



Research Article

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## A study on microbiological and physicochemical properties of homemade and small scale dairy plant buffalo milk yoghurts

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### ABSTRACT

In the present study, the physicochemical and microbiological qualities of commercially available yoghurt were investigated. For this purpose, sixty samples of homemade and forty samples of small scale dairy plants buffalo yoghurt were collected, and analyzed in triplicate. The composition of yoghurt samples ranged between for dry matter: 16.93-18.43%, 17.57-18.78%, for fat: 6.76-7.20%, 6.42-7.60%, for protein: 4.71-5.29%, 5.21-5.45%, for lactose: 3.08-3.25%, 3.11-3.21%, for ash: 0.91-0.93%, 0.91-0.94%, for titratable acidity (TA): 0.91-0.93, 1.07-1.12%, for pH: 4.15-4.17, 3.78-4.15, for the small scale dairy plants and homemade samples, respectively. The microbiological content of homemade and small-scale dairy plants yoghurt samples were investigated for the presence of total aerobic mesophilic bacteria (TAMB) *Lactococcus* spp., *Lactobacillus* spp., yeast and mould, coliform bacteria, *Staphylococcus aureus* (*S. aureus*), *Salmonella* spp. and *E. coli*. In general, yeast and mould and coliforms were higher in the homemade buffalo yoghurts. *S. aureus*, *Salmonella* spp. and *E. coli* were not found in any of the buffalo yoghurt samples. The results found do not meet the microbiological criteria of the Turkish Food Codex Communiqué for fermented milk products in terms of coliform and yeast and mould.

**Keywords:** Buffalo yoghurt; homemade yoghurt; physicochemical quality; microbiological quality

### INTRODUCTION

In general, yoghurt is a milk product, composed by using *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* (*S. thermophilus*) bacterial cultures in milk fermentation process[1]. The broad popularity of yoghurt is due to its nutritional value and therapeutic benefits upon consumption[2, 3]. There is an increasing demand for yoghurt, and standard yoghurt is the fastest growing dairy product on the market.

Yoghurt is conventionally produced from cowmilk; however milk from other animals (such as water buffaloes, yaks, goats, horses, and sheep) is also known to be used in the production of yoghurt. Among these, buffalo yoghurt has particular characteristics that distinguish it from the others, including superior industrial yield, high fat and non-fat dry matter contents in and unique sensory attributes. Buffalo yoghurt contains a higher amount of milk fat, protein and total solids, than the other yoghurts. It has a creamy consistency and rich flavour profiles. Communication, modernization, economic growth, education and developed industrialization have also increased demands for buffalo yoghurt, especially in larger cities. In Turkey, almost all of the buffalo milk at hand is processed into yoghurt, which is consequently the most demanded product in its local markets. Production by traditional methods is the most prominent part of the Turkey's Dairy Industry.

Buffalo yoghurt is commonly produced from fresh buffalo milk, without the need increasing the solids with evaporation or other, by small family-operated farms, for both domestic and commercial market needs. In many

cases, incubation conditions are relatively uncontrolled because manufacturing facilities are quite primitive in their authentic practices. Thus, differences in the starter bacterial compositions, incubation temperatures and sanitizing procedures, result in varying qualities of buffalo yoghurt. The traditional manufacturing of buffalo yoghurt is foremost based on knowledge and experience. Due to low quality of raw materials used, the hygienic quality, and without implementation of standard operation procedures, these traditional production methods are inefficient. The quality of buffalo yoghurt varies depending on the regions where milk was collected therefore there are important differences between traditional homemade productions and industrial dairy productions and the quality of products available in the market varies considerably. Generally these traditional production methods are proved to be inefficient[4].

Most of these products are sold in the market without proper quality control and there is a risk to microbial contamination during their shelf life. For this reason, enumeration of microorganisms in buffalo yoghurt should be made, in order to reflect the deficiencies in hygienic practices within homemade and small scale dairy industry products. Stricter sanitary standards are not implemented to regulate quality parameters of dairy producers. Particularly, buffalo yoghurt is prepared in unhygienic conditions and contains a lot of contaminants which may cause health hazards spontaneously.

Regarding the homemade method, non-homogenized, non-standardized, non-fortified raw buffalo milk is heated to boiling point and allowed to cool down to body temperature. Following that, 1-2 weight percent of buffalo yoghurt is added to the milk to be processed. The buffalo milk is then left to get fermentation until it clotted.

The vessel in which the buffalo yoghurt is kept during this souring period is usually not insulated; however, in the coolest areas (village or local home), the vessel is wrapped in cloth, or is put in hold to keep the souring temperature as stable as possible. Small-scale buffalo yoghurt production is, on the other hand, made by the rural dairy plants. These plants produce firmer yoghurt, which is generally preferred by the customers of local market chains. Their heated milk is cooled to inoculation temperature and poured into earthenware pots or plastic pots. Then, starter culture is inoculated into the milk in the container, and the milk is left to incubate at 42-43°C for 3-4 hours. After the incubation, buffalo yoghurt can be used for consumption. As it is not possible to sterilize earthenware pots in small scale dairy plants, they are instead, washed with hot water.

The unlabeled buffalo yoghurt is then sold in either plastic or earthenware pot containers at the local markets. The use of earthenware pots which gives the buffalo yogurt a unique flavour and a thicker texture, are typically preferred by consumers and restaurants. To date, there is minimal comparative research data to characterize the properties of buffalo yoghurt, in Turkey. However, the authentic, traditionally fermented buffalo yoghurt is still highly preferred by most restauranters and their diners, because their authentic cuisine relies on the accompaniment. As there is limited (comparative research) data on the characteristics of Buffalo yoghurt, especially in Turkey, for this purpose, samples of yoghurt from homemade and small scale dairy plants were collected and assessed for their physicochemical and microbiological qualities.

## MATERIALS AND METHODS

### Supplying yoghurt samples

This study was conducted in four periods. In each period, ten samples of buffalo yoghurt produced by different small-scale dairy plants were purchased from commercial markets, and fifteen samples of homemade buffalo yoghurt were purchased from local markets (Table 1). Collected samples were transported (under aseptic conditions in an ice-packed container) to the laboratory and evaluation commenced immediately.

### Physico-chemical analysis

All the yoghurt samples were analyzed in triplicate for total solid and ash (gravimetric), fat content (Gerber method), protein (kjeldahl methods), Lactose (the method of Fehling) and, titratable acidity were determined using the methods described by AOAC [5]. The pH of the yoghurt samples were measured directly using pH meter (Hanna pH meter No. 211).

Wheysyneresis was estimated by measuring the volume of separated yoghurt whey(ml/100 g) [6]. Apparent viscosity was determined by using a RV Brookfield viscometer (Stoughton, USA) [7].

### Microbiological analysis

Ten grams of yoghurt samples were homogenized using vortex (Model: 58816-123, VWR, ABD) stirrer with 90 ml sterile peptone water to obtain a  $10^{-1}$  dilution. Further (ten-fold) serial dilution was made using the same diluents until a dilution of  $10^{-8}$  was obtained. An aliquot of (0.1 ml) suitable dilution was spread plated onto sterile petri

dishes (of suitable media) in triplicates for the enumeration of different organisms. Plates containing 20-200 colonies were enumerated and the results were expressed as colony-forming units, per milliliter (CFU/ml) of yoghurt sample. All the tests conducted in the scope of this study were carried out according to the BAM[8].

**Table 1. Experiment plan of small scale dairy plants and homemade buffalo yogurt samples**

Periods	Samples		
	Small-scale dairy plants	Homemade	Total
January-March	10	15	25
April-June	10	15	25
July-September	10	15	25
October-December	10	15	25
Total	40	60	100

*Lactococcic* counts were carried out on M17 medium (Oxoid CM785) at incubation of 30°C under anaerobic conditions for 48 hours. *Lactobacilli* counts were performed on deMan Rogosa and Sharpe medium (MRS, Oxoid CM361) at the incubation temperature of 30°C under anaerobic conditions (5% CO<sub>2</sub>) for 72 hours. TMAB count was obtained by pour plating 1ml of each dilution into Plate Count Agar (PCA). The colonies were counted after 48 hours of incubation at 35°C[9]. The yeasts and moulds were determined using Potato Dextrose Agar medium (PDA) with 10% tartaric acid. The plates were incubated at 25-28 °C for 48 hours. The CFU's of yeasts and moulds were counted[10]. For coliform test, one ml of sample was plated onto Mac Conkey Agar media. The plates were incubated at 37 °C for 48 hours. Plates showing positive coliforms were subjected to the confirmed test using Brilliant Green Bile Lactose Broth in test tubes with Durham tubes. The test tubes were then incubated at 44 °C. The total and fecal coliforms were counted, respectively Desoxycholate Lactose Agar (DLA) and Violet Red Bile Lactose Agar (VRBLA) after 24 to 48 hours at 37 and 44°C for 48 hours. Each subcultured tube was confirmed positive. For the determination of *E. coli*, a loopful from each positive Lauryl Sulphate Tryptose (LST) tube was streaked on Eosin Methylene Blue Agar (EMB, Oxoid CM69) and incubated at 37 °C for 24-48 hours. Suspicious colonies (dark centered with or without a green metallic sheen) of *E. coli* on EMB Agar were subjected to confirmation tests (IMVIC). Indole (+), Methyl Red (+), Voges Proskauer (-) and Citrate (-) cultures after 24 to 48 hours incubation at 37°C were assessed as *E. coli* type 1[11]. *S. aureus* was grown on Baird Parker Agar (BPA) (Oxoid, CM0275) supplemented with egg yolk-tellurite emulsion (Merck, 1.03785) at 35 °C for 48 hours. The coagulase activity was performed by using Staphaurex Test[12]. Mannitol Salt Agar (MSA) was used for the isolation of *Salmonella* spp. count according to BAM[13]. 25 ml of each sample were pre-enriched in 225 ml of Buffered Peptone Water (BPW) for 24 hours at 37°C. 1 ml of each pre-enrichment culture was used to inoculate 10 ml of Muller-Kauffmann Tetrathionate (MKT) and Selenite-Cystine (SC) enrichment broths, which were incubated at 43 and 35-37°C respectively, for 24 and 48 hours. After thoroughly mixing each enrichment broth, a 3 mm (10 µl) loopful was removed and streaked onto Xylose Lysine Deoxycholate Agar (XLDA).

### Statistical analysis

All microbial counts were converted to the base-10 logarithm of the number of CFU's per ml of buffalo yoghurt samples (log<sub>10</sub>CFU/ml), and their means and standard deviations were calculated. Statistical analysis was conducted using the General Linear Model in SPSS software version 16.0. using one-way analysis of variance (ANOVA) and the means were compared across groups by Duncan's test. All analyses were carried out in triplicate and the significant differences were determined at (P < 0.05).

## RESULTS AND DISCUSSION

### Physicochemical properties of samples

The results of the physicochemical properties of the yoghurt samples obtained from homemade and small-scale dairy plants are presented in Table 2. Dry matter contents of buffalo yoghurts varied between 17.54-18.78% (w/w) for homemade, and 16.93-18.43% for small-scale dairy plants. Homemade yoghurt had higher contents of dry matter compared to the small scale dairy plants. Significant differences (p<0.05) were observed between the homemade yoghurt and small scale dairy plants yoghurt. These results of total dry matter concentration of buffalo yoghurt were higher than those reported by Younus et al.[14], while the values of the present study were lower than those found by Mahmood et al.[15]. Generally, a higher content of dry matter was detected in homemade buffalo yoghurts, which could be attributed to the increased milk concentration used over a longer time of uncontrollable and high temperature pasteurization.

The mean fat contents of homemade and small scale dairy plants buffalo yoghurt ranged between 6.42-7.60 and 6.76-7.20% respectively. Significant differences (P<0.05) were found between those mean values. Bano et al. [16] reported that buffalo milk yoghurt (whole buffalo milk %100) contained 6.45% fat which is much lower than our experimental value. El-Samragy and Samragy[17] reported that yoghurt contained 4.3% fat. According to the

Turkish Food Codex Communiqué of fermented dairy products [18], yoghurt should contain a minimum of 3 per cent fat in whole milk yoghurt, 0.15-3 per cent fat, in fat reduced, and a maximum of 0.15 per cent fat in fat free yoghurt. The variation in buffalo yoghurt fat could be because of the variations in raw milk, applications to reduce fat and practices such as adulteration of milk by adding water or mixing buffalo milk with ewes', goats' and cows' milks.

The concentrations of protein in the small scale dairy plants and homemade buffalo yoghurt were between 4.71-5.29% and 5.21-5.45 %, respectively. The differences in protein were significant between small scale dairy plants and homemade yoghurts ( $p < 0.05$ ). These values are close to those reported by other authors [19, 20]. While, the values of the present study were lower than found by Nahar et al. [21] whose results were higher than those obtained by another author [22]. According to the Turkish Food Codex Communiqué of fermented dairy products [18], yoghurt should contain minimum 5.6 per cent protein in whole milk yoghurt.

**Table 2. Physicochemical composition of small scale dairy plants and homemade buffalo yogurt samples from commercial shops and local markets (n=100) (mean  $\pm$  SD)<sup>A</sup>**

	Periods			
	January-March	April-June	July-September	October-December
Small scale dairy plants buffalo yoghurts				
TS <sup>1</sup> (%)	18,35 $\pm$ 0,12 <sup>c</sup>	16,93 $\pm$ 0,1 <sup>a</sup>	17,35 $\pm$ 0,06 <sup>b</sup>	18,43 $\pm$ 0,04 <sup>c</sup>
Fat (%)	7,20 $\pm$ 0,03 <sup>c</sup>	6,77 $\pm$ 0,05 <sup>a</sup>	6,76 $\pm$ 0,04 <sup>a</sup>	7,09 $\pm$ 0,04 <sup>b</sup>
Ash (%)	0,90 $\pm$ 0,03 <sup>a</sup>	0,93 $\pm$ 0,01 <sup>a</sup>	0,91 $\pm$ 0,02 <sup>a</sup>	0,91 $\pm$ 0,02 <sup>a</sup>
protein (%)	5,29 $\pm$ 0,02 <sup>d</sup>	4,71 $\pm$ 0,05 <sup>a</sup>	4,82 $\pm$ 0,05 <sup>b</sup>	5,20 $\pm$ 0,06 <sup>c</sup>
TA <sup>2</sup> (%)	0,91 $\pm$ 0,0 <sup>a</sup>	0,92 $\pm$ 0,0 <sup>b</sup>	0,93 $\pm$ 0,0 <sup>c</sup>	0,92 $\pm$ 0,0 <sup>b</sup>
pH	4,17 $\pm$ 0,0 <sup>c</sup>	4,16 $\pm$ 0,0 <sup>b</sup>	4,15 $\pm$ 0,0 <sup>a</sup>	4,16 $\pm$ 0,0 <sup>b</sup>
Lactose (%)	3,25 $\pm$ 0,02 <sup>c</sup>	3,17 $\pm$ 0,02 <sup>b</sup>	3,08 $\pm$ 0,02 <sup>a</sup>	3,15 $\pm$ 0,05 <sup>b</sup>
Homemade buffalo yoghurts				
TS <sup>1</sup> (%)	18,60 $\pm$ 0,24 <sup>c</sup>	17,54 $\pm$ 0,12 <sup>a</sup>	17,89 $\pm$ 0,07 <sup>b</sup>	18,78 $\pm$ 0,08 <sup>c</sup>
Fat (%)	7,60 $\pm$ 0,07 <sup>b</sup>	6,44 $\pm$ 0,13 <sup>a</sup>	6,42 $\pm$ 0,14 <sup>a</sup>	7,41 $\pm$ 0,32 <sup>b</sup>
Ash (%)	0,91 $\pm$ 0,02 <sup>a</sup>	0,94 $\pm$ 0,02 <sup>a</sup>	0,93 $\pm$ 0,02 <sup>a</sup>	0,91 $\pm$ 0,03 <sup>a</sup>
Protein (%)	5,38 $\pm$ 0,06 <sup>b,c</sup>	5,24 $\pm$ 0,02 <sup>a</sup>	5,36 $\pm$ 0,04 <sup>b</sup>	5,45 $\pm$ 0,04 <sup>c</sup>
TA <sup>1</sup> (%)	1,07 $\pm$ 0,01 <sup>a</sup>	1,08 $\pm$ 0,03 <sup>a</sup>	1,12 $\pm$ 0,01 <sup>b</sup>	1,10 $\pm$ 0,01 <sup>ab</sup>
pH	4,15 $\pm$ 0,03 <sup>c</sup>	3,84 $\pm$ 0,02 <sup>b</sup>	3,78 $\pm$ 0,02 <sup>a</sup>	4,00 $\pm$ 0,02 <sup>c</sup>
Lactose (%)	3,21 $\pm$ 0,05 <sup>b</sup>	3,13 $\pm$ 0,02 <sup>a</sup>	3,11 $\pm$ 0,01 <sup>a</sup>	3,16 $\pm$ 0,03 <sup>ab</sup>

<sup>A</sup>Values represented as average  $\pm$  standard deviation from triplicate determinations with three separately samples. <sup>1</sup>TS: Total solid, <sup>2</sup>TA: Titratable acidity. a, b, c: Means with different superscript within each column differ significantly by Tukey's test at  $P < 0.05$

The average amounts of lactose in homemade and small scale dairy plants buffalo yoghurt were 3.11-3.21%, 3.08-3.25%, respectively. The mean percentage of lactose was higher in small scale dairy plants buffalo yoghurt than in the homemade. The differences in lactose percent between homemade and small scale dairy plants buffalo yoghurt samples were significant ( $P < 0.05$ ). In our experiment the results agreed with the findings of Mahmood et al. [15], who found that the average value of dahi from buffalo milk was 4.46%. However, the concentration of lactose at the end of fermentation of buffalo yoghurt in our study was lower than the previous studies for buffalo yoghurt reported by authors [20, 22], and the lactose of small scale dairy plants buffalo yoghurt was higher than homemade buffalo yoghurt. This could be due to the slow metabolic activity of the bacteria at low temperatures.

The mean ash contents for small scale dairy plants and homemade buffalo yoghurt were 0.90-0.93% and 0.91-0.94%, respectively. Significant differences ( $P < 0.05$ ) in ash content were found among different homemade and small scale dairy plants buffalo yoghurt. The highest ash content was found in home-made (0.94%) and the lowest ash percent was seen in small scale dairy plants made from buffalo yoghurt (0.90%). Hussein et al. [23] (2011) determined that the average percentage of ash content in buffalo milk yoghurt was 0.89-0.92%. Anwer et al. [19] reported that buffalo milk yoghurt contained 0.82% ash which is much lower than our experimental value. Also, the value of ash observed in this study are in agreement with Nahar et al. [21] which reported that buffalo yoghurt contained 0.98 % ash.

Lactic acid is one of the major products of lactose degradation due to the bacterial fermentation. As shown in Table 2, small scale dairy plants and homemade buffalo yoghurt samples have lower values of titratable acidity in the January-March period, and higher values in the July-September period, respectively, 0.91-1.07%, and 0.93-1.12%. A significant change in titratable acidity between homemade and small scale dairy plants was observed in buffalo yoghurt samples ( $P < 0.05$ ). Younus *et al.* & Han *et al.* found that buffalo yoghurt contained 1.16% and 1.11% lactic acid which are similar to our experimental results [14, 20]. Enb *et al.* reported that buffalo milk yoghurts content were 1.23% and 1.22-1.33% respectively, and these lactic acids are higher than our results [22]. Consequently, the values of lactic acid content obtained for the buffalo yoghurt samples were in agreement with those described by Turkish Food Codex Communiqué of fermented dairy products which minimum acidity of 0.6 per cent is recommended [18]. In this study, increases in titratable acidity of yoghurt samples and increasing total solids of

contents were observed. Also the lactic acid content is in agreement with the lactic acid bacteria content (LABC). The increase of LABC leads to the increase in lactic acid content. The high lactic acid value of homemade buffalo yoghurt might be caused by the insufficient cooling process, after the fermentation stages.

The mean pH value of homemade buffalo yoghurt was 3.78-4.15 and in small scale dairy plants buffalo yoghurt, supplied from the retail stores was 4.15-4.17. Statistical analysis showed that significant difference ( $p < 0.05$ ) existed between pH value of different homemade and small scale dairy plants buffalo yoghurt samples. It was observed that there were significant differences ( $P < 0.05$ ) among the pH values of different local market homemade buffalo yoghurts. However, the homemade buffalo yoghurt was found to include a lower pH value than that of small scale dairy plants buffalo yoghurt. Han et al. and Anjum et al. found that the pH values of buffalo milk yoghurt samples were within the range of 3.89-4.3 and 4.32-4.44, respectively [20, 24]. Similar results were found by Ghadge et al. which revealed that pH value was 4.32 in controlled buffalo yoghurt samples [25]. The pH of homemade and small scale dairy plants buffalo yoghurt were within the accepted level whereas pH of homemade buffalo yoghurt was slightly higher than the small scale dairy plants samples. Because in homemade buffalo yoghurts proper fermentation conditions are not fully controlled, a large variation of pH in the end product is evident. A decrease in pH between the time interval of exhibiting/displaying and selling is caused by insufficient cooling. In addition, there is no proper system of culture dosage in homemade yoghurt which largely affects the acidity of the final yoghurt [26].

Whey separation or syneresis is regarded as a common defect on the surface of buffalo yoghurt and other set-style fermented dairy products alike. The results showed that minimum levels of syneresis were present in small scale dairy plants buffalo yoghurt, while maximum values were present in homemade buffalo yoghurt at 18.74 ml/100g and 21.22 ml/100g, respectively. The syneresis values of yoghurt samples were affected significantly ( $p < 0.05$ ) by homemade and small scale dairy plants. These changes are shown in Table 3. The physical attributes of yoghurts, including the lack of visual whey separation, are crucial aspects of the quality and overall sensory consumer acceptance [27]. The syneresis values from 22.8 to 29.64 per cent were reported for commercial yoghurt in Pakistan [14]. It was found that conditions of fast rates of acidification, compared with slower rates, gave high levels of whey separation [28]. The greater expulsion of whey from buffalo yoghurt samples is possibly linked to the more rapid acidification occurring.

**Table 3. Rheological properties of small scale dairy plants' and homemade buffalo yogurt samples (n=100) (mean  $\pm$  SD)<sup>A</sup>**

Periods	Small scale plant's buffalo yoghurts		Homemade buffalo yoghurts	
	Syneresis (ml/100g)	Viscosity (cp)	Syneresis (ml/100g)	Viscosity (cp)
January-March	18,74 $\pm$ 0,14 <sup>a</sup>	13980,00 $\pm$ 419,40 <sup>c</sup>	19,27 $\pm$ 0,73 <sup>a</sup>	14426,67 $\pm$ 325,63 <sup>c</sup>
April-June	19,58 $\pm$ 0,11 <sup>c</sup>	10650,00 $\pm$ 860,17 <sup>a</sup>	19,89 $\pm$ 0,82 <sup>ab</sup>	12484,67 $\pm$ 770,0 <sup>a</sup>
July-September	19,80 $\pm$ 0,1 <sup>d</sup>	12455,67 $\pm$ 880,44 <sup>b</sup>	21,22 $\pm$ 0,22 <sup>c</sup>	13370,00 $\pm$ 235,80 <sup>b</sup>
October-December	19,19 $\pm$ 0,09 <sup>b</sup>	13963,33 $\pm$ 273,38 <sup>c</sup>	20,75 $\pm$ 0,39 <sup>bc</sup>	14786,67 $\pm$ 120,97 <sup>c</sup>

<sup>A</sup>Values represented as average  $\pm$  standard deviation from triplicate determinations with three separately samples. a, b, c: Means with different superscript within each row differ significantly by Tukey's test at  $P < 0.05$

In our study, homemade yoghurt samples were determined a high acidification rate. The present results are in accordance with those reported by the authors that a higher acidification rate may result in a less developed protein network with fewer protein cross-links leading to a weaker gel that is more susceptible to syneresis [29, 30].

Viscosity, which is a very important quality parameter of yoghurt, is influenced by total solids, fat content and processing techniques [31]. The viscosity values of experimental samples (see Table 3) were lower than those found in the small scale dairy plants product. The viscosity values of small scale dairy plants yoghurt samples were found higher than homemade yoghurt samples, and there were significant differences between homemade and small scale dairy plants yoghurt samples ( $p < 0.05$ ). This result agrees with those reported by authors [19, 23, 32]. The increase in dry matter is very important. It can be noted that, homemade buffalo yoghurt samples had the highest total solids content and viscosity, whilst small scale dairy plants yoghurt samples had the lowest total solids and viscosity value.

### Microbiological properties

Results of microbiological properties of homemade and small scale dairy plant buffalo yoghurt samples were shown in Table 4. The population of *Lactococcus* range from 6.34 to 6.43 log CFU/ml and 7.47 to 7.53 log CFU/ml for small-scale dairy plants and homemade buffalo yoghurt samples, respectively. Statistical analyses showed that significant differences ( $P < 0.05$ ) existed among the homemade and small-scale dairy operators buffalo yoghurt samples. The total count of *Lactococcus* was higher in homemade samples when compared to small-scale dairies samples. In order to have good characteristics of yoghurt, the ratio of *Lactococcus* and *Lactobacilli* should be 1:1. In this study, yoghurt culture assessment showed that the small-scale dairy farmers and homemade samples were

mono-cultured (*Lactococcus*), indicating that it does not fulfill the quality criteria of yoghurt with respect to culture quality. Nevertheless, results were based on a limited number of industries, and further testing with larger numbers is necessary to confirm current results.

Small-scale dairy farmers and homemade buffalo milk yoghurt samples population of *Lactobacilli* range from 6.29 to 6.39 log CFU/ml and 7.31 to 7.49 log CFU/ml, respectively. In both homemade and small-scale dairy buffalo yoghurt samples, *Lactobacilli* counts in July-September were higher than the other months. Statistical analysis showed that significant differences existed between the different homemade and small scale dairy plants buffalo yoghurt samples ( $P < 0.05$ ). Also, in literature, the levels of *Lactobacilli* counts were within the range from 6.10 to 6.68 log CFU/ml in natural yoghurt [1]. It may be due to acidity production in yoghurt, good quality of raw material and good storage conditions.

The total TAMB count was found to be between 7.1 and 7.33 log CFU/ml in small-scale dairy plants buffalo yoghurt and 8.24-8.57 CFU/ml in homemade buffalo yoghurt samples. Statistical analyses for the TMAB counts showed significant differences ( $P < 0.05$ ) for small-scale dairy plants and homemade yoghurt samples during the July-September period. These values are in agreement with the study of Haj et al. who reported that with a TAMB of 7.27 to 7.68 log CFU/ml [33]. The main reason for these relatively high counts of TAMB can be ascribed to inadequate sanitary conditions during yoghurt processing, the lack of good hygienic practices (GHPs), storage time and dairy farmers not refrigerating their products.

Coliforms have probably got more attention than most other groups of bacteria on account of their importance as indicator organisms for predicting unhygienic conditions during production and processing. Coliforms are indicators of poor hygiene and possible contamination with microorganisms of faecal origin [34]. In present study, coliform counts were detected between from 2.36 to 2.78 log CFU/ml and 3.2 to 3.89 log CFU/ml in traditional small-scale dairy operators and homemade buffalo milk yoghurt samples, respectively. This might be the results of the poor hygienic conditions of the production period. The sources of product contamination are probably; unwashed hands of manufacturers, poor quality of water used to clean the earthen pots and exposure of the product to open air during production. The presence of coliform organisms in buffalo yoghurt samples (especially homemade ones) indicates the contamination of yoghurt during their production and handling, which may pose public health problems.

**Table 4. Microbiological properties of small scale dairy plants and homemade buffalo yoghurt samples. ( $\log_{10}$  CFU/ml) (n=100) (mean  $\pm$  SD)<sup>A</sup>**

	Periods			
	January-March	April-June	July-September	October-December
Small scale dairy plants buffalo yoghurts				
<i>Lactococcus</i> spp.	6.34 $\pm$ 0.02 <sup>a</sup>	6.37 $\pm$ 0.04 <sup>ab</sup>	6.43 $\pm$ 0.03 <sup>c</sup>	6.41 $\pm$ 0.03 <sup>bc</sup>
<i>Lactobacilli</i> spp.	6.29 $\pm$ 0.02 <sup>a</sup>	6.33 $\pm$ 0.04 <sup>ab</sup>	6.39 $\pm$ 0.04 <sup>b</sup>	6.38 $\pm$ 0.3 <sup>b</sup>
TMAB	7.12 $\pm$ 0.01 <sup>b</sup>	7.19 $\pm$ 0.03 <sup>b</sup>	7.33 $\pm$ 0.02 <sup>c</sup>	7.10 $\pm$ 0.5 <sup>a</sup>
Yeasts and Moulds	2.70 $\pm$ 0.35 <sup>b</sup>	2.08 $\pm$ 0.04 <sup>a</sup>	2.40 $\pm$ 0.14 <sup>ab</sup>	2.65 $\pm$ 0.16 <sup>b</sup>
Coliforms	2.78 $\pm$ 0.26 <sup>b</sup>	2.54 $\pm$ 0.28 <sup>ab</sup>	2.77 $\pm$ 0.07 <sup>b</sup>	2.36 $\pm$ 0.10 <sup>a</sup>
Homemade buffalo yoghurts				
<i>Lactococcus</i>	7.40 $\pm$ 0.01 <sup>a</sup>	7.46 $\pm$ 0.02 <sup>b</sup>	7.53 $\pm$ 0.01 <sup>c</sup>	7.43 $\pm$ 0.02 <sup>ba</sup>
<i>Lactobacilli</i>	7.31 $\pm$ 0.04 <sup>a</sup>	7.40 $\pm$ 0.02 <sup>b</sup>	7.49 $\pm$ 0.01 <sup>c</sup>	7.40 $\pm$ 0.03 <sup>b</sup>
TMAB	8.24 $\pm$ 0.03 <sup>a</sup>	8.35 $\pm$ 0.04 <sup>b</sup>	8.57 $\pm$ 0.02 <sup>c</sup>	8.25 $\pm$ 0.03 <sup>a</sup>
Yeasts and Moulds	5.38 $\pm$ 0.43 <sup>a</sup>	5.64 $\pm$ 0.19 <sup>a</sup>	6.31 $\pm$ 0.1 <sup>b</sup>	5.69 $\pm$ 0.21 <sup>a</sup>
Coliforms	3.81 $\pm$ 0.29 <sup>b</sup>	3.67 $\pm$ 0.19 <sup>b</sup>	3.89 $\pm$ 0.32 <sup>b</sup>	3.20 $\pm$ 0.17 <sup>a</sup>

<sup>A</sup>Values represented as average  $\pm$  standard deviation from triplicate determinations with three separately samples. a, b, c: Means with different superscript within each column differ significantly by Tukey's test at  $P < 0.05$

The presence of yeast and mould in yoghurts has a substantial effect on organoleptic properties and shelf life of the product. The average yeast and mould count per ml of small-scale dairy plants and homemade buffalo milk yoghurt were from 2.08 to 2.70 log CFU/ml and from 5.38 to 6.31 log CFU/ml, respectively. Statistical analysis showed that yeast and mould between small-scale dairy plants and homemade buffalo yoghurt samples varied significantly ( $P < 0.05$ ). These values were above the limits of 1 and 2 log CFU/ml for yeast and mould [35]. On average, the highest yeasts and moulds counts per ml were found in homemade buffalo milk yoghurt. It is observed that the average yeasts and moulds counts of homemade and small-scale dairy plants samples obviously exceeded the maximum recommended standards by the TFC [18]. Presence of yeasts and moulds was the indication of contamination. High rates of yeasts and moulds colonies were observed in homemade buffalo yoghurt samples. This contamination may be the result of traditional culture (starter) or contamination of post-production or logistic mishandling. These results might be due to the method of buffalo yoghurt manufacturing, inadequate post-fermentation hygiene, handling, especially prior to sale and packaging which are entirely based on the antiquated (traditional) system.

The results indicated that *E. coli*, *S. aureus* and *Salmonella* were not detected in any of the samples tested. The presence of *E. coli* in the environment is considered as evidence of recent contamination with mammalian or avian feces. Because many fecal-borne microorganisms are pathogenic in animals and humans, the presence of *E. coli* in water and foods is indicative of a potential hazard [36].

The presence of *Salmonella* in foodstuffs indicates an internationally accepted human health concern. Currently, *Salmonella* spp. remains a serious foodborne illness risk worldwide according to data of EFSA and FAO/WHO [37, 38].

*S. aureus* is a facultative anaerobic Gram-positive coccus; it is non-motile, catalase, coagulase positive and is the causative agent of staphylococcal food poisoning. *S. aureus* is potentially hazardous at >log 4 CFU/g [39].

Not to be determined *E. coli*, *S. aureus* and *Salmonella* in any of the samples tested on the other hand, the presence of coliform, yeast and mould may be due to the unhygienic environmental conditions, poor processing and mishandling in home conditions, or by air-borne contamination such as coughing and sneezing, (which is common during respiratory infection epidemics) or by similar symptomatic carriers, that come into contact with foods.

### CONCLUSION

This investigation was conducted to compare the microbiological and physicochemical properties of home-made and small-scale dairy buffalo yoghurt samples, because in-store stock qualities vary so drastically. According to the results of microbiological properties, small-scale dairy farmers buffalo yogurt samples are generally of better quality. It was observed an adequate growth of *Lactococci* and *Lactobacilli* bacteria in both home-made and small-scale dairy buffalo yoghurt samples. Additionally, coliform and yeast and mould counts were higher in homemade buffalo yoghurt samples than in small-scale dairy plants samples. High yeast and mould in yoghurt samples generally correspond to unhygienic cleaning techniques and/or inadequate storage conditions.

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