

## Role Of Small And Large Scale Producers In Transmission Of Food Poisoning Organisms Through Consumption Of Ice Cream

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### ABSTRACT

One hundred and twenty random samples of each of small scale and large scale ice cream lots were collected from groceries, peddlers and supermarkets in Zagazig City to be examined for their sanitary condition. Coliform counts /g. were  $4.3 \times 10^6$ ,  $4.9 \times 10^6$  and  $3.2 \times 10^6$  in plain, fruit and chocolate ice cream samples respectively in small scale samples mean while that of ice cream (cups) in large scale samples was  $1.4 \times 10^4$ . The most prevalent Coliform species isolated from examined small scale ice cream samples were *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Erwinia amylovora*, *Erwinia cycripedii*, *Klebsiella pneumoniae* and *Serratia marcescens*, while in case of large scale samples the most prevalent Coliform species were *Enterobacter cloacae*, *Enterobacter agglomerans* and *Klebsiella terrigena*. *E.coli* could not be detected in all samples. concerning the Staphylococci count the mean value in examined small scale ice cream samples was  $3.2 \times 10^4$ ,  $2.7 \times 10^5$  and  $1.5 \times 10^4$  in plain, fruit and chocolate ice cream samples respectively in small scale samples, while it was recorded as  $1.9 \times 10^2$ ,  $6 \times 10$  and  $4.9 \times 10$  in cups, bars and cones ice cream respectively in large scale samples. *Staphylococcus aureus* and *Staphylococcus epidermidis* were the predominated staphylococci isolated from the examined small scale ice cream samples, while in case of large scale ice cream samples *Staphylococcus aureus* was isolated only from cups ice cream samples. *Yersinia enterocolitica* could be detected only in 2.5% of examined chocolate ice cream samples.

### INTRODUCTION

Ice cream is frozen foam that consists of air cell dispersed in an aqueous matrix (1). Produced by freezing pasteurized mixture of milk solid other than fat, sugar, emulsifier and stabilizer. Flavour enrichment of ice cream is because of optional addition of fruit nuts, candies, syrups and other flavoring ingredients (2).

Ice cream is considered as one of the most favorite frozen dairy products all over the world. It is a delicious, highly nutritious food and has a therapeutic value for persons suffering from irritation and infection of mouth and throat due to its coldness (3). It is highly appreciable by all ages because it is considered as safe, enjoyable, energy giving and refreshing food because levels of water soluble vitamins and minerals are two to three times higher than those of full cream milk. Also, it is a valuable source of vitamin B. and contains twice vitamin A content as that of milk (4). But it is not a major nutritional significance among the general population possible exceptions are amongst children and old persons for whom ice

cream can be an important source of dietary energy (5).

Ice cream is a relatively safe product, but failure to pasteurize it and to prevent environmental contamination can render it unsafe, especially to infants and immunocompromised adults. It is protected from spoilage by very low temperature of preparation and storage; however major ingredients used to make these products are prone to spoilage and several ingredients are added after the last lethal process, (pasteurization has been completed) (6).

Ice cream is a milk based product which can be considered a good medium for microbial growth due to its nutrient content, almost neutral PH (PH 6-7) and long storage duration. several steps in the production of ice cream can cause microbiological hazards. However, pasteurization, freezing and hardening steps can eliminate most of these hazards. Pasteurization of milk can destroy most of the pathogens posed risk to public health. The potential microbiological hazards can still be found in the final products after pasteurization through the

addition of contaminated ingredients or improper handling. This is especially important in the preparation of soft ice cream as its final stage of the production is carried out at point of sale (2, 7).

## MATERIAL AND METHODS

### Collection of samples

A total of 240 random samples of small scale and large scale ice cream (120 for each type) were collected from different localities in Zagazig City, Sharkia governorate, Egypt. Each sample was obtained as it is sold in cups, biscuits, sticks or bars (large scale samples) or was taken by the spatula into sterile air tight jar of 100g. capacity containers which was filled to the top (little free air space) for small scale samples. Samples were transferred to the laboratory with a minimum delay in an insulated ice box at  $-4^{\circ}\text{C}$  then immediately examined.

### Preparation of samples

Each sample was left to stand in a thermostatically controlled water bath adjusted at  $44^{\circ}\text{C}\pm 1$  for not more than 15 minutes with periodical shaking till it was melted. The prepared sample was subjected to the following examination.

### Bacteriological examination

#### 1-Preparation of serial dilution (8)

After thoroughly mixing 11gm. From each sample were transferred into sterile, wide mouthed container, containing 99 ml of sterile water ( $40^{\circ}\text{C}$  to  $45^{\circ}\text{C}$ ), shake well until a homogeneous dispersion of 1:10 dilution was obtained, from which decimal serial dilutions were prepared.

#### 1-Enumeration and isolation of Coliforms (MPN/gm)

The count was determined using 3-tubes technique(8) streaks from positive tubes (acid and gas) were evenly spread onto MacConkey's agar plates. The plates were incubated for 24 hours at  $37^{\circ}\text{C}$ .and then the suspected colonies were picked up and isolated in a pure culture for further identification (9).

#### 2-Enumeration and isolation of Staphylococci (10)

One tenth ml from the previously prepared decimal dilutions of the examined samples was transferred and evenly spread on the dry surface of Baird-Parker agar medium plates (11) using a sterile bent glass rod. Inoculated plates were incubated at  $37^{\circ}\text{C}$  for 48 hours and Staphylococci count were calculated and recorded. suspected colonies were picked up and isolated in a pure culture for further identification (12).

#### 3-Isolation of Yersinia

Pre-enrichment on phosphate buffered saline and selective enrichment on bile oxalate-sorbose were carried out then a loopful of selective enrichment broth culture was seeded onto cefsulodin irgasan novobiocin (CIN) medium and incubated at  $25^{\circ}\text{C}$  for 24 hrs. suspected colonies were picked up and isolated in a pure culture for further identification (9,13,14).

## RESULTS AND DISCUSSION

The results achieved in Table 1 showed that total coliform count (MPN/gm.) of the examined small scale ice cream samples were ranged from  $4\times 10^2$  to  $1.1\times 10^8$ ,  $9\times 10^2$  to  $1.2\times 10^8$  and  $4\times 10^2$  to  $12\times 10^8$  with a mean value of  $4.3\times 10^6 \pm 0.28\times 10^6$ ,  $4.9\times 10^6 \pm 0.29\times 10^6$  and  $3.2\times 10^6 \pm 0.27\times 10^6$  for plain, fruit and chocolate ice cream respectively.

The obtained results are nearly similar to that previously reported (15). Higher (7, 16, 17). and lower values were reported (18, 19, 20).

Table 2 showed that total coliform count (MPN/gm.) of examined large scale ice cream samples was ranged from  $4.0\times 10^2$  to  $2.0\times 10^5$  with a mean value of  $1.4\times 10^4 \pm 6\times 10^3$  for cups ice cream only while it is negative in bars and cones ice cream samples.

Lower results were obtained by several authors (19, 20, 22). Coliforms were isolated in all examined ice cream samples (23), while in other study (20) failed to detect coliforms in examined ice cream samples.

**Table 1. Statistical analytical results of different bacterial counts in examined small scale samples**

Bacterial count/ml.	Plain ice cream			Fruit ice cream			Chocolate ice cream		
	Min .	Max .	Mean ±S.E.	Min .	Max .	Mean ±S.E.	Min .	Max .	Mean ±S.E.
Coliforms (MPN/gm):	$4 \times 10^2$	$1.1 \times 10^8$	$4.3 \times 10^6 \pm 0.28 \times 10^6$	$9 \times 10^2$	$1.2 \times 10^8$	$4.9 \times 10^6 \pm 0.29 \times 10^6$	$4 \times 10^2$	$12 \times 10^8$	$3.2 \times 10^6 \pm 0.27 \times 10^6$
Staphylococci	$2 \times 10$	$1 \times 10^5$	$3.2 \times 10^4 \pm 2.4 \times 10^4$	$9 \times 10$	$7.5 \times 10^5$	$2.7 \times 10^5 \pm 1.9 \times 10^5$	$4 \times 10$	$3 \times 10^5$	$1.5 \times 10^4 \pm 0.87 \times 10^4$

**Table 2. Statistical analytical results of different bacterial counts in examined large scale samples**

Bacterial count/ml.	ice cream (cups)			ice cream (bars)			ice cream (cones)		
	Min .	Max .	Mean ±S.E.	Min .	Max .	Mean ±S.E.	Min .	Max .	Mean ±S.E.
Coliforms (MPN/gm):	$4 \times 10^2$	$2 \times 10^5$	$1.4 \times 10^4 \pm 0.60 \times 10^4$	0	0	0	0	0	0
Staphylococci	$2 \times 10$	$1 \times 10^5$	$3.2 \times 10^4 \pm 2.4 \times 10^4$	$9 \times 10$	$7.5 \times 10^5$	$2.7 \times 10^5 \pm 1.9 \times 10^5$	$4 \times 10$	$3 \times 10^5$	$1.5 \times 10^4 \pm 0.87 \times 10^4$

Table 3 showed that *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Erwinia amylovara*, *Erwinia cycripedii*, *Klebsiella apneumoniae* and *Serratia Mmrcenscens* were isolated from the examined small scale ice cream samples in the percentages of 5%, 2.5%, 0.0, 5%, 17.5%, 7.5% and 2.5% from plain ice cream and of 2.5%, 2.5%, 7.5%, 5%, 10%, 5% and 2.5% from fruit ice cream, While in Chocolate ice cream isolated as 0.0, 5%, 0.0, 2.5%, 7.5%, 7.5% and 0.0 respectively.

The results obtained are nearly similar to that reported in several studies (20, 24, 25). While it is partially different with that obtained in other study (15).

The results in Table 4 showed that *Enterobacter cloacae*, *Enterobacter agglomerans* and *Klebsiella terrigena* were isolated from the examined large scale ice cream samples by the percentages of 5.0, 5.0 and 25.0% respectively. Partially different values were cited (20).

Coliforms being non-spore formers and consequently susceptible to pasteurization. Their presence post pasteurization 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 (26,27).

The occurrence of psychrotrophic enterobacteriaceae in milk products is of greater importance with regard to the quality and shelf life of dairy products. The species and the numbers of enterobacteriaceae present determine the potential quality impairment of the product, mainly due to extracellular proteolytic and lipolytic enzymes. These enzymes induce undesirable changes in milk proteins and milk fats. The destabilization of casein and the production of free fatty acids cause organoleptic defects in milk and its products, giving rise to a rancid and off-flavor (28, 29).

Table 3. Incidence of organisms isolated from the examined small scale samples

	Plain ice cream (N=40)		Fruit ice cream (N=40)		Chocolate ice cream (N=40)	
	+ve	%	+ve	%	+ve	%
<u>Coliforms:</u>	2	5.0	1	2.5	0	0.0
<i>Citrobacter freundii</i>						
<i>Enterobacter aerogenes</i>	1	2.5	1	2.5	2	5.0
<i>Enterobacter cloacae</i>	0	0.0	3	7.5	0	0.0
<i>Erwinia amylovora</i>	2	5.0	2	5.0	1	2.5
<i>Erwinia cycripedii</i>	7	17.5	4	10.0	3	7.5
<i>Klebsiella pneumoniae</i>	3	7.5	2	5.0	3	7.5
<i>Serratia marcescens</i>	1	2.5	1	2.5	0	0.0
<u>Staphylococci:</u>	6	15.0	5	12.5	4	10.0
<i>Staph. aureus</i>						
<i>Staph. epidermidis</i>	15	37.5	13	27.5	20	50.0
<u>Yersinia :</u>	0	0.0	0	0.0	1	2.5
<i>Yersinia enterocolitica</i>						
<i>Yersinia intermedia</i>	1	2.5	3	7.5	6	15

The results tabulated in Table 1 revealed that the examined small scale ice cream samples for Plain, Fruit and Chocolate samples were contaminated by Staphylococci, the level of contamination was ranged from  $2 \times 10^4 - 1 \times 10^5$  with a mean value of  $3.2 \times 10^4 \pm 2.4 \times 10^4$ ,  $9 \times 10^4 - 7.5 \times 10^5$  with a mean value of  $2.7 \times 10^5 \pm 1.9 \times 10^5$  and  $4 \times 10^4 - 3 \times 10^5$  with a mean value of  $1.5 \times 10^4 \pm 8.7 \times 10^3$  respectively for plain, fruit and chocolate ice cream samples. Relatively similar results were obtained (18), while higher (17, 27, 30) and lower values were recorded (20, 21, 22).

Table 2 showed that total staphylococci count in examined large scale ice cream samples was ranged from  $2 \times 10^4 - 1 \times 10^5$  with a mean value of  $3.2 \times 10^4 \pm 2.4 \times 10^4$ ,  $9 \times 10^4 - 7.5 \times 10^5$  with mean average  $2.7 \times 10^5 \pm 1.9 \times 10^5$  and  $4 \times 10^4 - 3 \times 10^5$  with mean average  $1.5 \times 10^5 \pm 8.7 \times 10^3$  for cups, bars, cones ice cream samples respectively. Lower values were detected (33), while higher values were recorded (22, 34).

According to identification of isolated staphylococci, *Staphylococcus aureus* was detected in 15, 12.5 and 10% of examined plain, fruit and chocolate ice cream

respectively, while 37.5%, 27.5% and 50% of examined samples were contaminated by *Staphylococcus epidermidis* (Table 3).

*Staphylococcus aureus* was detected in 60% of examined ice cream samples (35), while (36) found this M.O. in all examined samples.

The obtained results are coincided with the study which indicated found that all the examined samples were free from *Staphylococcus aureus* (37).

Table 4 showed that *Staphylococcus aureus* was detected only in 10% of cups ice cream samples. but it failed to be detected in bars and cones ice cream samples, while 25%, 30% and 10% of examined samples were contaminated by *Staphylococcus epidermidis* respectively. *Staphylococcus aureus* was detected in all examined samples in India (36).

Although several authors (19-21) didn't detect *Staphylococcus aureus* in their examined samples.

*Staphylococcus aureus* is found in a wide variety of habitats including human skin, where many strains are commensally that may be clinically significant or contaminants of

food (38). It is a frequent cause of human infections and cause gastrointestinal symptoms like nausea, emesis, abdominal cramps and diarrhea (39). Also it is considered the most frequently occurring major pathogen of cow's mammary gland (40, 41).

The presence of coagulase positive staphylococcosis, which is mainly *Staphylococcus aureus* when transmitted from man and animals, can lead to Staphylococcal food poisoning as a result of growth of the organism and release of the enterotoxins in the food. Enterotoxins production and secretion occurs especially when ice cream products are not properly prepared and stored. The presence of starch and proteins also encourages enterotoxin production by micro-organisms (42).

The results given in Tables 3 and 4 showed that *Yersinia enterocolitica* failed to be detected in any of examined plain or fruit ice cream samples but can be detected in 2.5% of examined chocolate ice cream while *Yersinia intermedia* can be detected in 2.5%, 7.5% and 15% in plain, fruit and chocolate ice cream respectively. Lower (43) and higher values were recorded (20,44).

*Yersinia* species failed to be detected in any of the examined large scale ice cream samples (20,36).

*Yersinia* organisms are Gram negative, psychrotrophic milk-borne enteric pathogens. These organisms are widespread in the environment and are indigenous to the gastrointestinal tracts of worm blooded animals including dairy cattle (45, 46).

**Table 4. Incidence of organisms isolated from the examined large scale samples**

Organisms	ice cream (cups) (N=40)		ice cream (bars) (N=40)		ice cream (cones) (N=40)	
	+ve	%	+ve	%	+ve	%
<u>Coliforms:</u>	2	5.0	0	0.00	0	0.00
<i>Enterobacter Cloacae</i>						
<i>Enterobacter agglomerans</i>	2	5.0	0	0.00	0	0.00
<i>Klebsiella terrigena</i>	10	25.0	0	0.00	0	0.00
<u>Staphylococci:</u>	4	10.0	0	0.00	0	0.00
<i>Staph.aureus</i>						
<i>Staph.epidermidis</i>	10	25.0	12	30.0	4	10.0
<u>Yersinia :</u>	0	0.0	0	0.00	0	0.00
<i>Yersinia enterocolitica</i>						
<i>Yersinia intermedia</i>	0	0.0	0	0.00	0	0.00

*Yersinia enterocolitica* has the distinction of surviving and multiplying in food held at refrigeration temperature, therefore milk and dairy products contaminated initially with even low levels of this bacterium may serve not only as a vehicle, but also as a medium for its proliferation (47). Milk and its products have been incriminated in several outbreaks of yersiniosis due to the psychrotrophic nature of *Y. enterocolitica* which is accompanied by increasing use of refrigeration in food preservation. A highly publicized food associated outbreak of yersiniosis among

school children occurred in Oneida, New York, due to consumption of contaminated chocolate milk (48).

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### الملخص العربي

دور منتجى المثلوجات اللبنية على النطاق الواسع والمحدود فى نقل ميكروبات التسمم الغذائى

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أجريت هذه الدراسة على ٢٤٠ عينة عشوائية جمعت من مصادر مختلفة سواء من صغار المنتجين (بدون اضافات والمنكهة بالفواكهة والمنكهة بالشيكلولائة) او من الانواع المنتجة على نطاق واسع (المعبأة فى اكواب والاستيك والكونو) والمعروضة للبيع بمدينة الزقازيق وزعت كالتالى ٤٠ عينة لكل نوع من عينات صغار المنتجين وكذلك عينات النطاق الواسع وقد اسفرت الدراسة عن النتائج الاتية:

تواجدت ميكروبات الكوليفورم فى عينات المثلوجات اللبنية لصغار المنتجين ( بدون اضافات والمنكهة بالفواكهة والمنكهة بالشيكلولائة) بنسبة ٤٠% و ٣٥% و ٢٢,٥% على التوالى وقد كان متوسط عدد تلك الميكروبات فى هذه العينات هو ٤,٣ × ١٠<sup>٦</sup> و ٤,٩ × ١٠<sup>٦</sup> و ٣,٢ × ١٠<sup>٦</sup> على التوالى. بينما لم يتم عزل ميكروبات الكوليفورم من عينات المثلوجات اللبنية ذات النطاق الواسع فيما عدا العينات التى تباع معبأة فى اكواب وذلك بنسبة ٣٥% وكان متوسط عددها ١,٤ × ١٠<sup>٤</sup>. كما تبين ان متوسط المكورات العنقودية فى عينات المثلوجات اللبنية بدون اضافات والمنكهة بالفواكهة والمنكهة بالشيكلولائة فى عينات صغار المنتجين هى ٣,٢ × ١٠<sup>٤</sup> و ٢,٧ × ١٠<sup>٤</sup> و ١,٥ × ١٠<sup>٤</sup> على التوالى بينما كانت فى عينات النطاق الواسع هى ١,٩ × ١٠<sup>٤</sup> و ٦ × ١٠<sup>٤</sup> و ٤,٩ × ١٠<sup>٤</sup> فى العينات المعبأة فى اكواب والاستيك والكونو على التوالى. هذا وقد تم عزل وتصنيف عترات ستروباكترا فرينداى وانتيروباكترا ابروجينز انتيروباكترا كلواكى و ابرونيا اميلوفارا و ابرونيا سيكريبيدى و كليسيلا نيمونى و سيراتيا مارسينسينز من عينات صغار المنتجين بينما تم عزل عترات انتيروباكترا كلواكى و انتيروباكترا اجلوميرانز وكليسيلا تيريجينا من عينات النطاق الواسع. وكذلك تم عزل ميكروب المكور العنقودى الذهبى من عينات صغار المنتجين بنسبة ٢٥% بينما تم عزلها بنسبة ٦,٦٧% من العينات ذات النطاق الواسع. كما امكن عزل ميكروب اليارسينيا انتيروكوليتيكا من عينات المثلوجات اللبنية لصغار المنتجين بنسبة ٢,٥% بينما لم يتم عزله من عينات المثلوجات اللبنية ذات النطاق الواسع .