doi:10.5455/vetworld.2013.400-404

ORIGINAL RESEARCH

Microbiological evaluation of yoghurt products in Qena city, Egypt

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Received: 11-10-2012, Accepted: 28-11-2012, Published online: 14-04-2013

How to cite this article:
El-Malt LM, Abdel Hameed KG and Mohammed AS (2013) Microbiological evaluation of yoghurt products in Qena city, Egypt, *Vet World* 6(7):400-404,doi:10.5455/vetworld.2013.400-404

Abstract

Aim: To examined Large and small scale yoghurts for presence of microorganisms of sanitary importance.

Materials and Methods: A total of 100 random samples from various dairy shops, street vendors and supermarkets located in Qena city were collected within 2008 - 2010.

Results: The recorded results show that (92%) and (70%) of the examined small and large scale yoghurt samples had *Psychrotrophic count* with an average of 3.9 x 10^4 and 6.8 x10^3 respectively. It was found that *Enterococci* were detected in (58%) and (40%) with an average count of 1.72 x10^4 and 2.0 x 10^3 for the examined small and large scale yoghourt samples. *S. aureus* were detected in (72%) and (36%) with an average of 8.5 x 10^3 and 9.41 x10^2 for both small and large scale yoghurt samples. Regarding yeast and moulds they were found in (94%) and (40%) with an average counts of 1.4 x 10^4 for small scale and 3.9 x 10^2 of large scale yoghurt samples. Most probable number (MPN) technique showed that [(38), (20)], [(35), (17)] and [(30), (6)] out of the total examined small and large scale yoghurt samples were contaminated with *coliform*, *Fecal coliform* and *E. coli* respectively. The incidence of *E. coli* biotype I and II for examined small scale yoghurt samples were (63.4%) and (36.6%), while for the examined large scale yoghurt samples were (16.7) and (83.3) respectively.

Conclusions: Yoghurt samples obtained from Qena city markets constitute a high risk hazard to consumers. So suggestive hygienic measures to safeguard the consumer health. As well as, the recommended sanitary practices for improving quality of small and large scale yoghurt must be applied.

Key words: *Enterococci*, large and small scale yoghurt, *Psychrotrophs*, *Staphylococcus aureus*

Introduction

Yoghurt is the most popular type of fermented milk in Egypt. The nutritive value of yoghurt is attributed to the fat content, sugar and casein. Therefore, yoghurt is recommended for sick and convalescent people. It is also inhibit the bacterial flora of intestine which may lead to constipation, autointoxication and colitis, as well as, it helps in the absorption of calcium and phosphorus [1].

Psychrotrophic bacteria are ubiquitous bacteria that are able to grow at refrigerated temperature, and their natural habitats are soil, water and animal. Some of *psychrotrophs* affect the quality of dairy products through their production of enzymes, in particular lipase and proteases which are heat stable leading to decrease the keeping quality of milk products. Some members of *Psychrotrophic* bacteria have been implicated as a causal agent of food poisoning [2].

Enterococci may have a distinctive role as indicators of poor factory sanitation owing to their relatively high resistance to drying, detergents, as well as to freezing temperature. Moreover, these organisms are also implicated in food poisoning outbreaks [3]. Several investigators have reported the occurrence of *Enterococci* in dairy products [4].

Coliforms are routinely used as indicator to the quality of the milk and milk products as some members of *coliforms* are responsible for the development of objectionable taints in milk and its products rendering them of inferior quality or even unmarketable [3].

Escherichia coli (*E. coli*) frequently contaminate food and it is a good indicator of fecal pollution [5]. Its presence in milk products indicates presence of other enteropathogenic microorganisms which constitute a public health hazard [5].

Staphylococcus aureus (*S. aureus*) in food article is an index of its contamination from personnel sharing in production and handling. Moreover, Enterotoxigenic *S. aureus* strains may find opportunity to grow and multiply in the food leading to food poisoning among consumers [6].

Presence of yeasts and moulds in milk and dairy products are undesirable even when found in few numbers as they resulting in objectionable changes that render the products of inferior quality [7].

This study aimed to evaluate the microbiological quality of small and large scale yoghurt in Qena city which may be contaminated with microorganisms of sanitary importance that gaining access to product from various sources during production, handling, distribution, and may be implicated in food poisoning.

Materials and Methods

The present study was carried out within April

Table-1. Statistical analytical results of microbiological examination of the examined yoghurt samples

Microbiological examinations	Small scale yoghurt					Large scale yoghurt				
	Positive samples		Counts/ g or ml			Positive samples		Counts/ g or ml		
	No./50	%	Min.	Max.	Average	No./50	%	Min.	Max.	Average
Psychrotrophic count	46	92	1.7×10^3	3.0×10^5	3.9×10^4	35	70	4.0×10^2	6.0×10^4	6.8×10^3
Enterococci count	29	58	2.5×10^2	1.6×10^5	1.7×10^4	20	40	1.0×10^2	1.5×10^4	2.0×10^3
S. aureus count	36	72	3.0×10^2	6.9×10^4	8.5×10^3	18	36	1.1×10^2	5.7×10^3	9.4×10^2
Yeast & mould counts	49	98	2.5×10^2	1.4×10^5	1.4×10^4	20	40	1.0×10^2	1.4×10^3	3.9×10^2

Table-2. Frequency distribution of positive yoghurt samples of *Coliforms*, *Fecal coliforms* & *E coli* based on their MPN

Count / gm	*Coliform*				*Fecal coliform*				*E. coli*			
	SSY		LSY		SSY		LSY		SSY		LSY	
	No./38	%	No./20	%	No./35	%	No./17	%	No./30	%	No./6	%
3 - <10	7	18.4	7	35	9	25.7	17	100	14	46.7	6	100
10–< 10^2	9	23.7	12	60	20	57.2	-	-	14	46.7	-	-
10^2 – < 10^3	8	21.1	1	5.0	4	11.4	-	-	2	6.6	-	-
10^3 – < 10^4	14	36.8	--	--	2	5.7	-	-	-	-	-	-
Total	38	100	20	100	35	100	17	100	30	100	6	100

SSY; Small scale yoghurt, LSY; Large scale yoghurt

2008-January 2010 in the Department of Food Hygiene, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

Collection of samples: One hundred random samples of small and large scale yoghurt "50 samples each" were collected from different dairy shops and supermarkets in their original containers ready for sale.

Preparation of serial dilutions [8].

1. *Psychrotrophic* count [9]:
2. *Enterococci* count [10]:
3. Total *coliforms*, fecal *coliforms* and *E. coli* count using MPN technique [11]:

• Presumptive and confirmed test for coliforms group [11]:
• Confirmed test for *E. coli* count [11]:
• Identification of *E. coli* recovered from the examined samples using [8]:

4. Enumeration and isolation of *S. aureus* [12]:

• Morphological characters for all isolate by staining reaction [8]:
• Biochemical reactions by catalase activity [13], anaerobic mannitol fermentation [14], coagulase test [15]:

5. Total yeast and mould counts [16].

Discussion

It is clearly evident from Table-1, that (92%) and (70%) of the examined small and large scale yoghurt samples were contaminated with psychrotrophs with a count/g was ranged from 1.7×10^3 to 3.0×10^5 with an average count of 3.9×10^4 /g for small scale yoghurt samples. Higher counts were obtained by [17]. While, large scale yoghurt samples had counts ranged from 4.0×10^2 to 6.0×10^4 with an average count of 6.8×10^3 /g. Higher counts were obtained by [18,19]. Lower count was recorded by [20].

The high incidence and counts of psychrotrophic bacteria detected in small scale yoghurt could be attributed to the absence of heat treatment, carelessness during processing, unsatisfactory handling or due to presence of large counts of psychrotrophs in raw milk used in processing. Additionally, most of psychrotrophic bacteria are destroyed by a mild heat treatment as pasteurization. So that, presence of these microorganisms in large scale producers implies post pasteurization contamination and/ or presence of heat resistant or spore former types as *Bacillus* and *Clostridium* [21].

It is clearly evident from Table-1, that (58%) and (40%) of the examined small and large scale yoghurt samples were contaminated with *Enterococci* with a count ranged from 2.5×10^2 to 1.6×10^5 with an average count of 1.7×10^4 /g for small scale yoghurt samples. The obtained results run nearly similar to that obtained by [22]. Lower count was recorded by [23]. Whereas, the *Enterococci* count was ranged from 1.0×10^2 to 1.5×10^4 with an average count of 2.0×10^3 /g for large scale yoghurt samples. Higher counts were obtained by [18, 20, 24]. Lower counts were recorded by [22, 25].

According to the limits proposed by the Egyptian standards [26], we found that (58%) and (40%) of the examined small and large scale yoghurt samples, respectively failed to comply with the limits, (Table 4). The existence of *Enterococci* in yoghurt is an indication of neglected sanitary control measures during production. Moreover, *Enterococci* count is considered more reliable than the *coliforms* count as an index of sanitary quality of yoghurt as they are able to survive the unfavorable microenvironment as the low pH value of yoghurt.

It is clear from the finding in Table-1, that (72%) and (36%) of the examined small and large scale yoghurt samples were contaminated with *S. aureus* with an average of 8.5×10^3 /g for small scale yoghurt samples and an average of 9.4×10^2 /g for large scale yoghurt samples. In case of the examined small scale yoghurt samples, higher counts were obtained by [27]. Lower counts were recorded by [17]. While incase of the examined large scale yoghurt samples, higher

Table-3. Frequency distribution of *E. coli* biotypes recovered from the examined yoghurt samples

Type of yoghurt sample	No. of samples examined	No. of isolated *E. coli*	*E. coli* biotype I		*E. coli* biotype II	
			No.	%	No.	%
Small scale producer	50	30	19	63.4	11	36.6
Large scale producer	50	6	1	16.7	5	83.3

Table-4. Summarized results of microbiological examination of yoghurt samples in compared with the Egyptian standards (Egyptian Organization for Standardization and Quality Control "EOSQC, [26])

Organisms	Standards	Small scale Yoghurt samples				Large scale yoghurt samples			
		Unacceptable		Acceptable		Unacceptable		Acceptable	
		No.	%	No.	%	No.	%	No.	%
Coliforms	Free	38	76	12	24	20	40	30	60
E. coli	Free	30	60	20	40	6	12	44	88
Staph. aureus	Free	36	72	14	28	18	36	32	64
Enterococci	Free	29	58	21	42	20	40	30	60
Yeasts & molds	Not more than 10 /gm	49	98	1	2	20	40	30	60

Table-5. Summarized Results of microbiological examination of yoghurt samples compared with the Food Standards [32]

Organisms	Standards	Small scale Yoghurt samples				Large scale yoghurt samples			
		Unacceptable		Acceptable		Unacceptable		Acceptable	
		No.	%	No.	%	No.	%	No.	%
Coliforms	Acceptable up to 10 /gm	31	62	19	38	13	26	37	74
E.coli	Free	30	60	20	40	6	12	44	88
Coagulase positive *S. aureus*	Acceptable up to 10/gm	36	72	14	28	18	36	32	64

counts were obtained by [20, 28]. Lower counts were recorded by [25, 27]. According to the limits proposed by the Egyptian standards [26], we found that (72%) and (36%) of the examined small and large scale yoghurt samples, respectively failed to comply with the limits, (Table-4).

Presence of *S. aureus* in yoghurt usually indicates contamination from food handlers through hand or arm lesions caused by *S. aureus* or by coughing and sneezing, which is common during respiratory infections or in symptomatic carriers that come in contact with food [29].

Concerning yeasts and moulds it was found in (98%) and (40%) of the examined small and large scale yoghurt samples with count/g was ranged from 2.5 x 10^2 to 1.4 x 10^5 with an average of 1.4 x 10^4 /g for small scale yoghurt samples. Higher counts were obtained by [28]. Lower counts were recorded by [30]. While, large scale yoghurt samples had counts ranged from 1.00 x 10^2 to 1.40 x 10^3 with an average of 3.9 x 10^2 /g (Table 1). Higher counts were obtained by [20, 27, 28]. Lower counts were recorded by [30].

According to the limits proposed by the Egyptian standards [26], we found that (98%) and (40%) of the examined small and large scale yoghurt samples, respectively failed to comply with the limits (Table-4). Yoghurt by nature is a high acid product, therefore it may be considered as a highly selective environmental favoring the growth of yeasts and moulds as spoilage microorganisms, as well as, their presence in yoghurt is being indicative of poor sanitary practices.

Concerning the *coliforms* results in Table-2, indicated that (38) and (20) samples out of the examined small and large scale yoghurt samples, respectively were contaminated with *coliforms*. The highest frequency distribution of *coliforms* in small scale yoghurt samples was (36.8%) and lied in the range of 10^3 - $<10^4$ cfu/g. Higher counts were obtained by [27]. Lower counts were recorded by [31]. Whereas the highest frequency distribution of *coliforms* in large scale yoghurt samples was (60%) and lied within the range of 10 - $<10^2$ cfu/g. Higher counts were obtained by [24, 27]. Lower counts were recorded by [31].

According to the limits proposed by Egyptian standards [26], we found that (76%) and (40%) of the examined small and large scale yoghurt samples failed to comply with the limits, (Table-4). Whereas according to the limits proposed by Food standards [32], we found that (62%) and (26%) of the examined small and large scale yoghurt samples, respectively failed to comply with the limits, (Table-5).

Presence of *coliforms* in such high incidence in small scale yoghurt samples declare neglect sanitary measures reflecting the using of poor quality raw milk, insufficient preheating process, also presence of other enteric pathogens. Moreover, coliforms as an indicator of post processing contamination in yoghurt manufacture has been established and recommended by public health authorities worldwide [33]. Such rate of contamination of the examined large scale yoghurt samples is indicative of post processing contamination as these organisms unable to survive the heat treatment applied during yoghurt manufacture.

These data were surprising when one considered what was stated by [34] that yoghurt wasn't a good medium for *coliforms* as the number of *coliforms* introduced by inoculation decreased rapidly and couldn't be isolated after the 4th day due to the combined

effect of lactic acid and peroxides produced by starter culture. On contrary, [35] proved the aciduric tendency of *coliforms* and found that pH 4.5 or 5 are quite favorable but not equal in encouragement *coliforms* proliferation.

Inspection of the results in Table-2, showed that (35) and (17) samples out of the examined small and large scale yoghurt samples, respectively were contaminated with *fecal coliforms*. The highest frequency distribution in small scale yoghurt samples was (57.2%) and lied in the range of 10 - $<10^2$ cfu/g. The highest frequency distribution of large scale yoghurt samples was (100%) lied in the range of 3 - <10 cfu/g. Higher counts were obtained by [20, 24].

Regarding *E. coli* it was found in (30) and (6) samples out of the total examined small and large scale yoghurt samples. The highest frequency distribution of small scale yoghurt samples was (46.7%) and lied in the range of 3 - <10 and 10 - $<10^2$ cfu/g. Higher result was obtained by [27] and Lower counts were recorded by [30]. However, the highest frequency distribution of large scale yoghurt samples was 6 (100%) and lied in the range of 3 - <10 cfu/g (Table-2).

Similar result was recorded by [28], higher counts were obtained by [24] and lower counts were recorded by [30].

According to the limits proposed by Egyptian standards [26] and Food standards [32], we found that all recorded samples were failed to comply with the limits, (Tables 4 & 5).

The variation between the obtained data and the results showed by other investigators can be attributed to the fact that the survival of *E. coli* in fermented dairy products is highly variable depending on starter cultures used, pH value, and temperature of storage and composition of the products. Such rate of contamination of the examined large scale yoghurt samples is indicative of post processing contamination as these organisms unable to survive the heat treatment applied during yoghurt manufacture.

By inspection of Table-3, we found that the frequency distribution of *E. coli* biotype I and Biotype II for the examined small scale yoghurt samples were (63.4%) and (36.6%). However, for the examined large scale yoghurt samples were (16.7%) and (83.3%), respectively. Higher result for *E. coli* biotype I was obtained by [23].

Conclusion

In light of the above, yoghurt samples obtained from Qena city markets constitute a high risk hazard to consumers. The finding of the study warrant the need to undertake safety measures to avoid potential threats and apply educational programs for dairy products producers about the risk of the contamination of this product, how to prevent or at least reduce this pathogens from the yoghurt and how to apply strict hygienic measures during production, storage and distribution of the yoghurt. Moreover regulation of small scale producing yoghurt in Qena should be a part of a strategy to enhance producing of save and high quality dairy products.

Authors' contribution

LME and KGAH conceived and designed the study. ASM collected and analyzed samples. KGAH drafted and revised the manuscript. All the authors read and approved the final manuscript.

Acknowledgments

Authors are sincerely grateful for the help provided by all members of the Department of Food Hygiene, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

Competing interests

Authors declare that they have no competing interest.

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