



## Scientific Research

## Investigating the chemical and microbial characteristics of some local yogurt samples prepared from traditional dairy products stores in Tuyserkan city

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## ARTICLE INFO

## ABSTRACT

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Considering the great desire of consumers to consume traditional dairy products and their reluctance to use factory products, this study was conducted with the aim of investigating different chemical and microbial characteristics of traditional yogurt samples offered in Tuyserkan city and compare it with Pasteurized yogurt. The chemical characteristics including percentage of dry matter, moisture, acidity, pH and fat and microbial characteristics including total bacterial count, coliforms, mold and yeasts, and *Staphylococcus aureus* were investigated and compared with the pasteurized sample as a control sample. The moisture and dry matter content of the samples varied from 87.8-84.33% and from 12.62 to 15.25%, respectively. The fat content of the yogurts produced was between 2.5-2.9 and thus within the range of semi-fat yogurts according to the national standard. No significant difference was observed between different samples in terms of moisture, dry matter and fat ( $P > 0.05$ ). The acidity (percentage by weight in terms of lactic acid) and pH value of various samples ranged from 0.87-1.81 and 3.68-47.4, respectively and were therefore fully in line with the national standard (at least 0.7). Different samples showed a significant difference in terms of pH and acidity ( $P < 0.05$ ). The results of microbial characteristics indicate the presence of mold and yeast in all samples (100%), coliform in 83.33% and *Staphylococcus aureus* in 50% of the samples. Regarding microbial characteristics, a significant difference was observed between traditional and industrial samples. Thus, the industrial sample showed the lowest microbial load compared to the traditional yogurt samples ( $P < 0.05$ ). Therefore, it is strongly recommended to control the incoming raw milk and observe the sanitary conditions during production, packaging and supply in the production of traditional products. The results of sensory evaluation showed better color and taste for the pasteurized sample and better texture and overall acceptance for the traditional samples.

## 1- Introduction

Due to the presence of nutrients including protein, calcium, magnesium, water-soluble vitamins including B<sub>2</sub> and B<sub>12</sub>, and fat-soluble vitamins including A and D, dairy products play a prominent role in meeting the essential needs of people's food basket and consuming 2 up to 3 portions per day from this group are recommended for all ages. Although, the per capita consumption of dairy products in Iran is deficient and according to the statistics, the per capita consumption of dairy products in 2011 in Iran and the United States was 66 and 253 liters, respectively. Due to the low per capita consumption of dairy products, most people prefer local dairy products to industrial ones for various reasons. Many consumers tend to consume local dairy products due to their belief in better taste and organoleptic characteristics, higher nutritional characteristics, more health benefits, lack of information about the preparation method of industrial products and cultural reasons [1]. Although, local dairy products are prone to high microbial contamination due to non-compliance with health requirements and related standards and the supply of products without thermal processes including pasteurization and sterilization. Thus, previous studies have shown that the contamination level of these products is dangerous for consumers. For example, Sami et al [2] analyzed 155 different dairy products including milk, yogurt, buttermilk and cheese in Isfahan. The results showed contamination more than the standard limit to coliform, *Escherichia coli*, and mold and yeasts in 15, 11, and 20 samples, respectively. Among the tested dairy products, the highest contamination with coliform, *E.coli*, and mold and yeasts were observed for buttermilk, and yogurt, respectively. Also, in another study by Rezaei Derzikla et al [3], who examined local cheeses for the presence of *S. aureus* bacteria, it was found that 28.2% of the cheese samples were positive for *S. aureus* coagulase. In another study conducted by Najafi et al [4] on raw milk and unpasteurized butter, 35% and 10% listeria

contamination were observed in those two products, respectively. Also, Khemati Mursa et al [5] examined local cheeses of Qazvin City for the presence of *Listeria monocytogenes*. The results showed that among 128 samples examined, 14 samples were contaminated with the above bacteria. Mohammadi and Khakipour [6] reported the high microbial contamination of local cheeses sold in Karaj City with *S. aureus*. Considering the mentioned cases, it seems that a general review is necessary regarding the preparation and supply of local dairy products.

Yogurt is a semi-solid dairy product that is prepared by fermentation of milk by *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus* bacteria. Yogurt production has started in Middle Eastern countries and spread all over the world. The conversion of lactose into lactic acid during the fermentation process makes it possible for people with lactose intolerance to consume dairy products. Also, consumption of yogurt leads to a decrease in the pH of the stomach, which has a positive effect in reducing the possibility of contracting various diseases. Therefore, consuming yogurt as a rich product have a significant contribution in meeting the essential needs of people [7]. Yogurt is known as a healthy food with high nutritional value due to its protein, calcium, vitamins, and probiotic bacteria content. During the fermentation process, free amino acids, peptides, oligosaccharides and lipoproteins are produced in yogurt, which contribute significantly to improving its nutritional value [8]. Due to the reduced lactose, favorable acidity and organoleptic properties, yoghurt has a high consumption among different dairy products. Thus, the purpose of this study is to investigate the microbial and chemical properties of several yogurt samples in Tuyserkhan City and its comparison with the pasteurized sample. Therefore, the obtained

results can help in awareness of consumers in choosing the type of dairy product to consume.

## 2-Material and methods

Sulfuric acid, isoamyl alcohol, sodium hydroxide, and phenolphthalein were prepared in laboratory grade and brand Eisen-Golden Laboratories (USA). PCA, YGC, VRBA and Baird Parker Agar culture media were obtained from Merck Millipore (Germany).

### Yoghurt samples supply

Local yogurt samples were purchased from six markets in Tuyserkan City in December 2023. Then, chemical and microbial tests were performed on the same day of sample supply. A pasteurized yogurt was also purchased to compare the results. The local yogurts were coded from numbers 1 to 6, and the pasteurized yoghurt was coded as No. 7.

### Moisture content

The moisture content of samples was measured by a digital moisture analyzer (AND-MX 50) at 110°C with three repetitions for each sample.

### Total solid

Yogurt's total solid was measured based on national standard number 11328 [9]. For this purpose, the yogurt sample was homogenized entirely by a glass stirrer, and then 5 grams of the sample was weighed inside the glass plate. The sample was placed in the oven at a temperature of 110 °C until it reached a constant weight. The total solid content was expressed based on % weight according to the Eq (1):

$$\frac{\text{weight of dried sample (gr)} - \text{weight of empty petri dish (gr)}}{\text{weight of sample (gr)} - \text{weight of empty petri dish (gr)}} \times 100 \quad \text{Eq(1)}$$

**Total solid (% weight)=**

### Fat content

The fat content (% weight) was measured based on the Gerber method according to the national

standard method No. 695 [10]. For this purpose, 10 mL of 90% sulfuric acid was poured into a butyrometer (Gerber, 8%), then 11.3 gr of yogurt was added to it, after adding 1 mL of isoamyl alcohol, the lid of the butyrometer was closed and stirred slowly. Then, it was placed in a centrifuge at 1200 rpm for 5 minutes. After removing the butyrometer from the centrifuge, the fat content was read on the graduated part of the butyrometer. Three repetitions were done for each sample.

### pH and acidity

A pH meter (GF-300) was used to measure PH, to this end, the device was calibrated by buffers 4 and 7, then the electrode was placed directly into the yogurt sample and the pH was read. The acidity was measured using the national standard No. 2852 [11] according to the following method. First, 10 grams of the homogenized sample was weighed and mixed well with 100 ml of distilled water. Then, a few drops of phenolphthalein were added to it and the titration was carried out with 0.1 normal NaOH until the appearance of a light pink color. The acidity (% weight of lactic acid) was calculated according to the Eq (2):

$$\text{Acidity (\% weight of lactic acid)} = \frac{100 \times 0.09 \times \text{NaOH normality} \times \text{used NaOH (mL)}}{\text{weight of sample (gr)}} \quad \text{Eq(2)}$$

### Microbial characteristics

Microbial characteristics of yogurt samples were evaluated based on national standard No. 2406 [12] by counting the total number of microorganisms using plate count agar culture medium at 30°C for 72 hrs, *S. aureus* from Brad Parker agar at 37°C for 24-48 hrs, coliform from Violet Red Bile agar culture medium at 30°C for 24-48 hrs, and yeast and molds by glucose chloramphenicol agar yeast culture medium at 25°C for 5 days. In order to investigate the microbiological tests, firstly, 10 grams of the sample was homogenized in sterile conditions, then 90 mL of Ringer's solution was

added and mixed well by vortex. Different serial dilutions ( $10^{-1}$ -  $10^{-4}$ ) were prepared from it and carried out by microbial surface cultivation.

### Sensory evaluation

Sensory evaluation was done by examining the characteristics of the samples, including color, smell, taste, texture, appearance, and total acceptability, by 15 trained panels using a 9-point hedonic pleasure test. Based on this, the number 9 indicates extreme interest, and the number 1 indicates extreme disinterest.

### Statistical analysis

All the tests were done in three repetitions, and the results were reported as mean  $\pm$  standard deviation. Statistical evaluation was done using completely random design. The Duncan's multi-range test was used for comparison of means at 5% probability level. Data analysis

was done using SAS software at different stages of storage.

## 3-Results and discussion

### Fat content

Fat content plays a significant role in the quality of yogurt by improving its texture and mouthfeel. The global average fat range in yogurt is between 0.5% to 3.5% [13]. The fat content of the yogurts produced in the study was between 2.5-2.9 in the range of semi-fat yogurt based on the national standard No.695 [10]. According to the Iranian national standard No. 695 [10], yogurts with a fat content of 0.5% to 1.5% are in the low-fat range, between 1.5% to 3% in the semi-fat range, and between 3% to 6% in the high-fat range. Samples 1, 4, and 6 showed higher fat content than other ones ( $P > 0.05$ ), however the difference was insignificant. The difference between the fat content of different samples is related to the non-standardization of the dry matter in traditional yogurt and the various fat removal from the original raw milk.

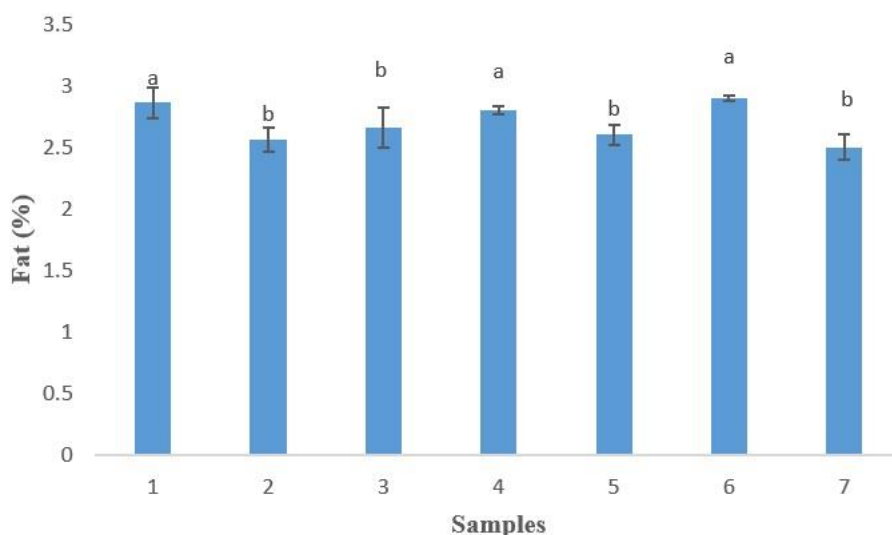


Fig 1 Fat content of different samples. (Different letters indicate significance ( $p > 0.05$ ))

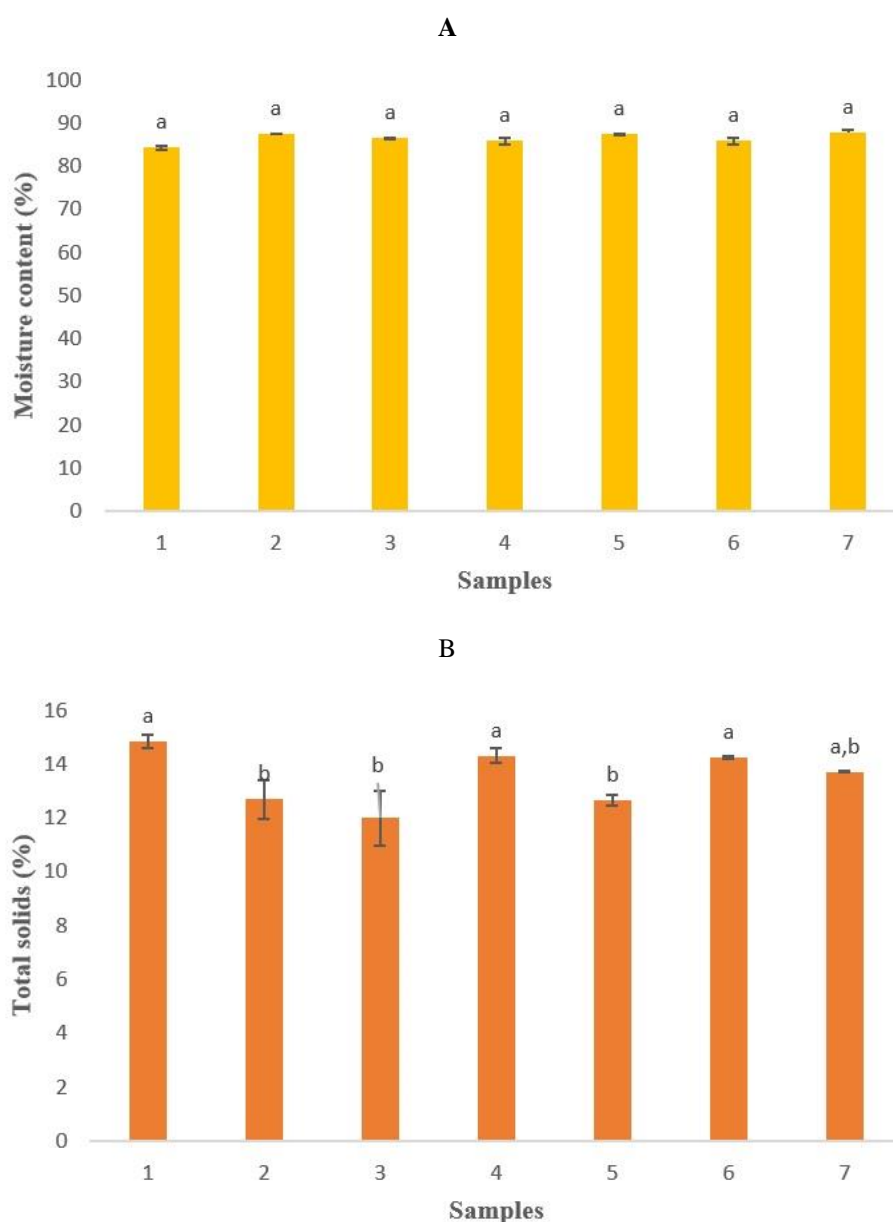
### Moisture content and total dry matter

The moisture content of different samples is shown in Figure 2 which, was between 84.33-87.8%. As can be seen, there is no significant

difference between different samples regarding moisture content ( $P > 0.05$ ). High moisture content means low solid content of yogurt, which is probably related to reducing fat

content in yogurt samples [7]. The amount of total solid of different samples was between 12 to 15.25%, which no significant difference was observed between samples ( $P > 0.05$ ). The total solid of yogurt is mainly related to protein, fat and lactose. Since the percentage of protein and lactose is almost the same in most kinds of milk, the difference in milk fat used and the amount of fat removal has the most significant effect on total solid. Based on this, samples with higher fat content show higher total solid, so samples numbers 1, 4, and 6 with the highest amount of

fat showed the highest total solid among other samples, however, this difference was not significant ( $P > 0.05$ ). The results agree with those of moisture content and fat. In addition, the preparation and formulation method of the product is also effective on dry matter. For example, in the industry, additives such as sodium caseinate, dried milk, whey, etc. are used to increase the amount of total solid. While, in the preparation of traditional yogurts, milk concentration is mainly used to increase the total solid.



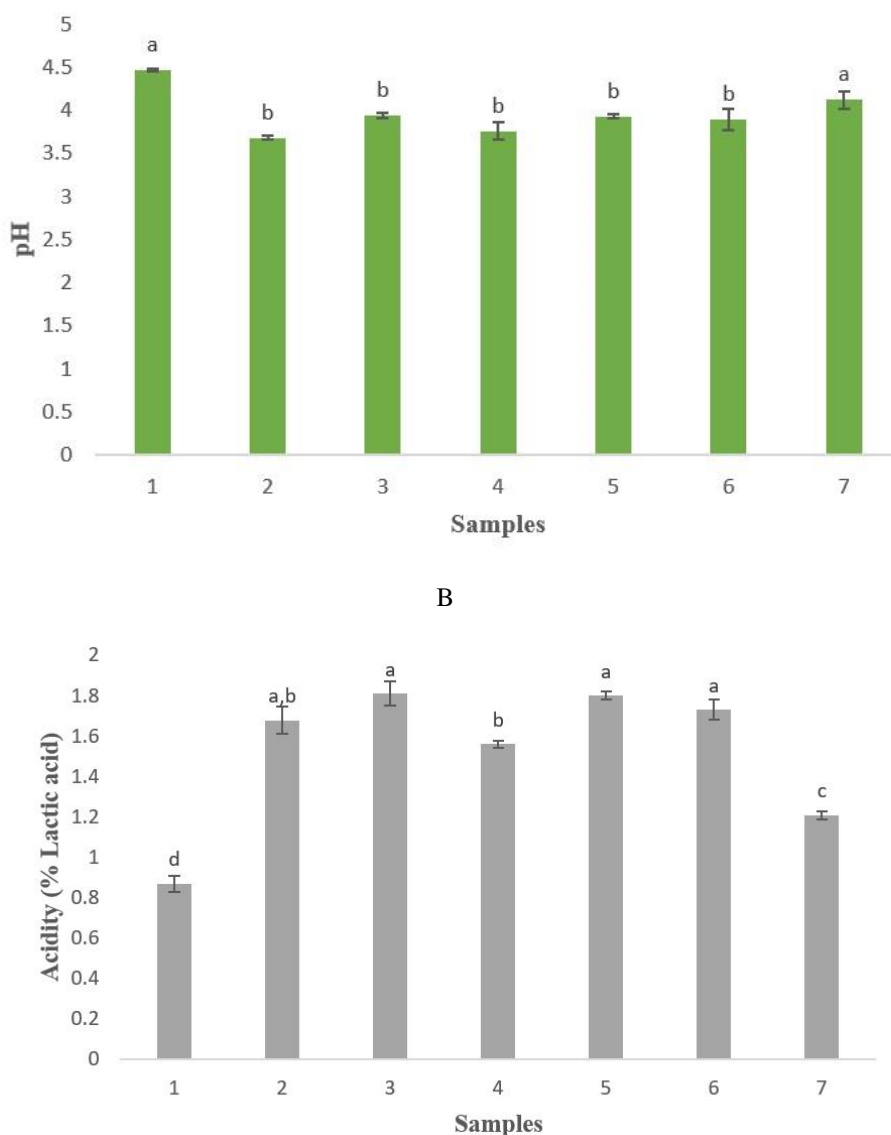
**Fig 2** Moisture content (A) and total solids (B) of different samples. Different letters indicate significance ( $p > 0.05$ )

### Acidity and pH

The pH of yogurt and fermented products is an indicator of acidity. Because yogurt has a complex buffering system, the relationship between titratable acidity and pH is not straightforward. The acidity (% weight of lactic acid) of different samples was between 0.87-1.81, which is entirely in line with the national standard (at least 0.7). Among dairy products, yogurt has a longer shelf life due to its lower pH and can be stored in the refrigerator for about 10 days [15]. The buffering capacity of yogurt, which is related to the presence of proteins, especially casein, phosphate salts, lactate and citrate, affects the development of acidity and pH, so the higher the buffering capacity of yogurt, the slower the pH drops. Although, the acidity increases more strongly. The pH of different yogurts was between 3.68-4.47, corresponds to the Iranian national standard No. 695 (maximum 4.6) [10]. As shown in Fig 3-A, both 1 and 7 samples indicated significantly ( $P < 0.05$ ) higher pH among different yoghurts, which the results are agreed with the acidity of those samples. The pH of milk plays an essential role in the texture and rheological characteristics of yogurt, so lactose (milk sugar) is used as an energy source for starter bacteria and is converted into lactic acid during metabolic reactions. By increasing the concentration of lactic acid and decreasing the pH of milk, the structure of casein micelles and denatured whey proteins is changed during heat treatment, and connections are established between them. The colloidal calcium phosphate plays a significant role in the production

mechanism of yogurt gel. When the pH of milk reaches 5.1-5.2 (the isoelectric pH of beta-casein), milk coagulation begins, and at pH 4.4-6.7, the coagulation of casein micelles is complete [16]. As can be seen, the pH of all samples was less than 4.5, which is entirely consistent with the observation of proper curd, and acceptable yogurt texture. After inoculation of milk with starter culture bacteria, *Streptococcus salivarius subsp. thermophilus* bacteria grew rapidly until its amount reached 90% of all bacteria. After 2 hours, *Streptococcus salivarius subsp. thermophilus* bacterium releases lactic, and formic acids and provides the conditions for the growth of *Lactobacillus delbrueckii subsp. bulgaricus*, and in this condition, this bacterium grows faster. Streptococcus causes the initial pH to decrease to 5, and in this case, the growth of *Lactobacillus delbrueckii subsp. bulgaricus* is accelerated, while the growth of *Streptococcus salivarius subsp. thermophilus* is prevented due to the accumulation of lactic acid produced. From this point on, *Lactobacillus delbrueckii subsp. bulgaricus* is responsible for reducing the pH. After 4 hours, the population of two bacteria is balanced. After the completion of fermentation, the acidity reaches 1.2-1.4% (pH 4.2-4.3), and in this condition, the total microbial count reaches  $2 \times 10^7$  CFU/mL. The desired consumer's acidity is 1.2-1.4%. Fermentation usually lasts 4 hours, during which both bacteria convert lactose into lactic acid. After preparing the final product, it is kept at a temperature of 5°C to slow the speed of chemical, microbial, and physical reactions [16].

A



**Fig 3** pH (A) and acidity (B) of different samples. Different letters indicate significance ( $p > 0.05$ )

### Microbial count

According to the Iranian national standard No. 2406 [12], the maximum limit for coliform, and mold and yeasts is 10, and 100 (CFU/g), respectively. The frequency distribution of total count of microorganisms, mold and yeasts,

coliform and *S. aureus* is shown in Table 1. As can be seen, 100% of the local yogurt samples showed total microbial count, and mold and yeasts. Coliform, and *S. aureus* were observed in 83.33%, and 50% of samples, respectively.

**Table 1** Distribution of total count of microorganisms, mold and yeast, coliform and *S. aureus* in yogurt samples

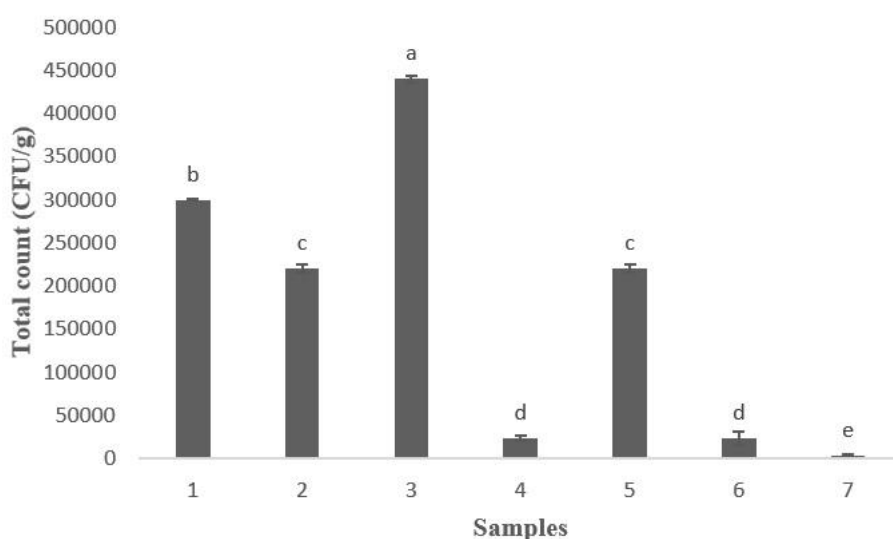
Sample type	Total microorganisms count	Yeast and Molds	Coliforms	<i>S. aureus</i>
Traditional yoghurt	100%	100%	83.33%	50%

### Microbial Total count

The total count of different samples is shown in Figure 4. The use of starter bacteria *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. Thermophiles* in yogurt have two prominent roles, which include the production of lactic acid and compounds effective in aroma and taste. However, the intensity of the yogurt flavor varies depending on the geographical region. Therefore, the production of aroma and flavor in yogurt by different strains of lactic acid has the most important effect on the characteristics of the final product. The fermentation temperature range is 42 to 44°C. When the temperature rises to 44°C, the metabolism's rate of the starter culture increases, and the yogurt becomes sweeter. The faster growth of the starter culture causes the faster curd formation. Immediately, after reaching the desired acidity, yogurt is refrigerated. This stage is very critical in the industry and plays a significant role in the taste of yogurt. According to the Codex standards of fermented products, the total number of microorganisms in yogurt, kefir, and koumiss should be less than  $10^7$  CFU/g, which is entirely consistent with the results obtained in this study. Thus, the highest total count of microorganisms is related to sample 3 with  $4.4 \times 10^5$  CFU/g, which is much less than the limit

in the national standard of Iran [16]. As can be seen, there is a significant difference among all samples regarding total microbial counts. The pasteurized yoghurt indicated the least total count, which was significantly ( $P < 0.05$ ) lower than those local samples.

Our results are entirely consistent with the study of Bassi et al [17], who showed the higher coliform, mold and yeasts in local yogurt samples compared to pasteurized one. This is related to the manual process of local yogurt production, and the type of yogurt packaging which is open-door, may lead to enter mold and yeast spores to the final product. Also, the average total count of microorganisms in yoghurt No. 6 was  $2 \times 10^5$  CFU/g, which is consistent with the study of Farinde et al [18] who reported the total microbial count of  $1.1 \times 10^5$ . The milk used for making yogurt should be heated to a point where all the pathogenic microbes, most of the vegetative cells, and endogenous enzymes are destroyed. Intense heat treatment of milk by destroying competing microorganisms provides the conditions for the growth of starter culture bacteria. If raw milk is stored in the cold for a long time (2-3 days), heat-resistant lipase enzymes are provided by psychotropic bacteria such as *Pseudomonas*, *Acinetobacter*, *Serratia*, *Achromobacter*, *Alkaligenes*, and *Moraxella*, which by affecting milk fat lead to lipolysis and increase of free fatty acids and yogurt bitterness [16].





**Fig 4** Total microorganism counts of different samples. Different letters indicate significance ( $p > 0.05$ )

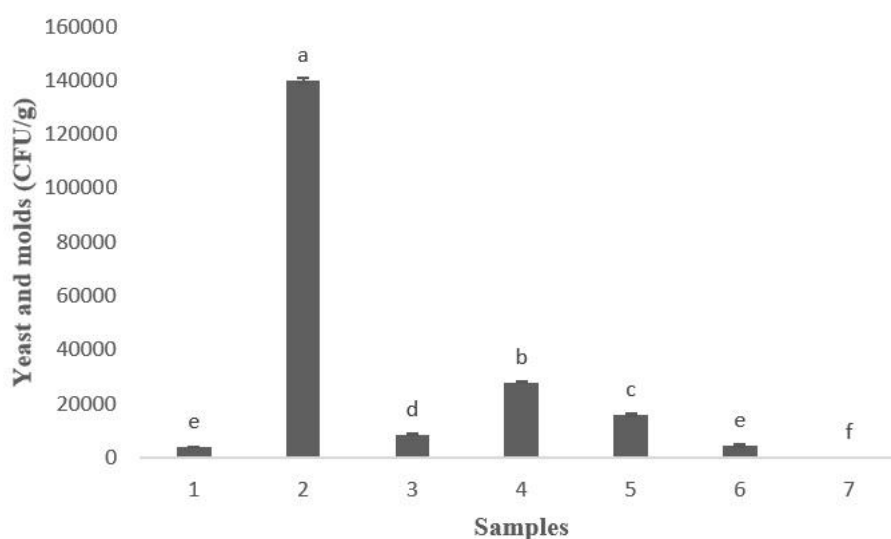
### Mold and yeasts

The amount of mold and yeasts count related to different samples is shown in Figure 5. As can be seen, the pasteurized sample was free of mold and yeasts, while local samples showed a high amount of mold and yeasts. Among local yogurts, the highest count of mold and yeasts was observed for yogurt No. 2 by  $1.4 \times 10^5$  CFU/mL, where the lowest count of mold and yeast was seen for samples numbers 1, and 6 indicated  $4.2 \times 10^3$  CFU/g, and  $4.6 \times 10^3$  CFU/g, respectively. Among different samples, only the pasteurized sample complied with the Iranian national standard No.695 [10], and the other samples showed non-compliance with the national standard [10] in terms of mold and yeasts, which declared the limit of mold and yeasts to be 100 colonies. Compared to other dairy products, yogurt has a longer shelf life due to its higher acidity and can be stored in the refrigerator for up to 10 days. Based on the type pH of yogurt, the main microbial spoilage in yogurt is related to acid-resistant microorganisms such as mold and yeasts, and it is mainly caused by molds in the form of surface spoilage [15]. The growth of mold in dairy products weakens the texture, changes the color, aroma, and taste, and causes a lot of economic losses. On the other hand, the more severe concern of mold growth is related to the presence of molds such as *Aspergillus*, *Fusarium*, and *Penicillium*, which make the product harmful to health through the production of mycotoxins such as aflatoxin, patulin, acratoin, etc. Since molds grow quickly at temperatures of  $-2$  to  $10$  °C, therefore, the use of cold treatment in the refrigerator have no effect on their growth [2]. Thus, during the stages of milking and preparing yogurt, the requirements of good production must be well followed by entering the minimum amount of mold spores to the product. Sami et al [2] showed that the type of yogurt production method (local and

pasteurized) is effective on the mold and yeasts contamination. They showed that the dairy products of Isfahan City were at a high level in terms of mold and yeasts, considering that supervisory organizations such as the Food and Drug Administration, and the Iranian Standard and Industrial Research Organization have continuously monitored only the industrial products, therefore, serious actions should be taken in this regard by determining the production requirements, and principles for quality products improvement. If the count of yeasts exceeds  $10^5$ - $10^6$  CFU/g, these effects will be evident on the product. Especially, galactose-positive yeasts can create many bubbles on the surface of fruit yogurt. The possibility of yeast contamination in fruit yogurts, which are common in European countries, is provided through mixers and the surface of packaging machines. Yeasts grow quickly and better than mesophilic and psychotropic bacteria in high acidity and low temperature. Concentrated yogurt is also very susceptible to contamination by yeasts due to exposure to air during concentration. To control yeast growth in Middle Eastern countries, adding potassium sorbate (maximum 300 mg/kg) or sodium benzoate (maximum 400 mg/kg) is common ways to control yeast growth. Molds are aerobic organisms and they need sufficient amount of oxygen to grow, so mold grows only on the surface of the product and can be identified by the appearance of yellow colonies on the surface. Common molds in fermented products include *Mucor*, *Rhizopus*, *Aspergillus*, *Penicillium* and *Alternaria*. Since aflatoxin contamination is caused by molds, the count of mold contamination in fermented products should not exceed 10 CFU/g. Aflatoxin can remain during the storage period of yogurt. Hasanin et al [19] showed that aflatoxin  $M_1$  is bound to casein micelles in yogurt and can be seen in detectable amounts after 3 weeks. On the contrary, aflatoxin  $B_1$  has a limited effect on the metabolic activities of yogurt starter culture

bacteria and is destroyed during fermentation. Other aflatoxin-producing molds such as *Aspergillus flavus*, and *Aspergillus parasiticus* (aflatoxin B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>) remain in yogurt and fermented products during the shelf life. Spores of *Aspergillus flavus* and *Aspergillus parasiticus* are highly resistant to storage conditions. According to the mentioned cases, to prevent the growth of mold and yeasts in fermented products, the following actions should be taken: 1) sanitary production

conditions during the production process should be established; 2) microbial control must be done during the yogurt and fruit yogurt packaging line; 3) the storage temperature should be below 4°C; 4) inoculation containers, tanks, and cold stores must be clean and disinfected; 5) in the production of fruit yogurts, the fruit must be heat treated before being added to the yogurt [16].



**Fig 5** Count of mold and yeasts of different samples. Different letters indicate significance ( $p > 0.05$ )

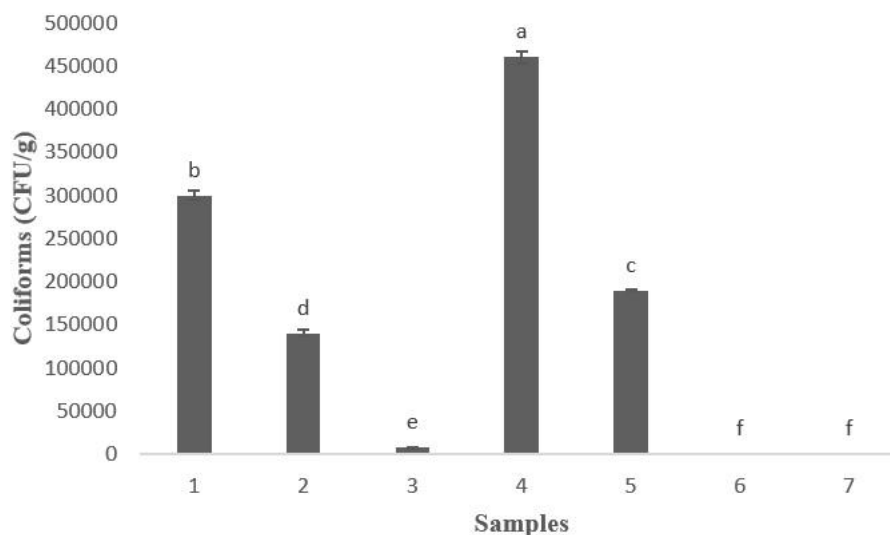
### Coliforms

The coliform content of different samples is given in Figure 6. As can be seen, the pasteurized sample was free of coliform, while local ones showed a high amount of coliform. Only one local sample (No. 6) was free of coliforms. While, the content of coliform in other yogurts was significantly high ( $P < 0.05$ ). According to the Iranian national standard No. 2406 [12], the maximum coliform limit is 10 (CFU/g) colonies. As can be seen, four samples showed a high amount of coliform among six samples, and in total, the amount of coliform was 83.33%, which is a high content. The presence of coliform was an indication of fecal contamination in food. As an indicator of fecal contamination, it shows the level of compliance with sanitary conditions in product production. Considering the boiling of yogurt milk in local

yogurt packaging, the presence of coliform in the product is related to cross-contamination and non-compliance with sanitary conditions during product production. In general, coliforms cannot tolerate high acidity and therefore are rarely seen in fermented products with low pH. In a study, Masa et al [20] showed that *E.coli* can survive in yogurt at a pH higher than 4.5 to 7 days. Also, coliforms usually do not resist antibiotics produced by yogurt starter bacteria. Also, cross-contamination and subsequent fermentation by *E.coli* O157:H7 pose a high consumer risk. Although *E. coli* O157:H7 is affected by the high acidity of yogurt, it can survive in cold storage. On the other hand, due to the short shelf life of yogurt compared to other fermented dairy products such as cheese, and considering that yogurt is available for consumption a few hours after being refrigerated, it is not possible to simply

reduce the amount of total forms. It was sufficient in acidic conditions [19]. As the results show, 83.33% of the samples showed coliform, which, considering the high number, it is highly recommended to observe the

hygienic conditions during production in local yogurt markets.



**Fig 6** Coliforms of different samples. Different letters indicate significance ( $p > 0.05$ )

### *Staphylococcus aureus*

The count of *S. aureus* related to different samples is shown in Figure 7. As can be seen, the pasteurized sample was free of *S. aureus*, while some local samples showed a high level of *S. aureus*. Three samples were free of *S. aureus* (50%) among six traditional yogurts. Among the three samples that contained *S. aureus*, sample No. 2 significantly showed a very high contamination with *S. aureus* ( $P < 0.05$ ). Due to the traditional method of making yogurt and the lack of control of incoming raw milk, the possibility of livestock contamination with various diseases, including mastitis, is high. To reduce staphylococcal contamination in milk, it is necessary to treat and control mastitis in livestock farms. It is also required to observe the hygiene principles during the production, transportation, and storage of raw milk. Due to its acidic pH, yogurt limits the growth of pathogenic bacteria. So, bacteria such

as *Listeria monocytogenes*, *salmonella*, *coliform*, and *S. aureus* have a meager chance of growing in yogurt. The growth of these bacteria is prevented immediately after 24 hours of yogurt production. The survival rate of pathogenic organisms during yogurt fermentation depends on the intensity of contamination, and the pH of the final product. Yogurts with a mild pH above 4.5 allow salmonella to grow for up to 10 days. Yogurt starter culture bacteria prevent the growth of *S. aureus* to a high extent. Yogurt starter culture bacteria show a robust antagonistic effect against *L. monocytogenes* bacteria, however, some strains of this bacterium can develop in acidic conditions, which can remain in yogurt during the product's storage period. Depending on the degree of contamination, the growth of *Yersinia enterocolitica* is partially or entirely prevented in yogurt and other fermented products. Survival of *Campylobacter jejuni* and *Salmonella* in fermented products is very scarce [16].

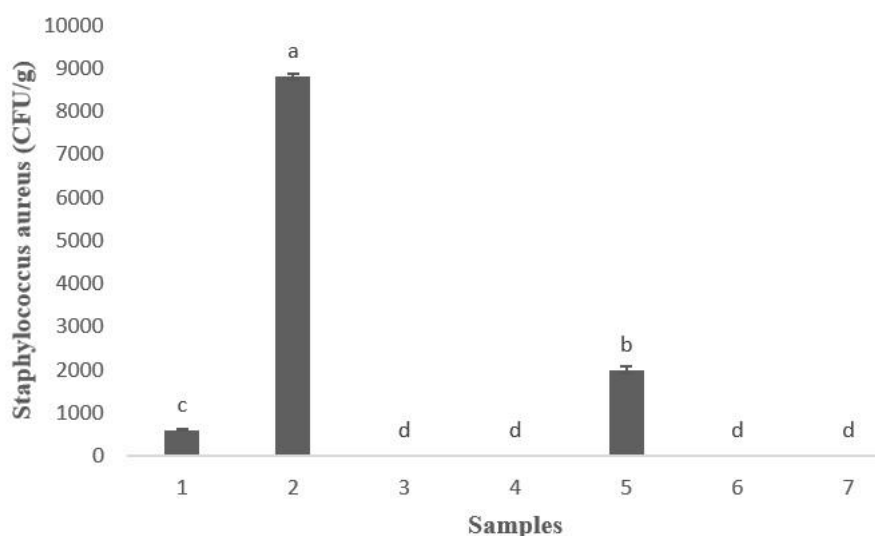


Fig 7 Count of *S. aureus* of different samples. Different letters indicate significance ( $p > 0.05$ )

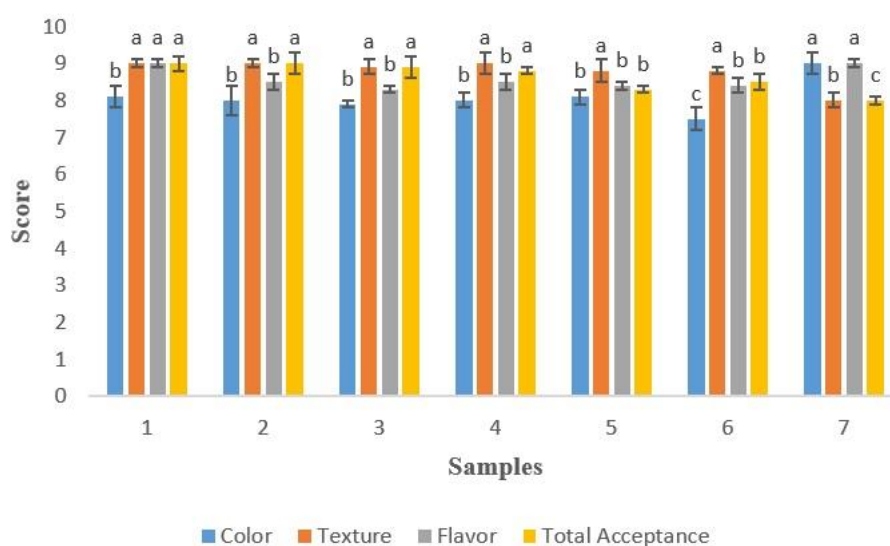
### Sensory evaluation

The sensory evaluation of different samples was evaluated by 9-point pleasantness test by examining the factors of color, texture, taste, and overall acceptance by 15 panelists, and the results are shown in Figure 8. The results indicated higher texture and acceptance for local yogurts than pasteurized ones. The texture of all traditional samples showed a significantly ( $P < 0.05$ ) higher scores than that of the pasteurized one. The reason for this observation is related to performing homogenization in pasteurized yogurt and not performing it in local yogurts. The presence of a layer of cream on the surface of local yogurts, which is related to the migration of fat to the surface of yogurt due to the difference between the density of fat and the water phase of milk, causes the accumulation of fat on the surface of yogurt. Consumers have a better mouthfeel than those samples in comparison with pasteurized samples. In the pasteurized products, the homogenization operation was carried out at high pressures of 2000-2500 psi, which resulted in a significant reduction in the diameter of the fat globules, as a result, prevented its migration to the surface of the yogurt, and according to the stock's law, the stability of this colloidal system increases. While in local yogurts, due to

their large size, the fat globules quickly migrate to the surface of the yogurt and form a layer of cream by accumulating in that part. The comparison of local and pasteurized yoghurts significantly ( $P < 0.05$ ) showed a better color for pasteurized yogurt. The reason for this phenomenon is the homogenization process in pasteurized yogurt, which due to the proper distribution of fat particles in the continuous phase is associated with better and more light diffusion and showed a whiter color for this yogurt than local yogurt samples. Regarding the taste factor, the pasteurized sample and one of the local yogurt samples showed a significantly better taste than the other samples. Among the traditional samples, sample No.1 had a better taste than other samples, which is due to the higher pH of this sample compared to other samples. The higher score of the pasteurized sample regarding the taste parameter is related to the homogenization process in this sample, which creates a better taste due to the uniform distribution of fat. Also, yogurt's pH is higher and its sweet taste is considered an important parameter from the point of view of consumers. Acidity plays an important role in quality parameters of yogurt from the point of view of taste, consumer acceptance and product shelf life. The production of lactic acid under controlled conditions during fermentation plays an essential role in the development of yogurt

gel structure. On the contrary, the developing acidity after fermentation is not desirable since it causes loss of whey, textural problems, and sour taste, which masks the flavor formed during fermentation. Therefore, the control of acidity after fermentation plays an essential role in the quality of the final product. The minimum level of acidity in the retail market of the product is determined by the International Dairy Association to be 0.7%. Although Codex standards have reported acidity at 0.6% [14]. Considering that the industrial sample is pasteurized after preparation, by controlling the growth of the bacteria that initiates the fermentation process, and the production of lactic acid, the shelf life of the product has progressed slowly, and therefore the development of sour taste in the pasteurized samples compared to the sample- Traditional events are postponed. In general, the appearance of the desired aroma and taste in yogurt is related to the production of compounds including aldehyde, acetone, acetone and diacetyl, which are produced by the initiator bacteria during the fermentation process. Therefore, any change in the intensity of fermentation such as the amount of inoculation, time and temperature of the

fermentation process can affect the aroma and taste of yogurt. For example, due to the thermophilic nature of yogurt starter bacteria, the inoculation temperature is 41-43°C. Temperatures higher or lower than this range cause changes in the aroma and taste of yogurt. Also, the amount of inoculation should be considered 2.5 to 3%. By increasing the amount of starter culture, the effective compounds in flavor are covered by increasing the production of lactic acid, and in this sense, it leads to the weakening of the sensory properties of yogurt. Also, rapid cooling of yogurt after fermentation causes negative effects on the sensory properties of yogurt [14]. According to the mentioned cases, since in the production of industrial products, the production steps are well controlled by the operator and engineers in the factory, therefore the product has relatively similar conditions in terms of sensory characteristics. In the production of traditional products in yogurt, due to the lack of monitoring and measurement equipment and continuous controls, a different product will be obtained in terms of sensory characteristics.



**Fig 8** Sensory evaluation results of those traditional and pasteurized yogurts

#### 4-Conclusion

Based on the obtained results, the chemical properties of yogurt, including pH, acidity, moisture, total solids and fat, are entirely

following the Iranian national standard no 695 [10]. However, the examination of microbial characteristics showed that 83% and 50% of the traditional yogurt samples were contaminated with coliform and *S. aureus*, respectively, and 100% were contaminated with mold and yeasts. Due to the traditional method of making yogurt and the lack of control of incoming raw milk, the possibility of livestock contamination with various diseases, including mastitis, is high. To reduce *staphylococcal* contamination in milk, it is necessary to treat and control mastitis in livestock farms. It is also required to observe the hygiene principles during the production, transportation and storage of raw milk. According to the mentioned cases, to prevent microbial contamination in fermented products, observing good production conditions, controlling incoming raw milk or obtaining raw milk from certified milk receiving centers, performing optimal heat treatment in yogurt packaging, using clean and disinfected containers, using proper packaging for final products with sealed lids, cleaning the place and compliance personal hygiene leads to a decrease in the amount of microbial contamination, especially cross-contamination in yogurt. Based on the obtained results, it seems that since the industrial units have continuously monitored the quality of incoming raw milk and the requirements of good production have been well implemented in the factory and according to the thermal process on the final product and the use of aseptic packaging. Thus, the contamination in the final product is significantly lower than in the local samples. Finally, it is suggested that to protect the public health, consumers should be informed about the process of preparing industrial products, and advised to use industrial dairy products.

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## بررسی ویژگی‌های شیمیایی و میکروبی چند نمونه ماست محلی تهیه شده از فروشگاه‌های عرضه محصولات لبنی سنتی شهرستان تویسرکان

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اطلاعات مقاله	چکیده
<p><b>تاریخ‌های مقاله:</b></p> <p>تاریخ دریافت: ۱۴۰۲/۱۱/۸</p> <p>تاریخ پذیرش: ۱۴۰۳/۲/۲۶</p>	<p>با توجه به تمایل بالای مصرف کنندگان به تهیه محصولات لبنی سنتی و تردید آنها به استفاده از محصولات کارخانه‌ای این مطالعه با هدف بررسی ویژگی‌های مختلف شیمیایی و میکروبی نمونه‌های ماست سنتی عرضه شده در شهرستان تویسرکان و مقایسه آن با نمونه ماست پاستوریزه صورت پذیرفت. ویژگی‌های شیمیایی شامل ماده خشک، رطوبت، اسیدیته، pH و چربی و ویژگی‌های میکروبی شامل شمارش کلی میکروارگانیسم‌ها، کلی فرم، کپک و مخمر و استافیلوکوکوس اورئوس مورد بررسی قرار گرفته و با نمونه پاستوریزه به عنوان نمونه شاهد مقایسه شد. محتوای رطوبت و ماده خشک نمونه‌ها به ترتیب از ۸۴/۳۳٪ تا ۸۷/۸٪ و ۱۲/۶۲ تا ۱۵/۲۵٪ متغیر بود. محتوای چربی ماست‌های تولید شده بین ۲/۵-۲/۹ در محدوده ماست نیم چرب بر اساس استاندارد ملی ماست قرار داشت. بین نمونه‌های مختلف از نظر رطوبت، ماده خشک و چربی تفاوت معنی داری مشاهده نشد (<math>P &gt; 0/05</math>). میزان اسیدیته (درصد وزنی بر حسب اسید لاکتیک) و pH نمونه‌های مختلف به ترتیب بین ۱/۸۱ - ۰/۸۷ و ۴/۴۷ - ۳/۶۸ بود که کاملاً با استاندارد ملی ماست (حداقل ۰/۷ می‌باشد) مطابقت داشت. نمونه‌های مختلف از نظر pH و اسیدیته تفاوت معنی داری را نشان دادند (<math>P &lt; 0/05</math>). نتایج ویژگی‌های میکروبی بیانگر حضور کپک و مخمر در ۱۰۰٪، کلی فرم در ۸۳٪/۳۳ و استافیلوکوکوس اورئوس در ۵۰٪ نمونه‌ها بود. در خصوص ویژگی‌های میکروبی تفاوت معنی داری بین نمونه‌های سنتی و صنعتی مشاهده شد. به طوری که نمونه صنعتی کمترین بار میکروبی را نسبت به نمونه‌های ماست سنتی نشان داد (<math>P &lt; 0/05</math>). از این رو کنترل شیر خام ورودی و رعایت شرایط بهداشتی حین تولید، بسته بندی و عرضه در تولید محصولات سنتی اکیدا توصیه می‌گردد. نتایج ارزیابی حسی رنگ و طعم و مزه بهتری برای نمونه پاستوریزه و بافت و پذیرش کلی بهتری را برای نمونه‌های سنتی نشان داد.</p>
<p><b>کلمات کلیدی:</b></p> <p>ماست، لبنیات سنتی، استاندارد ملی، ویژگی‌های شیمیایی، ویژگی‌های میکروبی.</p>	
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