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Physiochemical and microbiological characteristics of commercial and handmade traditional Jordanian yoghurt drink (Shanineh)

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ABSTRACT

Aims: Shanineh is a traditional native buttermilk beverage in Jordan, where it is consumed to a great extent during spring and summer seasons. This study was carried out to evaluate the microbiological and physiochemical properties of handmade Shanineh compared to that commercially produced by dairy plants in Jordan.
Methodology and results: A total of 280 Shanineh samples consisted of 100 handmade samples from different dairy farms (workshops), 170 handmade samples from local markets, and 10 commercially produced samples as controls. Physiochemical characteristics including pH, fat, specific gravity, solid non fat, total solid, salt and viscosity and microbiological quality including coliforms and yeast and mold were evaluated. The results revealed that the physiochemical and hygienic quality of commercially produced Shanineh (control) complied with Jordanian standards, and it was significantly different ($P \leq 0.05$) from the markets or workshops samples in all parameters, Moreover, Shanineh samples that were handmade in small farms or distributed to the markets did not comply with standards because it is produced under uncontrolled conditions. 100% of the workshop samples and 47% of the market samples gave positive results for coliform with a value ranged between (<1 -320 CFU/g), while all of the analyzed samples were highly contaminated with yeast and mold with highly significant difference from the control sample.
Conclusion, significance and impact of study: Handmade Shanineh did not meet the Jordanian standard in term of physiochemical and microbiological parameters and had low hygienic quality compared to commercial one, sources of contaminant should be verified and prevented to ensure the safety of this drink at point of consumption and to decrease the incidence of its health risk on consumers.

Keywords: Shanineh, Physiochemical properties, Microbiological quality

INTRODUCTION

Fermented milk products usually include Yoghurt, Labneh, Acidophilus milk, Raieb, Butter milk, Cultured butter milk, Kefir and Koumiss (Ahmed *et al.*, 2014).Drinking yoghurt is one of the fermented milk products that is categorized as stirred yoghurt of low viscosity and this product is consumed as a refreshing drink (Roseline *et al.*, 2006). Shanineh is a Jordanian yoghurt drink traditionally manufactured by mixing whole or fat free yoghurt that are produced either from cow, sheep or goat milk with water and salt, and consumed especially during hot seasons (Jordanian standard: JS 1648-2005). Similar beverages to Shanineh may usually known as Ayran in Turkey; Lassi and Dahi in Southern Asia; Viili, Tafil and Filmjolk in Scandinavian countries, Doogh in iran; and Laban drink in most Arab countries (Roseline *et al.*, 2006; Hatamikia *et al.*, 2016). However, these products differ from Shanineh in dilution ratio, rheological characteristics, fat content and sensory properties (Roseline *et al.*, 2006). Nowadays, Shanineh is produced in large scales by many Jordanian dairy companies or it can be made at small homemade scale.

Fermented milk products are exclusively cultured dairy products that can be produced either from skim, whole or slightly concentrated milk (Abdalla and Nabi, 2010). Microbial fermentation is usually aims to preserve food products, enhance its nutritive value and to destroy some undesirable factors. Significant changes may occur during fermentation process causing desirable biochemical changes resulting in the development of new aroma, flavor, taste and texture which results in increasing the sensory quality, palatability and acceptability of the product (Sodini *et al.*, 2006; Assefa *et al.*, 2008).

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Therefore, fermented products require specific and appropriate microorganism- lactic acid bacteria -(LAB)- in order to form their specific flavor and texture (Abdalla and Nabi, 2010; Negussie *et al.*, 2012).The action of LAB, that should be viable and active, results in lowering the pH of these products with or without coagulation (Ahmed *et al.*, 2014). The low pH of fermented milk products is reported to increase the bioavailability of some minerals that are required for optimum growth and maintenance of bones, such as calcium, phosphorus and magnesium compared to milk (Ahmed *et al.*, 2014). Furthermore, the proteolysis and acidification process that occur in fermented milk was reported to increase its digestibility comparing to raw milk, this as a result of fine flocculation of caseins that are formed during fermentation. Thus, Consumption of fermented milk play a vital role in significantly increasing immune responses (Roseline *et al.*, 2006; Varga, 2007; Abdalla and Abdel Nabi, 2010). For instance, reports have indicated that consumption of fermented milk products have been associated with alleviation the symptoms of diarrhea and chronic constipation, lowering the risk of colon cancer and lowering blood sugar, blood lipid, and blood pressure levels (Abdalla and Abdel Nabi, 2010). Nutritionally, fermented milk products have also considered an excellent source of protein, riboflavin (vitamin B2), thiamin (vitamin B1), vitamin B12, folate, niacin and zinc (Roseline *et al.*, 2006; Gebreselassie *et al.*, 2016).

Many types of microorganisms (MOs) may contaminate the fermented milk products and cause its spoilage and affect the health status of customer. These MOs includes coliforms, molds, yeasts, as well as various important pathogenic bacteria including enterotoxigenic strains of *Staphylococcus aureus* , enteropathogenic strains of *Escherichia coli*, *Salmonella* spp., *Listeriamonocytogenes*, *Campylobacterjejuni* and *Yersiniaen terocolitica* (Varga, 2007; Ahmed *et al.*, 2014; Hatamikia *et al.*, 2016) .

Shanineh can be produced easily with low cost because it does not require an expensive equipment, but it plays an important role in local behavior and identity of the consumer, the transfer of cultural tradition from one generation to another, and the interaction of this tradition with the rest of the world (Assefa *et al.*, 2008; Rafiq *et al.*, 2014). In traditional lactic acid fermented foods, it is very common to use and follow controlled natural fermentation processes, (Assefa *et al.*, 2008), but there is may be many problem of contamination and adultration during processing (Kristoffersen and Gould, 1966).

Therefore, the objectives of this study were to evaluate the physicochemical attributes and the extent of microbial contamination and microbial quality of shanineh, and to assess its quality and safety conformation with standards, specifications, and regulatory compliance, since this drink have been gaining a great attention and consumption from consumers in Jordan due to its high nutritional value especially vitamins and calcium and their probiotic characteristics compared to soft drinks.

MATERIALS AND METHODS

Sample collection

A total of 280 Shanineh samples (i.e., made from full fat yoghurt) collected during winter of the year 2016, consisted of 100 handmade samples that were collected from dairy farms, 170 handmade samples that were collected from markets and 10 samples were purchased from one brand of modern dairy factory, Zarqa, Jordan.

Collected samples were transported under aseptic conditions in an ice packed container to the laboratory and held at less than 4 °C, and analyzed for chemical and microbiological characteristics which carried out immediately. All parameters were analyzed in duplicate. Results were compared with Jordanian standards and technical working standards of one famous dairy product factory in Jordan as shown in Table 1.

Table 1: Jordanian standard and technical standard for Shanineh product.

Parameter	Jordanian Standard*	Technical standard**
pH	-	3.7-4.2
Fat	≥1.5	≥1.5
Specific gravity	-	1.027-1.030
Solid non fat	>5.5 %	5.5%
Total solid	≥7%	7.7%
Salt	-	0.7-1.1 %
Viscosity	-	14-20%
Coliform CFU/g	0	0
Yeast and mold CFU/g	0	0

* Jordanian standard: JS 1648/2005
** Technical working standards were taken according to dairy factories in Jordan

Physiochemical analysis

pH value of the examined samples was applied according to Kirk and Sawyer (1991). Shanineh sample was taken in to a beaker, electrodes along with temperature probe was inserted to sample. The constant reading appeared on pH meter base was noted and recorded as pH value of shanineh by using Jenway 3540 pH meter (Bibby Scientific Ltd, Staffordshire, UK) which was first calibrated using standard buffer solutions of pH 7.0 and pH 4.0 .

Lipids content was determined by Gerber method according to (Yam *et al.*, 2014). Ten mL ± 0.2 of sulphuric acid (specific gravity 1.820-1.825 g/mL at 15-21 °C) was added into a clean dry Gerber tube. Shanineh was mixed thoroughly and 11.3 mL were measured and added into the tube. Then (1-2 mL) of amyl alcohol was added and the tubes were stoppered. The contents were mixed thoroughly and a centrifugation process was made at 1100 rpm for 4 - 5 min. The fat content was read from the tube after it was soaked in a water bath at 65˚ C for 4 min.

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Total solid content was determined according to the modified method of AOAC (1990). Three grams of the sample were taken and added into dry clean aluminium dish. The dish was heated using steam bath for 10-15 min and transferred in an oven at 70 °C overnight, the samples were cooled in desiccators and re-weighed quickly. Continuous heating and weighing were repeated until the difference between two readings was less than 0.1 mg.

Specific gravity was measured using a lactometer and a thermometer according to (Yilma *et al.*, 2007), NaCl evaluations were measured according to the method cited in (Yam *et al.*, 2014). Viscosity of the samples were determined according to (Sodini *et al.*, 2006), solid non fat was evaluated using a DMA2001 Milk Analyzer (Miris Inc., Uppsala, Sweden).

Microbiological analysis

Preparation of food homogenate and decimal dilutions were applied according to (APHA, 2004). Total coliform count with completed test for *E. coli* was determined according to (BAM, 2002) Violet Red Bile Lactose Agar (VRBA) (Oxoid, UK) were used, then the plates were incubated at 32 °C for 24 h, typical dark red colonies (> 0.5 mm in diameter) were considered as coliforms. The conformation of test was done by transferring up to five typical colonies from each plate to tubes of 2% Brilliant Green Lactose Bile Broth (BGLBB) (Oxoid, UK). After 24 h of incubation at 32 °C the presence of gas bubbles means the presence of coliform. Total yeast and mold count was applied according to (ISO, 1994). A dehydrated media, Potato Dextrose Agar (PDA) from (Hi Media Laboratories Ltd., M096) was used. Cultural characteristics observed after incubation at 20 - 25 °C for 5 days.

Statistical analysis

Data were analyzed using SPSS v 16 (SPSS Inc. Released 2007). LSD -test was used to compare between treatments, correlations between dependent parameters, in addition to descriptive statistics for all parameters.

RESULTS AND DISCUSSION

Physiochemical analysis

Results of physiochemical analysis including pH, specific gravity, total solid, solid nonfat, salt, viscosity and fat are listed below in Table 2.

pH

Table 2 presents pH results of the examined samples. Results showed that pH readings ranged from 3.25 to 3.65 for the market samples, which was lower and significantly different (P≤0.05) from the mean pH readings that found in the workshops samples which was 3.63 with

Table 2: Statistical analytical results of pH in the examined samples of Shanineh.

	Treatment/	Mean ± SD**
pH	Markets	3.45^{b*}±0.13
	Dairy farm	3.63^{a} ±0.08
	Control	4.15^{b} ±0.10
Specific gravity	Markets	1.020^{a}±0.052
	Dairy farm	1.020^{a}±0.034
	Control	1.270^{b}±0.021
Solid non fat	Markets	3.74^{a}±0.96
	Dairy farm	5.96^{b}±0.38
	Control	5.70^{b}±0.25
Total solid	Markets	5.66^{a}±1.04
	Dairy farm	7.15^{b}±1.01
	Control	8.95^{c}±0.90
Salt	Markets	0.51b±0.177
	Dairy farm	0.38a±0.20
	Control	0.85c±0.09
Viscosity	Markets	14.28a±2.39
	Dairy farm	15.87^{a}±0.64
	Control	18.40^{b}±0.53
Fat	Markets	1.45^{b}±0.32
	Dairy farm	0.85^{c}±0.20
	Control	2.40^{a}±0.13

*Means followed with the same letter do not differ significantly according to LSD test
**SD: Standard Deviation

a minimum value of 3.5 and maximum value of 3.75. These low pH values for Shanineh for both workshops and markets was attributed to the conversion of lactose into organic acids (i.e., acetic acid and lactic acid) during fermentation (Ahmed *et al.*, 2014; Negussie *et al.*, 2012; Gebreselassie *et al.*, 2016). pH values, however, is considered lower than the technical standard that was attributed to lack of control over processing conditions, which results in increasing the microbial count and increasing the reduction of pH values, and this may be considered a health threatening food vehicle due to its direct consumption(Gulmez *et al.*, 2003). Similar results were reported by (Ahmed *et al.*, 2014; All and Dardir, 2009; Workul *et al.*, 2015). On the other hand, greater pH values were reported by Gebreselassie *et al.* (2016) with pH value ranging from 4.43 to 4.5. Lower pH values were reported by Roseline *et al.*, (2016) for similar products in Nigeria (i.e., Nono) with pH mean value of 2.87. These

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differences may be due to the variation in temperature and time of incubation of these products, and may also due to the high microbial count that found in Nono compared to other products.

Our results further indicated significant differences between the pH values for the samples that were collected from the market compared to that collected from the workshops were its lower in the markets. Results were attributed to the fact that samples that were taken from the workshops become fresher and distributed directly after processing while that in the markets may become older during displaying and its may mixed directly with the older batch upon receiving in the markets container which may be filled directly without previous cleaning. Additionally, result of uncontrolled handling and inappropriate storage condition of prepared Shanineh that are presents in the markets that may be resulted in increased microbiological contamination may resulted in the decrease in the products pH (Ahmed *et al.*, 2014).

Specific gravity

The specific gravity means for the samples taken from the markets as shown in Table 2 was 1.020 with a value ranged between 1.012-1.029 while the mean value for that taken from workshops equal 1.020 with a range between 1.017-1.028. It's obvious that there are no significant differences between the markets and the workshop, but they will significantly differ from the control sample.

Solid non fat and total solid

The solid non fat content for all of the samples taken from the workshops were within the required limits in all samples comparing to the Jordanian standards, but lower than that in the samples taken from the markets (Table 2). It was also found that there is significant differences (p<0.05) between workshops and markets samples were the total solid was higher in the workshops. These results were attributed to the adulteration process that may occur in some markets by addition of water in the storage container which makes dilution to the solid non fat content, total solid and viscosity (Gulmez *et al.*, 2003). Similar results were found in this study as shown in Table 2, were in both total solid and viscosity the mean value in the workshop samples (7.15, 15.87), respectively were higher than that in the markets samples (5.66,14.28) respectively. Yam *et al.*, 2014 found that total solid in Chal (fermented camel milk) was between 4.1- 5.0.

During long time of fermentation the microbial growth increase and this is results in degradation of protein and converting it to volatile content which results in lowering the solid non fat content and decreasing the viscosity in the markets samples comparing to that in workshops (Ahmed *et al.*, 2014). Highly positive correlation between solid non fat, total solids and the pH value (0.58) and (0.58) respectively were found in this study.

Gebreselassie *et al.* (2016) recorded that Water adulteration lowers the specific gravity and increases the freezing point of milk; normal whole milk has specific gravity range of 1.026 to 1.032 while its freezing point is minus 0.54 °C. Lactometer reading of milk was applied in cooperative units for checking the milk for adulteration. The purpose was to know whether water was added to the milk or not and to decide on rejection criteria (Gulmez *et al.*, 2003).

Salt content

The mean value for the salt content in all of the samples either from markets (0.51%) or workshops (0.38%) were lower than the required technical working specification limits, and there were no significant differences between them, but it's similar to that found in Yam *et al.*, 2014) for chal product and lower than that found in ayran product which was higher than 1% in (Gulmez *et al.*, 2003).

Fat content

The mean value for the fat content of the market samples was 1.45 while that for workshops was 0.85 with no significant difference between them, but both of them were not comply the Jordanian standards. Similar results were found by (Gebreselassie *et al.*, 2016) were the average fat content of all homemade buttermilk samples was 1.17 ± 0.68 g/100 g and was not significantly influenced by either buttermilk type or incubation time and was within the range (0.3–2.5 g/100 g). This is can be explained by the fact that microorganisms can't utilize fat so its value were not changes and because all of the buttermilk samples are made from whole fat yoghurt (Yilma *et al.*, 2007).

Microbial analysis

Coliforms

The mean value for the coliform count for the samples from the markets were lower than that from the workshops (78, 45) CFU/g respectively, were its ranged between 10-320 CFU/g in workshops samples with 100% positive results and between 0-230 CFU/g in market samples with 47% positive results. Ahmed *et al.* (2014) was found that 70% of the buttermilk samples that analyzed were contaminated with coliform. (Hatamikia *et al.*, 2016) reported that 53.3% of commercial ayran samples were not acceptable due to the presence of viable coliform. The high total coliform count in both treatments may be a consequence of the low level of hygiene maintained during the processing and presence of many sources of contamination either from handlers, contaminated utensils, inefficient pasteurization, bad cooling or using highly contaminated water in process (Gebreselassie *et al.*, 2016; Hatamikia *et al.*, 2016). Results that found in the markets may be also due to the bad cooling effect of the storage container and indicate low degree of cleanliness from one batch to another. Also the exposure of product while they are displayed for sale in bowls can serve as source of contamination. Similar

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results were found in Abdalla and Abdel Nabi, 2010). Higher results were found in Ahmed *et al.*, (2014) with a value of 8.8 × 102 CFU/g.

It's also revealed that coliform were lower in workshops comparing to that of markets although that's in workshop were more fresh, this is because the increasing growth of mold and yeast and increasing acidity during time which results in lowering the coliform content (Ahmed *et al.*, 2014).

Yeast and mold

All samples either from a workshop or markets were contaminated with yeast and mold with a very high count, which does not meet the Jordanian standards. Yeast and mold were found as the main contaminant source of fermented milk products, 100% of the buttermilk samples that were found to be contaminated with mold and yeast. Ahmed *et al.* (2014) found that yeast and mold content in butter milk samples were around 4.21 × 104 CFU/g and 2.17 × 104 CFU/g respectively. Yeasts may exist on the surface of production equipment, such as mixing vessels and filling machines that have been poorly cleaned and sanitized this explain the high count of yeast and mold.

Both coliform and mold and yeast contaminated secondarily to Ayran may not have ability to germination unlike for yeast and mold, possibly due to high acidity. Homemade Ayran samples consist of less total solid content due to supplementation of yoghurt with excess water. (Gulmez *et al.*, 2003; Ahmed *et al.*, 2014).

Table 3: Statistical analytical results of the microbiological parameters in the examined samples.

Microorganisim	Sample type	Number of Positive samples	% of Positive samples	Mean ± SD**
Coliform CFU/g	Markets samples	80	47%	45^{b*}± 75
	Dairy farms samples	100	100%	78^{b}±95
	Control	0	0	<1 ^{a}
Yeast and molds CFU/g	Markets samples	170	100%	-
	Dairy farms samples	100	100%	-
	Control	0	0	0

*Means followed with the same letter do not differ significantly according to LSD test
**SD: Standard Deviation

For the control samples that were produced by dairy plant, it was found that all of the results are within the required standards and specification and it's were significantly differed (p≤ 0.05) in all parameters from that in work shops or dairy markets as shown in Table 3, this is because the production inside the factory were under the monitoring of quality control department. So industrially produced Shanineh were founded to be safer for customer, similar results were recorded in (Gulmez *et al.*, 2003) for ayran samples.

CONCLUSION

For the Shanineh that are produced in dairy plants, the results were found to meet the required standards and specification and it was significantly different (p ≤ 0.05) in all parameters both physio-chemical and microbiological from the handmade Shanineh samples that were from workshops and markets.

From the current results we may conclude that handmade Shanineh were highly contaminated and had low hygienic quality compared to commercial one due to low sanitary measures that were taken during production and due to bad handling, storage and distribution.

This poor quality of handmade shanineh considered threat to the health, so certain precautions should be taken in consideration in order to protect the customer health and avoid infections from it. This could be achieved by increase the governmental monitoring on these farms and dairy market, also by increasing the awareness of public mainly the household workers and buyers in the markets. Making an obligatory GMP (good manufacturing practice) training for each person work in this feiled may be required also.

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