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Safety and Microbiological Quality of Frozen Dairy Desserts sold in Alexandria City

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Ice cream, Aerobic mesophilic count, coliforms count, Staph.aureus, flavored ice cream, mold count

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ABSTRACT

This study aimed to evaluate the safety and microbiological quality of frozen dairy desserts sold in different shops and supermarkets in Alexandria city. One hundred samples of frozen dairy desserts; eighty samples of ice cream of 4 different flavours (Vanilla, Chocolate, Mango and Strawberry) 20 of each and 20 samples of frozen yoghurt were collected randomly from Alexandria city. All samples were subjected to sanitary and microbiological evaluation. Regarding to Methylene Blue Reduction test results revealed that 15(75%), 10(50%), 7(35%) and 2(10%) of examined of ice cream samples (Vanilla, Chocolate, Mango and Strawberry) were qualified as Grade (1); respectively. While samples with Grade (2) were 2(1%), 1(5%), 0(0%) and 1(5%) of examined samples, respectively. While 2(10%), 2(10%), 6(30%) and 2 (10%) of the four different flavours were Grade (3) with poor quality respectively. Also 1(5%), 7(35%),7(35%) and 15(75%), of samples were grade (4), respectively. 17(85%) frozen yoghurt samples were categorized as Grade (4) and 3(15%) showed grade(3) for methylene blue reduction test; mean values of aerobic mesophilic count (cfu/g) in examined flavoured ice cream (Vanilla, Chocolate, Mango and Strawberry) were $1.5x\ 10^5 \pm 6.1x10^4$, $9.5x10^6 \pm 6.4x10^5$, $4.8x\ 10^6 \pm 2.4x10^6$ and $5.1x\ 10^7 \pm 1.8x\ 10^7$, respectively. Also, mean values of coliforms count (cfu/g) in vanilla, chocolate, mango, strawberry and frozen yoghurt were $2.5x10^2$ ±7.1x10, $6.6x\ 10^2 \pm 2.5x10^2$, $9.9x10^3 \pm 6.3x10^3$, $4x10^3$ ±$1.4x10^3$ and $1.5x\ 10^2 \pm$ 2.9x1, respectively; while, the respective mean values of *Staph. aureus* count (cfu/g) were $1.6x\ 10^2$ ±7.0x 10, 3.4x102±$9.4x10^2$, $1.9x10^3$±$1.5x10^3$, $7.4x10^2$±$2.9x10^2$ and $3.33x\ 10^2 \pm 1.2x10^2$, respectively. Finally, mean values of mold count (cfu/g) were $2.2x10^2 \pm 8.4x10$, $9.6x10^2 \pm 4.4x10^2$, $3.2x10^3$±$1.3x10^3$, $3.7x10^3$±1x $10^3$and $1.3x10^4 \pm 6.4x10^3$in examined Vanilla, chocolate, Mango, Strawberry ice cream samples and frozen yoghurt; respectively. The present investigation revealed that the communally prepared ice cream products sold at local markets in Alexandria city are unsatisfactory for human consumption. At retail shops, improper storage temperature and prolonged storage time affects the microbiological quality of ice-cream. To improve quality of ice cream, Good manufacturing practice (GMP) is mandatory especially at all post pasteurization steps, use of good quality raw materials and automatic machines to minimize handling, adoption of good sanitation practices and application of the HACCP principles in the system along with education of workers on personal hygiene.

1. INTRODUCTION

Frozen Dairy Desserts are congealed dairy products produced by freezing a heat treated mixture of milk, cream, milk solids, sugars, stabilizers, emulsifiers and flavouring. In recent years, consumption of such products increased gradually all over the world.

According to the FSANZ (2006) ice cream means the product obtained by freezing a pasteurized mix prepared from milk and /or other products derived from milk with or without the addition of nutritive sweetening agents, fruit and fruit products, eggs and egg products, coffee, cocoa, chocolate, condiments,

spices, ginger and nuts and it may also contain bakery products such as cake or cookies as a separate layer and/or coating. It is a highly appreciable not only by children and adults but also by sick and convalescent people especially after operation in the region of mouth and throat.Bacteriological quality of ice cream reflects hygienic practice in production and is an indication of good practice in production. Milk is another ingredient of ice cream that is vulnerable to bacterial contamination including E.coli, Klebsiella, Salmonella spp., and *Staph. aureus* (Marjan 2014). Primary sources of microbial contamination of ice cream includes water and raw milk, and other raw materials were secondary sources including flavoring agent, coloring substances, sweeting agents, handling and from contaminated air during processing (Ahmed *et al.*, 2009). Contaminated ice cream with pathogens can cause food borne disease, but pasteurization and freezing of ingredients eliminates most microbiological hazards (Campbell, 2015). Frozen yoghurt is a dessert that combines the texture of ice cream with the nutritive and healthy properties of yoghurt (Rezaei, et al., 2011). Its process consists of mixing all ingredients to make natural stirred yoghurt with stabilizers/emulsifiers and sugar, then freezing the mix in a conventional ice cream freezer (Tamime and Robinson 2007). Davidson *et al.*, (2000) reported that frozen yoghurt could serve as an excellent vehicle for the incorporation of probiotic bacteria as Streptococcus salivarius species thermophilus and Lactobacillus delbrueckii ssp. Bulgaricus as well as Bifidobacterium longumand Lactobacillus acidophilus. Abdelazez *et al.*, (2017) reported that functions of the frozen yogurt containing viable bifidobacterial cells are recognized and favored by the people of all ages. Staphylococcusaureus is a major food borne pathogen due to its capability to produce a wide range of heat stable enterotoxins that when ingested through contaminated dairy products could cause food human with varying severity (Peles *et al.*, 2005). Detection of E. coli in food is an indicative of fecal contamination and presence of other dangerous pathogenic microorganisms which can compromise the health and wellbeing of consumers. In addition to hygienic indicator, some strains of E. coli are directly pathogenic to humans like shiga toxin-producing Escherichia coli O157:H7 (ETEC O157) which can cause severe enteric infections (Atnafie *et al.*, 2017).Yeasts and moulds isolated from ice cream have been mainly associated with those of cane sugar obtained from inadequately treated raw sugar cane (Roberts *et al.*, 1998). The other ingredients such as sugar, emulsifiers and flavourants may have been contaminated by some heat resistant micro-organisms that include yeasts and moulds which may contaminate the product. The microbiological quality of ice cream during retail marketing mainly depends upon the postproduction handling of the product as well as efficiency and sanitary conditions during frozen storage (Nelapati et al. 2009).

The aim of this study was to evaluate the safety and microbiological quality of frozen dairy desserts (ice cream and frozen yoghurt) sold in Alexandria city and their potential risks to public health.

2. MATERIAL AND METHODS

2.1. Collection of the samples:

One hundred samples of frozen dairy desserts represented as eighty ice cream samples of different flavours (vanilla, chocolate, mango and strawberry) 20 of each and 20 samples of frozen yoghurt were collected randomly from supermarkets, dairy shops and street vendors at Alexandria city. All samples were subjected to sanitary evaluation and microbiological evaluation to judge and give a decision that the products were fit for consumption.

2.2. Sanitary evaluation of frozen dairy dessert samples.

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

2.3. Microbiological evaluation of examined frozen dairy desserts samples.

2.3.1. Preparation of serial dilution according to (APHA, 1992).

2.3.2. Aerobic mesophilic count according to ISO (4833: 2003) method.

2.3.3. Total Coliforms count (cfu/g) adopted by ICMSF, (2002).

2.3.3.1. Isolation and Identification of isolated coliforms according to (APHA, 1992).

2.3.3.2. Serological identification of isolated E. coli according to (MacFaddin, 2000).

2.3.4. Staphylococcus aureus count according to (Baird parker, 1962).

2.3.5. Total Mold count according to (Baily and Scott, 1998) and identification of the isolated moulds (Ramirez, 1982), Samson et al., (1995) and Pitt and Hocking, (2009).

3. RESULTS AND DISCUSSION

Egyptian Microbiological Standards for Ice cream 1185/ (2005) Total bacterial count <150.000 cfu /g

Total coliforms <10 cfu /g E. coli absent in 1 g Staph.aureus (coagulase- positive) absent in 1 g

4.1. Sanitary evaluation of ice cream:

4.1.1. Methylene blue reduction test:

Methylene blue reduction test (Benson, 2002) depends upon the ability of bacteria in milk to grow and to consume the dissolved oxygen, which reduce the oxidation reduction potentials in the medium.

Methylene blue reduction test used to measure the ability of microorganisms that present in frozen dairy desserts to grow in dilution kept at temperature ranged from 20-30 ºC, as the samples will be kept in a frozen temperature until the time of consumption (Armnanios, 2017).

It is evident from the results of samples of ice cream and frozen yoghurt that reported in table (1) that 15(75%), 10(50%), 7(35%) and 2(10%), of examined of ice cream samples (vanilla, chocolate, mango and strawberry) Grade (1) that showed that methylene blue reduction time for methylene blue test were over 4 hours that were good quality samples. Grade (2) the reduction time from 2.5-4 hours showed that 2(10%), 1 (5%), 0 (0%) and 1 (5%), of examined of samples were with a good quality samples, respectively. While 2 (10%), 2(10%), 6 (30%) and 2 (10%); respectively were Grade (3) with poor quality. The reduction time less than 30 minutes showed that 1 (5%), 7 (35%), 7 (35%) and 15 (75%); respectively of examined ice cream samples was with a grade (4) bad quality.

The result of examined samples of ice cream were of poor hygienic quality leading to fast dye reduction. Lower results were reported by Armnanios, (2017). Results illustrated in table (1) showed that 17(85%) of frozen yoghurt samples lie in grade (4) showed less than 30 minutes dye reduction time for methylene blue reduction test and 3 (15%) showed grade (3) with reduction time from 30 minutes to 2.5 hours and this is mainly due to high number of microorganisms present in the added starter rather than microbial contamination. Methylene blue reduction test is not a very reliable method for detection of contamination in dairy products (Igumbor *et al.*, 2002).

4.2. Microbiological evaluation of examined frozen dairy desserts:

Ice cream, a milk based product is good medium for microbial growth due to high nutrient value, and almost neutral pH value (pH ~6-7) and long storage duration (Hankin and Hanna, 1984) and (Bell, C. & Kyriakides, 1998) of ice-cream it is considered as a good media for microbial growth and is of public health hazard.

4.2.1. Aerobic mesophilic count:

Aerobic mesophilic counts are mainly due to spoilage and lactic acid producing bacteria and pathogenic microbes are used as indicator for quality of milk and milk products (Gressi *et al.*, 2008).

Results in Table (2) revealed values of aerobic mesophilic count were ranged from 8.8 $\times 10^2$ to 1.2 $\times 10^6$ with mean value of $5x10^5 \pm 6.1x10^4$ and ranged from 8.7× 10^3 to 1.3 $\times 10^8$ with mean value of $9.5x10^6 \pm 66.4x10^5$ for vanilla and chocolate flavored ice cream samples, while for mango and strawberry flavoured ice cream were ranged from 10 $\times 10^3$ to 3 $\times 10^7$ with mean value of $4.8x10^6 \pm 62.4x10^6$ and ranged from 10×10^3 to2.8×10^8 with mean value of $5.1x10^7 \pm 61.8x10^7$, respectively. This is may be due to usage of low quality fruits and avoid pasteurizing fruit flavoured ice cream to prevent any taste change.

The Egyptian Standards (2005) stated that the limit for aerobic mesophilic count of ice cream as <1.5 $\times 10^5$cfu /g. The obtained results were exceeded the legal limit in 5(25%), 10(50%), 14(70%) and 18(90%) of examined vanilla, chocolate, mango and strawberry ice creams; respectively.

Higher results were reported by El-Sharef *et al.*, (2006) and Shekarouf and Jafarpour (2006). While, lower results were reported by Yusuf *et al.*, (2013), Hamed, (2016), Jannat *et al.*, (2016), Kahraman and Kolanciyan, (2016), Abo El-Makarem, (2017) and Armnanios, Hala, (2017). Micro-organisms have been found to contaminate ice cream mix and proliferate as a result of temperature abuse, leading to food poisoning (Tomislav *et al.*, 2012).

Table (1): Grading of examined Frozen Dairy Dessert Samples according to Methylene Blue Reduction Time.

Grade	Reduction time	Flavoured ice cream								Frozen yoghurt	
		Vanilla		Chocolate		Mango		Straw berry			
		No.	%	No.	%	No.	%	No.	%	No.	%
I	Over 4 hours	15	75	10	50	7	35	2	10	0	0
II	2.5- 4 hours	2	10	1	5	0	0	1	5	0	0
III	0.5 -2.5 hours	2	10	2	10	6	30	2	10	3	15
IV	Less than 30 minutes	1	5	7	35	7	35	15	75	17	85
	Total	20	100	20	100	20	100	20	100	20	100

Table (2): Statistical analytical results of aerobic mesophilic count (cfu/g)in the examined frozen dairy dessert samples:

Product	No.of samples	Positive sample		Minim-um	Maxim-um	Mean ± SEM	Samples within permissible limit	
		No	%				No	%
Ice cream								
Vanilla	20	20	100	$8.8x10^2$	$1.2x10^6$	$1.5x10^5 \pm 6.1x10^4$	15	75
Chocolate	20	20	100	$8.7x10^3$	$1.3\ x10^8$	$9.5x10^6 \pm 6.4x10^5$	10	50
Mango	20	20	100	$10x10^3$	$3.0x10^7$	$4.8x10^6 \pm 2.4x10^6$	6	30
Strawberry	20	20	100	$10x10^3$	$2.8x10^8$	$5.1x10^7 \pm 1.8x10^7$	2	10

4.2.2. Coliforms count in the examined frozen dairy desserts:

Coliform organisms are the main microorganisms that reflect hygienic status of final product and effectiveness of hygienic practices in ice cream production. It is evident from table (3) that 12(60%), 15(75%), 20(100%), 20(100%) and 16(80%) of examined ice creams flavored vanilla, chocolate, mango, strawberry and frozen yoghurt were contaminated with coliforms, respectively. Results also revealed that the mean values of coliform count (cfu/g) of examined ice creams flavoured vanilla, chocolate, mango, strawberry and frozen yoghurt were $2.5x10^2 \pm 7.1x10$, $6.6x10^2 \pm 2.5x10^2$, $9.9x10^3 \pm 6.3x10^3$, $4x\ 10^3 \pm 1.4x\ 10^3$ and $1.5x\ 10^2 \pm 2.9x\ 10$, respectively.

Examined samples showed high contamination with coliforms in mango, followed by strawberry, chocolate ice creams, frozen yoghurt and vanilla flavoured ice creams. These findings are higher than those reported by El-Ansary (2015). Higher results were reported by Hossain and Kober, (2009), Ahmed *et al.,* (2009) and Vigil *et al.,* (2009).

The c count were more than <10/g which is the permissible limit for coliforms count in ice cream according to the Egyptian Standards, (2005). Results presented in table (3) indicated that only 6(30%), 4(20%), 3(15%), 1(5%) and 1(5%) of examined vanilla, chocolate, mango, strawberry ice creams and frozen yoghurt; respectively were complying with the limit of Egyptian Standards, (2005) for coliform organisms in ice cream.

Table (6) showed that frequency distribution of isolated coliforms from examined flavoured ice creams with vanilla, chocolate, mango and strawberry were *Citrobacte rdiverses* (16.67, 20, 15 and 10%), *Citrobacter freundii*(8.33, 13.33, 5 and 10%), *Enterobacter cloaca* (25, 20,15 and 25%), *Enterobacter aerogenes* (8.33, 13.33, 15and 10%), *Enterobacter agglumerans* (8.33, 13.33, 5and 5%), *Escherichia coli* (0, 6.67, 10 and 15%), *Klebsiella spp*. (25, 6.67, 30 and 15%) and *Serratia liquificans* (8.33, 6.67, 5 and 10%), respectively. While the respective incidence of *Citrobacter diverses, Citrobacter freundii, Enterobacter cloaca, Enterobacter aerogenes, Enterobacter agglumerans, Escherichia coli, Klebsiella spp. and Serratia liquificans*in frozen yoghurt samples were (12.50, 6.25, 18.75, 12.50, 12.50, 6.25, 25 and 6.25%).

Table (3): Statistical analytical results of Coliforms count (cfu/g) in the examined frozen dairy dessert samples:

Product (Ice cream)	No. of samples	Positive sample		Minimum	Maximum	Mean ± SEM	Samples within permissible limit	
		No	%				No	%
Vanilla	20	12	60	1.0x10	$9.3x10^2$	$2.5x10^2 \pm 7.1x10$	6	30
Chocolate	20	15	75	2. 0x10	$6.8x10^3$	$6.6x10^2 \pm 3.5x10^2$	4	20
Mango	20	20	100	1. 0x10	$1.2x10^5$	$9.9x10^3 \pm 6.3x10^3$	3	15
Straw berry	20	20	100	2.7x10	$2.2x10^4$	$4.0x10^3 \pm 1.4x10^3$	1	5
Frozen Yoghurt	20	16	80	1x10	$4.9x10^2$	$1.5x10^2 \pm 2.9x10$	1	5

Table (4): Statistical analytical results of Staphylococcus aureus count (cfu/g) in examined frozen dairy dessert samples.

Product (Ice cream)	No. of samples	Positive sample		Minimum	Maximum	Mean ± SEM	Samples within permissible limit	
		No	%				No	%
Vanilla	20	8	40	3. 0x10	$6.2x10^2$	$1.6x10^2 \pm 7.0$ x10	15	75
Chocolate	20	12	60	4. 0x10	1.0 x10^3	3.4 x$10^2 \pm 9.4$ x10	8	40
Mango	20	15	75	2. 0x10	2.2 x10^4	$1.9x10^3 \pm 1.5x10^3$	5	25
Straw berry	20	20	100	3. 0x10	$6.0x10^3$	$7.4x10^2 \pm 2.9x10^2$	3	15
Frozen Yoghurt	20	14	70	1. 8x10	$1.6x10^3$	$3.33x10^2 \pm 1.2x10^2$	2	10

Table (5): Statistical analytical results of Mold count (cfu/g)in examined frozen dairy dessert samples.

Product (Ice cream)	No. of samples	Positive sample		Minimum	Maximum	Mean ± SEM		
		No	%				No	%
Vanilla	20	15	75	3 x10	$1.3x10^2$	$2.2x10^2 \pm 8.4x10$		
Chocolate	20	17	85	3 x10	$7.6x10^3$	$9.6x10^2 \pm 4.4x10^2$		
Mango	20	18	90	3 x10	$1.9x10^4$	$3.2x10^3 \pm 1.3x10^3$		
Straw berry	20	20	100	11 x10	$1.4x10^4$	$3.7x10^3 \pm 1x10^3$		
Frozen Yoghurt	20	19	95	6. 3 x10	$9x10^4$	$1.3x10^4 \pm 6.4x10^3$		

Table (6): Frequency distribution of Coliform organisms isolated from positive examined frozen dairy dessert samples.

Isolated Coliform species	Vanilla		Chocolate		Mango		Strawberry		Frozen Yoghurt	
	No	%	No	%	No	%	No	%	No	%
Citrobacterdiversus	2	16.67	3	20	3	15	2	10	2	12.50
Citrobacterfreundii	1	8.33	2	13.33	1	5	2	10	1	6.25
Enterobacter cloaca	3	25	3	20	3	15	5	25	3	18.75
Enterobacter aerogenes	1	8.33	2	13.33	3	15	2	10	2	12.50
Enterobacter Agglumerans	1	8.33	2	13.33	1	5	1	5	2	12.50
Escherichia coli	0	0	1	6.67	2	10	3	15	1	6.25
Klebsiella spp.	3	25	1	6.67	6	30	3	15	4	25
Serratialiquefaciens	1	8.33	1	6.67	1	5	2	10	1	6.25
Total	12	100	15	100	20	100	20	100	16	100

The presence of coliform organisms is taken as an indication that other pathogenic organisms may also present in the sample (Trabulsi *et al.,* 2002). Presence of Coliforms contamination post pasteurization in ice creams indicated faulty heat process during preparation. Results illustrated in table (7) showed that Serodaignosis of isolated E. coli from positive examined frozen dairy desserts 2(22.22%) were O111: H2 (Enterohaemorrhagic E. coli), 1(14.28%) were O127: H6, (Enterotoxigenic E. coli), 2(22.22%) were O26:H11 (Enterohaemorrhagic E. coli), 1(11.11%) were O55: H7 (Enteropathogenic E. coli) and1(11.11%) were O91: H21 (Enterohaemorrhagic E. coli); while there were 2 unidentified species.

Table (7): SerologicalKey for Identification of E. coli isolated from positive examined dairy desserts samples

E.coli serotypes	Serodaignosis		Strain characterization
	No	%	
O111 : H2	2	22.22	EHEC
O127 : H6	1	11.11	ETEC
O26 : H11	2	22.22	EHEC
O55 : H7	1	11.11	EPEC
O91 : H21	1	11.11	EHEC
Untypable	2	22.22	-
Total	9	100	

Enterohaemorrhagic E. coli (EHEC) O157:H7strains cause severe illness, such as haemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Reuben *et al.,* 2013). E.coli strain O155: H7 strain is enteropathogenic E.coli (EPEC) causing infantile diarrhea which is watery and mucoid without blood. E. coli, O127: H6 strain is enterotoxigenic E. coli (ETEC) which is responsible for watery diarrhea during visiting to warmer countries due to production of heat labile toxin (Cholera like toxin) and heat stable toxin (Diarrheal toxin). Similar strains were reported by Fadel and Ismail (2009) with different incidences. El-Sharef *et al.,* (2006) reported an incidence of 1.3% from homemade ice creams sold in Libya was contaminated by E. coli O157: H7.

4.2.3. Staphylococcus aureus count in the examined frozen dairy desserts:

Staphylococcus aureus carry many genes that encode a variety of pyrogenic toxins (PTs), the most important is Staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin (TSST) (Afifi *et al.*, 2011). The PTs interact with several cellular targets to produce disease, such as food poisoning and toxic shock syndrome (Omoe et al, 2002). Staphylococcal Food Poisoning (SFP) is a mild intoxication occurring after the ingestion of food containing staphylococcal enterotoxins (SEs) (Chiang et al, 2008).

Obtained results in tables (4) revealed that staph. aureus organisms could be detected in 8(40%), 12(60%), 15(75%), 20(100%) and 14(70%) in examined vanilla, chocolate, mango, strawberry ice creams and frozen yoghurt, respectively with a mean values of $1.6x\ 10^2 \pm 7.0x10$, $3.4x\ 10^2 \pm 9.4\ x10$, $1.9x10^3 \pm 1.5x10^3$, $7.4\ x\ 10^2 \pm 2.9\ x\ 10^2$ and $3.33x10^2 \pm 1.2x10^2$ (cfu/g) in examined vanilla, chocolate,

mango, strawberry ice creams and frozen yoghurt, samples; respectively.

These results were lower than those of Armnanios, Hala, (2017) and Barman *et al.,* (2017). On the other hand, higher than results reported by Sathiaderan and Antony, (2012) and Jadhav and Rout, (2014). *Staph. aureus* can survive better in frozen products like ice creams and can elaborate enterotoxins leading to food poisoning outbreaks (Joshi *et al.,* 2004).The possible source of this microorganism in ice cream could be from hands, nose, skin and clothes of handlers. Coughing, talking and sneezing produce droplets which could be settle on ice cream during transportation, storage and retailing (Ojokoh, 2006) which may act as a source of contamination.

Egyptian Standards (2005) stipulates that ice cream must be free from staph. aureus. Results obtained in this study revealed that 15 (75%), 8 (40%), 5 (25%), 3 (15%) and 2 (10%) of examined vanilla, chocolate, mango, strawberry ice creams and frozen yoghurt were complying with the Egyptian Standards (2005) (absent in 1 g).

Milk and dairy food have frequently been implicated in staphylococcal food poisoning and contaminated raw milk is often involved (Peles *et al.*, 2005). Occurrence of staph. aureus in examined ice cream samples 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).

4.2.4. Mold count in the examined frozen dairy dessert:

Fungi can be particularly troublesome, because they can adapt to the environment of the food and can be difficult to detect on conventional plating media within the standard incubation times (Ledenbach *et al.,* 2009). However, mold could be many other colors ranging from grey to orange to brown (WHO, 2009).Common sources of moisture, condensation on surfaces due to excessive humidity, lack of ventilation, or low temperature. Steam or excess moisture in the air from baths/showers and cooking, water leakage, such as from a roof or plumbing leak, a cracked basement, or flooding increase growth of mold (WHO, 2009).

Illustrated data in table (5) showed that mold could be detected in 15(75%), 17(85%), 18(90%), 20(100%) and 19(95%) of examined vanilla, chocolate, mango, strawberry ice creams and frozen yoghurt, respectively. Results obtained revealed that examined vanilla, chocolate, mango, strawberry ice creams and frozen yoghurt samples were with minimum count (cfu/g) of 3x10, 3x10, 3x10, 11x10 and 6.3x10, respectively. And maximum counts (cfu/g) were $1.3x10^2$, $7.6x\ 10^3$, $1.9x10^4$, $1.4x\ 10^4$ and $9x\ 10^4$; respectively with a meanvalue of $2.2x10^2 \pm 8.4x10$, $9.6x10^2 \pm 4.4x10^2$, $3.2x10^3 \pm 1.3x10^3$, $3.7x10^3 \pm 1\ x\ 10^3$ and $1.3x10^4 \pm 6.4x10^3$, respectively.

Higher mold count was revealed in frozen yoghurt, strawberry and mango ice cream samples. This may be due to neglected pasteurization of these types to avoid flavour change. Fruit flavored ice cream containing a higher number of molds than other flavours and this may be due to fruit spoilage. Heat resistant fungi are widely distributed in soil and are consequently associated with spoilage problems in products containing fruits (yoghurt and ice-cream) which are readily contaminated by soil, e. g. strawberry, grapes, and other fruits (Aydin *et al.*, 2016). Results in present study are higher than those reported by Caglayanlar *et al.,* (2009), Mathews *et al.*, (2013) and Abou-EL Khair *et al.,* (2014).

Results in table (8) revealed that the incidence of mold in examined vanilla, chocolate, mango, strawberry ice creams and frozen yoghurt as; Alternaria alternaria (33.3, 31.58, 31.8, 93.3 and 47.4%), Aspergillus niger (20, 15.79, 13.6, 16.7, and 0%), Aspergillus flavus (20, 15.79, 9.1, 12.5, 0%), Aspergillus ochraceus (0, 0, 4.5, 4.2and 15.9%), Aspergillus parasiticus (0, 0, 4.5, 4.2 and 0%), Cladosporium spp. (6.67, 0, 9.1, 0 and 0%), Mucor (6.67, 10.52, 0, 0 and 10.5%), Penicillium (13.3, 26.32, 27.2, 29.1 and 26.3%). Alternaria alternaria spp. was found in all mold samples followed by Penicillum then A.niger, Otherwise in (Ojokoh, 2006), Aspergillus spp. was obtained from all the samples analyzed while Rhizopus spp. was isolated from three samples and Neurospora spp. from two samples. The presence of molds such as Aspergillus, Penicillium and Mucor spp. was detected by (Vibha *et al.,* 1996) in the samples of ice cream available in Mumbai region. These molds may get entry in ice cream from equipments, utensils, human hands and atmosphere.

To control mold contamination in ice cream, it is recommended that high sanitary conditions, health education and proper storage facilities can minimize the higher prevalence of this micro-flora in the product.

Table (8): Incidence of isolated molds in the examined frozen dairy dessert samples.

Isolates	Vanilla		Chocolate		Mango		Strawberry		Frozen yoghurt	
	No	%	No	%	No	%	No	%	No	%
Alternariaalternaria	5	33.3	6	31.58	7	31.8	8	93.3	9	47.4
Alternarianiger	3	3	3	15.79	3	13.6	4	16.7	0	0
Aspergillus flavus	3	3	3	15.79	2	9.1	3	12.5	0	0
Aspergillus ochraceus	0	0	0	0	1	4.5	1	4.2	3	15.9
Aspergillus parasiticus	0	0	0	0	1	4.5	1	4.2	0	0
Cladosporium Spp.	1	6.67	0	0	2	9.1	0	0	0	0
Muccor	1	6.67	2	10.52	0	0	0		2	10.5
Penicillium	2	13.3	5	26.32	6	27.2	7	29.1	5	26.3
Total	15	100	19	100	22	100	22	100	19	100

5. CONCLUSION

In conclusion our results revealed that ice cream and frozen yoghurt sold in Alexandria city were contaminated with E. coli and staph. aureus pathogenic microorganisms these isolates constitutes a public health hazard to consumers. Once the ice cream becomes contaminated, freezing temperature could not make the product safer later. At retail shops, improper storage temperature and prolonged storage time affects the microbiological quality of ice-cream. To improve quality of ice cream, Good manufacturing practice (GMP) is mandatory especially at all post pasteurization steps, use good quality raw materials and automatic machines to minimize handling, adoption of good sanitation practices and application of the HACCP principles in the system along with education of workers on personal hygiene will definitely improve the quality of ice cream.

6. REFERENCES

Abdelazez, A., Muhammad, Z., Zhang, O., Zhu, Z., Abdelmotaal, H., Sami, R., Meng, X. 2017. BioMed Res. Int. Article ID 6438528, 10 pages

Abo El-Makarem, H.S. 2017.Microbial quality of street-vended ice cream. J. Vet.Med. Res. 24 (1): 299-307.

Abou-El Khair, E., Salama, A.R.H., Radwan, A., Khalafallah, A., Arafa, H. 2014. Bacteriological quality of packaged ice cream in Gaza city, Palestine J. Food and Nut. Sci.2(3): 68-73.

Afifi, N.A., Sadek, A., Aggour, M.G., El-Tamawy, A.A., Nemr, M.Mm., 2011. Molecular Characterization of Staphylococcus Aureus Enterotoxins in Milk and Some Dairy Products. Egyptian Journal of Medical Microbiology, 20 (1): 107-116.

Ahmed, K., Hussain, A. Imran. Qazalbash, M.A., Hussain W., 2009. Microbiological quality of ice cream sold in Gilgit town. Pak. J. Nutr., 8 (9): 1397–1400 .

Amer, A.A., Amer, R., El-Leboudy., A. A. 2012. Sanitary and microbiological status of ice cream sold at Alexandria Governorate. 6th Scientific, 20-22 Nov, 2012, Alexandria, Egypt.

American Public Health Association (APHA) 1992.Standard Methods for the Examination of Dairy products 15th Ed.New York.

Armnanios, H.F.M. 2017. Sanitary Evaluation of Frozen Desserts sold at Local Markets. Ph.D a thesis Faculty of Veterinary Medicine, Alexandria University.

Atnafie, B., Paulos, D., Abera, M., Tefera, G., Hailu, D., SurafelKasaye, S., Amenu, K. 2017. Occurrence of Escherichia coli O157:H7 in cattle feces and contamination of carcass and various contact surfaces in abattoir and butcher shops of Hawassa, Ethiopia. BMC Microbiol. 17:24.

Aydin A., B.H. Ulusoy, O. Ergun 2016. A survey on heat resistant moulds in heat treated milk, milk products and fruit juices. Archiv fur lebensmittelhygiene. (56):49-72 . http://www.schaper-verlag.de.

Baily, W.R. Scott, E.G. 1998. Diagnostic Microbiology. A Textbook for the Isolation and Identification of Pathogenic Microorganisms.The C.V. Mosby Company Saint Louis.

Baird-Parker, A.C. 1962.An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. J. Appl. Bacteriol. 25: 12-19.

Barman, A. Kumar, P. Kumar Roy, S. Ray, R. Kumar; B. Rani, B. Kumar Singh, 2017. Evaluation of microbiological quality of Ice-cream available in Kolkata and its Suburbs. The Pharma Innovation J. 6(8): 377-380

Bell, C. ,Kyriakides. 1998. Listeria : Apractical approach to the organism and its control in foods. Blackie Academic &Porfessional. London. 1998.

Benson, J.K. 2002. Microbiological applications. Laboratory manual in general Microbiol.8th edition.1-478.

Caglayanlar, G.E., Kunduhoglu, B., Coksoyler, N. 2009. Comparison of the Microbiological Quality of Packed and Unpacked Ice Creams Sold in Bursa, Turkey. J. Arts Sci. 12: 93-102.

Campbell, M. A. 2015. An Examination of Microbial Population in Different Brands and Flavors of Ice cream. A Thesis M. V. Sc. The University of Mississippi, Oxford.

Chiang, Y.C., Liao, W.W., Fan, C.M., Pai, W.Y., Chiou, C.S., Tsen, H. Y. 2008. PCR detection of Staphylococcus enterotoxins N, O, P,Q,R,U and survey of SE types in S. aureus isolates from food poisoning cases in Taiwan. Int. J. Food Microbiol., Jan 15;121(1): 66-73.

Davidson, R.H., Duncan, S.E., Hackney, C.R., Eigel, W.N., Boling, J.W., 2000. Probiotic culture survival and implications in fermented frozen yogurt characteristics. Journal of Dairy Science, 83, 666 – 673.

Egyptian Standard .2005.Milk and dairy products. Part 1: ice cream. Egyptian Organization for Standardization and Quality Control. EOS: 1185.

El-Ansary, M. A. 2015. Hygienic quality of vanilla ice cream sold atlocal market. Alex. J. Vet. Sci., 44(1): 54–58.

El-Sharef, N., Ghenghesh, K.S,Gnan, Y.A.S., Rahouma, A. 2006.Bacteriological Quality of Ice cream in Triboli, Libya. Food Control, 17:637-641.

Fadel, H.M., Jehan Ismail . 2009. prevalence and Significance of Staphylococcus aureus and Enterobacteriaceae species in selected dairy products and handlers. Int. J. Dairy Sci. 2: 1- 9.

FSANZ (Food Standards Australia New Zealand) 2006.A Risk Profile of Dairy products in Australia. Food Standards Australia New Zealand Report.

Gressi, M., Lanzoni, M., Pompli, A., Lazzarini, R. I., Malleuzzi, D., Ricco, B. 2008.Detection of microbiological concentration in ice cream using the impedance technique.BiosensBioelectron, Jun.15-23 (1) .

Hamed, O. H. M. 2016.Evaluation of the Microbioical Quality of Flavoured Ice Cream in Khartoum State.M.V.Sc. a Thesis, Sudan University of Science and Technology, College of Graduate Studies.

Hankin, L., Hanna, J.G. 1984. Quality of ice cream and ice milk. Bull ContecticuitAgricExper Stat. 818: 6.

Harrigan, M. F., McCance, M. E. 1976.Laboratory methods in food and dairy microbiology. Academic Press, London, England.pp. 193.

Hossain, M.T., Kober, A. H. 2009. Microbiological Quality of Ice Cream Available in Chittong. Bangladesh J. Microbiol, Volume 25, Number 2, December 2008, pp 135-136.

Igumbor, E.O.; Obi, C.L., Te.Mingo .2002. Bacteriological examination of pasteurized milk and milk products sold in Harar. Zimbabw J. Food Sci. Technol.39-129.

International Commission for the Microbiological Specifications of Foods (ICMSF) 2002. Microorganisms in Foods 7 Microbiological Testing in Food Safety Management Authors.

International Standard Organization (ISO, 4833-2003). Horizontal method for the enumeration of microorganism's colony-convert technique at 30 ec.International Standard Organization, Gerara, Switzerland.

Jadhav, A.S., Raut P.D. 2014. Evaluation of microbiological quality of ice creams marketed in Kolhapur City, Maharashtra, India. Int. J. Curr. Microbiol.App.Sci., 3(9) 78-84.

Jannat, B., Hasan, Md., Gomes, M., Uddin, M. J., AlSanjee, S., Fatema, K., Datta, S. 2016. Comparative Analysis of the Microbiological Quality between Industrially Produced and Street Vended Ice Creams Offered for Public Consumption in Dhaka City. Bangl. J. Biores. Commun. 2(2): 259-263.

Joshi, D.R., Shah, P.K., Manandhar, S., Sharma, S., Banmali, P. 2004. 'Microbial quality of ice cream sold in Kathmandu', J. Nepal Health Research Council, 2, pp. 37-40 .

Kahraman, T., Kolanciyan, A.M. 2016. Microbiological Quality of Ice cream sold in Istanbul.Turkey Vet. . 65 (3): 111-115.

Ledenbach, Loralyn, H., Robert, T., Marshall, 2009.Microbiological Spoilage of Dairy products. Compendium of the Microbiological Spoilage of Foods and Beverages, Springer-Verlag New York, 2010, p. 41.

MacFaddin, J. F. 2000. Biochemical tests for identification medical bacteria. Warery Press Inc, Baltimore, Md. 21202 USA.

Marjan, S., Kanta, Das, K., Munshi, K., Noor, R. 2014. Drug-resistant bacterial pathogens in milk and some milk products. Nutrition and Food Science. 44:241-248.

Mathews, S., Ngoma, L., Gashe, B., Mpuchane, S. 2013. General microbiological quality of ice cream and ice pop sold in Gaborone, Botswana. Ethno Med., 7(3): 217-226.

Nelapati, S., Krishnaiah, N., Kiranmayi, B. 2009.Physio-chemical and microbiological quality of ice creams soldin and around Greater Hyderabad MunicipalCorporation. J. Vet. Public Health, 7(2): 121–124.

Ojokoh, A.O. 2006. Microbiological examination of ice cream sold in Akure, Pakistan. J. Nutr., 5(6): 536–538.

Omoe, K., Ishikawa, M., Shimoda, Y., Hu, D.L., Ueda, S., Shinagawa K. 2002. Detection of seg, seh and sei genes in Staph.aureus isolates and determination of enterotoxin productivities of S. aureus isolates harboring seg, seh or sei genes. J. Clin. Microbiol. 40: 857-862.

Peles, F., Wagner, M., Varga, L., Hein, I., Rieck, P., Gutser, K., Kereszturi, P., Kardos, G., Turscani, I., Beri, B., Szabo, A. 2005.Coaggulase positive staphylococci and staphylococcus aureus in food products marketed in Italy. Int. J. Food Microbial., 98: 73-79.

Pitt, J.I., A.D. Hocking .2009. Fungi and Food Spoilage, 3rd edn.Springer, New York.

Ramirez, C. 1982. Manual and atlas of the Penicillium. El Sevir Biomedical Press, Amsterdam, pp 187.

Reuben, R.C., Owuna, G. 2013. Antimicrobial Resistance Patterns of Escherichia coli O157:H7 from Nigerian Fermented Milk Samples in Nasarawa State, Nigeria. International Journal of Pharmaceutical Science Invention; Volume (2) Issue 3: PP 38-44.

Rezaei, R., Khomeiri, M., Kashaninejad.,Alami, M. 2011. Effects of guar gum and arabic gum on the physicochemical, sensory and flow behavior characteristics of frozen yoghurt. International Journal of Dairy Technology. 64 (4): 563-568.

Roberts,T.A., Pitt, I.J., Farkas, J., Grau, F.H .1998. Milk and dairyproducts. In:Microorganisms in Foods 6.ICMSF.London: BlackieAcademic&Proffesional, pp. 521-528, 559-563.

Rostamzad, A., Rostamneia, N. 2016. Prevalence of the Panton-Valentine Leukocidin Gene in Clinical Isolates of Staphylococcus aureus Isolated from Hospitals the Ilam Province of Iran. J. Clin. Microb.Infec. 3(1).

Samson R.A., Hoekstra, E.S., Frisvad, J.S. ;Filtenborg, O. 1995. Introduction to food-borne fungi (4th edition); CenrtaalbureauVoorSchimmelculturesBaarn Delft.

Sathiaderan, A., Antony, B. 2012. Bacteriological quality of ice-cream marketed in Thrissur town, Kerala, India. Vet. World 5(12): 738-741. American Public Health Association.

Shamila-Syuhada, A.K., Rusul, G.,Wan-Nadiah,W.A., Chuah, L.O. 2016. Prevalence and Antibiotics Resistance of Staphylococcus aureus Isolates Isolated from Raw Milk Obtained from Small-Scale Dairy Farms in Penang, Malaysia. Pakistan Vet. J. 36(1): 98-102.

Shekarfroush, S. , B. Jafarpour, 2006. Comparison of microbial contamination in the traditional ice cream with Iranian Food Standards in Shiraz, Iranian. J. Food. Sci. Tech., 2: 11-16.

Tamime, A.Y., Robinson, R.K. 2007. Background, standards and marketing of frozen yogurt: Yoghurt: Science and Technology (ed. AY Tammie and RK Robinson). 3nd ed. CRC Press, Boca Raton, FL. p. 392-393.

Tomislav, P., Samarzija D, Zamberlin, S. 2012. Psychro-trophicbacteria and milk and dairy products qualityMljekarstvo, 62 (2): 77-95.

Trabulsi, L.R., Keller, R., TardelliGromes, T.A. 2002.Typical and a typical enteropathogenic Escherichia coli. Emerging Infect. Dis., 8(5): 508-513.

Vigil, J., Jiang, D., Chen, J., Palumbo, L., Galbadage, T., Brown, L., Yiang, J., Koo, H., DuPont, W., Ericsson, C., Adachi, A., DuPont, L. 2009. Coliform and Escherichia coli Contamination of Desserts Served in Public Restaurants from Guadalajara, Mexico, and Houston, Texas, Am J Trop Med Hyg 80(4): 606-608.

Vibha, K., Sherikar, A. A., Majee, S. 1996. Microbiological profile of various ice creams sold in Mumbai region. Indian J. Dairy Sci. 1996; 49(9):619-624.

WHO guidelines for indoor air quality, 2009. Dampness and Mold, World Health Organization. (http://www.euro.who.int/document/E92645.pdf .

Yusuf, M.A., Abdul Hamid, T.H. 2013.Assessment of the Bacteriological Quality of Ice cream offered for Public consumption in Bauchi, Nigeria. Journal of Pharmacy 3:.25-30.