Investigation of Physicochemical, Microbiological, and Rheological Properties and Volatile Compounds of Ewe and Cow Milk Yoghurt

M. Karami¹

ABSTRACT

Yoghurt, with viscous texture and containing volatile compounds, has special appeal for the consumer. In this study, to produce such a yoghurt without any additives, two types of yoghurt from ewe and cow milk were prepared. The samples were evaluated for physicochemical and volatile compounds and viscosity on the first, 7th, and 14th days of shelf life. In addition, the enumeration and viability of *Lb. bulgaricus* and *Str. thermophilus* were studied. Ewe yoghurt had more dry matter, protein, and acid production ability compared to cow yoghurt, with lower pH. The number of lactobacilli was greater in ewe yoghurt which caused increase in acidity and decrease in pH value in ewe yoghurt. The number of lactobacilli and streptococcus decreased during shelf life of both yoghurts. The viscosity of ewe yoghurt was greater than cow yoghurt and it increased during 14 days of storage. Regarding volatile compounds, acetaldehyde and diacetyl decreased during shelf life while ethanol increased in both cow and ewe milk yoghurt.

Keywords: Lactobacilli, Shelf life, Viscosity.

INTRODUCTION

Yoghurt is one of the fermented dairy products that is produced using activity of starter microorganisms in milk. Some bacteria are used for production of yoghurt, among thermophilus them Streptococcus (Str. thermophilus) and Lactobacillus delbrueckii subsp. bulgaricus (Lb. bulgaricus) are general starters. These starters ferment lactose and produce lactic acid. Yoghurt must have, at least, 10^7 live bacteria (Lee and Lucey, 2010). Lactic Acid Bacteria (LAB) have numerous applications for preservation of food, because they are safe and natural microflora of some foods (De Martinis et al., 2001). Some researchers relate the beneficial effects of yoghurt to starter cultures, but there are some claims about the functional properties of probiotic bacteria in yoghurt (Lomax and Calder, 2009). It has been reported that yoghurt starters can resemble probiotics and have functional properties (Guarner *et al.*, 2005; Lahtinen *et al.*, 2006).

Acidity, free fatty acids, flavor producing components (Diacetyl, Acetaldehyde and Acetone), sensorial and nutritional properties are the main factors for quality assessment of voghurt. These factors depend on the type, chemical properties, processing conditions and additives of milk (Tamime and Robinson, 2001). Flavor of yoghurt is basically formed by volatile (butyric, acetic, propionic) and nonvolatile (lactic, pyruvic, oxalic) acids as well as miscellaneous carbonyl compounds (acetaldehyde, diacetyl, acetoin and acetone) via the fermentation or thermal degradation of some milk constituents (Ott et al. 1997; Tamime and Robinson, 2001; Saint-Eve et al., 2006).

Ewe milk has higher total solids and principal nutritional components in

¹ Department of Food Science and Technology, Faculty of Food Science and Technology, Bu-Ali Sina University, Hamedan, Islamic Republic of Iran. e-mail: mkarami@basu.ac.ir

comparison with cow milk. This type of milk has high nutrients, density, viscosity, refractive index, and acidity, whereas its freeze point is lower than cow milk. Its total solids are 19% while it is 12-13% for cow milk (Park et al., 2007). It means that fat and protein of ewe milk is higher, thus it is the preferential milk among dairy products, especially yoghurt (Tamime and Robinson, 2001). Ewe milk contains higher amounts of protein than cow milk and, because of its pleasant creamy-sour flavor, is considered by many to be better than cow yoghurt (Erkaya and Sengul, 2011). In ewe milk, lipids have higher physicochemical properties, main caseins are equivalent in this two types of milk, but calcium and inorganic phosphorus of ewe milk micelle is higher while heat resistance and β -case in content of it is lower (Ruiz-Sala et al., 1996). Fat globule diameter at ewe milk is 3.5 µm that is smaller than that of cow milk. The levels of low and medium-chain fatty acids are higher in ewe milk, e.g. Caproic $C_{6:0}$ (2.9 versus 1.6), Caprilic C_{8:0} (2.6 versus 1.3), Capric C_{10:0} (7.8 versus 3) and Lauric C_{12:0} (4.4 versus 3.1) (Park et al., 2007). Due to some factors such as seasonal variation and low industrial processing, the sporadic and annual production of ewe milk has been limited to about 50 kg (Hilai et al., 2011).

Final odor and flavor of yoghurt is formed by some principal components including nonvolatile acids (Lactic and Pyruvic acid), volatile acids (Butyric and acetic), carbonylic (Acetaldehyde, Diacetyl, Acetone) and miscellaneous compounds (such as amino acids that are produced during thermal processing) (Tamime and Robinson, 2001). It seems that some amounts of lactic acid. acetaldehyde and diacetyl with proper ratio are necessary for final aroma and flavor of yoghurt (Erkaya and Sengul, 2011). Some researchers suggested the following amounts of flavorous compounds for yoghurt:

Acetaldehyde (2-41 mg kg⁻¹), Diacetyl (2-2.3 mg kg⁻¹), Acetone (2.2-28.2 mg kg⁻¹) and Ethanol (0.2-9.9 mg kg⁻¹) (Condurso *et al.*, 2008; Kaminarides *et al.*, 2007).

The most studied volatile component by researchers is acetaldehyde (Erkaya and Sengul, 2011). Acetaldehyde has been reported as the key aromatic component of yoghurt and due to the kind of milk, its quality, physicochemical properties, type of starter, incubation time and shelf life, the carboxylic substances of yoghurt change (Ott *et al.*, 1997). Chua *et al.* (2017) indicated that the ratio of casein/whey protein is the main detrimental quality parameter for yoghurt, and with addition of whey protein to caseins ratio, the yoghurt becomes firmer and stiffer and volatile compounds change.

Unfortunately, little information about bacteriology, main component, viscosity and volatile compounds and their relation in ewe and cow milk yoghurt is available. Therefore, in this study, we aimed to: (1) Investigate the growth pattern of starters in ewe and cow milk yoghurt and compare them during shelf life, Analyze and compare (2)principal components of both milk types, (3) Measure viscosity, which is one of the main factors affecting acceptability of yoghurt, and determine its relation with other components discussed, and (4) Evaluate volatile compound, such as acetaldehyde, acetone, ethanol and diacetyl content of both yoghurts during shelf life and relate to other factors.

MATERIALS AND METHODS

Cow and ewe milk were prepared with following characteristics from dairy farms of Ghorveh City (Kurdistan, Iran). (Table1).

The analysis of these components is similar to some reported studies (Bonczar *et al.*, 2002; Haenlein and Wendorff, 2006).

Table 1. Characteristics of the studied cow and ewe milk.

Milk type	Acidity	pН	Lactose	Freeze point	Density	SNF	Protein	Fat
Cow milk	14 °D	6.6	4.0 %	-0.557	1.032	8.4 %	3.2%	4.0 %
Ewe milk	22 °D	6.7	4.2 %	-0.578	1.038	12.0 %	6.2%	6.0%

Starter Cultures

In this study, Express 01 starter culture (CHR-Hansen, Denmark) was used. This commercial starter contains *Lb. bulgaricus* and *Str. thermophilus* with equal ratio and is used for set type yoghurt. According to manufacturer recommendation, 0.1 U kg⁻¹ of this starter culture is enough for 500-750 kg milk to produce an acceptable set-yoghurt.

Culture Media

MRS Agar (Vancouver, Canada) was used for enumeration of *Lb. bulgaricus* as anaerobic pour-plate at 45°C for 72 hours and M17 Agar (Vancouver, Canada) was used for *Str. thermophilus* as aerobic pour-plate at 37°C for 72 hours (Vancouver, Canada). These cultures are used as bacterial medium for determination of yoghurt starters' growth and viability (Lawrence *et al.*, 2015).

Yoghurt Manufacture

Yoghurt manufacture is performed using the method of Tamime and Robinson (2001). First, milk samples were heated to reach to 90°C and then remained 10 minutes at this temperature. For inoculation, milk was cooled to 43°C and added 0.5% starter culture, mixed to be homogenous and filled in polystyrene package. Then, samples were incubated at 43°C to reach pH value of 4.5 and were immediately cooled to 10°C and stored at 5°C before physicochemical, bacteriological, and rheological analysis were performed. To attain pH of 4.5, ewe milk must be incubated for 3 hours and cow milk for 3.5 hours.

Physicochemical Determinations

pH of the samples was measured using a pH-meter (metrohm, Swiss), and titratable acidity of samples was determined with titration of milk with 1/9N NaOH using

phenolphthalein as an indicator and reported as Dornic degree (Fadela *et al.*, 2009).

Fat and lactose content of the samples were determined by Gerber and Bertrand methods (Bonczar *et al.*, 2002).

Protein content of the two types of yoghurt were analyzed using the crude nitrogen content of the samples and then multiplied by 6.38 to obtain protein content, and moisture content was estimated using oven drying methods (AOAC, 1990).

Volatile Compounds

Volatile Compounds (VCs) of ewe and cow milk, including Acetaldehyde, Acetone, Ethanol and Di-Acethyl were determined using Guler and Gursoy-Balci (2011) method with some modifications. An Agilent model 6890 Gas Chromatography (GC) (Agilent, Palo Alto, CA, USA) was used for identification of VCs and a GC-Mass equipped with HP-5MS column: 5% Phenyl Methyl Silox 325 c (30 m×250 µm id×0.25 film thickness) μm for measurement. A sample of 5 g was used for preparing 500 µL injected sample volume. The VCs were separated at following conditions: syringe size was 2.5 mL-hs, initial oven temperature was 80°C for 5 minutes and with 10°C min⁻¹ ramp increased to 180°C and held 25 minutes at this temperature. The injection valve temperature was 200°C and detector temperature was 200°C, Helium gas was as carrier with flow of 1.4 mL min⁻¹. the injection method to GC was split. Peaks were identified by comparing the mass spectra with the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) library, version 0.2 L, and their retention times were compared with authentic standards (Merck, Hohenbrunn, Germany and Fluka, Sigma-Aldrich, Switzerland). Peak areas (arbitrary units) were calculated from the total ion current. External standard technique was used for quantification of constituents. For this purpose, authentic standards of acetaldehyde, acetone, diacetyl, and ethanol were accurately weighed and dissolved in 10 g of double distilled water. Five sets of concentrations were prepared in the range of $1-40 \ \mu g^{-1}$. To calculate a mean peak area for each standard compound, results obtained from the collections of standards were used. Using known amount of standard and its peak area, the amounts of each compound in the sample were calculated. All collections were made in triplicate.

Statistical Analysis

SPSS computer program (version 13.0 for windows) was used for statistical processing (Coakes, 2006). Effects of storage days (1^{st} , 7^{th} , 14^{th} days) and the types of milk (ewe and cow milk) on chemical composition, VCs (Acetaldehyde, Acetone, Di-Acetyl and Ethanol) microbial population and viability, and viscosity were evaluated by Bonferroni Repeated- Measures Analysis of Variance. The paired comparisons of means were made using the Duncan test (P< 0.05).

RESULTS AND DISCUSSION

Main Constituents

The results of physicochemical analysis of ewe and cow milk yoghurt during shelf life $(1^{st}, 7^{th} \text{ and } 14^{th} \text{ days})$ have been indicated on Figure 1.

Results indicated that pH of both yoghurts decreased during shelf life from 1 to 14 days (P< 0.05). During this shelf life, acidity of samples increased. Data analysis indicated that highest pH belonged to the first day and lowest one to the 14^{th} day of shelf life. Promotion of acidity and decline of pH can be related to the activity of starters' bacteria and the fermentation of lactose to lactic acid (Tamime and Robinson, 2001). As indicated on Figures 1 and 2, the slopes of pH decrease and acidity increase are higher for ewe milk yoghurt in comparison with cow milk yoghurt. Data indicated that at the first

day, acidity of ewe milk yoghurt was greater than cow milk yoghurt (P< 0.05). This pattern remained steady up to 14th day of shelf life. The difference between pH and acidity of ewe milk yoghurt and cow milk yoghurt can be related to the initial acidity of ewe milk, so that its acidity was 22 °D, while the acidity of cow milk was 14 °D at this time. This variance can be due to higher nitrogen content of ewe milk. Higher protein in ewe milk can play a role as higher buffer capacity and more starter activity and acidity. At higher buffer capacity, more acid can be produced, while the media is stable, without sensible change (Urbach, 1995). In other words, growth and extension of bacteria depends on protein content (especially amino acids) of the media. Fermentation length and shelf life have great effect on pH decrease and acid production relate to time and the kind of milk (Akin and Akin, 2007). Similar pH and acidity changes have been reported in other studies (Bonczar et al., 2002; Karademir et al., 2002; Ott et al., 1997; Stelios and Emmanuel, 2004). Physicochemical results of this study were in agreement with other studies (Bonczar et al., 2002; Stelios and Emmanuel, 2004). In both yoghurts, the amount of fat, protein, and lactose showed little decrease. Fat and protein decline can be due to lipolysis and proteolytic changes. Little decrease in fat, protein, and lactose were reported in other studies (Hussain, 2004). Total solids did not change significantly, but some minor decrease happened that could be due to lactose fermentation and its conversion to other volatile compounds and acids (Bano et al., 2011). Conversely, some other studies indicated that total solids increase during shelf life as a result of water vaporization from unpacked yoghurt surface (Ismail and Salem, 2006).

Microbiological Results

Analysis of microbiological results of ewe and cow milk yoghurt is presented in Figure 2.

1152

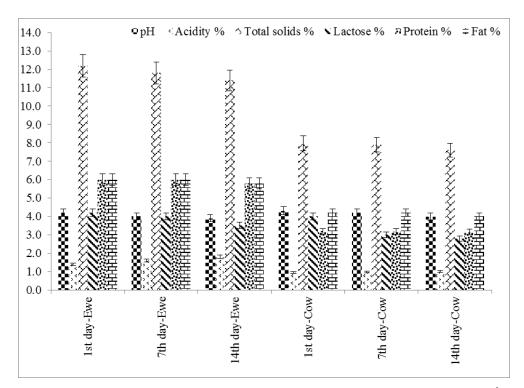


Figure 1. Physicochemical properties of ewe and cow milk yoghurt on different days (1st, 7th and 14th days).

Both Lb. bulgaricus and Str. thermophilus grow synergistically at their medium (Tamime and Robinson, 2001). Continuous growth during shelf life and the number of viable bacteria at the consumption are critical factors for healthy properties of the final product. As results indicated (Figure 2), the population of lactobacilli was greater than streptococci in both yoghurt types. In addition, Ewe milk yoghurt had more lactobacilli, it may be due to its higher total solids. After 7th day of shelf life, a little decrease was observed in microbial population.

It has been shown that there is a direct relation between microbial population and bufferic capacity of yoghurt. This hypothesis has been emphasized in other studies (Zare *et al.*, 2011). Beside this, vitamins, amino acids, and minerals promoted the growth of starters (Tamime and Robinson, 2001). These results indicate why the starters at ewe milk yoghurt are greater than cow milk yoghurt. The higher protein and total solids of this yoghurt increase its bufferic capacity.

The higher protein in ewe milk yoghurt leads to its higher amino acids content. Amino acids are the needed raw materials for growth of starters. Lactobacillus is responsible for lactic acid and aromatic compounds production, so, its higher population can be the reason for higher acidity of ewe milk yoghurt (Tamime and Robinson, 2001). Streptococci population, in both yoghurt types, were lower than lactobacilli as one logarithmic cycle, this finding was in agreement with that of Fadela et al. (2009). This difference may be due to the fermentation temperature. The best temperature for growth of lactobacilli is 42°C, while it is 38°C for streptococci (Akin and Akin, 2007). Strepcococci population was higher in ewe milk yoghurt, probably due to higher fat and protein level in this yoghurt type that can stimulate streptococci growth (Tamime and Robinson, 2001). However, in another study, it was shown that addition of protein (especially cysteine amino acid) can reduce the growth of Streptococcus thermophilus and prompt

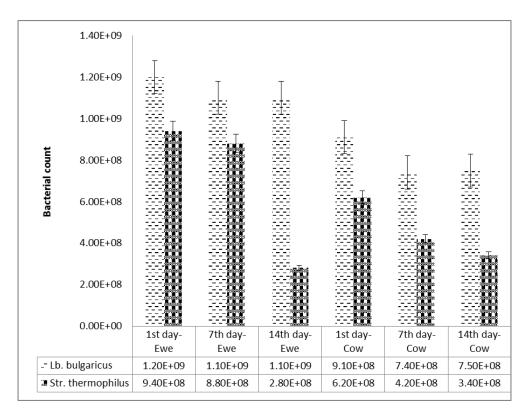


Figure 2. Microbiological analysis of ewe and cow milk yoghurt.

Lactobacillus bulgaricus growth (Urbach, 1995). In other words, Streptococcus thermophilus is more sensitive to lactic acid accumulation and is wasted rapidly (Michael et al., 2010), this reason can justify the decline of this microorganism during the study. Although it has been found that yoghurt consumption has beneficial effects on human health, the number of live bacteria voghurt is critical for in healthy characteristics (Tamime et al., 1984). In this regard, yoghurt's live bacteria must be higher than 10^7 CFU g⁻¹ to comply healthy needs of man (Fadela et al., 2009). But this population decreases during yoghurt shelf life and in retail and diminishes its healthy effects. In this study, live bacteria of ewe milk yoghurt after 14 days were higher than 10^8 CFU g⁻¹, especially lactobacillus that was 10^9 CFU g⁻¹ at the 1st and 7th days of shelf life. Thus, ewe milk yoghurt is better for the growth of the mentioned starters and maybe probiotics. Milk constituents and shelf life have direct role on bacterial count of yoghurt, especially lactobacilli count

(Fadela *et al.*, 2009; Michael *et al.*, 2010). As a result of some factors such as diminished oxygen supply, pH variation, protein network constraint and bacterial metabolites, bacterial population decrease during shelf life. Consequently, yoghurt can be maintained longer, although its probiotic characteristics may be deteriorated.

Viscosity Measurement Results

Ewe and cow milk yoghurt viscosity analysis are shown in Figure 3. As indicated in Figure 5, there is meaningful difference between ewe and cow milk yoghurt viscosity that can be related to the difference between the main constituents of these milk types (Park, 2007). Protein and fat are the most effective factors on milk viscosity and, their contents in ewe milk are high. Thus, when the protein content is low, the yoghurt texture turns into less firm (Biliaderis *et al.*, 1992; Tamime and Robinson, 2001). It has been reported that with increasing the total

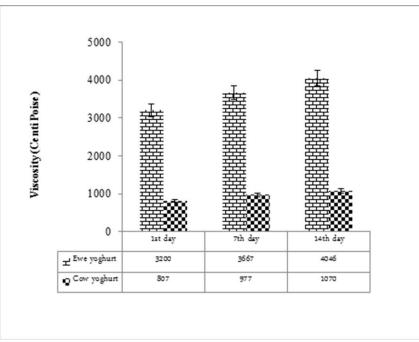


Figure 3. Viscosity of ewe and cow milk yoghurt during the shelf life.

solids of the used milk, the yoghurt will be more viscose (Zare et al., 2011). In this study, there were differences between protein and total solids of ewe and cow milk voghurt. The high protein of ewe milk yoghurt made the texture firm. Also, high protein and total solids of ewe milk yoghurt are equivalent to its higher water holding capacity, and greater apparent viscosity (Hilai et al., 2011). With continuing shelf life from the 1st to 14th days, viscosity of both yoghurt types increased. This increase may be due to starter's metabolites and change of protein configuration, junctions and protein-protein linkage at 3-D protein network of yoghurt (Burkus and Temellis, 2005).

One of other factors to describe the viscosity change is related to starters that can produce exo-poly saccharides. These compounds are produced from lactose that can be effective on product viscosity and texture (Tamime and Robinson, 2001). Mainly, lactobacilli are responsible for Exo-polysaccharides (EPS) production. As indicated in the microbiological analysis section, their population in ewe milk yoghurt was more than cow milk yoghurt, which mean more EPS production at ewe milk yoghurt and its higher viscosity. Similar results have been reported by other authors (Fadela *et al.*, 2009; Haenlein *et al.*, 2006; Vasiljevic *et al.*, 2007).

Volatile compounds

Volatile compounds analysis of ewe and cow milk yoghurt is reported in Figure 4. There was no detectible amount of acetone in the analyzed samples, thus it was not reported in the results. Its little amount may be due to detection limit of the used method (Guler et al., 2009). Such results have been reported about lack of other volatile compounds in yoghurt (Xanthopoulos et al., 1994), but Vagenas and Roussis (2012) indicated that ewe milk contained several methyl esters while cow milk did not. Also, Erkaya and Sengul (2011) identified a total of 34 volatile compounds in cows, buffaloes, ewes and goats' yoghurts during their storage at 4°C, including aldehydes, ketones, alcohols. esters. acids. terpenes. hydrocarbons and sulfur compounds. Like their research, in this study, acetaldehyde,

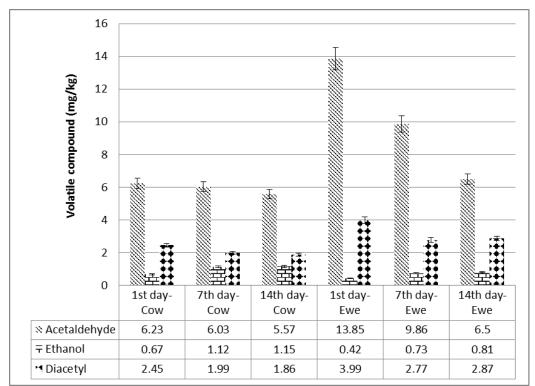


Figure 4. Volatile compounds of cow and ewe milk yoghurt during shelf life.

diacetyl and acetoin, were the major compounds of yoghurt and were detected in all yoghurts.

Acetaldehyde is one of the most important compounds in relation to yoghurt flavor. Acetaldehyde and other flavorful carbonylic produced compounds may be from metabolic pathways or other precursors other than lactose, such as valine, pyruvate, threonine and acetyl phosphate (Erkaya and Sengul, 2011). Although amino acids and peptides may indirectly contribute to yoghurt flavor, but they have ability to produce flavorful compounds via complex reactions. As a result of thermal processing, some free amino acids such as Methionine, Valine and Phenylalanine are produced and converted to flavorful compounds. One of the main acetaldehyde producing pathways is from threenine precursor that in this metabolic pathway, threonine, is transformed to acetaldehyde and glycine via Threonine aldolase. The amount of this amino acid in milk is trace, but increases as a result of proteolysis by Lb. bulgaricus

(Guler et al., 2009). Also, because of higher bufferic capacity and acid production of ewe milk, Lb. bulgaricus overcomes, that can produce more acetaldehyde in comparison with Str. thermophilus (Brazuelo et al., 1995). As shown in Figure 4, VCs contents depended on the voghurt shelf life. At the first day, acetaldehyde had its highest value, then decreased slowly. Higher acetaldehyde content of ewe milk yoghurt may be related to production of acetaldehyde from amino acids degradation, especially Threonine, by Threonine Aldolase. This degradation was mostly done by Lb. bulgaricus. Other studies indicate that high acid yoghurts have more acetaldehyde (Guler and Gursoy-Balci, 2011). Results indicated that this pattern was repeated in our study. While the acidity of ewe milk yoghurt is higher, its acetaldehyde content is more, too. Thus, the high content of free amino acids and acidity cause high level of acetaldehyde in ewe milk yoghurt. During the shelf life, acetaldehyde content decreased. This was with low gradient in cow milk yoghurt, but in ewe milk yoghurt it had more gradient. Always, acetaldehyde content decrease with time, (Tamime and Robinson, 2001). In addition, acetaldehyde easily converts to ethanol by alcohol dehydrogenase enzyme. Alcohol dehydrogenase enzyme is produced by Str. thermophilus and has highest yield at low pH. Acetaldehyde oxidizes easily to acetate at low pH. This reduction of VCs, such as acetaldehyde, may be the reason for decreased acceptability during maintenance of yoghurt. One of the other reasons for acetaldehyde reduction was related to its high volatility and low boiling point that cause its escape from yoghurt surface during shelf life. This reduction has been proved by other studies (Guler et al., 2009; Guler and Gursoy-Balci, 2011).

The highest diacetyl content was detected at the first day of ewe milk yoghurt, though it decreased with continuing shelf life. But in cow milk yoghurt, it increased somewhat at 7th day and decreased again at 14th day. Ekinci and Gurel (2007) reported that the diacetyl content of yoghurt was considerably increased until 7th day and then decreased at the end of the storage. Some researchers have reported that starters do not produce diacetyl (Xanthopoulos et al., 1994), but some others found that diacetyl is generated by pure culture of Lb. bulgaricus (Beshkova et al., 1998). Diacetyl content decreased during shelf life in both yoghurt types. As shown, milk type and shelf life affected diacetyl content. Similar results are reported by others (Bonczar et al., 2002; Guler and Gursoy-Balci, 2011). Some researchers reported that the decrease in diacetyl amount in all voghurts by the end of the storage could be due to hydrolysis by microbial enzymes to form other substances (Erkaya and Sungul, 2011). Diacetyl conversion to acetone was dependent on lipolytic activity of starter bacteria (McSweeney and Sousa, 2000). But we could not detect acetone, maybe because of low diacetyl content or low enzymatic activity of the used starters. Totally, the carbonylic compounds level (acetaldehyde and diacetyle) decreased during shelf life, this can be due to extra starter bacteria activity, evaporation of the compounds from the yoghurt surface, or the activity of starters hydrolyzing enzymes (Beshkova *et al.*, 1998; Tamime and Robinson, 2001). According to the results, in contrast with the two compounds, ethanol content increased during shelf life. Similar results have been proposed by some others (Guler *et al.*, 2009; Guler and Gursoy-Balci, 2011; Bonczar *et al.*, 2002).

CONCLUSIONS

Lactobacilli had better viability than streptococci in ewe milk yoghurt, because of its higher total solids and protein. With regard to higher lactobacilli population in ewe milk yoghurt, its acidity was higher than cow milk yoghurt. At the end of shelf life, the microbial population of ewe milk yoghurt was greater than 10⁸ CFU g⁻¹, thus, it can be healthy and beneficial for human. With regard to viability of bacteria in ewe milk yoghurt, it is possible to use it as a proper milk for probiotic yoghurt production. In addition, ewe milk yoghurt had higher viscosity than cow milk yoghurt during shelf life, thus it is possible to produce a viscose yoghurt. Ewe milk yoghurt had more total solids, protein, and fat. Its higher protein content caused the production of more volatile compounds. In this regard, acetaldehyde has the main role. Higher aromatic compounds of ewe milk yoghurt can create more consumer acceptability.

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مقایسه خواص فیزیکوشیمیایی، میکرویی، رئولوژیکی و ترکیبات فرار ماست حاصل از شیر میش و گاو

م. کرمی

چکیدہ

ماست با قوام بالا و حاوی ترکیبات طعمی فرار، از نظر مصرف کنندگان مقبولیت بیشتری دارد. در این تحقیق دو نوع ماست حاصل از شیر گاو و میش تهیه شد. نمونه های حاصل از نظر خصوصیات فیزیکوشیمیایی، محتوای اسید لاکتیک باکتریها، ویسکوزیته و ترکیبات فرار در روزهای اول، هفتم و چهاردهم انبارداری بررسی و مقایسه شدند. بعلاوه، شمارش و تشخیص باکتریهای لاکتوباسیلوس بولگاریکوس و استرپتوکوکوس ترموفیلوس انجام شد. ماست حاصل از شیر میش، ماده خشک، پروتئین و قدرت تولید اسید بیشتری را نسبت به ماست گاو نشان داد. همچنین مقدار HT نمونه ماست میش انبارداری ۴۱ روزه کاهش یافته و ویسکوزیته نمونه ماست میش در تمام دوره بیشتر از ماست گاو بوده و انبارداری ۴۱ روزه کاهش یافته و ویسکوزیته نمونه ماست میش در تمام دوره بیشتر از ماست گاو بوده و انبارداری ۴۱ روزه کاهش یافته و ویسکوزیته نمونه ماست میش در تمام دوره بیشتر از ماست گاو بوده و انبارداری ۴۱ روزه کاهش یافته دو نوع ماست افزایش یافت. در ارتباط با ترکیبات فرار، مقادیر استالدهید و دی استیل در طول انبارداری کاهش یافت درحالی که اتانول دراین دوره دره دو ماست گاو و میش افزایش یافت.