

BACTERIOLOGICAL STUDIES ON ICE-CREAM IN KAFR EL-SHEIKH GOVERNORATE

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ABSTRACT

Ice cream is a nutritionally enriched dairy product manufactured from combinations of ingredients. Quality of ice cream depends on ingredients used, processing hygiene, handling, and storage. One hundred and fifty samples of ice cream (50 each of large scale producers, small scale producers and street vendors) were collected from different local supermarkets, shops and street vendors at Kafr El-Sheikh governorate for bacteriological examination. The mean total aerobic bacterial count were $1.07 \times 10^3 \pm 1.26 \times 10^2$, $1.40 \times 10^4 \pm 1.62 \times 10^3$ and $4.55 \times 10^4 \pm 4.79 \times 10^3$ cfu/g for large scale, small scale and street vendors ice cream samples respectively. While coliforms were detected in 8 (16%) large scale, all (100%) small scale and street vendors ice cream samples with average count 4.38 ± 0.14 , $1.82 \times 10^3 \pm 2.43 \times 10^2$ and $8.37 \times 10^3 \pm 1.28 \times 10^3$ MPN/g, respectively. All the examined small scale and vendors ice cream samples did not comply with the Egyptian Standard of ice cream for coliforms (<10 MPN/g). Staphylococci were detected in 28 (56%) large scale, all (100%) small scale and street vendors ice cream samples with a mean value of $1.08 \times 10^2 \pm 1.51 \times 10$, $2.37 \times 10^3 \pm 3.26 \times 10^2$ and $7.27 \times 10^3 \pm 1.06 \times 10^3$ cfu/g, respectively. Coagulase +ve Staph. aureus and salmonellae could not be isolated from any examined ice cream samples, that complied with the Egyptian Standard. It is concluded that there is a great need for continuous monitoring of small scale producers and street vendors of ice cream to ensure relatively safe product.

INTRODUCTION

Ice cream is a nutritionally enriched dairy product consumed particularly in summer as well as throughout all year and continues to be dominant interest of large segments of population specially children. The ingredients of ice cream may be various combinations of milk, cream, evaporated or condensed milk, dried milk, coloring and flavoring materials, fruits, nuts, sweetening agents, eggs, egg products¹ and stabilizers. Any of these may contain microorganisms and affect the quality of the product as judged by its bacterial load or its content of various specific species of bacteria (*Yaman et al., 2006*).

A typical ice cream contains 12% fat, 11% non-fat milk solids, 15% sugar and about 1% minor ingredients, the rest being water, moreover air is a major ingredient and account for about half of the volume of the final product (*Brain and Allan, 1982*).

As ice cream is a milk based product, it represent excellent medium for the growth of many microorganisms due to its nutrient (lactose, protein, ... etc) and almost neutral pH of 6-7 (*Kanbakan et al., 2004*). Some of these microorganisms may be of public health importance causing diseases e.g. cholera, typhoid, bacillary dysentery (*Richard and Micheal, 1989*). Contaminated ice cream were implicated in several gastrointestinal outbreaks in a number of countries in Asia, Europe, North America (*Dijuretic and Wall, 1996; Chug, 1997 and Dijuretic et al., 1997*) and Africa (*Yusuf Al-Jezani, 2008*).

Quality of ice cream depends on extrinsic factors that include manufacture procedures as well as intrinsic factors that include the proportion of ingredients used. Primary sources of microbial contamination of ice cream include water, raw milk, fresh and dried fruit and eggs, whereas secondary sources include flavoring, coloring substance, stabilizer, utensils, handling and from contaminated air during processing. (*Lampert, 1987 and Norma and Ana, 2000*).

Although pasteurization, freezing and hardening steps in production of ice cream can eliminate most of the microbial hazards, numerous health hazards are still persistent due to various conditions such as presence of spore forming microorganisms (*Alexander and Rothwel, 1970*) or due to post pasteurization contamination (*Verma, 1974*).

This study presents analysis of bacteriological criteria of ice cream samples collected from large scale producers, small scale producers and street vendors in Kafr El-Sheikh governorate.

MATERIALS AND METHODS

1. Collection of samples:

One hundred and fifty samples of ice cream (50 each of large scale producers, small scale producers and from street vendors) were collected from different local supermarkets, shops and street vendors at Kafr El-Sheikh governorate. The collected samples were transferred as soon as possible to the laboratory in an insulated ice box at 4°C with a minimum of delay where they were prepared and bacteriologically examined.

2. Preparation of samples:

Each sample was prepared and serially diluted according to *A.P.H.A., 1992*.

3. Bacteriological examination:

3.1. Total aerobic bacterial count “TABC” :

Total aerobic bacteria were counted according to *A.P.H.A., 1992* and the colony forming unit per gram (cfu/g) sample was calculated.

3.2. Coliforms count “MPN/g”:

For counting coliforms in samples under test, 3-tubes most probable number (MPN) technique was applied according to (*Harrigan, 1998*) using lauryl sulphate-tryptose broth (LST). The coliforms count (MPN/g) was calculated using the probability table (*DeMan, 1980*).

3.3. Enumeration of total staphylococci and isolation of *Staphylococcus aureus* according to *Smith and Baird Parker, 1964*.

Suspected *Staph. aureus* colonies was black shiny colonies with white margins and surrounded by clear zone extending onto opaque medium were picked up and inoculated into tubes containing 5 ml of Brain Heart Infusion Broth (BHI). Inoculated tubes were incubated at 37°C for 18 hours and used for further identification.

3.3.1 Identification of *Staphylococcus aureus*: (according to *A.P.H.A., 1992*):

3.4 Isolation of Salmonellae: (according to *I.C.M.S.F., 1982*)

3.4.1 Identification of suspected salmonellae: (according to *Baily and Scott 1998*)

RESULTS

Table (1): Statistical analytical results of total aerobic bacterial count of the examined ice cream samples.

Ice cream samples	No. of examined samples	Positive samples		Total aerobic bacterial count (cfu/g)		
		No.	%	Minimum	Maximum	Mean ± SEM
Large scale producers	50	50	100	5.0×10	3.5×10^3	$1.07 \times 10^3 \pm 1.26 \times 10^2$ c
Small scale producers	50	50	100	1.4×10^3	6.7×10^4	$1.40 \times 10^4 \pm 1.62 \times 10^3$ b
Street Vendors	50	50	100	2.9×10^3	2.0×10^5	$4.55 \times 10^4 \pm 4.79 \times 10^3$ a
Total	150	150	100	5.0×10	2.0×10^5	$2.02 \times 10^4 \pm 2.26 \times 10^3$

cfu : colony forming unit EM : Standard error of mean. Means without a common letter (a , b, c) differ significantly (P<0.05).

Table (2): Statistical analytical results of total coliforms count of the examined ice cream samples.

Ice cream samples	No. of examined samples	Positive samples		Coliforms count (MPN/g)		
		No.	%	Minimum	Maximum	Mean ± SEM
Large scale producers	50	8	16	4.0	7.0	4.38 ± 0.14 c
Small scale producers	50	50	100	4.0×10	5.0×10^3	$1.82 \times 10^3 \pm 2.43 \times 10^2$ b
Street Vendors	50	50	100	1.5×10^3	5.0×10^4	$8.37 \times 10^3 \pm 1.28 \times 10^3$ a
Total	150	108	72	4.0	5.0×10^4	$4.8 \times 10^3 \pm 5.8 \times 10^2$

MPN : Most probable number SEM : Standard error of mean. Means without a common letter (a , b, c) differ significantly (P<0.05).

Table (3): Statistical analytical results of total staphylococcal count of the examined ice cream samples.

Ice cream samples	No. of examined samples	Positive samples		Total staphylococcus count (cfu/g)		
		No.	%	Minimum	Maximum	Mean \pm SEM
Large scale producers	50	28	56	1.0×10^1	3.5×10^2	$1.08 \times 10^2 \pm 1.51 \times 10^0$ c
Small scale producers	50	50	100	1.0×10^2	1.2×10^4	$2.37 \times 10^3 \pm 3.26 \times 10^2$ b
	50	50	100	1.1×10^3	4.8×10^4	$7.27 \times 10^3 \pm 1.06 \times 10^3$ a
Street Vendors						
Total	150	128	85.33	1.0×10^1	4.8×10^4	$3.8 \times 10^3 \pm 4.6 \times 10^2$

cfu : colony forming unit SEM : Standard error of mean. Means without a common letter (a , b, c) differ significantly (P<0.05)

Table (4): The examined ice cream samples that complied with the Egyptian standard (*E.O.S.Q, 2005*)

Ice cream samples	Samples complied with the Egyptian standard							
	Total aerobic bacterial count		Coliforms count		Coagulase +ve staphylococci *		Salmonellae**	
	No.	%	No.	%	No.	%	No.	%
Large scale producers	50	100	50	100	50	100	50	100
Small scale producers	50	100	0	0	50	100	50	100
Street Vendors	49	98	0	0	50	100	50	100
Egyptian standard	< 1.5×10^5 cfu/g		< 10 MPN/g		Free from pathogens			

* coagulase +ve *Staphylococcus aureus* could not be detected from any of the examined ice cream samples .

** *Salmonellae* could not be detected in any of the examined ice cream samples

Fig. (1): Frequency distribution of the examined ice cream samples based on their total aerobic bacterial count (cfu/g)

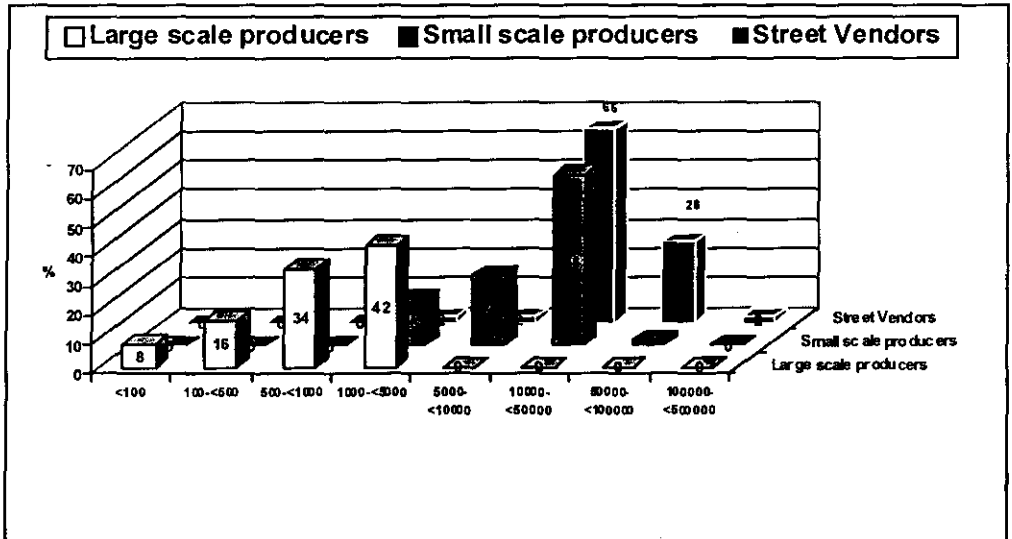


Fig. (2): Frequency distribution of the examined ice cream samples based on their total coliforms count (MPN/g).

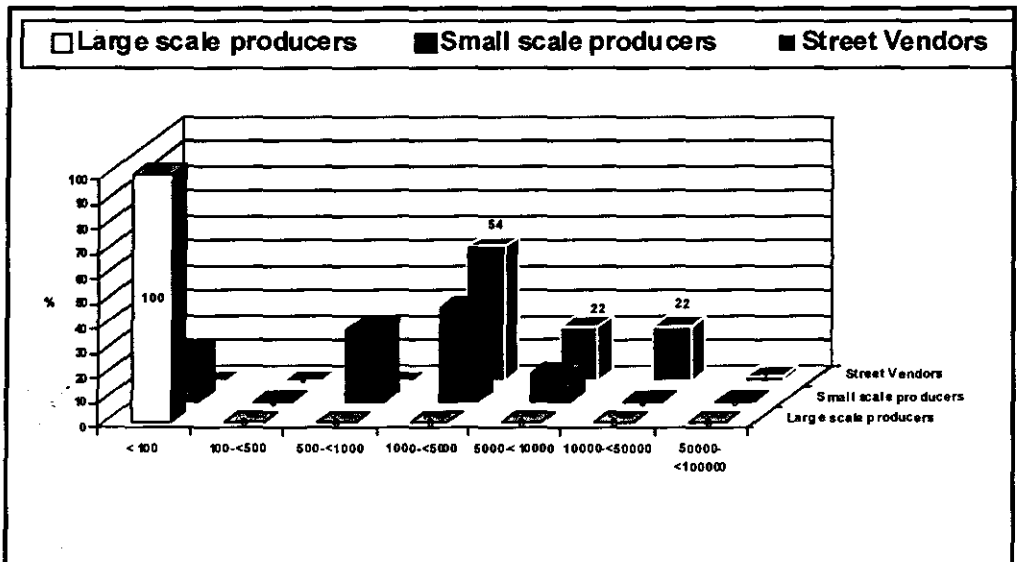
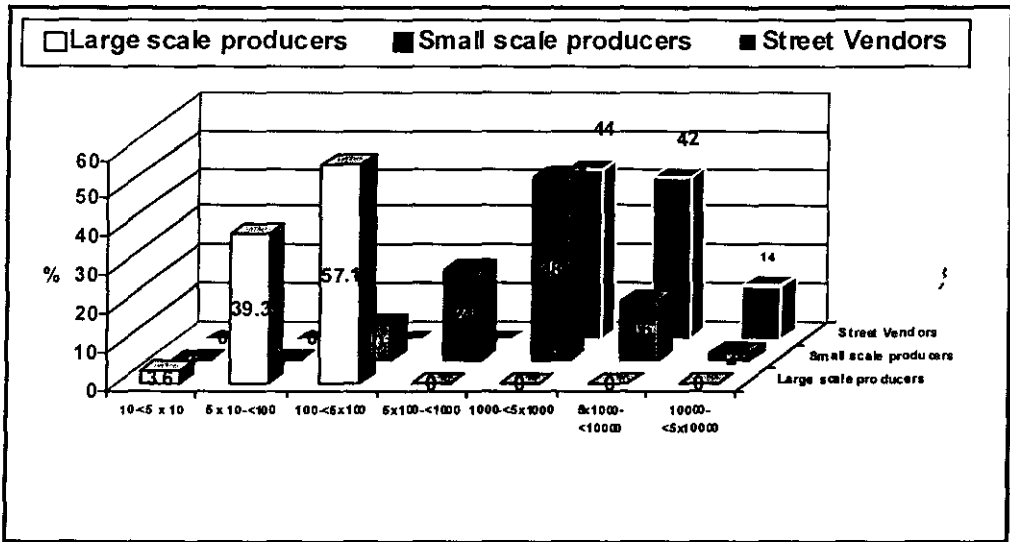


Fig. (3): Frequency distribution of the examined ice cream samples based on their staphylococcal count (cfu/g).



DISCUSSION

Results recorded in Table (1) reveal that all the examined ice cream samples were positive for total aerobic bacteria with a count range (and mean \pm SEM) of 5×10 to 3.5×10^3 ($1.07 \times 10^3 \pm 1.26 \times 10^2$), 1.4×10^3 to 6.7×10^4 ($1.40 \times 10^4 \pm 1.62 \times 10^3$) and 2.9×10^3 to 2.0×10^5 ($4.55 \times 10^4 \pm 4.79 \times 10^3$) cfu/g in ice cream samples collected from large scale producers, small scale producers and from street vendors respectively. Most of the examined large scale producers samples (42%) had TABC ranged from 10^3 - $<5 \times 10^3$ cfu/g followed by 34% at range of 5×10^2 - $<10^3$, while the highest frequency distribution (58%) of small scale producers ice cream samples lies within the range of 10^4 - $<5 \times 10^4$ cfu/g, also 66% of street vendors samples had TABC at the range 10^4 - $<5 \times 10^4$ cfu/g (Fig. 1).

The total aerobic bacterial count in any food article is not only a sure indicative of its safety for consumption, but is an important test for judging the hygienic condition under which the food has been processed and handled (*Saad, 1996*).

According to the Egyptian standard of ice cream (*E.O.S.Q., 2005*), all the examined ice cream samples were within the allowable limits of $<1.5 \times 10^5$ cfu/g for total aerobic bacterial count except only one sample from street vendors that had higher total aerobic bacterial count (Table 4).

Regarding coliforms, the results presented in Table (2) show that 8 (16%) samples out of the fifty examined ice cream samples from large scale producers were contaminated with coliforms with a count ranged from 4.0 to 7.0 MPN/g and a mean value of 4.38 ± 0.14 MPN/g. Considering small scale and street vendors ice cream samples, coliforms bacteria could be detected in all of the examined samples (100%), with count varied from 4.0×10 to 5.0×10^3 MPN/g and 1.5×10^3 to 5×10^4 with an average of $1.82 \times 10^3 \pm 2.43 \times 10^2$ and $8.37 \times 10^3 \pm 1.28 \times 10^3$ MPN/g . All the positive large scale producers ice cream samples (100%) had coliforms count $<10^2$ MPN/g, but 38% of the positive small scale samples had coliforms counts ranged from 10^3 - $<5 \times 10^3$ MPN/g also 54% of street vendors samples had coliforms count lies within the range of 10^3 - $<5 \times 10^3$ MPN/g (Fig. 2).

Coliform bacterial group in ice cream has been considered important in bacteriological analysis due to their significance as indicator organisms for pinpointing the unhygienic conditions during processing, handling and distribution (*Abdel-Haleem, 1995*). The Egyptian Standard

(*E.O.S.Q, 2005*) stipulates that coliforms count should not exceed 10 microorganisms/g in ice cream. All the examined ice cream samples (100%) obtained from small scale and street vendors had count higher than that recommended by (*E.O.S.Q, 2005*). On the other hand, all (100%) large scale ice cream samples had coliforms count complied with the recommended standards (Table 4).

The high coliforms count obtained in small scale and street vendors ice cream samples in this investigation reflects, unhygienic practices during manufacture and distribution. This may be due to poor ingredients, insufficient heat treatment, contaminated water and carelessness of employer who lack necessary knowledge of personal hygiene, product handling and sanitation routines (*Richards,2002 and Kanbakan et al.,2004*).

The results recorded in Table (3) reflect that staphylococci were detected in 28 (56%) out of the fifty examined ice cream samples from large scale producers, with total count ranged from 1.0×10^2 to 3.5×10^2 cfu/g and a mean value of $1.08 \times 10^2 \pm 1.51 \times 10^2$ cfu/g. While staphylococci could be detected in all (100%) of the examined ice cream samples collected from small scale producers and street vendors, with a count ranged from 1.0×10^2 to 1.2×10^4 and 1.1×10^3 to 4.8×10^4 cfu/g with an average of $2.37 \times 10^3 \pm 3.26 \times 10^2$ and $7.27 \times 10^3 \pm 1.06 \times 10^3$ cfu/g (Table3). Most of the large scale producers positive samples (57.1%) had staphylococcal counts at the range 10^2 - $<5 \times 10^2$ cfu/g, while the highest frequency distribution of small scale producers samples (48%) had counts ranged from 10^3 - $<5 \times 10^3$ cfu/g also 44% of street vendors samples had staphylococcal count lies within the range of 10^3 - $<5 \times 10^3$ cfu/g followed by 42% at the range 5×10^3 - $<10^4$ (Fig. 3).

Presence of staphylococci in ice cream represents a good indicator of the personal hygiene of workers with respiratory infection and suppuration (*Harvey and Gilmour, 1990 and Kamat et al., 1991*). From the achieved data presented in Table (3), it is evident that staphylococci were existed in all of the examined ice cream samples collected from small scale producers and street vendors. This could be attributed to the contamination of the mix from ingredients used, employees and from carelessness during distribution. Moreover, presence of staphylococci in 56% of the examined ice cream samples from large scale producers may indicate post pasteurization contamination from food handlers (*Hobbs and Gelbert, 1982*).

The growth of staphylococci in food products represents a potential public health hazard since many strains of *Staph. aureus* produce enterotoxins which cause food poisoning if ingested. Enterotoxin production and secretion occurs especially when ice cream are not properly prepared and stored, also the presence of starch and proteins encourages enterotoxin production by microorganisms (*Jay, 1992*).

Results show that coagulase +ve *Staphylococcus aureus* were not detected from any of the examined ice cream samples. According to the Egyptian Standard of ice cream (*E.O.S.Q., 2005*), the product should be free from pathogenic microorganisms, it is clear that all the examined ice cream samples from large scale, small scale producers and street vendors were free from *Staph. aureus* and comply with the Egyptian Standards (Table 4).

The presence of salmonella in ice cream may possibly be due to either fresh eggs or egg powder used in ice cream production or contamination of pasteurized ice cream premix during transport in tanker trailers that had previously carried non-pasteurized liquid eggs (Hennessy et al., 1996). The presence of *Salmonella* spp. may pose a great risk of public health.

Salmonellae could not be detected from any of the examined ice cream samples; this result agrees with the Egyptian standard (E.O.S.Q., 2005) which states that ice cream should be free from pathogenic microorganisms (Table 4).

Statistical analysis of data show that mean counts of total aerobic bacteria, coliforms and total staphylococci are significantly differ between the examined types (large scale producers, small scale producers and from street vendors) of ice cream samples at $P < 0.05$ (Tables 1, 2, 3).

From the results and discussion of the present study, it is evident that the majority of the examined small scale producers and street vendors ice cream samples were not satisfactory according to Egyptian standard while ice cream from large scale producers were of superior quality it is concluded that there is a great need for continuous monitoring of small scale producers and street vendors of ice cream, educating about good sanitary practices and periodical checking of ice cream should be carried out by health authorities to ensure relatively safe product.

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دراسات بكتريولوجية على الأيس كريم في محافظة كفر الشيخ

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الآيس كريم من منتجات الألبان الواسعة الانتشار بين فئات كثيرة وأعمار مختلفة من المستهلكين. تحت ظروف الإنتاج المختلفة وكذلك التوزيع والتداول قد يتعرض الآيس كريم للتلوث الميكروبي مما يشكل خطورة على صحة المستهلك ، لذلك أجريت هذه الدراسة على مئة وخمسون عينة آيس كريم (50 إنتاج مصانع كبيرة ، 50 إنتاج مصانع صغيرة ، 50 من الباعة الجائلين) جمعت من السوبر ماركت ، المحلات المختلفة والباعة الجائلين بمحافظة كفر الشيخ لتقييمها بكتريولوجياً. وجد أن متوسط العدد الكلى للبكتريا الهوائية في الآيس كريم لعينات إنتاج مصانع كبيرة، إنتاج مصانع صغيرة و الباعة الجائلين $1.07 \times 10^3 + 1.26 \times 10^2 \times 1.4 \times 10^4 + 1.62 \times 10^3$ و $4.55 \times 10^4 + 4.79 \times 10^3$ خلية/جم على التوالي. وبالنسبة لعدد الميكروب القولوني لذات العينات كان المتوسط العددي $0.14 + 4.38$ ، $1.82 \times 10^3 + 2.43 \times 10^2$ و $8.37 \times 10^3 + 1.28 \times 10^3$ خلية /جم. كما سجلت نتائج العد الكلى للميكروب المكور العنقودي متوسط عددي $1.08 \times 10^2 + 1.51 \times 10$ ، $2.37 \times 10^3 + 3.26 \times 10^2$ و $7.27 \times 10^3 + 1.06 \times 10^3$ خلية/جم لذات العينات على التوالي. اعتماداً على عدد الميكروب القولوني المعوى فإن كل عينات الآيس كريم المجمعة من صغار المنتجين وكذلك الباعة الجائلين لا تتفق مع المواصفات القياسية المصرية ، بينما عينات إنتاج المصانع الكبيرة تتفق مع هذه المواصفات. بينما أوضحت النتائج عدم تواجد الميكروب المكور العنقودي الذهبي وكذلك السالمونيلا في جميع العينات التي تم فحصها. هذا وقد نوقشت أسباب تواجد تلك الميكروبات في الآيس كريم وكذلك الشروط الصحية الواجب إتباعها للحفاظ على صحة المستهلك.