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Evaluation of Bacteriological Quality of Packed Ice Creams Sold In Retail Stores in Tripoli City, Libya

Mohamed R. Alsagher*, Izdehar M. Alahdab, Sahar N. Alkhwildi, Najat Elmaghrhi, Ayda Ghalboon and Raja M. Moman

Department of Microbiology and Immunology, Faculty of Pharmacy, University of Tripoli, Tripoli / Libya

*Corresponding author: Prof. Mohamed R. Alsagher

Abstract		Original Research Article

Ice cream is considered as one of the most favorable media for growing of bacterial species and as potential source of food poisoning. The main objective of the present study was to determine the bacterial loads of ice cream. A total of thirty samples of fourteen packed ice cream brands were collected from different retail stores in Tripoli city of Libya. Thereafter, their bacteriological quality were assessed and studied comparatively. Total Bacterial viable count (TBVC), total coliform count (TCC) were determined in plate count agar (PCA). Mannitol salt agar (MSA) and Eosin methylene blue (EMB) agar, *Salmonella-Shigella* agar and *Listeria* agar were used for *Staphylococcus aureus*, *E. coli*, *Salmonella spp*. and *Listeria spp*. count respectively. The results revealed that all samples had TBVC of cfu/g ranging from 1×10^4 to 8×10^4, in which 7 (23.3%) of the ice cream samples fell within the acceptable limit and met the standard plate count according to the Libyan Standard for Ice Cream (LSIC, 1997). By contrast, 7 samples (23.3%) showed zero TBVC. Moreover, 16 sample (53.3%) showed contamination with bacteria in a numbers too numerous to be counted (TNTC). However, on the basis of total coliform count, 20 samples (66.7%) showed high level of contamination. In conclusion, the majority of ice creams have been contaminated with pathogens in particularly *Escherichia coli*. Hence, it is recommend that a greater attention is needed to apply strict microbiological quality control to ensure the safety of final products of ice creams by improving the quality of production technology and sanitation strategies.

Keywords: Bacterial contamination, packaged ice cream, coliform count *Salmonella*, *E. coli*.

INTRODUCTION

Ice cream is one of nutritionally enriched dairy product and commonly consumed as frozen milk and an excellent source of food energy. In addition of milk, ice cream contains a variety of ingredients such as, cream, fat, sugar, emulsifier, stabilizer, colors, an optional enrichment flavors [1], calcium, phosphorous and minerals [2]. As a milk based product with high nutrients value, almost neutral pH value (pH 6-7) ice cream is considered as a good media for microbial growth leading to microbial contamination of the product. Microbial contamination can occur at various stages of manufacturing and during addition of ingredients. Moreover, operators carrying certain diseases are considered as a major source for contaminating the ice cream with potential pathogens especially during handling of the product with contaminated hands. Additionally, factors such as tools, equipment, water, workers, environment, packaging materials, transportation and distribution may contribute to the transmission of microbes into ice cream [3]. Moreover, improper maintenance of machines used for production of soft ice cream can play an important role in microbial contamination especially due to biofilm formation [4-7]. High quality ice cream is associated with the stages of production [8, 9] in which several factors including cleaning and disinfection, hygiene of storage area, hygienic design and personnel training have to be implemented. High bacteria counts and potential public health hazard may occur due failure to adhere to these factors [9]. Hence, strict microbiological quality control must be applied on ice cream manufacturing in order to builds quality into the product assurance the highest possible of error free from any of the pathogens [2].

OBJECTIVE

The aim of this study was to evaluate the bacteriological quality of selected ice cream marketed

in Tripoli city and to assess the potential of this frozen product to pose risk to public health.

MATERIALS AND METHODS
Sample collection
In this study, 30 ice cream samples were collected randomly from different retail stores in Tripoli city of Libya. The samples comprised of fourteen packed/industrial ice cream brands; three samples each was collected for each brand over a period of three months and were assessed for their bacteriological quality.

Total bacterial viable count (TBVC)
Ten grams of ice-cream sample was weighed and diluted in 90 ml of buffer peptone water. One ml of buffered solution was serially diluted in normal saline up to dilutions of (10^{-3}) and (10^{-4}). 100 µl of each dilution was plated out in triplicate onto plate count agar media and incubated at 37°C for 48 h; typical growing colonies were then counted and identified.

Isolation and identification of pathogenic bacteria
TBVC and total coliform count were determined in plate count agar (PCA). Mannitol salt agar (MSA) and Eosin methylene blue (EMB) agar were used for Staphylococcus aureus and E. coli count respectively. For the detection of Salmonella and Listeria, enrichment was done using Selenite F broth and Listeria Enrichment Broth and then plated on Salmonella-Shigella agar and Listeria agar respectively. Typical colonies were selected and their identity was confirmed by standard biochemical reactions [10]. All types of culture media are manufactured by (OXOID-Unipath Ltd. Basings STOKE, Hampshire, U.K.).

RESULTS & DISCUSSION
The bacteriological quality of ice cream consumed by public in Tripoli city was investigated in this study and four bacteria were isolated; Escherichia coli, Staphylococcus aureus, Salmonella spp., and Listeria spp. All the 30 samples of ice cream examined showed positive for one or two types of bacteria, the table 2.1 presents the distribution of all types of bacteria among different types of ice cream. Viable microbial population obtained by standard plate count reflects the microbiological quality of product examined. The results in table 2.1 show that all samples had TVBC of cfu/g ranging from 1×10^4 to 8×10^4, in which 7 (23.3%) of the ice cream samples fell within the acceptable limit and met the standard plate count according to LSIC which are $\le 3 \times 10^4$ and ≤ 10 cfu/ml respectively [11]. By contrast, 7 samples (23.3%) showed zero TVBC. Moreover, 16 sample (53.3%) showed contamination with bacteria in numbers too numerous to be counted (TNTC). These is out of the limit set by the Libyan Standard for Ice Cream (LSIC, 1997) and Bureau of Food and Drugs (BFAD), which are $\le 3\times10^4$ and 2.5×10^5 cfu/ml respectively. Higher percentage of bacterial contamination (80%), TVBC ranged between

3×10^1 and 5×10^8 cfu/ml and the MPN values of coliforms between 0.0 and 11/ml found in ice cream samples collected from different manufactures were reported in the only single study carried out by El-Sharef, et al., [11] in Tripoli city. However, on the basis of total coliform count by MPN technique (table 2.2), bacterial contamination was detected in all samples, in which 20 samples (66.7%) showed high level of contamination (>2.400 MPN/g). This result is beyond limits set by LSIC, and still also beyond the limits set by bureau of Food and Drugs (BFAD), which is 2.5×10^5 cfu/g and 100 MPN coliforms [12]. Bacterial load not more than 10^5 bacteria in ice cream samples reflect good hygiene [23], however values above 10^5 are unacceptable [13]. In this study, the highest count (8×10^4) and the lowest count (1×10^4) were recorded for Pepeto-chocolate ice cream (sample number 28) and Naseem (type bundok) ice cream (sample number 8) respectively. Similar results for other branded ice creams were recorded by Okojoh [14], Moshood and Tengku [15] and Edward et al., [16]. High value of standard plate count (10^6 cfu/g) has also been reported by [17]. The difference in standard plate count from sample to sample of tested ice creams presented in this investigation may be attributed to the different sanitary practices adopted in different ice cream plants and outlets during manufacture, packaging, storage and distribution of the product and also due to addition of optional ingredients after pasteurization of the mix (color, essence, fruit, nut, sauce, etc.). All of the samples tested in this study were negative for Staph. aureus and Listeria spp., while E. coli was detected in 20 ice cream samples (66.67%) and Salmonella spp. in 12 ice cream samples (40%). In the testing for Salmonella, a study conducted by Silveira et al., [18], and Hoffmann et al., [19] after investigating the hygienic-sanitary quality of ice cream resulted positive for Salmonella spp. in 75% of the samples revealing higher value than obtained in this study, also Emad Abou-El Khair, et al., [20] represented that, the highest level of contamination (56%) was occurred with coliform E. coli. According to (TFC, 2011), the presence of Salmonella spp. and Listeria monocytogenes in 25 g of ice cream is not acceptable. In this study 10 g was used in which Salmonella spp. were detected in 40% of ice cream samples. The present work had a result different to the study conducted by Emad Abou-El Khair, et al., [20] and Tolga Kahraman and Kolanciyan on 2016 [22] in which all of tested samples were found negative for Salmonella spp., while only small percentage (0.67%) of samples was found contaminated with Listeria monocytogenes.

The assessment of TBVC has clearly provided information that the ice cream sample of Extreme (sample number 27) was of the superior quality, followed by Yasmina; type. Ella-Ya-Ella (sample number 16) and then Extreme-gold (sample number 27) because the counts were less than the recommended

microbiological standard of US Food and Drug administration [21].

CONCLUSION

In the general context of this study, it can be concluded that, the majority of selected ice cream samples have an unacceptable level of bacterial contamination and did not present satisfactory microbiological quality giving an indication of improper sanitary condition in Tripoli, and may play a significant role in the transmission of potentially vital bacteria causing different diseases, and might pose risks to the consumers' health. Therefore, it's recommend that a greater attention is needed to apply strict microbiological quality control to ensure that the ice cream product received by the consumer will be pure, healthful and of the quality claimed. Accordingly, implementation of effected legislation and proper public educational programs towards maintaining strict hygienic control regarding; production, handling, processing, distribution and storage of ice cream are of great importance in order to protect consumers against health hazard might be raised from contaminated products. Thus, controlling the microbial quality of ice creams is a prime responsibility of the government health authorities to take intensive investigation towards this issue to improve the hygienic quality of ice cream in all steps, post pasteurization and at retail level. The

ice creams tested in the current study were commonly contaminated with some strains of pathogenic bacteria. From the four common bacterial strains (Escherichia coli, Staphylococcus aureus, Salmonella spp., and Listeria spp.) that expected to contaminate the collected samples of ice cream, only two strains of bacterial species were highlighted. The active participation of these two species in descending order of percentage as E. coli (66.67%) and Salmonella spp. (40%) and no growth colonies were observed for Staphylococcus aureus and Listeria spp. The isolation of E. coli, and Salmonella spp. suggests that ice cream could be a source of infection to humans particularly for children and vulnerable elderly people by members of the Enterobacteriaceae [16]. Finally, it is strongly recommended that more studies on this subject would be required on other Libyan cities to report with certainty the percentage and types of pathogenic bacteria causing cross infection among Libyan people due to consumption of ice creams.

Table-2.1: Bacteriological quality of ice cream marketed in Tripoli city, Libya

№	Sample		Coliforms (MPN/g)	TBVC (cfu/gx10^4)	Isolated bacteria			
	Name	Type			E. coli	Salmonella spp.	Staph. aureus	Listeria spp.
1	نسيم	طريفه	> 2.400	0	+	+	-	-
2		لبنى	460	9	+	+	-	-
3		سندباد	> 2.400	0	+	-	-	-
4		عملاق	> 2.400	0	+	-	-	-
5		ممتاز	> 2.400	TNTC	+	-	-	-
6		رامي	> 2.400	TNTC	+	-	-	-
7		نافع	43	TNTC	+	-	-	-
8		بندق	9	1	-	+	-	-
9		فرحان - شوكولاتة	28	0	-	+	-	-
10		فرحان - فراولة	> 2.400	0	-	+	-	-
11		ممتاز	> 2.400	TNTC	+	-	-	-
12	شهرزاد	فستق	43	TNTC	-	-	-	-
13		موجه	> 2.400	TNTC	-	+	-	-
14		فانيليا	> 2.400	9	-	-	-	-
15		البرج	> 2.400	TNTC	+	+	-	-
16	ياسمينه	ياعيلة عيله	7	0	-	-	-	-
17		قنين	240	TNTC	+	-	-	-
18		بحبوح	> 2.400	TNTC	+	-	-	-
19	ثالجه	Turbo	> 2.400	3	+	+	-	-
20		Super Cano	> 2.400	TNTC	+	-	-	-
21	بطريق- كرانش		> 2.400	20	-	+	-	-
22	هناء بنكهة الشكولاته		> 2.400	TNTC	+	-	-	-
23	فيلادلفيا		> 2.400	TNTC	+	+	-	-
24	دولفي		> 2.400	37	+	+	-	-
25	سامبا- المذاق		> 2.400	TNTC	+	-	-	-
26	Extreme		< 3	0	-	-	-	-
27	Extreme-gold		28	TNTC	-	-	-	-
28	Pepeto-chocolate		480	80	+	-	-	-
29	Pepeto-strawberry		> 2.400	TNTC	+	-	-	-
30	Twister		> 2.400	TNTC	+	+	-	-

TNTC, too numerous to count (i.e. bacteria in a numbers over 300 cfu)

Table-2.2: Most Probable Number (MPN) per 1g of sample and 95% confidence limit

№	Sample		Number of positive tubes in three sets of tubes with three different dilutions			MPN/g	MPN Limit	
	Name	Type	0.1	0.01	0.001		Lower	Upper
1	نسيم	طريفه	3	3	3	> 2.400		
2		لبنى	3	3	1	460	71	2.400
3		سندباد	3	3	3	> 2.400		
4		عملاق	3	3	3	> 2.400		
5		ممتاز	3	3	3	> 2.400		
6		نافع	3	1	0	43	7	210
7		رامي	3	3	3	> 2.400		
8		بندق	2	0	0	9	1	36
9		فرحان - شوكولاتة	3	0	0	28	4	120
10		فرحان - فراولة	3	3	3	> 2.400		
11		ممتاز	3	3	3	> 2.400		
12	شهرزاد	فستق	3	1	0	43	7	210
13		موجه	3	3	3	> 2.400		
14		فانيليا	3	3	3	> 2.400		
15		البرج	3	3	3	> 2.400		
16	ياسمينه	عيله	1	1	0	7	1	21
17		فينين	3	3	0	240	36	1.300
18		بحبوح	3	3	3	> 2.400		
19	ثالجه	Turbo	3	3	3	> 2.400		
20		Super Cano	3	3	3	> 2.400		
21	بطريق- كرانش		3	3	3	> 2.400		
22	هناء بنكهة الشكولاته		3	3	3	> 2.400		
23	فيلادلفيا		3	3	3	> 2.400		
24	دولفي		3	3	3	> 2.400		
25	سامبا- المذاق		3	3	3	> 2.400		
26	Extreme		0	0	0	< 3	0.5	9
27	Extreme-gold		3	3	0	240	36	1.300
28	Pepeto-chocolate		3	3	3	> 2.400		
29	Pepeto-strawberry		3	1	1	75	14	230
30	Twister		3	3	3	> 2.400		

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