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Original research

Effect of transglutaminase and buffalo milk incorporation on textural parameters and starter cultures viability of strained yogurt

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ABSTRACT

The popularity of strained yogurt has risen worldwide in recent years because of factors such as the product's extended shelf life and its easy handling and transport. The texture of strained yogurt is an important characteristic which determines the acceptability of the product. Transglutaminase enzyme (TG) is an applicable approach to improve textural characteristics of food products thru formation of isopeptide bonds between proteins. Furthermore, by incorporating buffalo milk in the product formulation, a strained yogurt with a denser and creamier texture could be produced. The aim of the present study was therefore to scrutinize the effect of TG enzyme treatment on textural characteristics of strained yogurt prepared from a mixture of cow and buffalo milk. Strained yogurt samples were produced with different amounts of buffalo milk replaced with cow milk (0, 25, 50, 75 and 100% v/v) and using TG enzyme (0 and 0.015% w/v) and evaluated during 21 days of storage at the refrigerator temperature (4°C). Generally, the amount of texture parameters increased with increasing the concentration of TG enzyme and buffalo milk replacement. With the passage of storage time, hardness and adhesiveness increased and cohesiveness decreased significantly ($p < 0.001$). Among the variables, only storage time had a significant effect on survival rate of lactic acid bacteria; with passage of storage time, their survival rate decreased significantly ($p < 0.001$). In conclusion, it was found that by using TG enzymatic treatment (0.015%) and 100% buffalo milk, it is possible to produce a strained yogurt with the best textural and sensorial characteristics.

Keywords: Condensed yogurt; TG; Texture; Acceptability; Storage period

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1. Introduction

Yogurt is one of the oldest famous foods in the world, which has nutritional value and special therapeutic benefits in the human diet (Mehmood et al., 2008). It is produced via milk acidification and gel formation by yogurt starter bacteria including *Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (Vénica et al., 2018). Yogurt can be made from the milk of various animals such as cow, goat, buffalo, sheep and camel. The milk of different animals is different in terms of composition, which affects the textural and rheological characteristics of the final product (Petridis et al., 2014). Buffalo milk can be used alone or in combination with other milks to produce yogurt. Since buffalo milk has higher amounts of fat, protein, total solids, fat-free solids, calcium and phosphorus than other animal milks, the yogurt produced from this milk shows unique textural and sensory characteristics along with a higher production yield and dry matter content (Yilmaz-Ersan et al., 2017).

Condensed yogurt or strained yogurt is a fermented milk product, the popularity and consumption of which have increased in recent years. It is now widely produced on the industrial scale and by removing a part of the serum from yogurt to obtain a product with the amount of 8-11% fat and 23-25% total solids. Similar products produced in other countries are yogurt cheese or Labne (Middle East), labna, labni, lebni, labani or labneh (Arabian countries), Torba or Sezme (Turkey), Seba (Bulgaria), Skyr (Ireland), Yimmer (Denmark), Chaka and Shreikhand (India) (Dinkci, 2012; Aloğlu & Öner, 2013). Strained yogurt is consumed as a main food at breakfast in many Middle Eastern countries such as Iraq, Iran and Lebanon (Özer & Robinson, 1999). The texture of

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strained yogurt is an important characteristic, determining the acceptability of the product. Ideally, it should have a smooth, pasty and semi-solid texture (Aloğlu & Öner, 2013). Fermentation of buffalo and sheep milk creates a smooth texture with increased consistency, while the milk of other animals such as cows tends to create soft gel consistency (Petridis et al., 2014).

Proteins are of great importance for forming the network structure of fermented milk products, particularly yogurt. The structure of proteins can be modified by chemical, physical and enzymatic methods. Among them, enzyme reactions are used as practical methods in various branches of the food industry; compared to chemical methods; they minimize the risk of forming toxic compounds (Şanlı et al., 2011). The microbial transglutaminase enzyme (TG, E.C.2.3.2.13) is one of the transferase enzymes that catalyzes the acyl transfer reaction between gamma carboxyamide groups of peptides around glutamine residues and first-type amines including epsilon-amine of lysine residues in a specific protein (Motoki & Seguro, 1998). These reactions lead to the formation of new intra- and inter-molecular covalent bonds and can improve the function and structure of proteins (Guyot & Kulozik, 2011). The TG enzyme exists widely in nature and is found in the tissue of mammals, fish and plants. Owing to the cost-effective production of TG enzyme by microorganisms, especially by *Streptoverticillum* strain, it is possible to use this enzyme in industrial food production. The advantages of using microbial transglutaminase enzyme are lower extraction and purification costs and non-calcium-dependent catalytic function (Milanović et al., 2007). This enzyme, with the molecular weight of 37368 daltons and 331 amino acids, has the highest efficiency at the pH between 5 and 9 and temperature between 37 and 50°C (Farrokh & Baghaei, 2017). It is known as a safe additive in the food industry and, nowadays, is the only enzyme that is used on a commercial scale to create covalent bonds in proteins. Today, extensive researches have been carried out on the use of TG enzyme in foods particularly in dairy products, such as yogurt (Jooyandeh & Mortazavi, 2018), ice cream (Danesh et al., 2017), Kefir (Beirami et al., 2021), and cheese (Torabi et al., 2021; Danesh et al., 2018).

There is no research related to investigation on simultaneous effects of TG enzyme and amount of buffalo milk substitution on the quality of strained yogurt. Dinkci (2012) studied the effect of TG on the physicochemical, microbiological and sensory properties of strained yoghurt and found that although textural and microbial parameters of the strained yoghurts were not affected by the enzyme, the water holding capacity was improved and the acidity decreased. Furthermore, TG had no significant effect on the sensory attributes. Romhei et al. (2014) reported that addition of buttermilk powder (2% W/W) and transglutaminase treatment (1 unit.g^{-1} protein), either individually or in combination, improved textural and organoleptic properties of fat-free buffalo yogurt. Yilmaz-Ersan et al. (2017) described the textural and sensorial properties of buffalo milk yogurt and buffalo milk yogurt mixed either with cow's or cow + ewe's milk. Their results showed that the yogurt made with buffalo milk alone offered superior characteristics than mixed milk yogurts.

In our previous work, the impact of TG enzyme and amount of buffalo milk substitutions with cow milk on physicochemical characteristics of strained yogurt were evaluated. Here, the simultaneous effects of TG enzyme treatment and the type of milk (amount of cow or buffalo milk) on the textural characteristics, starter bacteria counts and acceptability of strained yogurt were studied during 21 days of storage under refrigeration conditions.

2. Material and Methods

2.1. Materials

Raw cow and buffalo milks (with 3.25 and 6.6% fat, respectively) were obtained from the animal husbandry research station of Agricultural Sciences and Natural Resources University of Khuzestan. YF-L811 yogurt culture in lyophilized powder form (from CHE Hansen Co., Denmark), culture medium MRS-Agar (from Merck Co., Germany) and Transglutaminase (Activa YG, Ajinomato Co., France) contains *Streptoverticillium morbaeaense* with an activity level of 100 units per gram of protein were used for strained yogurt preparation. The ingredients of the TG enzyme powder used included lactose, yeast extract, maltodextrin, vegetable oil, and TG enzyme.

2.2. Preparation method of yogurt samples

Before yogurt preparation, the fat of cow and buffalo milk was initially adjusted to 2.5%. Thereafter, buffalo milk was mixed with cow milk at different amounts of 0, 25, 50, 75 and 100% substitutions. Afterwards, the milk samples (5 kg each) were subjected to heat treatment at 90°C for 15 min (Yademellat et al., 2017). Then, TG enzyme was added at the amounts of 0 (control sample) and 0.015% (w/v) to the milk at 45°C and the samples were kept for 60 min in an incubator (Binder, made in England) at the aforementioned temperature. Subsequently, heat treatment (80°C for 1 min) was performed in order to inactivate the TG enzyme (Oner et al., 2008). Then, the milk temperature was reduced to the inoculation temperature (45°C) and 3% of yogurt starter was added. In this step, the fermented milk samples were kept in the incubator (44°C) until the pH reached to 4.6. After the fermentation stage, the samples were cooled and placed in the refrigerator at 4°C for 2 h. Then, the yogurt samples were transferred into cotton bags for concentrating and kept at the same temperature of 4°C for 10-14 h. Finally, the condensed yogurt samples were packed in disposable sterile polystyrene containers (by UV waves) and kept at 4°C (Dinkci, 2012).

2.3. Texture analysis test

The texture test of yogurt samples was performed using a texture measuring device TA.XT.PLUS (Micro stable system, made in England) according to the Jooyandeh (2009) with some changes. For this purpose, an aluminum cylindrical probe with the diameter of 36 mm was used. The speed of the probe before, during and after the test, was set to 1 mm/s and texture parameters including hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness were recorded.

2.4. Microbial test

In order to count the number of lactic acid bacteria in the samples, 1 gr of each yogurt sample was initially mixed well with 9 ml of sterile physiological serum; then, serial dilutions were performed for each of the samples. In the following, the selected dilutions were cultured on MRS Agar culture medium with using pour plate method and the tested plates were incubated at 37°C for 72 h under anaerobic conditions. After this period, the plates with 30 to 300 colonies were counted (Lollo et al., 2013).

2.5. Acceptability of the product

45 untrained panelists (selected from the students of the university) were employed to assess the acceptability of the strained yogurt samples. Sensory properties of yogurt samples were evaluated after being fixed to room temperature at 20°C. All the samples were coded with random 3-digit numbers, and the 9-point hedonic scale (1=the least and 9=the most) was applied. Bottled water was provided to clean the palate between samples in the hedonic test (Petridis et al., 2014).

2.6. Statistical analysis

In the present study, according to different replacement amounts of buffalo milk with cow milk (at 5 levels of 0, 25, 50, 75 and 100%) and TG enzyme concentrations (at 2 levels of 0 and 0.015%), totally, 10 yogurt treatments were prepared and the textural and microbial characteristics of the samples during 21 days of storage (in 7-day courses) were evaluated. Data analysis was performed through completely randomized design in factorial format using SPSS software (version 20). The mean values of treatments were compared using Duncan's test at the 5% level. The graphs were drawn and reported using Excel software.

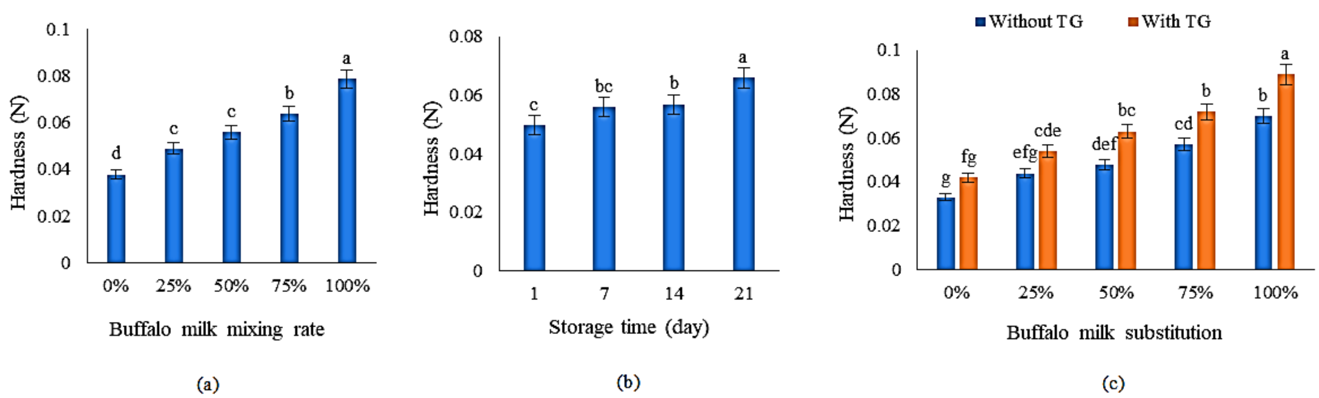

Fig. 1. Effect of buffalo milk substitution (a), storage time (b) and the interaction of buffalo milk substitution and TG enzyme (c) on hardness of strained yogurt samples.

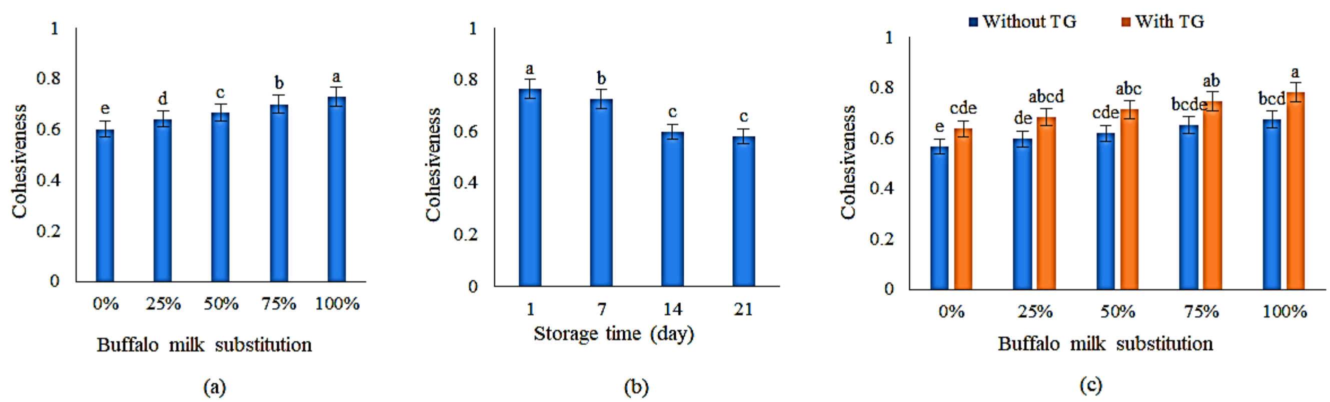

Fig. 2. Effect of buffalo milk substitution (a), storage time (b) and the interaction of buffalo milk substitution and TG enzyme (c) on cohesiveness of strained yogurt samples.

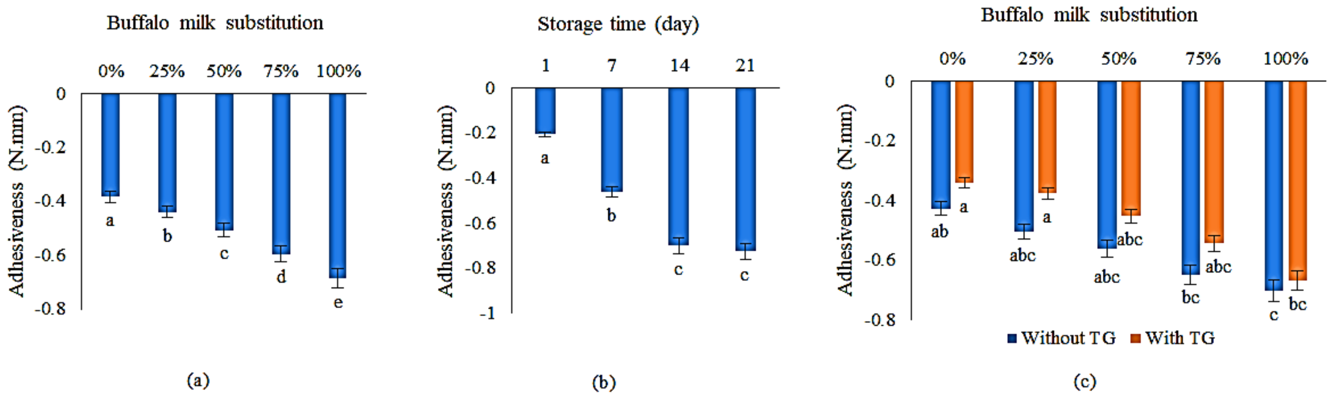

Fig. 3. Effect of buffalo milk substitution (a), storage time (b) and the interaction of buffalo milk substitution and TG enzyme (c) on adhesiveness of strained yogurt samples.

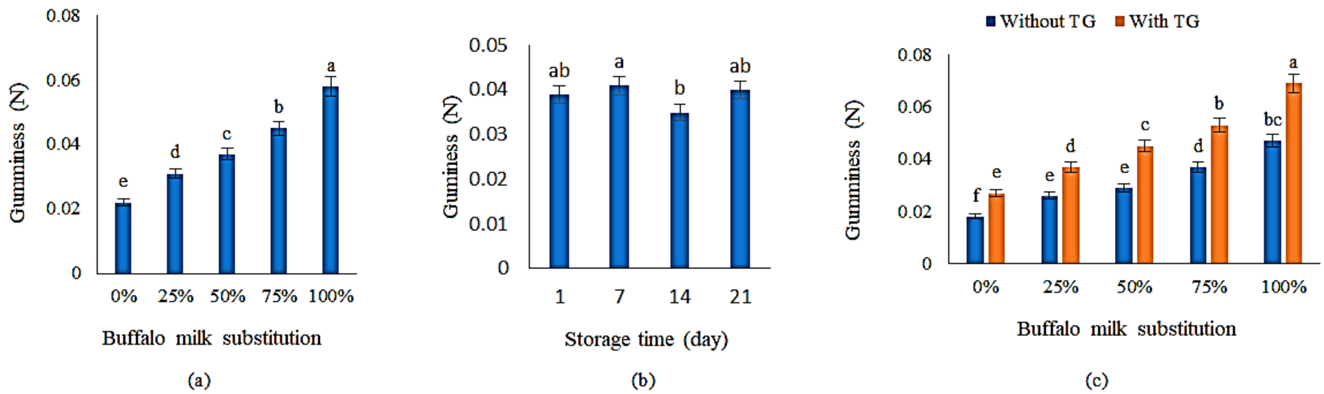

Fig. 4. Effect of buffalo milk substitution (a), storage time (b) and the interaction of buffalo milk substitution and TG enzyme (c) on gumminess of strained yogurt samples.

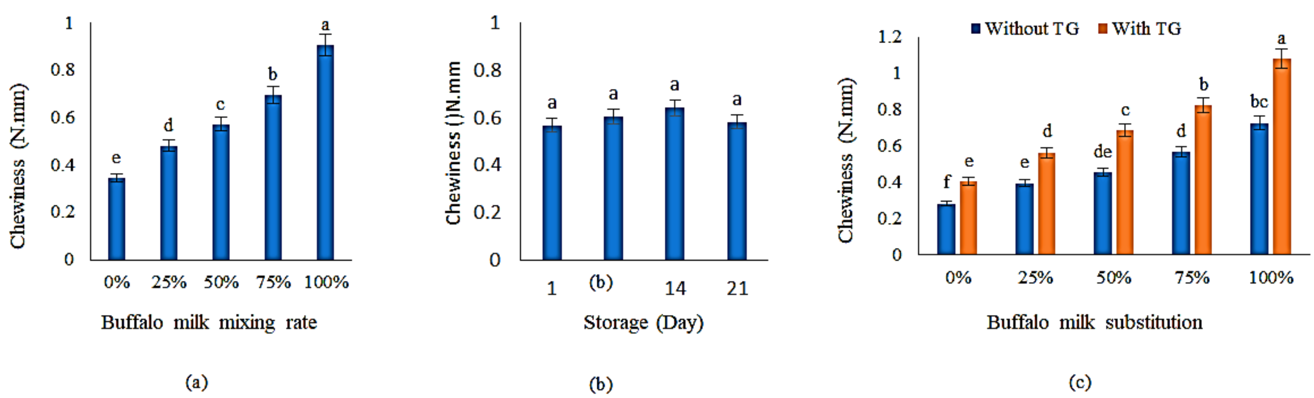

Fig. 5. Effect of buffalo milk substitution (a), storage time (b) and the interaction of buffalo milk substitution and TG enzyme (c) on chewiness of strained yogurt samples.

3. Results and Discussion

3.1. Evaluating textural characteristics

3.1.1. Hardness

Based on the results, the effect of substitution amount of buffalo milk, TG enzyme concentration and storage period, as well as the interaction of buffalo milk substitution levels and enzyme treatment on the hardness of milk were significant ($p < 0.001$). However, other interactions were not significant on this parameter. Increasing the replacement amount of buffalo milk significantly increased the hardness of samples, although no significant difference was observed between the samples containing 25% and 50% buffalo milk (Fig. 1a). Treatment of milk with TG enzyme (0.015% w/v) enhanced the hardness of strained yogurts from 0.050 to 0.064 N. During the storage period, the hardness of the samples increased significantly (Fig. 1b). Among all of the treatments, strained yogurt contained 100% buffalo milk and 0.015% TG enzyme (0.089 N) and control (sample without buffalo milk and TG enzyme, with 0.033 N) had the highest and the lowest hardness values, respectively (Fig. 1c).

According to the results, with increasing the replacement amount of buffalo milk, the amount of hardness increased. The reason can be attributed to the increase in the amount of protein and milk solids. Hardness is defined as the force needed to achieve specific deformation (Akgun et al., 2016). Based on the results, samples containing enzyme had a higher hardness than those without enzyme, since the formation of cross-linkages caused by the TG enzyme decreases the permeability of the gel and, as a result, a more stable and stronger structure is created (Şanlı et al., 2011). Domagata et al. (2013) also found a direct and strong relationship between TG treatment and texture hardness. According to the results, the hardness of the samples increased during the storage period. Jooyandeh et al. (2015) reported that the hardness of all yogurt samples increased with the passage of storage time, which is in line with the results of the present study. Moreover, Karazhyan and Salari (2011) showed an increase in the hardness of yogurt samples during 21 days of storage. They declared that increasing the hardness is directly related to increasing the amount of protein and solids. In the study conducted by Hasnai Zaferani et al. (2017), it was revealed that buffalo yogurt, having more protein, solids, and fat percentage, had a higher hardness as compare with cow yogurt. In the present study, the highest amount of hardness was also assigned for the sample containing 100% buffalo milk. In fact, a higher protein content increases the degree of cross-linkages of the gel network and consequently, the structure of the gel become denser and harder (Yilmaz-Ersan et al., 2017).

3.1.2. Cohesiveness

According to the results, the effects of all three variables, i.e., the replacement amount of buffalo milk, TG enzyme concentration and storage period, as well as the interaction of the replacement amount of buffalo milk and TG enzyme on the cohesiveness of the samples were significant ($p < 0.001$), but other interactions were not significant in terms of this characteristic. As the replacement amount of buffalo milk increased, the cohesiveness increased (Fig. 2a). Like hardness, treatment of milk with TG enzyme (0.015% w/v) enhanced the cohesiveness of strained yogurts and this parameter in samples prepared with or without TG was 0.713 and 0.622, respectively. The cohesiveness of the strained yogurt samples decreased significantly during the storage period. But this reduction was not significant between the 14th and 21st days of storage (Fig. 2b). Among all of treatments, strained yogurt having 100% buffalo milk and 0.015% TG enzyme with 0.783 had the highest cohesiveness and the control sample with 0.568 had the lowest cohesiveness (Fig. 2c).

Cohesiveness index is defined as the degree of deformation of the material before it breaks, directly depending on the strength of the internal bonds of the product (Akgun et al., 2016). Lauber et al. (2000) declared that the reason for increasing the force required to break the yogurt gel is associated with the oligomerization of proteins or the establishment of cross-links between milk proteins by TG enzyme. As it predicted, cohesiveness of the samples treated with enzyme was higher. In similar results, Ilicic et al. (2014) reported that probiotic strained yogurt samples treated with enzymes had higher cohesiveness than the untreated ones. Furthermore, as the storage period increased, the cohesiveness of the samples decreased. The reason can be attributed to increasing the proteolytic activity of lactic acid bacteria during the storage period, which leads to the hydrolysis of proteins and, therefore, the texture compression of the samples decreases (Moayedzadeh et al., 2015). Decreasing the cohesiveness index with increasing the storage time has also been indicated in the study conducted by Torabi et al. (2020) about the synbiotic processed white cheese treated with TG enzymes. In contrast with our results, Dinkci (2012) reported that with the passage of storage time, the cohesiveness of the strained yogurt samples containing the enzyme increased. In addition, they found that TG enzyme had no effect on the cohesiveness of the strained yogurt samples. The reason was attributed to mixing method before the sample packaging. The results of Yilmaz-Ersan et al. (2017) showed that the cohesiveness index of yogurt samples obtained from buffalo milk alone was higher than the mixture of buffalo milk with milk of other animals and they declared this was due to the higher amount of solid matter and protein. In the present study, the highest value of cohesiveness was observed in the sample containing 100% buffalo milk.

3.1.3. Springiness

According to the results, the effects of all three variables, i.e., the replacement amount of buffalo milk, TG enzyme concentration and storage period, as well as all their interactions on the springiness of samples, were not significant ($p > 0.05$).

3.1.4. Adhesiveness

The results demonstrated that the effects of all three variables, i.e., the replacement amount of buffalo milk, TG enzyme concentration and storage period ($p < 0.001$), as well as the interaction of the replacement amount of buffalo milk and enzyme ($p < 0.05$) on the adhesiveness values of the samples, were significant, but other interactions were not significant on this characteristic. By increasing the replacement amount of buffalo milk, the adhesiveness of yogurt samples increased significantly (Fig. 3a). In examining the effect of enzyme concentration, the adhesiveness values were determined as -0.476 N.mm in the sample containing 0.015% enzyme and -0.569 N.mm in the sample without enzyme. During the storage period, the adhesiveness of the samples increased significantly, although no significant difference was observed between the 14th and 21st days of storage (Fig. 3b).

By scrutinizing the interaction of buffalo milk replacement and enzyme concentration, it was found that the sample containing 100% buffalo milk and without enzyme had the highest amount of adhesiveness which was -0.704 N.mm. Besides, the lowest value of adhesiveness was -0.34 N.mm for the sample containing 0.015% enzyme and without buffalo milk (Fig. 3c).

Adhesiveness is the work required to overcome the attraction forces between food surface and other surfaces (Park, 2007). According to the results, adhesiveness was less in the samples treated with TG enzyme. Foods that have a more open protein matrix have more adhesiveness (Jooyandeh et al., 2017). Since the establishment of cross-links by the TG enzyme leads to the compact structure of the protein network, it is probably the reason that the adhesiveness of the samples treated with the TG enzyme was less than that of the untreated samples. In different results, Shi et al. (2017) demonstrated that the adhesiveness of the samples increased with increasing the intensity of TG enzyme treatment (10 units per gr of protein for 5, 7.5 and 10 min). In another study, Domagata et al. (2013) reported that the degree of texture adhesiveness of yogurt samples containing 3 units of enzyme per gr of protein was lower than the samples made with 2 units of TG enzyme. They did not recommend adding more than 3 units of enzyme, since using the higher amounts of enzyme was not effective in improving the hardness and adhesiveness of yogurt. According to the results, just like the hardness characteristic, the adhesiveness of the samples increased with increasing the mixing amount of buffalo milk and during the storage period.

3.1.5. *Gumminess*

Gumminess refers to the force required to crush semi-solid food until it is ready to be swallowed, which is the product of hardness and cohesiveness (Park, 2007). Based on the results, the amount of buffalo milk substitution ($p < 0.01$), TG enzyme concentration ($p < 0.001$) and storage period ($p < 0.05$) as well as the interaction of the amount of buffalo milk substitution and enzyme concentration ($p < 0.001$) on the gumminess of the samples were significant, but regarding to this characteristic, other interactions were not significant. By increasing the replacement amount of buffalo milk, the gumminess of the samples increased significantly (Fig. 4a). In examining the effect of enzyme concentration, the gumminess was determined to be 0.046 N in the sample containing 0.015% of enzyme and 0.031 N in the sample without enzyme. Scrutinizing the gumminess during the storage period indicated that the gumminess of the samples increased until the 7th day of the storage and, then, decreased and increased until the end of the storage period. In terms of the above-mentioned characteristic, there was no significant difference between the samples on the 1st and 21st days of storage (Fig. 4b). Amongst treatments, strained yogurt contained 100% buffalo milk and 0.015% TG enzyme with 0.069 N had the highest gumminess, while the control sample with 0.018 N had the lowest gumminess (Fig. 4c).

Gumminess, like the hardness index of the samples, increased with increasing the replacement amount of buffalo milk and enzyme. This is due to increasing the texture strength of the yogurt samples. Gouch et al. (2009) indicated that gumminess and hardness increased significantly in the samples treated with TG enzyme. They declared that the higher amount of these parameters in samples containing enzyme may be due to ε-(γ-Gla) Lye crosslinks created by TG enzyme. In the present study, the gumminess of yogurt samples increased initially up to 7th day of storage, then decreased up to 14th days and finally increased at the end of storage period. The highest value of this parameter was observed on the 7th day of storage. Making changes in the arrangement and connections of proteins with each other can explain this issue.

3.1.6. *Chewiness*

Chewiness is defined as the work required chewing solid food until it is ready to be swallowed (Park, 2007). Based on the results, the effects of the replacement amount of buffalo milk, TG enzyme concentration ($p < 0.001$) as well as the interaction of the replacement amount of buffalo milk and enzyme ($p < 0.001$) on the chewiness of the samples were significant, but storage period ($p > 0.05$) and other interactions were not significant on this characteristic. Increasing the replacement amount of buffalo milk significantly increased the chewiness index (Fig. 5a). In examining the effect of TG enzyme concentration, the chewiness values were determined as 0.713 N.mm in the sample containing 0.015% enzyme and 0.486 N.mm in the sample without enzyme. Scrutinizing the chewiness of yogurt samples during the storage period demonstrated that the chewiness index, like the hardness insignificantly increased until the 14th day of storage, but then slightly decreased at the end of the storage period (Fig. 5b). Among all of treatments, strained yogurt having 100% buffalo milk and 0.015% TG enzyme with 1.083 N.mm had the highest chewiness and the control sample with 0.283 N.mm had the lowest chewiness (Fig. 5c).

According to the results, the chewiness of the yogurt samples was similar to the hardness of the samples in such a way that the chewiness increased with increasing the replacement amount of buffalo milk and enzyme. In contrary with our results, Dimitreli et al. (2017) reported that increasing the replacement amount of buffalo milk decreased the hardness and chewiness of cheese samples. They stated that the reason could be the high fat content of buffalo milk. While Hasani Zaferani et al. (2017) declared that buffalo yogurt has more strength and texture stability than cow yogurt due to its higher fat content.

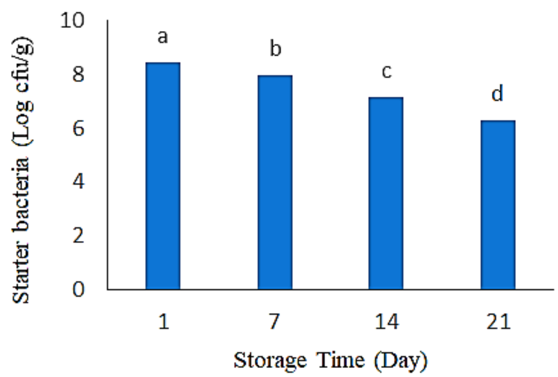

Fig. 6. The effect of storage time on the survival of starter culture bacteria.

3.2. *Examining the growth changes of yogurt starter bacteria*

Based on the results of statistical analysis, the concentration of TG enzyme and the amount of buffalo milk replacement and their interactions had not significant influence on the survival of starter bacteria ($p > 0.05$), while the storage time had significant effect ($p < 0.001$). With the passage of storage time, the survival rate of starter cultures decreased significantly. The highest count of lactic acid bacteria (8.45 log cfu/g) was on the 1st day of storage and the lowest rate (6.30 log cfu/g) was on the 21st day of storage (Fig. 6). The reason can be attributed to the increase in acidity that controls the growth of bacteria or acts as an antibacterial agent (Ismail et al., 2017). In agreement with this study, Akgun et al. (2016) and Ramdhani (2018) reported noticeable decrease in the count of lactic acid bacteria with passing the storage time.

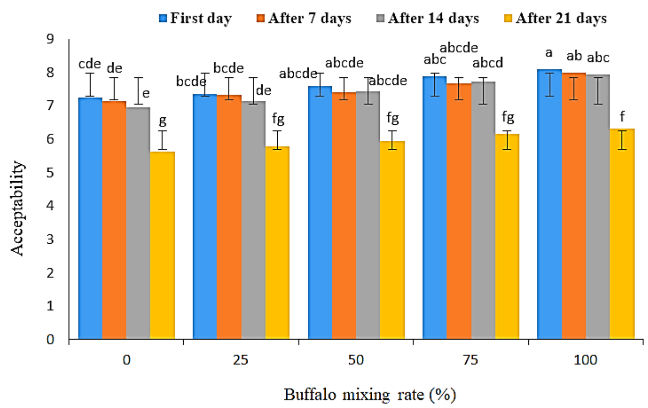

Fig. 7. The effect of storage time and buffalo milk substitution on the acceptability of strained yogurt samples during 21 days of cold storage.

3.3. *Acceptability of the product*

The results of sensory analysis of the strained yogurt samples during 21 days of the cold storage are shown in Fig. 7. Among strained yogurt samples, sample prepared with 100% buffalo milk had the highest acceptability and sample having 0% buffalo milk had the lowest score. However, no significant differences were found between strained yogurts containing 50, 75 and 100% buffalo milk and the mean scores during the storage were 7.11, 7.36 and 7.58, respectively. Several researches revealed that as compare with cow milk, buffalo milk is superior ingredient for making different kind of dairy products since it imparts a rich aroma and taste into the products (Yilmaz-Ersan et al., 2017; Hanif et al., 2012; Jooyandeh et al., 2022).

As it shown in Fig. 7, the impact of storage period on the sensory score was more than the amount of milk substitutions. At the end of storage time, i.e. after 21 days of storage, the acceptability of all the strained yogurt samples having different substitution levels reduced dramatically which was mainly due to yogurt sourness and syneresis. The mean values of scores of strained yogurt samples at the beginning and after 7, 14 and 21 days of storage were 7.64, 7.51, 7.44 and 5.97, respectively. The decrease in acceptability score of strained yogurt during the storage period has been reported by other researches (Dinkci, 2012; Jooyandeh et al., 2022).

Results also revealed that treatment of milk with TG enzyme significantly ($p < 0.05$) improved acceptability scores of the all samples having different buffalo milk levels at various storage period. Based on results, sample prepared with 100% buffalo milk and 0.015% TG concentration had the best acceptability score. As it previously mentioned, cross linkages between proteins by TG enzyme results in higher yogurt gel stability and a lower whey separation. Furthermore, there is a direct relation between total solids and protein of utilized milk with the acceptability score of the prepared yogurt. In agreement with our results, Tofiq Mahmood (2022) reported the higher acceptability in yogurt sample treated with microbial TG. Hanif et al. (2012) also reported that the yogurt prepared with buffalo milk has higher rheological and sensorial properties as compared with cow yogurt.

4. Conclusion

According to the results, the replacement amount of buffalo milk and TG enzyme concentration did not have a significant effect on the survival rate of lactic acid bacteria, but had a positive effect on the textural characteristics of the strained yogurt samples, in such a way that the best results were observed in the samples containing 100% buffalo milk and 0.015% enzyme. In fact, by creating covalent bonds between milk proteins, the TG enzyme increases the water holding capacity and stability in the yogurt gel which is an effective strategy for improving the textural characteristics of the product. Panelists scored satisfactorily acceptable a strained yogurt rich in buffalo milk concentration (75-100%) and treated with TG enzyme, with an adequate smooth and gelatinous texture. However, there were no significant differences between strained yogurts containing more than 50% buffalo milk. Buffalo milk has a high nutritional value and its worldwide production has significantly increased during the recent years. Therefore, its incorporation into different foods particularly fermented dairy products is suggested to produce the products with unique textural and sensorial properties.

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Conflict of interest

The authors declare no conflict of interest.

References

Akgun, A., Yazici, F., & Gulec, H. A. (2016). Effect of reduced fat content on the physicochemical and microbiological properties of buffalo milk yoghurt. *LWT-Food Science & Technology*, *74*, 521-527.

Aloğlu, H. Ş., & Öner, Z. (2013). The effect of treating goat's milk with transglutaminase on chemical, structural, and sensory properties of labneh. *Small Ruminant Research*, *109*(1), 31-37.

Beirami, F., Hojjati, M., & Jooyandeh, H. (2021). The effect of microbial transglutaminase enzyme and Persian gum on the characteristics of traditional kefir drink. *International Dairy Journal*, *112*, 104843.

Danesh, E., Goudarzi, M., & Jooyandeh, H. (2017). Effect of whey protein addition and transglutaminase treatment on the physical and sensory properties of reduced-fat ice cream. *Journal of Dairy Science*, *100*, 5206–5211.

Danesh, E., Goudarzi, M., & Jooyandeh, H. (2018). Transglutaminase-mediated incorporation of whey protein as fat replacer into the formulation of reduced-fat Iranian white cheese: physicochemical, rheological and microstructural characterization. *Journal of Food Measurement & Characterization*. *12*(4), 2416-2425.

Dimitreli, G., Exarhopoulos, S., Antoniou, K. K., Zotos, A., & Bampidis, V. A. (2017). Physicochemical, textural and sensory properties of white soft cheese made from buffalo and cow milk mixtures. *International Journal of Dairy Technology*, *70*(4), 506-513.

Dinkci, N. (2012). The influence of transglutaminase treatment on functional properties of strained yoghurt. *Journal of Animal & Veterinary Advances*, *11*(13), 2238-2246.

Domagała, J., Wszołek, M., Tamime, A. Y., & Kupiec-Teahan, B. (2013). The effect of transglutaminase concentration on the texture, syneresis and microstructure of set-type goat's milk yoghurt during the storage period. *Small Ruminant Research*, *112*(1-3), 154-161.

Farrokh, S. A., & Baghaei, H. (2017). Investigation of adding microbial transglutaminase enzyme on physico-chemical, textural and sensory properties of chicken steak. *Research & Innovation in Food Science & Technology*, *5*(4), 359-368. (In Persian)

Gauche, C., Tomazi, T., Barreto, P. L. M., Ogliari, P. J., & Bordignon-Luiz, M. T. (2009). Physical properties of yoghurt manufactured with milk whey and transglutaminase. *LWT-Food Science & Technology*, *42*(1), 239-243.

Guyot, C., & Kulozik, U. (2011). Effect of transglutaminase-treated milk powders on the properties of skim milk yoghurt. *International Dairy Journal*, *21*(9), 628-635.

Hanif, M. S., Zahoor, T., Iqbal, Z., Ihsan-ul-Haq, A., & Arif, M. (2012). Effect of storage on rheological and sensory characteristics of cow and buffalo milk yogurt. *Pakistan Journal of Food Sciences*, *22*(2), 61-70.

Hasani Zaferani, S., Aminifar, M., & Moslehi shad, M. (2017). The study on relationship between microstructure and technological properties of yoghurt produced from different types of milk during storage. *Iranian Journal of Biosvstems Engineering*, *48*(4), 457-465. (In Persian)

Iličić, M. D., Milanović, S. D., Carić, M. Đ., Dokić, L. P., & Kanurić, K. G. (2014). Effect of transglutaminase on texture and flow properties of stirred probiotic yoghurt during storage. *Journal of Texture Studies*, *45*(1), 13-19.

Ismail, M. M., Ghoneem, G. A., EL-Boraey, N. A. L., Tabekha, M. M., & Elashrey, H. F. (2017). Effect of mixing soy milk with buffalo or cow milk on the chemical composition, microbial properties and sensory evaluation of yoghurt. *Journal of Biotechnology Research*, *3*(7), 56-65.

Jooyandeh, H. (2009). Effect of fermented whey protein concentrate on texture of Iranian white cheese. *Journal of Texture Studies*, *40*(5), 497-510.

Jooyandeh, H., & Mortazavi, A. (2018). Impact of addition of milk powder and microbial transglutaminase (TG) on the sensory and textural properties of set yoghurt. *Food Processing & Preservation Journal*, *10*(2), 121-136. (In Persian)

Jooyandeh, H., Alizadeh Behbahani, B., Noshad, M., & Sffari Samani, E. (2022). Impact of transglutaminase enzyme on some characteristics of strained yoghurt prepared from cow and buffalo milk mixture. *Food Processing & Preservation Journal*, *14*(2), 17-34. (In Persian)

Jooyandeh, H., Danesh, E., & Goudarzi, M. (2017). Effect of microbial transglutaminase on physical, rheological, textural and sensory properties of light ice cream. *Iranian Food Science & Technology Research Journal*, *13*(4), 469-479. (In Persian)

Jooyandeh, H., Mahmoodi, R., Samavati, V., & Hojjati, M. (2015). Effect of cold enzymatic treatment of milk by transglutaminase on textural properties of yogurt. *Journal of Food Science & Technology (Iran)*, *13*(1), 91-99. (In Persian)

Karazhyan, H., & Salari, R. (2011). Comparison of physiochemical, rheological and sensory properties of yoghurt made from fresh Cow's milk and powdered milk. *Journal of Innovation in Food Science and Technology*, *3*(2), 11-19. (In Persian)

Lauber, S., Henle, T., & Klostermeyer, H. (2000). Relationship between the crosslinking of caseins by transglutaminase and the gel strength of yoghurt. *European Food Research & Technology*, *210*(5), 305–309.

Lollo, P. C. B., de Moura, C. S., Morato, P. N., Cruz, A. G., de Freitas Castro, W., Betim, C. B., ... & Amaya-Farfan, J. (2013). Probiotic yogurt offers higher immune-protection than probiotic whey beverage. *Food Research International*, *54*(1), 118-124.

Mehmood, S. T., Masud, T., Mahmood, T., & Maqsud, S. (2008). Effect of different additives from local source on the quality of yoghurt. *Pakistan Journal of Nutrition*, *7*(5), 695-699.

Milanović, S. D., Carić, M. Đ., Đurić, M. S., Iličić, M. D., & Duraković, K. G. (2007). Physico-chemical properties of probiotic yoghurt produced with transglutaminase. *Acta periodica technologica, 38*, 45-52.

Moayedzadeh, S., Khosrowshahi asl, A., & Zomorodi, S. (2015). Effect of transglutaminase on proteolysis and rheological properties of nonfat yoghurt. *Iranian Food Science & Technology Research Journal*, *11*(4), 325-336. (In Persian)

Motoki, M., & Seguro, K. (1998). Transglutaminase and its use for food processing. *Trends in Food Science & Technology*, *9*(5), 204-210.

Oner, Z., Karahan, A. G., Aydemir, S., & Aloglu, H. S. (2008). Effect of transglutaminase on physicochemical properties of set-style yogurt. *International Journal of Food Properties*, *11*(1), 196-205.

Özer, B. H., & Robinson, R. K. (1999). The behaviour of starter cultures in concentrated yoghurt (labneh) produced by different techniques. *LWT – Food Science & Technology*, *32*(7), 391-395.

Park, Y. W. (2007). Rheological characteristics of goat and sheep milk. *Small Ruminant Research*, *68*(1-2), 73-87.

Petridis, D., Dimitreli, G., Vlahvei, K., & Deligeorgakis, C. (2014). Effects of buffalo and cow milk mixtures enriched with sodium caseinates on the physicochemical, rheological and sensory properties of a stirred yogurt product. *Journal of Food Research*, *3*(6), 54-69.

Ramdhani, J. P. (2018). Effect of transglutaminase addition to chemical, physical, and culture survivability of yogurt during storage period. *E3S Web of Conferences*, *67*, 03042.

Romhei, E., Abdel-Hamid, M., & Awad, A. A. (2014). The addition of buttermilk powder and transglutaminase improves textural and organoleptic properties of fat-free buffalo yogurt. *Dairy Science & Technology*, *94*(3), 297-309.

Şanlı, T., Sezgin, E., Deveci, O., Şenel, E., & Benli, M. (2011). Effect of using transglutaminase on physical, chemical and sensory properties of set-type yoghurt. *Food Hydrocolloids*, *25*(6), 1477-1481.

Shi, J., Li, D., & Zhao, X. H. (2017). Quality attributes of the set-style skimmed yoghurt containing enzymatic cross-linked or thermal polymerized whey protein isolate. *CyTA-Journal of Food*, *15*(1), 34-40.

Tofiq Mahmood, M. K. (2022). Physicochemical and sensory properties of buffalo's milk yogurt treated with microbial transglutaminase (mTGase) enzyme. *ProEnvironment*. *15*(50), 170-177.

Torabi, F., Jooyandeh, H., & Noshad, M. (2021). Evaluation of physicochemical, rheological, microstructural, and microbial characteristics of synbiotic ultrafiltrated white cheese treated with transglutaminase. *Journal of Food Processing & Preservation*, *45*, e15572.

Torabi, F., Jooyandeh, H., Noshad, M., & Barzegar, H. (2020). Texture, color and total acceptance of synbiotic ultrafiltrated white cheese treated with microbial transglutaminase enzyme during storage period. *Iranian Journal of Food Science & Technology*, *17*(98), 135-145. (In Persian)

Vénica, C. I., Wolf, I. V., Suárez, V. B., Bergamini, C. V., & Perotti, M. C. (2018). Effect of the carbohydrates composition on physicochemical parameters and metabolic activity of starter culture in yogurts. *LWT - Food Science & Technology*, *94*, 163-171.

Yademellat, M., Jooyandeh, H., & Hojjati, M. (2017). The effect of application of Persian and Balangu-Shirazi gums on textural properties of low-fat stirred yogurt. *Journal of Food Research*, *27*(4), 171-181. (In Persian)

Yilmaz-Ersan, L., Ozcan, T., Akpinar-Bayizit, A., & Delikanli-Kiyak, B. (2017). The characterization of the textural and sensory properties of buffalo milk yogurts. *International Journal of Advances in Science Engineering & Technology*, *5*(3), 37-42.