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Sanitary Evaluation of Frozen Dessert Sold at Local Markets

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ABSTRACT

One hundred samples of ice cream were collected randomly from Alexandria province. All samples were subjected to sanitary evaluation, chemical analysis and microbiological examination. Results revealed that the mean values of total bacterial count in examined packed ice cream samples with flavour of (choccolate, stawberry and vanilla) were ($3.6x10^4 \pm 4.8x10^3$), ($5.7 \times 10^5 \pm 4.5 \times 10^4$) and ($1.5x\ 10^4 \pm 6.6.\times10^3$) respectively. While in Street vended mix ice cream the mean value of total bacterial count were $1.3x10^6 \pm 1.3x10^5$. Results also revealed that percent of total psychotropic bacterial count of examined samples of packed ice cream with different flavour (chocolate, strawberry and vanilla) were 100% for all examined types of ice cream samples respectively, while the mean values ± SEM were ($1.9 \times 10^3 \pm 5.3 \times 10^2$); ($5.1 \times 10^4 \pm 3.1 \times 10^4$) and ($6.9 \times 10^3 \pm 1.3 \times 10^3$) respectively. All unpacked ice cream street vended Mix were positive for psychotropic Microorganisms with a mean values of ($1.3 \times 10^5 \pm 2.4 \times 10^4$). Results also revealed that 20 (80%) of examined unpacked street vended ice cream Mix were contaminated with coliform organisms, with a mean value of ($3.1 \times 10^4 \pm 1.1 \times 10^4$). While the mean values of coliform/gm were ($1.2x10^3 \pm 1.1x10^2$) and ($6.8x10^3 \pm 2.5x10^3$) and ($9.5x10^2 \pm 1.37x10^2$) of packed ice cream with different flavours (chocolate, strawberry and vanilla), respectively. The mean value of Staphylococcus aureus were $3.0 \times 10^3 \pm 1.3 \times 10^2$, $1.3 \times 10^4 \pm 4.5 \times 10^3$ and $2.3 \times 10^3 \pm 6.1 \times 10^2$, respectively, in examined packed ice cream samples. The mean value of Staphylococcus aureus in unpacked street vended ice cream was $2.6 \times 10^3 \pm 5.1 \times 10^2$. Also the results revealed that the incidence of Enterococcus organisms in examined samples of packed ice cream flavored vanilla was 5 (20%). The mean value of Enterococcus organisms/g was $4.1 \times 10^2 \pm 1.9 \times10^2$ in examined strawberry ice cream samples. On the other hand 12 (48%) of examined unpacked street vended ice cream Mix samples were contaminated with enterococci organisms with mean value of $1.8 \times 10^3 \pm 6.9 \times 10^2$.

1. INTRODUCTION

Ice creams, a dairy product with high nutritional value and the presence of easily useable fats, carbohydrate and protein is an ideal environment for spoilage by microorganisms (Frazier and Westhoff, 1978). Ice cream is a nutritionally enriched congealed dairy product consumed by all age groups particularly children, during summer (El-Sharef et al., 2006). It is a combination of milk, sweetener, stabilizers, emulsifier and flavoring agents, egg products, coloring additives and hydrolyzed products of starch (Marshall and Arbuckle, 1996). Any of these ingredients may account for the various specific species of bacteria (Yaman et al., 2006).The high content of nutrients like lactose of ice cream, proteins and its neutral pH (6-7) make it an excellent growth medium for microbes, some of which may cause serious disease and outbreaks like cholera, typhoid and bacillary dysentery in human beings (Ahmed et al., 2009). Bacteriological quality of ice cream reflects

hygienic practice in production and is an indication of good practice in production. It is an indication of food which determines the bacteriological quality of ice creams (Ambily and Beena, 2012).The primary sources of microbial contamination of ice-cream include water and raw milk (raw materials where secondary sources include flovering agents, coloring substances, sanitizer utensils, handling and from contaminated air during processing (Khalil et al., 2009). Ice cream is liable to contamination with different types of microorganisms from different sources during production, processing handling and storage which lead it to be unfit for consumption and may also represent a public health hazard. (Azadnia et al., 2011). The critical part is majority of ice cream consumers are young children including those of vulnerable age (Whitney et al., 2002).

The microbiological characteristic of the ice cream during retail marketing can also be determined by the post-manufacture management of the item as well as efficiency and sanitary environment during frozen storage (Warke et al., 2000).Therefore the aim of this research is to determine the sanitary and bacteriological quality of ice cream offered for public consumption.

2. MATERIAL AND METHODS:

2.1. Collection of the samples:

One hundred samples of ice cream, (75) samples from packed ice cream flavored by chocolate, strawberry and vanilla (25) of each, as well as (25) samples from unpacked street-vendor ice cream mix. The samples were collected randomly from supermarket, patisseries shops and street vendors at Alexandria province. All examined collected samples were obtained as it sold to public and dispatched directly to the laboratoryin an ice box as soon as possible with a minimum of delay to be prepared and examined. All samples were subjected to sanitary evaluation and microbiological examination to judge and give a decision that the products were fit for consumption.

2.1.1. Preparations of ice cream samples:

Ice cream samples were transferred aseptically in a representative portion, not less than 200 grams in sterile polyethylene bag in an ice box at -4 ^{o}C. The sample was divided into two portions; the first was subjected to sanitary evaluation by methylene blue reduction test and chemical analysis while the second portion was subjected to microbiological examination. Ice cream samples were left to melt in a thermostatically controlled water bath at 44^{o}C for more than 15 minutes. Each sample was then thoroughly mixed using a sterile stirrer before being examined.

2.1.2. Sanitary evaluation and chemical analysis of ice cream samples.

Methylene blue reduction test (Harrigan and McCance, 1976).

Fat, sugar and total solids contents of ice cream samples were determined by using Milk Scan (Lacto-scan) (Lacto-star mini 3510 Germany) (IDF Standard, 141: 1996).

2.1.3. Microbiological evaluation of examined ice cream samples.

a) Preparation of ice cream samples according to (APHA, 2003).

b) Total aerobic bacterial count according to ISO (4833: 2003) method.

c) Total psychrotrophic bacterial count: (According to ISO, 2002).

d) Coliform count (MPN/ml adopted by ICMSF, 2002).

Morphological Identification of isolated coliforms according to (APHA, 1992).

e) Staphylococcus aureus count (Baird parker, 1962).

Confirmation of the isolated suspected Staphylococcus aureus organisms (Sonnewirth, and Jarett, 1980).

f) Enumeration of Enterococci (Hartman et al., 2001).

Confirmation of Enterococci according to APHA (1992).

Statistical analysis: The SPSS pocket program for windows was used for the statistical analysis. Values of different parameters were expressed as the Mean ± Standard Error (±SE).

3- RESULTS AND DISCUSSION

Table (1): Grading of the examined ice cream samples according to methylene blue reduction test.

Grade	Reduction time (hours)	Packed ice cream samples						Unpacked samples (Street vended)	
		Chocolate		Strawberry		Vanilla			
		No.	%	No.	%	No.	%	No.	%
1	Over 4	5	24	3	12	7	28	0	0
2	2.5 – 4	6	24	7	28	6	24	0	0
3	0.5 – 2	10	40	10	40	9	36	7	28
4	Less than 0.5	4	12	5	20	3	12	18	72
	Total	25	100	25	100	25	100	25	100

Table (2): Statistical analytical results of fat content of examined samples of ice cream of different flavors.

Flavor/type	No. of examined samples	Positive samples	%	Mean ± SEM	Permissible Limit, ES (2005)	Samples within permissible limit	
					Not less than 6 %	No.	%
Chocolate	25	25	100	6.32±0.36		25	100
Strawberry	25	25	100	6.26±0.54		25	100
Vanilla	25	25	100	6.47±0.85		25	100
Street vended Mix (Unpacked)	25	12	48	5.42±0.34		12	48

Mean with different letters is significant at P<0.05
SEM= Standard error of mean

Table (3): Statistical analytical results of total solids content of examined samples of ice cream of different flavors.

Flavor/type	No. of examined samples	Minimum	Maximum	Mean ± SEM	Permissible Limit ES (2005	Samples within permissible limit	
					Not less than 32 %	No.	%
Chocolate	25	32.01	34.25	33.88±0.89		25	100
Strawberry	25	30.23	33.57	32.75±0.47		20	80
Vanilla	25	31.17	33.75	32.62±0.23		22	88
Street vended Mix (Unpacked)	25	28.91	30.21	29.81±0.94		15	60

Mean with different letters is significant at P<0.05 SEM= Standard error of mean

Table (4): statistical analytical results of sugar content of examined samples of ice cream of different flavors

Flavor/type	No. of examined samples	Minimum	Maximum	Mean ± SEM	Permissible Limit ES (2005)	Samples within permissible limit	
						No.	%
Chocolate	25	7.97	9.81	8.92±0.41	Not more than 12 %	25	100
Strawberry	25	8.91	10.50	9.04±0.17		25	100
Vanilla	25	8.21	10.65	9.15±0.21		25	100
Street vended Mix (Unpacked)	25	11.51	14.01	13.32±1.14		9	36

Mean with different letters is significant at P<0.05 SEM= Standard error of mean

Table (5): Statistical analytical results of Total bacterial count of examined samples of ice cream of different flavors.

Type	No. of examined samples	Positive samples		Minimum	Maximum	Mean ± SEM	ES permissible limit	Samples within permissible limit	
Packed ice cream		No	%					No	%
Chocolate	25	25	100	2.5×10^3	9.6×10^4	$3.6 \times 10^4 \pm 4.8 \times 10^3$	$< 1.5 \times 10^5$/g	25	100
Strawberry	25	25	100	3.5×10^4	9.7×10^5	$5.7 \times 10^5 \pm 4.5 \times 10^4$		12	48
Vanilla	25	25	100	9.0×10^3	2.3×10^4	$1.5 \times 10^4 \pm 6.6 \times 10^3$		25	100
Street vended Mix	25	25	100	1.2×10^5	3.4×10^6	$1.3 \times 10^6 \pm 1.3 \times 10^5$		7	28

Mean with different letters is significant at P<0.05 SEM= Standard error of mean

Table (6): Statistical analytical results of Total pychrotrophic bacterial count of examined samples of ice cream of different flavors.

Flavor/type	No. of examined samples	Positive sample		Minimum	Maximum	Mean ± SEM
		No.	%			
Chocolate	25	25	100	1.8×10^2	1.0×10^4	$1.9 \times 10^3 \pm 5.3 \times 10^2$
Strawberry	25	25	100	2.0×10^3	7.6×10^4	$5.1 \times 10^4 \pm 3.1 \times 10^4$
Vanilla	25	25	100	7.0×10^2	2.3×10^4	$6.9 \times 10^3 \pm 1.3 \times 10^3$
Street vended Mix(Unpacked)	25	25	100	7.6×10^3	4.1×10^5	$1.3 \times 10^5 \pm 2.4 \times 10^4$

Mean with different letters is significant at P<0.05. SEM= Standard error of mean

Table (7): statistical analytical results of Coliforms count of examined samples of ice cream of different flavors.

type	No. of examined samples	Positive sample		Minimum	Maximum	Mean ± SEM	Permissible Limit ES (2005	Samples within permissible limit	
		No	%				< 10/g	No	%
Chocolate	25	14	56	3.0×10^2	1.6×10^3	$1.2 \times 10^3 \pm 1.1 \times 10^2$		11	44
Strawberry	25	17	68	2.0×10^3	3.6×10^4	$6.8 \times 10^3 \pm 2.5 \times 10^3$		8	32
Vanilla	25	13	52	3.0×10^2	4.3×10^3	$9.5 \times 10^2 \pm 1.37 \times 10^2$		12	48
Street vended	25	20	80	1.7×10^3	1.3×10^5	$3.1 \times 10^4 \pm 1.1 \times 10^4$		5	20

Mean with different letters is significant at P<0.05. SEM= Standard error of mean

Table (8): incidence of isolated Coliforms from the examined ice cream of different flavors samples.

Isolates	Chocolate		Strawberry flavor		Vanilla flavor		Street vended	
	No.	%	No.	%	No.	%	No.	%
Citrobacter diversus	1	4	3	12	2	8	-	-
Citrobacter freundii	-	-	-	-	2	8	3	12
Edwardsiella tarda	1	4	2	8	2	8	2	8
Enterobacter agglomerans	1	4	1	4	-	-	-	-
Enterobacter cloacae	2	3	1	4	2	8	2	8
Escherichia coli	6	24	4	16	3	12	10	40
Klebsiella ozaenae	1	4	2	8	-	-	2	8
Proteus mirabilis	2	8	3	12	2	8	-	-
Providencia alcalifaciens	-	-	-	-	-	-	3	12
Providencia rettgeri	1	4	3	12	1	4	3	12
Serratia liquefaciens	1	4	2	8	3	12	-	-
Serratia marcescens	2	8	1	4	-	-	2	8

Table (9): Statistical analytical results of Staphylococus aureus count of examined samples of ice cream of different flavors.

Type	No of examined samples	Postive samples		Minimum	Maximum	Mean ± SEM	Permissible Limit ES (2005)	Samples within permissible limit	
		No	%				free from staph. aureus	No	%
Chocolate	25	13	52	1.2×10^2	1.8×10^4	$3.0 \times 10^3 \pm 1.3 \times 10^2$		12	48
Strawberry	25	16	64	3.9×10^2	5.3×10^4	$1.3 \times 10^4 \pm 4.5 \times 10^3$		9	36
Vanilla	25	15	60	7.0×10^2	7.5×10^3	$2.3 \times 10^3 \pm 6.1 \times 10^2$		10	40
Street vended	25	24	96	1.3×10^3	8.0×10^4	$2.6 \times 10^3 \pm 5.1 \times 10^2$.		1	4

Mean with different letters is significant at P<0.05 SEM= Standard error of mean

Table (10) showed that the incidence of Enterococcus organisms in examined samples of ice cream

Type	No of examined samples	Positive samples		Minimum	Maximum	Mean ± SEM
		No	%			
Vanilla	25	5	20	2.0×10^2	2.0×10^3	$4.1 \times 10^2 \pm 1.9 \times 10^2$
Chocolate	25	0.0	0.0	0.0	0.0	0.0
Strawberry	25	0.0	0.0	0.0	0.0	0.0
Street vended	24	12	48	1.0×10^3	3.0×10^3	$1.8 \times 10^3 \pm 6.9 \times 10^2$

4. DISCUSSION

Ice cream is considered the most popular Egyptian dairy products.

4.1. Sanitary evaluation of ice cream:

4.1.1. Methylene blue reduction test:

It is evident from the results reported in in Table (1) that 5 (24%); 3 (12%); 7 (28%) of examined samples of packed ice cream which flavoured with (Chocolate, strawberry and vanilla) were Grade (1) that Showed methylene blue reduction time for methylene blue test over (4 hours) or more, that was with grade (1) and (good quality samples), While 5 (24%), 7 (28%) and 6 (24%) samples from chocolate, strawberry and vanilla packed ice cream samples that showed reduction time from (2.5 - 4 hours) that were with a (fair quality) for flavored ice cream samples respectively. On the other hand 10(40%) ; 10(40%) and 9(26%) out (25) sample with reduction time of (0.5-2 hours) were with a (poor quality), while 4(12%) ; 5(20%) and 3 (12%) with reduction time of less than (30 minutes) were with a bad quality, Grade (4) for all previous flavoured ice cream samples.

The results of unpacked street vended ice cream samples that reported in Table (4) that 0 (0%); 7 (28%) and 18 (72%) samples showed that Methylene blue reduction time of Methylene blue test were over 4 hours grade (1) quality and (2.5 – 4) hours, grade (2) quality and (0.5 – 2) hours, grade (3) and less than (0.5) hours grade (4) respectively. The results of unpacked samples of ice cream were of poor hygienic quality leading to fast dye reduction. The obtained results were nearly in agreement with those reported by Ayoub (1992).

Methylene blue reduction test used to measure the ability of microorganisms that present in ice cream to grow in dilution kept at temperature ranged from 20-30oc, as the ice cream will be kept in a frozen temperature until the time of consumption. The quality of ice cream is dependent on many factors including storage temperature. Currently, the industry standard for ice cream storage is 28.9°C. Ice cream production costs may be decreased by increasing the temperature of the storage freezer thus lowering energy costs. (Buyck et al., 2011).

4.1.2. Chemical composition: Ice cream contains mainly milk fat (about 10-16%; depending on the standard), non-fat milk solids (about 9-12%), sugar (sucrose) (9-12%), water (55 - 64%) and 0.20- 0.50% stabiliser and/or emulsifier (Silliker et al. 1980).

Results obtained from the chemical analysis of ice cream are presented in Table 2, 3 and 4. Illustrated that the the mean value of fat content of examined ice

cream samples of chocolate, strawberry, vanilla and street vended Mix were 6.32 ± 0.36, 6.26 ± 0.54, 6.47 ± 0.85 and 5.42 ± 0.34, respectively. While the mean value of total solids contents were 33.88 ± 0.89, 32.75± 0.47, 32.62 ± 0.23 and 29.81± 0.94, for examined ice cream samples of chocolate, strawberry, vanilla and street vended Mix, respectively. And the mean value of sugar contents were 8.92 ± 0.41, 9.04 ± 0.17, 9.15 ± 0.21 and 13.32 ± 1.14 for examined ice cream samples of chocolate, strawberry, vanilla and street vended Mix, respectively. From results explaned in table 2 and 4 it is clear that all examined ice cream samples of chocolate, strawberry and vanilla were within permissble limit of ES, 2005 for fat and sugar content. Results recorded in table 2 for fat content cleared that 12(48%) of street vended ice cream were within permissble limit of ES, 2005; Results recorded in table 3 for total solids content cleared that 25(100%), 20(80%), 22(88%) and 15(60%) were within permissble limit of ES, 2005. From the results recorded in table 4 for sugar content it is clear that 9(36%) of examined street vended ice cram were in agreement with ES, 2005. Total soluble solids of the ice cream are contributed by the addition of sweetener into ice cream, besides that, sweetener also gives essential bulk, texture, and body to ice cream (International Dairy Federation, 1998).

The richness in nutritive constituents of ice-cream has been realized by all but the production and handling of this food is very complex and associated with problems (El-Sharef et al., 2006).

4.2. Bacteriological examination of examined ice cream samples

Ice-cream, a milk-based product, is a good media for microbial growth due to high nutrient value, almost neutral pH value (pH ~6-7) and long storage duration of ice-cream (Bell and Kyriakides, 1998). Possible sources of microorganisms in ice-cream have been reported to include raw materials used for the composition of ice-cream mix such as separated milk and milk powder cream, flavouring and colouring substances and stabilizers (Gomez ,1999). However, pasteurization, freezing and hardening steps in the production can eliminate most of the microbiological hazards. However, the potential microbiological hazards found in the final products can still be introduced after pasteurization through adding contaminated ingredients and improper handling procedures (Marshall, 1998).The results indicated the unhygienic conditions prevailing during distribution or sale particularly the ice cream where products are sold in open containers.

4.2.1. Total bacterial count :

The total aerobic counts are a good indicator of general hygiene, permitting the appreciateion of microbial pollution and the general quality of the product. The microbiological quality of milk and dairy products is influenced by the initial flora of raw milk, the processing conditions, and post-heat treatment contamination (Houghtby et al., 1992).The represented data in table (5) revealed that the average total bacterial count in examined packed ice cream samples in examined packed ice cream samples with flavour of (choccolate,stawberry and vanilla) were ranged from ($2.5x10^3$)to ($9.6\ x10^4$) and ($3.5x10^4$) to ($9.7x10^5$) and ($9.0x10^3$) to ($2.3x10^4$) with a mean values of ($3.6x10^4 \pm 4.8x10^3$), ($5.7 \times 10^5 \pm 4.5 \times 10^4$) and ($1.5x\ 10^4 \pm 6.6.\times10^3$) respectively. While in Street vended mix ice cream the total mesophilic count is ranged from 1.2×10^5 to 3.4×10^6 with mean value of $1.3x10^6 \pm 1.3\ x10^5$

These obtained were revealed that 25 (100%) of examined unpacked street vended ice cream mix were contaminated with a percent of 100% Table (5).

Results revealed in Table (5) Revealed that there were a significant difference ($p < 0.05$) in total bacterial count (TBC) between the packed ice cream flavored with chocolate, strawberry and vanilla ice cream. While there is no significant different between packed and unpacked street vended ice cream Mix.

Results obtained from Table (5) were represented to show the comparison between the results obtained from microbiological examination for examined packed and unpacked ice cream samples and the Egyptian standard (2005) were indicated that 25 (100%), 12 (48%) and 25 (100%) of packed ice cream sample of the different flavors (chocolate, strawberry and vanilla ice cream), and 7 (28%) of unpacked ice cream street vended Mix. Respectively.

Higher count of total bacterial than 1.5×10^5 C FU/g. This is the legal limit to Egyptian Standards (2005). Higher results were reported by El-Sharef et al., (2006), Yaman et al., (2006) , Amer (2012) and Ghadimi et al., (2017). While lower results were reported by Yusuf et al., (2013), Hamed (2016),

Jannat et al.,(2016) and Abo El-Makarem (2017). The presence of high bacterial load indicates faecal contamination of the ice cream, inadequate processing and post-pasteurization contamination Koneman et al., (1994).

The ice cream has been classified as a high risk potential hazard which has been implicated in numerous outbreaks of food poisoning (Baraheem et al., 2007). Contamination of ice cream causes several outbreaks of gastroenteritis diseases in a number of countries in diseases in a number of countries in Asia, Europe, Africa and North America (Yaman et al., 2006).

4.3.2. Total psychotrophic bacterial count:

The results revealed in Table (6) showed that percent of total psychotropic bacterial count of examined samples of packed ice cream with different flavours chocolate, strawberry and vanilla were 100% for all examined types of ice cream samples respectively, while the range of total psychotropic bacterial count of examined ice cream of different flavour were (1.8×10^2) to (1.0×10^4); (2.0×10^3) to (7.6×10^4) and (7.0×10^2) to (2.3×10^4) respectively. On the other hand mean values ± SEM were ($1.9 \times 10^3 \pm 5.3 \times 10^2$); ($5.1 \times 10^4 \pm 3.1 \times 10^4$) and ($6.9 \times 10^3 \pm 1.3 \times 10^3$) respectively. The percent of total positive examined samples of unpacked ice cream street vended Mix was also 100% with an average of (7.6×10^3) to (4.1×10^5) with a mean values of ($1.3 \times 10^5 \pm 2.4 \times 10^4$). Psychrotrophic microorganisms are therefore the major contaminants and pathogens associated with ice cream and other foods that are served in frozen or chilled state (Caglayanlar et al. 2009). The presence of these organisms in pasteurized ice cream could be due to their ability to survive the pasteurization process as in the case with spore formers, (Osamwonyi et al. 2011). Lower results were obtained by Mathews et al., (2013). Possible sources of these microorganisms in ice cream have been reported to include raw materials used for the composition of ice cream mix such as separated milk and milk powder, cream, flavouring, colouring substances and stabilizers (Caglayanlar et al., 2009). The major psychrotrophic bacteria found in milk and milk products include species of Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Chromobacterium, Citrobacter, Clostridium, Corynebacterium, Flavobacterium, Lactobacillus, Microbacterium, Moraxella, Serratia, Streptococcus, Pseudomonas, Aeromonas, Enterobacter, Klebsiella, Staphylococcus, Micrococcus, Yersinia, Listeria and Escherichia (Eneroth et al., 2000).

The results presented in Table (6) revealed that there is a significance difference ($p < 0.5$) in total psychotropic counts between packed ice cream flavored by strawberry and the unpacked type. On the other hand there is no significant difference between packed and unpacked ice cream flavored with chocolate and vanilla. Since most of the ice cream is consumed by children and is popular amongst adults, the study of the microbial status of this product is very useful (Hazhir et al., 2006)

4.2.3. Total Coliform count:

Coliform organisms are commonly used to reflect the hygienic status of final products and effectiveness of hygienic practizes in production of ice cream decreasing trend of unsatisfactory rates of ice cream (Kambamanoli-Diamou,(2000). The presented data in Table (7) revealed that 14 (56%), 17 (68%) and 13 (52%) of examined packed ice cream samples flavored chocolate, strawberry and vanilla were contaminated with coliforms organisms respectively.

The contamination levels with coliform/gm were varied from (3.0×10^2) to (1.6×10^3) and (2.0×10^3) to (3.6×10^4) and (3×10^2) to (4.3×10^3) respectively. With a mean values of ($1.2 \times 10^3 \pm 1.1 \times 10^2$) and ($6.8 \times 10^3 \pm 2.5 \times 10^3$) and ($9.5 \times 10^2 \pm 1.37 \times 10^2$) respectively.The most contaminated examined ice cream samples were strawberry, followed by chocolate and vanilla flavour packed ice cream respectivi. The most contaminated examined samples were strawberry, followed by chocolate and vanilla flavor packed ice cream.

Results obtained in Table (7) showed that 20 (80%) of examined unpacked street vended ice cream Mix were contaminated with coliform organisms, with a level varied from (1.7×10^3) to (1.3×10^5) to with a mean value of ($3.1 \times 10^4 \pm 1.1 \times 10^4$). Higher counts were reported by Mohamed (2011) and Jannat et al., (2016) while lower counts were reported by Azadnia et al (2011), Osamawonyi et al., (2011), Surva et al., (2015) and Abo El-Makarem (2017). Similar counts were recorded by Amer (2012) and Gihan Mohammed et al., (2013). Higher incidence of Coliforms is examined ice cream samples were reported by Khalil et al., (2009). Lower incidences of Coliform were

reported by Baraheem et al., (2007). Similar incidences of coliform were reported by Abou Elkhair et al., (2014).The presence of Coliform bacteria indicates the presence of faecal contamination in food. This suggests the possibility of presence of other enteropathogenic bacteria in the food (Baraheem et al., 2007).The Egyptian Standards, (2005) for ice cream have proposed à limit of total coliform to be less than 10MPN/g. Based on the Egyptian specification < 10/g coliform organisms in ice cream Egyptian standard (2005).As the data presented in table (7) that indicated that 11 (44%), 8 (32%) and 12 (48%) of packed ice cream samples and 5 (20%) of unpacked street-vended ice cream samples, respectively had higher count of total coliform thatn < 10/g which is the legal limit Egyptian standards, (2005).

Data presented in Table (7) revealed that there is à significant difference ($p < 0.05$) in total coliform count between packed ice cream flowered by strawberry and the respective unpacked type. On the other hand there is no significance difference between packed and unpacked ice cream street vended ice cream Mix.The difference between the presence investigation results and the previous studies may be attributed to sampling techniques, sources of sampling, handling of samples and media used. It is evident from the results that coliforms organisms contaminate high percent of street vended ice cream Mix. This contamination could be attributed to poor hygienic quality measures of ingredients, neglecting of strict sanitization techniques and prolonged storage period of the ice cream. Coliform being non- spore formers should be susceptible to pasteurization. Their post pasteurization presence in ice-cream may be due to faulty heat process or to post pasteurization contamination by handlers with poor sanitary practices. The level of presence of these organisms in food has been described as index of food hygiene (Hobbs and Golbert ,1982). The presence of high level of fecal coliforms contamination represents a public health risk due to the possible presence and transmission of pathogens such as enteropathogenic Escherichia coli, may also be present in the ice cream (Windrantz and Arias 2000).

Results illustrated in Table (8) showed that all the enteric organisms that isolated from packed ice cream with the different flavours of chocolate, strawberry and vanilla were Citrobacter diversus (4%, 12%, 8%), Citrobacter freundii (0.0%, 0.0%, 8%), Edwardsiella tarda (4%, 8%,8%) , Enterobacter agglomerans (4%, 4%, 0.0%), Enterobacter cloacae (8%, 4%, 8%), Escherichia coli (24%, 16%, 12%), Klebsiella ozaenae (4%, 8%,0.0%); Proteus mirabilis (8%, 12%,8%),Providencia alcalifaciens (0.0%, 0.0%,0.0%), Providencia rettgeri (4%, 12%, 4%), Serratia liquefaciens (4%, 8%,12%) and Serratia marcescens (8%, 4%,0.0%),respectively. On the other hand the results illustrated in Table (5) cleared that the enteric organisms isolated from unpacked street vended ice cream were Citrobacter diverse (0.0%); Citrobacter freundii (12%); Edwardsiella tarda (8%); Enterobacter agglomerances (0.0%); Klebsiella ozaenae (8%); proteus mirabilis (0.0%); providencia alcalifaciens (12%); providencia rettgeri (12%); serratia liquifaciens (0.0%) and serratia marcescens (8%) respectively. The most isolated strains were detected by Baraheem et al., (2007); Abou-Elkair et al., (2014) and Abo El-Makarem (2017).

This result showed that most of all examined samples were contaminated with coliform organisms this could be through light upon the neglecting of hygienic measures during processing, packaging, storage and distribution. E. coli is considered as an indicator of faecal contamination of foods and emergence of some pathogen strains and causes severe concern (Aslani and Alikhani, 2009). High rate of Escherichia coli were found in packed ice cream and most high percent 40% for unpacked street vended ice cream similar results were detected by Abou El- Khair (2014). Higher results were obtained by Abo El-Makarem (2017). Lower incidence of E.coli were reported by Bagudu (2015) and Ghadimi et al., (2017). E.coli is among many pathogenic microorganisms which can get access to milk and dairy products and is considered as a reliable indicator of contamination by manure, soil, and contaminated water (WHO, 2004). Unsafe sources, contaminated raw food items, improper food storage, poor personal hygiene during food preparation, inadequate cooling and reheating of food items, and a prolonged time lapse between preparing and consuming food items have been identified as contributing factors for outbreaks of food borne diseases FAO, (2004). E. coli are often used as an indicator microorganisms, and high populations of E. coli imply a risk that other enteric pathogens may be present in the sample (Arafa and Soliman, 2013). Citrobacter freundii has been found among intestinal infection. Klebsiella

pneumoniae is associated with more severe disease and greater patient mortality than other Gram-negative bacteria (Yu et al., 2007).

4.2.4. Staphylococcus aureus Count:

Staph. aureus is considered the third most important cause of disease in the world among the reported foodborne illnesses (Boerema et al., 2006).The bacterium Staph. aureus is a most important agent of dairy products quality-deterioration worldwide (Asao et al. 2003). Milk and dairy products play an important role, since enterotoxigenic strains of Staph. aureus have been frequently isolated in them (Pelisser et al., 2009).

Nevertheless Staph. aureus can still be destroyed through heat treatment when present in milk and other food products (Banwart, 1998). Although heat may kill Staph. aureus cells the enterotoxin may persist because in food, it is more heat stable than the micro-organism (Banwart, 1998). A recent survey revealed that staphylococcus aureus was involved in 15% of recorded foodborne illnesses caused by dairy products in eight developed countries (Debuyser et al., 2001).

Results obtained in Table (9) Revealed that Staphylococcus aureus organisms could be detected in 13 (52%); 16 (64%) and 15 (60%) of examined packed ice cream samples flavourd of Chocolate, strawberry and vanilla respectively. The minimum count of staphylococcus aureus was 1.2×10^2, 3.9×10^2 and 7.0×10^2, while the maximum count was 1.8×10^4, 5.3×10^4 and 7.5×10^3 respectively. With a mean value of $3.0 \times 10^3 \pm 1.3 \times 10^2$, $1.3 \times 10^4 \pm 4.5 \times 10^3$ and $2.3 \times 10^3 \pm 6.1 \times 10^2$ respectively in examined packed ice cream samples. Lower counts were obtained by Abo El-Makarem (2017). Higher contamination level were recorded by Ghadimi et al., (2017). Lower incidence were reported by Rahimi (2013) and Abd El Tawab et al. (2016).The most contaminated ice cream samples were strawberry followed by vanilla and chocolate ice cream. Table (9) revealed that the staphylococcus aureus could be detected in 24 (96%) of unpacked street vended ice cream Mix. The minimum count of staphylococcus aureus in unpacked street vended ice cream was 1.3×10^3 while the maximum was 8.0×10^4 with a mean value of $2.6 \times 10^3 \pm 5.1 \times 10^2$. The result obtained from table (9) revealed that 12 (48%), 9 (36%) and (10) 40% of packed ice cream samples within permissible limit while 13(52%), 16 (64%) and 15 (60) were failed to be parallel with the Egyptian Standard, (2005).

The possible sources of these bacteria in ice-cream could be from nose where it is commonly found in hands, skin and clothing of handler Hobbs and Golbert (1982).

IDF (1996) set the number of Staph. aureus in frozen milk based products should not exceed 100/g, Staph aureus is leading cause of foodborne intoxication (minimum infection dose $10^5 – 10^7$/gor 1-20ug enterotoxin per/person (Luca et al., 1997). De Buyser et al. (2001) reported that Staphylococcus aureus was by far the most frequent pathogen associated with food pathogen outbreaks (85.5% of the outbreaks) in France.

Egyptian standard (2005) stipulates that ice cream must be free from Staph. aureus. The obtained results were revealed that all positive ice cream samples contain counts more than legal standards. Data presented in Table (9) revealed that there is à significant difference ($p < 0.05$), in Staphylococcus aureus count between packed ice cream flavored chocolate, strawberry, and vanilla. While no significant difference between packed and unpacked ice cream samples.Staph. aureus is potentially hazardous at $> 10^4$ cfu/ml (Han et al., 2005).The Present of results revealed in Table (9) shows that the mean with a significance difference ($p < 0.05$) in staphylococcus aureus count between packed ice cream flavored by strawberry and respective unpacked type.

While there is no significance difference between packed and unpacked street vended ice cream Mix. The presence of Staph. aureus in ice cream can be due to poor hygiene practices of handlers. This microorganism, is naturally found on the hands, nasal cavity and skin of humans (Rostamzad and Rostamneia , 2016), therefore droplets containing

microbe might be produced during coughing, talking and sneezing, which could settle on ice cream (Shamila-Syuhada et al., 2016). The growth of Staph. aureus in foods presents a potential public health hazard because many strains of Staph. aureus produce enterotoxins (SEs) that cause food poisoning if ingested (Boerema et al., 2006).

It is, therefore, imperative that education and training in good food hygiene practices should be imparted to all food handling workers (Pal, 2012).

4.2.5. Enterococci count:

The reasons for the prevalence of enterococci in dairy products has long been considered a result of unhygienic conditions during milk collection and processing, together with their resistance to pasteurization temperatures and their adaptability to different substrates and growth conditions (Giraffa, et al.,1997), The major responsible for human enterococcal infections is E. faecalis followed by E. faecium and with a much lower incidence by other enterococcal species (Mundy, et al., 2000) . In the last decade Enterococci have been reported as the second most common cause of wound and urinary tract infection and the third most common cause of bacteraemia (De Fa´tima Silva Lopes et al., 2005).

Data recorded in Table (10) showed that the incidence of Enterococcus organisms in examined samples of packed ice cream flavored vanilla was 5 (20%). On the other hand all examined samples of chocolate and strawberry flavor were Enterococcus organisms negative. The level of contamination with Enterococcus organisms/g was varied from (2.0×10^2) to (2.0×10^3) with a mean value of $4.1 \times 10^2 \pm 1.9 \times 10^2$ in examined strawberry ice cream samples. On the other hand 12 (48%) of examined unpacked street vended ice cream Mix samples were contaminated with enterococci organisms with level varied from (1.0×10^3) to (3.0×10^3) with a mean value of $1.8 \times 10^3 \pm 6.9 \times 10^2$ respectively. The most contaminated samples were strawberry while chocolate and vanilla flavored ice cream samples were enterococcus organisms free.

Data presented in Table (10) revealed that there is a significant difference ($p < 0.05$) in Enterococci count between packed ice cream flavored by strawberry and the respective unpacked type. While there is no significant difference between packed and unpacked ice cream flavored with chocolate and vanilla. Higher incidence were oreported by Afshin and Saeid (2011) and Yusuf et al., (2013) . Nearly Similar results were reported by Yaman et al., (2006) and Abo El-Makarem (2017). Lower counts were reported by Afshin and Saeid (2011).The ability of Enterococcus species to survive a range of adverse environments (Van den Berghe et al., 2006) allows multiple routes of cross-contamination of enterococci in causing human disease, including those from food.

The organisms may enter the milk and dairy products either directly from human or animal faces or indirectly from contaminated sources, exterior of the animal and/or from the milking equipments and utensils Gelsomino et al., (2001). Recently, enterococci have become one of the most common nosocomial pathogens, with patients having a high mortality rate of up to 61 % (De Fa´tima Silva Lopes et al. 2005).

In conclusion, the results of the present investigstion revealed that the communully prepared ice cream products solda at local markets and street vendors in Alexandria Governorate are unsatisfactory for human consumption. So these microorganisms constitute a public health hazard to consumers. Since ice cream harbors many potent pathogens, its microbial quality has always been crucially important to public health.

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