acidity percentage, protein percentage, and syneresis percentage. Manufacturing yoghurt with varying percentages of glutathione-treated transglutaminase enzyme resulted in differences in pH, acidity percentage, SNF%, fat percentage, protein percentage, and syneresis percentage values. pH, a crucial factor in the production of yoghurt and dairy products since it dictates both bacterial and enzymatic activity, dropped between 4.67 and 4.62 in various treatments (Laye et al., 1993).

The percentage of acidity rose from 0.81 to 0.91 during the storage period. These acidity levels resulted from the conversion of lactose to lactic acid by bacteria and enzymes. There was a difference in fat and protein contents accordingly, which led to quality aspects during storage. O'Neil et al. (1979), concluded that acidity rose with an increase in storage time. The percentage of fat stayed rather constant during the storage period. However, there was a slight drop from 6.59% to 6.56%, which had no bearing on the storage conditions (Aprodu et al., 2011).

Transglutaminase enzyme addition in buffalo milk yoghurt revealed non-significant differences across all treatments from the control sample, which was made by adding 1.5g of gelatin per 300 ml (Table 2). All of the fatty acids showed observable alterations in comparison to the controlled samples. Throughout the 21-day storage period, all treatments saw a drop in saturated, monounsaturated, and polyunsaturated lipids.

Table 2. Fat and Protein Contents of The Yoghurt Made with Transglutaminase

Treatments	Samples	Day 0	Day 7	Day 14	Day 21	Mean
Fat %	To	8.2±0.07	8.1±0.03	8.1±0.03	8.1±0.01[a]	8.18±0.01[ab]
	T1	8.6±0.03	8.5±0.06	8.5±0.09	8.5±0.11[ab]	8.58±0.03[ab]
	T2	8.8±0.06	8.8±0.10	8.8±0.67	8.8±0.11[ab]	8.82±0.67[ab]
	T3	8.9±0.06[a]	8.90±0.06[a]	8.9±0.12[b]	8.5±0.06[ab]	8.81±0.07[a]
	T4	9.3±0.03[a]	9.3±0.11[a]	9.3±0.03[b]	8.9±0.03[a]	9.22±0.09[a]
Protein %	To	10.54±0.06[a]	10.55±0.06[a]	10.57±0.12[b]	10.58±0.06[ab]	10.56±0.07[a]
	T1	10.91±0.03[a]	10.93±0.11[a]	10.95±0.03[b]	10.96±0.03[a]	10.93±0.09[a]
	T2	11.34±0.06[a]	11.38±0.11[a]	11.38±0.13[ab]	11.39±0.06[b]	11.37±0.09[a]
	T3	11.65±0.03	11.67±0.06	11.69±0.09	11.71±0.11[ab]	11.68±0.03[ab]
	T4	11.91±0.06	11.94±0.10	11.96±0.67	11.99±0.11[ab]	11.95±0.67[ab]

Glutathione-treated transglutaminase enzyme was used to make the yoghurt, and the results were examined for fat percentage, pH, SNF percentage, acidity percentage, protein percentage, and syneresis percentage. The production of yoghurt using varying concentrations of transglutaminase enzyme treated with glutathione led to measurable differences in pH, acidity levels, SNF percentage, fat content, protein concentration, and syneresis percentages. pH, a crucial factor in producing yoghurt and dairy products since it also determines bacterial and enzymatic activity, dropped between 4.57 and 4.54 in various treatments (Table 3). During the storage period, the percentage of acidity rose from 0.77 to 0.82. The observed acidity levels were attributed to the conversion of lactose into lactic acid, facilitated by bacterial and enzymatic activity. O'Neil et al. (1979) concluded that acidity levels increased progressively with extended storage duration. Transglutaminase enzyme addition in cow milk yoghurt revealed non-significant differences across all treatments from the control sample, which was made by adding 1.5g of gelatin per 250 ml. All of the fatty acids showed discernible alterations when compared to the control samples. Throughout the 21-day storage period, all treatments saw a drop in saturated, monounsaturated, and polyunsaturated lipids (Laye et al., 1993).

When microbial transglutaminase is added to milk (from buffalo, cows, and goats) used to make yoghurt, the product's quality (chemical, rheological, and organoleptic qualities) is significantly improved. The quality of the yoghurt produced from goat milk treated with MTGase was discovered to be enhanced by this enzyme. The enzyme that cross-links milk protein is responsible for the improvement that has been seen. In order to produce yoghurt with a suitable texture, good shelf life, and fewer post-acidification changes during storage, it is advised to create yoghurt from buffalo, cow, and goat milk heated to 72˚C for 15 seconds and treated with 0.4, 0.5, and 0.75 g MTGase L-1, respectively (Eom et al., 2017).

The main proteins found in milk, such as casein and whey, are made up of a sequence of amino acids, some of which are required and some of which are not, in varying amounts. According to the current study, glutamic acid is one of the main amino acids found in buffalo milk yoghurt, and the transglutaminase enzyme had no effect on its concentration in the yoghurt when it was added at varying amounts. However, after 28 days of storage, its concentration somewhat dropped. The buffalo milk yoghurt's minor amino acid in each treatment (T0, T1, T2, T3, and T4) was shown to be methionine (See Table 4).

Table 3. pH and Syneresis of The Yoghurt Made with Transglutaminase

Treatments	Samples	Day 0	Day 7	Day 14	Day 21	Mean
pH	To	4.5±0.07^{d}	4.48±0.06^{db}	4.45±0.23^{a}	4.44±0.09^{b}	4.46±0.07^{d}
	T1	4.48±0.03^{a}	4.47±0.18^{ab}	4.45±0.03^{a}	4.44±0.03^{b}	4.46±0.03^{a}
	T2	4.44±0.24^{a}	4.43±0.11^{c}	4.41±0.27^{a}	4.4±0.03^{b}	4.42±0.03^{a}
	T3	4.43±0.08^{c}	4.41±0.09^{a}	4.4±0.03^{ab}	4.39±0.04^{ab}	4.40±0.06^{b}
	T4	4.44±0.02^{b}	4.44±0.13^{a}	4.39±0.15^{b}	4.38±0.01^{a}	4.41±0.03^{b}
syneresis%	To	1.08±0.01^{bb}	1.07±0.03^{a}	1.13±0.09^{ab}	1.14±0.03^{ab}	1.10±0.04^{b}
	T1	1.12±0.08^{c}	1.13±0.09^{a}	1.14±0.03^{ab}	1.15±0.04^{ab}	1.13±0.06^{b}
	T2	1.18±0.02^{b}	1.19±0.13^{a}	1.21±0.15^{b}	1.22±0.01^{a}	1.2±0.03^{b}
	T3	1.21±0.03^{a}	1.22±0.18^{ab}	1.26±0.03^{a}	1.27±0.03^{b}	1.24±0.03^{a}
	T4	1.23±0.24^{a}	1.23±0.11^{c}	1.26±0.27^{a}	1.28±0.03^{b}	1.25±0.03^{a}

Table 4. Total solids and sensory aspects of the yoghurt made with transglutaminase

Treatments	Samples	Day 0	Day 7	Day 14	Day 21	Mean
Total Solids %	To	22.6±0.01[a]	22.54±0.007[b]	22.53±0.007ab	22.52±0.007[ab]	22.54±0.009[ab]
	T1	23.56±0.009[a]	23.52±0.003[a]	23.53±0.009[a]	23.48±0.01[ab]	23.52±0.009[ab]
	T2	24.46±0.01[a]	24.44±0.003[b]	24.48±0.01[ab]	24.35±0.009[a]	24.68±0.006[a]
	T3	24.94±0.24[a]	24.94±0.11[c]	24.90±0.27[a]	24.85±0.03[b]	24.90±0.03[a]
	T4	25.98±0.08[c]	25.71±0.09[a]	25.67±0.03[ab]	25.64±0.04[ab]	25.75±0.06[b]
Sensory Evaluation (Overall Acceptability)	To	7.86±0.06[a]	7.43±0.06[a]	6.96±0.12[b]	6.93±0.06[ab]	6.93±0.07[a]
	T1	7.56±0.03[a]	6.93±0.11[a]	6.63±0.03[b]	6.03±0.03[a]	6.03±0.09[a]
	T2	7.3±0.06[a]	6.50±0.06[a]	6.80±0.12[b]	6.16±0.06[ab]	6.16±0.07[a]
	T3	7.36±0.03[a]	6.63±0.11[a]	6.60±0.03[b]	6.36±0.03[a]	6.36±0.09[a]
	T4	7.56±0.06[a]	7.33±0.11[a]	7.13±0.13[ab]	6.93±0.06[b]	6.93±0.09[a]

Incubating skim milk at 80°C or above for 1 minute significantly increases the cross-linking of caseins in the milk. This outcome has been attributed to the cross-linking of casein to denatured whey proteins. In fact, the electrophoresis demonstrates that casein and β -lactoglobulin are cross-linked. However, the degradation of the transglutaminase inhibitor after heating may be the source of this. Caseins cross-linked successfully in our tests where we did not heat the milk and employed a transglutaminase concentration greater than the inhibitor concentration. Whey proteins were not cross-linked in this experiment. This implies that the only factor causing the cross-linking of whey proteins is heating the milk, not the inhibitor's breakdown (O'Sullivan et al., 2001). According to the current study, glutamic acid is one of the main amino acids found in buffalo milk yoghurt, and the transglutaminase enzyme had no effect on its concentration in the yoghurt when it was added at varying amounts (Sulong et al., 2024).

However, after 21 days of storage, its concentration somewhat dropped. Because of inhibition, methionine was shown to be the minor amino acid in buffalo milk yoghurt across all treatments. The inhibition of transglutaminase was discovered during milk cross-linking tests. The findings indicate a molecular weight of 3.5 kDa and demonstrate that the inhibitory action may be extracted from the milk proteins using simple precipitation, ultrafiltration, or dialysis. The inhibitor determines this. Without affecting the functionality of cross-linked proteins or food products, these inhibitors can be employed to control the degree of cross-linking (Eom et al., 2017). Diseases induced by in vivo transglutaminase activity (T0, T1, T2, T3, and T4) may also benefit greatly from the use of transglutaminase inhibitors. Transglutaminase's impact on the microstructure is evident since the enzyme can create covalent bonds between protein molecules. Compared to samples without MTGase, the gel formed considerably faster after MTGase was added. As stated differently, the cross-linking of peptide-bound glutamine and lysine residues in treated samples resulted in the formation of a pronounced high molecular weight polymer.

Whey and casein proteins, which are the main proteins found in milk, are made up of a sequence of amino acids, both essential and non-essential, in varying amounts. Glutamic acid is one of the main amino acids found in buffalo milk yoghurt, and the current investigation showed that the transglutaminase enzyme had no effect on its concentration in yoghurt after it was added in varying doses. However, after 21 days of storage, its concentration somewhat dropped. In all treatments (T0, T1, T2, T3, and T4), arginine was shown to be the minor amino acid in the cow milk yoghurt.

Comparing all treatments to control samples throughout the course of the 21-day storage period, non-significant differences were also seen in all other amino acids. Similar to the cow milk yoghurt made using the Transglutaminase enzyme, glycine and cysteine displayed non-significant variations with values below 0.0 mg/g (Stancheva et al., 2011). Transglutaminase enzyme addition in cow milk yoghurt revealed non-significant differences across all treatments from the control sample, which was made by adding 1.5g of gelatin per 250 ml. All of the fatty acids showed discernible alterations compared to the control samples. Throughout the 21-day storage period, all treatments saw a drop in saturated, monounsaturated, and polyunsaturated lipids. Whey and casein proteins, which are the main proteins found in milk, are made up of a sequence of amino acids, both essential and non-essential, in varying amounts (Intansari et al., 2023).

When the primary protein of whey, β-lactoglobulin, is thermally denatured, its secondary and tertiary structures change, exposing its hydrophobic residues to the solvent and causing aggregation and intermolecular interaction. Covalent and non-covalent bonds are often in charge of the gel's three-dimensional structure. The rate-limiting stage in the aggregation process, which primarily involves the interaction with β-lactoglobulina, is thought to be the unfolding of the protein, which exposes and activates free slphydryl groups (Kok & Hutkins, 2018). Glutamic acid is one of the main amino acids found in buffalo milk yoghurt, and the current investigation showed that the transglutaminase enzyme had no effect on its concentration in yoghurt after it was added in varying doses.

Sensory Evaluation

For 21 days, yoghurt samples were assessed. Panellists completed an evaluation using a 9-point hedonic scale sensory assessment grade card to record their scores (Meilgaard et al., 2007). Colour, flavour, look, and general acceptability were all shown to be non-significantly different across all treatments (T0, T1, T2, T3, and T4). However, T2 outperformed all other treatments in terms of colour and appearance. Similarly, as the enzyme concentration rises, the flavour becomes somewhat more bitter (Kumar & Mishra, 2004). Taste dropped in T1, T2, T3, and T4 from 9.01% to 7.01%. However, after 28 days of storage, its concentration somewhat dropped. In all treatments (T0, T1, T2, T3, and T4), arginine was shown to be the minor amino acid in the cow milk yoghurt (Table 4). The yield stress (s0) and consistency index (K) of the solutions subjected to the transglutaminase treatment at temperatures more than 85 C were significantly higher ($p < 0.05$) than those of the control. The highest rise in these values occurred at 95 C, indicating that heat denaturation increased the exposure of lysine and glutamine residues to the enzymatic activity. Thus, sensory evaluation was used to define the difference in the sensory aspect in the quality comparison of yoghurt, but it needs modification according to consumer perceptions (Alagbe, 2023).

Given that β -lactoglobulin and α-lactalbumin undergo reversible structural changes based on the temperature and duration of heat exposure, the reduced reaction rates at temperatures below 95 C are expected (Jung et al. 2016). Comparing all treatments to control samples throughout the course of the 28-day storage period, non-significant differences were also seen in all other amino acids. Similar to the cow milk yoghurt made using the Transglutaminase enzyme, glycine and cysteine displayed non-significant variations with values below 0.0 mg/g (Akdeniz & Akalin, 2019).

CONCLUSIONS

Fat, protein, acidity, and ash content were higher in yoghurt prepared by buffalo milk and lower in cow milk yoghurt. Due to halal sources, consumers' growth toward yoghurt consumption was higher. The yoghurt prepared from the blend of buffalo and cow milk had better texture and taste and was more acceptable to consumers. TGase inhibits the syneresis and prevents the putrefaction process, thereby prolonging life.

Yoghurt can be used as a vehicle for halal market products. The enzyme transglutaminase has no negative effects on any kind of milk, and it can be used to substitute gelatin because its use in Islamic nations is contradicted by its production source. Transglutaminase enzyme will, therefore, be a cheap and accessible source for curdling, strengthening gel matrix, or as a nutraceutical ingredient in foods for human health. Yoghurt with TGASE has shown an antagonist effect against a large number of pathogenic organisms. The enzymatic treatment of milk proved beneficial to retard the syneresis phenomenon during yoghurt storage at 4OC. It improved water holding capacity during centrifugation. The post-acidification procedure and stability of yoghurt samples were both effected by cross-linking of transglutaminase with milk protein. Thus, this treatment proved an effective tool for improving the functional properties of yoghurt.

LIMITATION & FURTHER RESEARCH

The post-acidification procedure and stability of yoghurt samples were both effected by cross-linking of transglutaminase with milk protein. Halal and kosher food production is a big challenge in dairy industries, so alternative plant-based gelling agents must be identified as transglutaminase enzyme is costly, so the trend must be shifted toward easily available, economical and efficient gelling agents. Thus, this treatment proved an effective tool for improving the functional properties of yoghurt. Consumer concerns about the authenticity of Halal and Kosher food and non-food products have grown. Therefore, the gelatin species' origin must be detected and quantified to ensure its integrity regarding Halal and Kosher issues.

Future studies may explore the incorporation of probiotic cultures and whey protein concentrates into yogurt, as these additions have the potential to improve both its overall acceptability and therapeutic values. Therefore, the viability of probiotic bacterial culture in yoghurt must be determined. In order to claim a product as a probiotic, it should contain more than 106cfu viable probiotic bacteria per gram. The viability of the probiotic organisms is the primary factor in the use of probiotic yoghurt.

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