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Microbiological and Physicochemical Characteristics of Plain Set Yoghurt Manufactured by Traditional Plants in Khartoum State, Sudan

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Authors' contributions

This work was carried out in collaboration between both authors. Author MOMA designed the study, performed the statistical analysis and wrote the final draft of the manuscript. Author WAGH managed the literature searches, wrote the first draft of the study and managed the analysis of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was conducted to evaluate the microbiological and physicochemical characteristics of plain yoghurt manufactured by traditional plants in Khartoum State.
Methodology: Fifty samples of plain set yoghurt were collected from traditional plants in Khartoum State (Khartoum, Omdurman and Khartoum North towns) at day 1 of manufacture in sterile plastic containers and transported in ice box (4ºC) to the laboratory for microbiological and physicochemical examination.
Results: The results showed that the area of sampling significantly affected the microbiological quality of yoghurt except *Staphylococcus aureus*. Samples from Khartoum had high count of total viable bacteria (TVB), coliform bacteria, lactobacilli and yeasts and moulds counts (Log $_{10}$ 9.76±0.129, Log$_{10}$ 5.95±0.0166, Log $_{10}$ 4.92±1.284 and Log $_{10}$ 4.87±0.067 cfu/g, respectively). TVB and coliform bacteria counts were high in samples from plant B (Log $_{10}$ 9.83±0.117 and Log $_{10}$ 6.02±0.207 cfu/g, respectively), while *S. aureus* count was high in samples from plant E (Log $_{10}$

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4.29±0.039) and lactobacilli and yeasts and moulds counts were high in samples from plant D (Log $_{10}$ 5.25±1.777 and Log $_{10}$ 4.89±0.046 cfu/g respectively). Fat, total solids (TS) and ash contents were high in samples from Omdurman (4.00%±0.946, 12.29%±1.351 and 0.76%±0.039, respectively), while protein content was high in samples from Khartoum (3.63%±0.371) and pH was high in samples from Khartoum North (5.50±0.397). Fat, TS and ash content were high in samples from plant E (4.82%±0.451, 13.48%±0.473 and 0.77%±0.030, respectively), while protein content was high in samples from plant B (3.80±0.251) and pH was high in samples from plant C (5.49±0.397).
Conclusion: Yoghurt from traditional plants was contaminated with microorganisms that may causes diseases to human, so legislations should be adopted to prohibit such plants.

Keywords: Microbiological; physicochemical; traditional plants; yoghurt.

1. INTRODUCTION

Fermented foods are of great importance since they provide and preserve vast quantities of nutritious elements, in a wide diversity of flavors, aromas and textures, which enrich the human diet. Over 3500 traditional fermented foods exist worldwide, which were developed as a means of preserving nutrients [1]. The nature of fermented products is different from one region to another depending on the local indigenous microflora, which in turn reflects the climatic conditions of the area. Traditional fermented dairy products in regions with a cold temperature climate contain mesophilic bacteria such as *Lactococcus* and *Leuconostoc* species, while thermophilic bacteria such as *Lactobacillus* and *Streptococcus* prevail in regions with hot, subtropical or tropical climates [2]. Dairy products are consumed as such or used in preparation of many food items such as pastries, pies and cakes to provide specific functional properties [3]. Yoghurt is a milk product obtained by the fermentation of milk by the action of symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus,* resulting in the reduction of pH with coagulation. These starter micro-organisms are normally viable, active and abundant in the product to the date of minimum durability. If the product is heat-treated the requirement for viable micro-organisms does not apply [4].

Yoghurt is a complete food product that possesses some biochemical and bacteriological characteristics that make it extremely useful in human diets [5]. Traditional yoghurt contains high amount of fat and protein which play an important role in the formation of its sensory properties [6]. The quality of yoghurt curd, similarly to other products obtained as the result of milk fermentation processes, depends on the quality and composition of the applied bacterial cultures. Appropriate proportions used in the bacterial culture precondition their mutual development and, hence, the proper course of the milk protein coagulation process following the acidification of the environment resulting in the formation of the casein gel of ordered network structure [7]. In Sudan, yoghurt is mainly manufactured in authorized dairy plants which comply with the standards set by the government. However, some traditional plants exist illegally and manufacture yoghurt for the market, and these plants may not follow the legal standards, so these products might be hazardous to the consumers. This study is conducted to determine the physicochemical and microbiological characteristics of yoghurt manufactured by traditional plants in Khartoum State.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Fifty samples of yoghurt were collected from traditional plants in Khartoum State [20 samples from Khartoum (10 samples from each of plants B and D); 20 samples from Omdurman (10 samples from each of plants A and E); and 10 samples from Khartoum North (10 samples from plant C)]. The samples were randomly collected at day 1 of manufacture and transported in an ice box (4℃) to the laboratory for analysis immediately upon arrival, otherwise the analyses were carried within 24 hr.

2.2 Physicochemical Analysis of Yoghurt

Fat content was determined by Gerber method [8], while the total protein content was determined by Kjeldahl method [8]. The total solids content was determined by oven drying method [8], while ash content was determined by incinerating the samples at 550℃ for 4 hr

followed by cooling and weighing [8]. The pH was determined using digital pH meter (Super Fit, India). Before use, the pH meter was calibrated with buffer standard solution of pH 4 and 10.

2.3 Microbiological Examination

2.3.1 Preparation of serial dilutions

Eleven grams of yoghurt were dissolved in 99 ml of sterile distilled water to make 10^{-1} dilution, then 1ml from the above-mentioned dilution (10^{-1}) was aseptically transferred to 9 ml sterile distilled water. This procedure was repeated to make serial dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. From each dilution, 1 ml was transferred to Petri dish in duplicate [9].

2.3.2 Total viable bacteria count

Pour plate method was used for the enumeration of total viable bacterial count, 1 ml of sample was deposited in the appropriate dilution in sterile Petri dishes and incubated at 32±1℃ for 48±3 hr [9].

2.3.3 *Staphylococcus aureus* count

Mannitol salt agar was used for the enumeration of *S. aureus*, 1 ml from each sample decimal dilution was streaked on the surface of pre-solidified mannitol salt agar medium and incubated at 37℃ for 48 hr [10].

2.3.4 Coliform bacteria count

MacConkey agar was used to determine the coliform count according to Christen et al. [11]. The plates were incubated at 37℃ for 48 hr.

2.3.5 Lactobacilli bacteria count

Decimal dilutions of the sample were streaked on solidified sterile MRS medium, and plates were incubated at 37℃ for 48±3 hrs [12].

2.3.6 Yeast and mould count

The total count of yeasts and moulds was determined according to Frank et al. [12] using yeast extract agar. The plates were incubated at 25℃ for 5 days.

2.4 Statistical Analysis

Statistical Analysis Systems (SAS, ver. 9) was used for data analysis, and the effect of area from which samples were collected and the plant on physicochemical and microbiological characteristics were determined by general linear model (GLM) procedure. Mean separation was carried out using Duncan multiple range test ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characteristics of Plain Yoghurt

Table 1 presents the effect of area from which samples were collected on the physicochemical characteristics of yoghurt. The area of sampling significantly affected all physicochemical characteristics. Fat, TS and ash contents were significantly ($P<0.001$) higher in samples collected from Omdurman (4.00±0.946%, 12.29±1.351% and 0.76±0.039% respectively), while the protein content was significantly ($P<0.001$) higher in samples collected from Khartoum (3.63±0.371%), and pH was significantly ($P<0.001$) higher in samples collected from Khartoum North (5.50±0.397). All physicochemical characteristics were significantly affected by the manufacturing plant. The fat ($P<0.001$), TS ($P<0.001$) and ash ($P<0.05$) contents were significantly higher in samples collected from plant E (4.82±0.451%, 13.48±0.473% and 0.77±0.030% respectively) and lower in samples from plant B for fat (2.69 ±0.145%), plant C for TS (10.17±0.403%) and ash (0.63±0.031%) contents. The protein content was significantly ($P<0.05$) higher in samples collected from plant B (3.80±0.251%) and lower in samples collected from plant E (2.89±0.166%). The pH was significantly ($P<0.05$) higher in samples collected from plant C (5.49±0.397%) and lower in samples collected from plant B (4.09±0.952) (Table 2). The highest fat content was in plant E in Omdurman area (4.82±0.451%), while the lowest fat content was in plant C in Khartoum North (3.08±0.214%). The highest and lowest protein contents were in plant B in Khartoum (3.80±0.251%) and plant E in Omdurman (2.89±0.166%). The total solids and ash contents were high in plant E in Omdurman (13.48±0.473 and 0.77± 0.030%, respectively), while the lowest contents were in plant C in Khartoum North (10.17±0.403% and 0.63±0.031%, respectively). The pH value ranged between 4.09±953 in plant B in Khartoum and 5.50±0.397 in plant C in Khartoum North (Table 3). The higher fat content in Omdurman is in line with the findings of Abdalla and Adam [13] and disagree with that reported by Younus et al.

[14] and Tarackci and Kucukoner [15]. The fat content of yoghurt in all plants except plant B complies with Sudanese Standards [16] which stated that the minimum fat content should be 3%. The protein content of samples from Khartoum area is similar to that reported by Kucukoner and Tarakci [17] and Elbakri and ElZubair [18]. The lower total solids content in Khartoum North disagrees with the findings of Mohammad and ElZubeir [19] and Younus et al. [14]. In Khartoum North, the ash content is in agreement with the findings of Elbakri and ElZubair [18] and Mohammad and ElZubeir [19]. The pH in Khartoum North is similar to the findings of Khan et al. [20]. In plant E, high fat content may be due to variation in the composition of milk used in manufacture. The result is in line with that reported by Haj et al. [21] and disagrees with the findings of Yuonus et al. [14]. The high content of protein in plant B is in agreement with that reported by Tarakci and Kucukoner [15]. The lowest total solids in plant C is lower than that reported by Ahmad et al. [22] for unbranded yoghurt. Higher ash content in plant E is in accord with the findings of De Silva and Rathnayaka [23] and Eissa et al. [24]. The results of pH in plant B is in accordance with that reported by De Silva and Rathnayaka [23], and high pH in plant C is similar with that reported by Khan et al. [20].

3.2 Microbiological Characteristics of Traditional Yoghurt

Table 4 presents the microbiological characteristics of yoghurt from three areas. All microorganisms under study were significantly affected by the area from which samples were collected except S*. aureus*. Samples from Khartoum were highly contaminated with TVBC (Log 9.76±0.129), coliform bacteria count (Log 5.95±0.016), and yeasts and moulds count (Log 4.87±0.067) and high lactobacilli count (Log 4.92±1.284), while samples from Khartoum North were least contaminated (Log 9.61±0.085, Log 5.86±0.061 and Log 4.78±0.029 for TVBC, coliform bacteria and yeast and moulds count respectively). Table 5 presents the microbiological characteristics of yoghurt collected from different traditional plants. Samples from plant B were highly ($P<0.001$) contaminated with TVB (Log 9.83±0.117) and coliform bacteria (Log 6.02±0.207), while samples from plant D had higher count of lactobacilli (Log 5.25±1.777) and yeasts and moulds (Log 4.89±0.046). However, least contaminated plant with TVB (Log 9.61±0.085), coliform bacteria (Log 5.86±0.061), and yeasts and moulds (Log 4.78±0.030) was plant C. The highest TVB and coliform bacteria counts were in plant B in Khartoum (Log 9.83±0.117 cfu/g and Log 6.02±0.207cfu/g, respectively), while the lowest counts were in plant C in Khartoum North (Log 9.61±0.085 and Log 5.86±0.061 cfu/g respectively). *S. aureus* count ranged between Log 4.23±0.066 cfu/g in plant B in Khartoum and Log 4.29±0.039 cfu/g in plant E in Omdurman. The highest lactobacilli and yeasts and moulds counts were in plant D in Khartoum (Log 5.25±0.178 and Log 4.89±0.046 cfu/g, respectively), while the lowest lactobacilli count was in plant A in Omdurman (Log 4.50±0.046 cfu/g), and the lowest yeasts and moulds count (Log 4.78±0.029 cfu/g) was in plant C in Khartoum North (Table 6). The high TVBC may be attributed to the traditional method of manufacture under poor hygienic conditions. The results are higher than those reported by Yuonus et al. [14] and Akabanda et al. [25], and in line with De Silva and Rathnayaka [23]. Coliform bacteria count in three plants is in line with the findings reported by Abdalla and Hussain [26] and Kucukoner and Tarakci, [17]. High content of bacteria might suggest unhygienic processing of yoghurt [27]. *S. aureus* is high and this may be due to contamination post processing and handling. The result disagrees with that reported by Abdalla and Hussain [26] and Eissa et al. [24]. The result of lactobacilli count disagrees with that reported by Abdalla and Hussain [26]. High yeasts and moulds count may be due to absence of sanitary conditions during processing**,** and the result is in line with that reported by Abdalla and Hussain [26] and El- Ansary [28]. The high TVBC in samples from plant B is in disagreement with that reported by Mohammad and ElZubeir [19], and lower than that reported by Attita Allah et al. [29]. Detection of coliform may be due to poor hygiene and absence of sanitation under traditional conditions. High count of coliform bacteria is in disagreement with that reported by El Baradei et al. [30], higher than the findings of Younus et al. [14]. *S. aureus* in some plants is lower than that reported by Abdalla and Hussain [26], and disagree with the findings of El-Ansary [28]. Lactobacilli count in some plants is lower than that reported by Savadogo et al. [31]. The result of yeasts and moulds count in some plants is lower than that reported by Tarakci and Kucukoner [15]. However, the results are in agreement with those reported by Abdalla and Hussain [26], and disagree with that reported by El-Ansary [28].

Table 1. Physicochemical characteristics of yoghurt samples collected from Khartoum, Khartoum North and Omdurman (Mean±SD)

Physicochemical characteristics	Area			*P*
	Khartoum	Omdurman	Khartoum North	
Fat (%)	3.29±0.621[b]	4.00±0.946[a]	3.08±0.214[c]	<0.0001
Protein (%)	3.63±0.371[a]	2.92±0.455[b]	3.01±0.402[b]	<0.0001
Total solid (%)	11.22±0.566[b]	12.29±1.351[a]	10.17±0.403[c]	<0.0001
Ash (%)	0.69±0.035[b]	0.76±0.039[a]	0.63±0.031[c]	0.0023
pH	4.12±0.666[c]	4..76±0.081[b]	5.50±0.397[a]	0.0014

Means in each row bearing similar superscripts are not significantly different (P>0.05), SD=Standard deviation

Table 2. Physicochemical characteristics of yoghurt samples collected from different traditional yoghurt plants (Mean±SD)

Physicochemical characteristics	Traditional plant					*P*
	A	B	C	D	E	
Fat (%)	3.19±0.476[c]	2.69±0.145[d]	3.08±0.214[c]	3.39±0.107[b]	4.82±0.451[a]	<0.0001
Protein (%)	2.95±0.629[c]	3.80±0.251[a]	3.01±0.402[c]	3.46±0.395[b]	2.89±0.166[c]	<0.0001
Total solid (%)	11.11±0.762[c]	10.91±0.564[c]	10.17±0.403[d]	11.53±0.368[b]	13.48±0.473[a]	<0.0002
Ash (%)	0.74±0.044[b]	0.68±0.039[c]	0.63±0.031[d]	0.70±0.028[c]	0.77±0.030[a]	0.0345
pH	4.75±0.105[b]	4.09±0.952[c]	5.49±0.397[a]	4.14±0.023[c]	4.77±0.049[b]	0.0410

Means in each row bearing similar superscripts are not significantly different (P>0.05), SD= Standard deviation

Table 3. Physicochemical characteristics of yoghurt samples collected from different traditional yoghurt plants in the three areas (Mean±SD)

Area	Physicochemical characteristics					
	Plant	Fat (%)	Protein (%)	Total Solids (%)	Ash (%)	pH
Omdurman	A	3.19±0.476	2.95±0.629	11.11±0.762	0.74±0.044	4.75±0.105
	E	4.82±0.451	2.89±0.166	13.48±0.473	0.77±0.030	4.77±0.049
Khartoum	B	2.69±0.145	3.80±0.251	10.91±0.564	0.68±0.039	4.09±0.953
	D	3.89±0.107	3.46±0.395	11.53±0.368	0.70±0.028	4.14±0.023
Khartoum North	C	3.08±0.214	3.01±0.005	10.17±0.403	0.63±0.031	5.50±0.397

Table 4. Microbiological quality of yoghurt samples collected from Khartoum, Khartoum North and Omdurman (Mean±SD)

Microorganisms	Area from which samples were collected			*P*
	Khartoum	Omdurman	Khartoum North	
TBC	9.76±0.129[a]	9.68±0.090[b]	9.61±0.085[c]	<0.0001
Coliform	5.95±0.016[a]	5.89±0.058[b]	5.86±0.061[b]	0.0025
S. aureus	4.26±0.063[a]	4.28±0.043[a]	4.27±0.038[a]	1.542
Lactobacilli	4.92±1.284[a]	4.28±0.098[ab]	4.49±0.064[b]	0.0425
Yeasts and moulds	4.87±0.067[a]	4.81±0.063[b]	4.78±0.029[c]	<0.0001

Means in each row bearing similar superscripts are not significantly different (P>0.05), SD=Standard deviation

Table 5. Microbiological quality of yoghurt samples collected from different traditional plants in the three towns (Mean±SD)

Microorganisms	Traditional plant					*P*
	A	B	C	D	E	
TVBC	9.69±0.115[b]	9.83±0.117[a]	9.61±0.085[c]	9.69±0.102[b]	9.67±0.057[b]	<0.0001
S. aureus	4.27±0.046[a]	4.23±0.066[b]	4.27±0.038[a]	4.28±0.047[a]	4.29±0.039[a]	.0002
Coliform bacteria	5.89±0.077[b]	6.02±0.207[a]	5.86±0.061[b]	5.88±0.049[b]	5.88±0.030[b]	0.0002
Lactobacilli bacteria	4.50±0.115[b]	4.59±0.033[b]	4.49±0.064[b]	5.25±1.777[a]	4.61±0.026[b]	0.0344
Yeasts and moulds	4.81±0.081c[d]	4.85±0.078[b]	4.78±0.030[d]	4.89±0.046[a]	4.82±0.038[c]	0.0347

Means in each row bearing similar superscripts not significantly different (P>0.05), SD=Standard deviation

Table 6. Microbiological quality of yoghurt samples collected from different traditional plants in the three areas (Mean±SD)

Area	Plant	TVB	Coliform	*S. aureus*	Lactobacilli	Yeasts and moulds
Omdurman	A	9.70±0.115	5.90±0.077	4.27±0.046	4.50±0.115	4.81±0.081
	E	9.67±0.057	5.88±0.030	4.29±0.039	4.61±0.026	4.82±0.038
Khartoum	B	9.83±0.117	6.02±0.207	4.23±0.066	4.59±0.033	4.85±0.078
	D	9.69±0.102	5.88±0.049	4.28±0.047	5.25±0.178	4.89±0.046
Khartoum North	C	9.61±0.085	5.86±0.061	4.27±0.039	4.61±0.026	4.78±0.029

4. CONCLUSION

The results of physicochemical and microbiological characteristics of traditional yoghurt indicated that the area from which samples were collected significantly affected the physicochemical and microbiological characteristics of yoghurt except for *S. aureus* count which was not affected. The plant manufacturing the product significantly affected both physicochemical and microbiological characteristics of yoghurt. Yoghurt from traditional plants was contaminated with microorganisms that may causes diseases to human, so legislations should be adopted to prohibit such plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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