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Microbiological evaluation of small and large scale produced ice cream in Assiut city

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ABSTRACT

This study aimed to compare between the microbiological criteria of small and large scale produced ice cream consumed in Assiut city, Egypt. A total of 70 small and large scale produced ice cream samples (35 each) were collected from available retailer ice cream shops and supermarkets in Assiut city and evaluated microbiologically. The incidences of aerobic bacteria count (APC), psychrotrophes, *Enterococci*, coliform, faecal coliform counts, *Escherichia coli*, yeasts and moulds counts were 100, 100, 97.14, 65.71, 31.43, 22.86, 100 and 65.71% in small scale produced ice cream samples respectively; while, in large scale produce ice cream samples, the incidences were 100, 74.29, 42.86, 45.71, 31.43, 11.43, 54.29 and 100%, respectively. *Staphylococcus aureus* was negative in both type of ice cream samples while coagulase negative *Staphylococci* (CNS) and anaerobes were detected in small scale produce ice cream samples in incidences of 11.43 and 80% respectively, the incidences were 8.57 and 57.14% for large scale produced ones, respectively. Two isolates of *Escherichia coli* O157:H7 and *Stx*1 and *Stx*2 were detected in some *Escherichia coli* strains isolated from small scale produced ice cream samples. Comparison of the microbiological criteria of the two types of ice cream samples with Egyptian Standards was also done. Sources of these microorganisms, their economic and public health importance as well as the suggestive control measures were discussed.

INTRODUCTION

Ice-cream is considered one of the most favorite dairy products for voluminous segments of the population not only for younger but also for older. It is a good supply for calcium, phosphorous, milk proteins, fat, lactose and essential vitamins **(Balthazar et al. 2017).**

Unfortunately, the microbiological quality

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of ice cream can be low, as it is a good growth-medium for microbes due to its nutrients and to its almost neutral pH of 6-7. There are numerous reports on human pathogens incidence in ice cream as *Staphylococcus aureus*, *Listeria monocytogenes, Bacillus cereus*, pathogenic Streptococus species, *Escherichia coli especisally Escherichia coli* O157:H7, *Salmonella spp*., *Yersinia enterocolitica* and **(Warke et al. 2000; Kanbakan et al. 2004; Hosseini-Naveh et al. 2019 and Yan et al. 2022).**

Consumption of ice cream contaminated with *Escherichia coli* O157:H7 can be fatal due to possibility of haemolytic uraemic syndrome incidences **(Jenkins et al. 2020).**

Ice cream is considered a good medium for growth of psychrotropic microorganisms that can cause spoilage of milk and dairy product because they are able to produce extracellular or intracellular thrmoresistant enzymes (proteases, lipases and phospholipases) **(Cempírková and Mikulová, 2009).**

Ice cream is produced in Egypt on two scales: a small scale, where it is made in ice cream shops, and a large scale, where it is manufactured in the large factories of the producing companies. Moreover, during its production, contamination of the products occurs, whether the production is on a small scale or on a large scale, and this contamination may be with some microbes that cause human diseases such as *Staphylococcus aureus* and *Escherichia coli* O157:H7. Therefore, the aim of this study is to determine and compare the microbiological status of ice cream produced on a small and large scale with a comparison of those results with the Egyptian Standard specifications No, 1185-1 (2005) and isolate some human pathogens such as *Staphylococcus aureus* and *Escherichia coli* O157:H7.

MATERIALS and METHODS

Samples collection: a total of 70 small and large-scale produced ice cream samples (35 each) were collected from ice cream shops and supermarkets in Assiut city, Egypt. Each sample was collected in its container as soled to the consumers in sterilized polyethylene bags and transferred rapidly in ice box as soon as possible to the laboratory for microbiological examination.

Sample Preparation : ice cream samples were melted in a thermostatically controlled water bath at a temperature of up to 40°C for not more than 15 minutes and mixed well. Ten ml of melted samples were diluted in 90 ml sterile saline to make 1/10 dilution then subsequent ten-fold serial dilution was made up to 10^{-6}/ml dilution using sterile saline then 0.1 ml of each corresponding dilution was used for enumeration of different microbial parameter.

Aerobic plate count, total bacterial count using standard plate count agar, according to ISO 4833-2 :2013.

Psychrotrophic count, using standard plate count agar, according to ISO 1740 :2019.

Enterococci count by spreading method, using KF agar medium, according to **Hartman et al. (2001).**

Total coliforms, fecal coliforms and Escherichia coli count, using MPN/ml Methods, according to **Feng et al. (2020).**

Identification of the isolated *Escherichia coli* strains, by using biochemical testes (Indole, Methyl Red, Voges-Proskauer, Citrate utilization and Urease tests), according to **Mahon and Lehman. (2019).**

Isolation and identification of *Staphylococcus aureus*, using nutrient broth containing 10% NaCl and mannitol salt agar, according to **A.P.H.A. (2001).**

Total yeasts and molds counts, using malt extract agar, according to **Tournas et al. (2001).**

Detection of anaerobes, by using the Stormy Fermentation test, according to **Cruickshank et al. (1975).**

Molecular detection of Shiga toxins (*Stx*1 and *Stx*2) genes and *fli*C specific gene for *Escherichia coli* O175:H7 in some *Escherichia coli* strains isolated from small and large scale

ice cream samples by application of PCR assay:

Application of PCR assay was performed in reference Lab., Animal Health Research Institute, Egypt, by the following molecular procedure:

DNA extraction: DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer.

Oligonucleotide Primers. Primers used were supplied from Metabion (Germany) are listed in the following table

Target gene	Primers sequences	Amplified segment (bp)	Amplification (35 cycles)				Final extension	Reference
			Primary denaturation	Secondary denaturation	Annealing	Extension		
*Stx*1	ACACTG-GATGATCTCAGTGG CTGAATCCCCCTCC ATTATG	614	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Dipineto *et al.*, 2006
*Stx*2	CCATGACAAC-GGACAGCAGTT CCTGTCAACTGAG-CAGCACTTTG	779						
Escherichia coli O157:H7 fliC	GCGCTGTCGAGTTC-TATCGAGC CAAC-GGTGACTTTATCGC CATTCC	625	94˚C 5 min.	94˚C 30 sec.	57˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Fratamico *et al.*, 2000

PCR amplification: For *Stx*1 and *Stx*2 genes, duplex PCR assay was used. Primers were utilized in a 50- µl reaction containing 25 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 15 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler. For *Escherichia coli* O157: H7 *fli*C gene, uniplex PCR was used. Primers were utilized in a 25-µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 40 µl of the duplex PCR products and 20 µl of the uniplex PCR products were loaded in each gel slot. Generuler 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Statistical analysis of data: statistical analysis was done with GraphPad Prism software packaged for windows version 9.3.1 (GraphPad-Software, LLC, USA). Descriptive statistics (mean and standard error) were calculated. Shapiro-Wilk's test was applied to establish normality or non-normality of data distribution. Comparative statistics between the two types of ice cream were applied by using unpaired *t*-test to compare the mean values between the two types for parametric variables (normal data distribution) while, Mann-Whitney *U* test was used to compare the difference between the two groups for non-parametric variables (no normality in data distribution**) (Lantz et al. 2016).** In addition, *Chi*-square test was used to compare the categorical variables between both types. Significant result was considered when *P* value was < 0.05 (**McHugh, 2013**).

RESULTS

Table 1. Statistical analytical results of microbiological examination of small and large scale produced ice cream samples (n = 35).

Microbiological examination	Small scale produced ice cream						Large scale produced ice cream					
	Positive samples		Min.	Max.	Average	±SEM	Positive samples		Min.	Max.	Average	±SEM
	No./35	%					No./35	%				
APC (CFU/ml)	35	100	1.9×10^3	3.04×10^5	9.17×10^4	1.32×10^4	35	100	10^2	4.8×10^5	1.06×10^5	2.19×10^4
Psychrotrophs (CFU/ml)	35	100[a]	10^2	1.92×10^5	4.74×10^{4b}	7.64×10^3	26	74.29[a]	$< 10^2$	2.48×10^5	3.86×10^{4b}	1.11×10^4
Enterococci (CFU/ml)	34	97.14[a]	$< 10^2$	2.5×10^5	3.18×10^{4b}	8.41×10^3	15	42.86[a]	$< 10^2$	4.71×10^4	2.64×10^{3b}	1.51×10^3
Coliform (MPN/ml)	23	65.71	<3.0	1.1×10^3	3.3×10^2	7.94×10	16	45.71	<3.0	1.1×10^3	2.18×10^2	6.66×10
Faecal coliform (MPN/ml)	11	31.43	<3.0	2.4×10^2	1.79×10	7.91	11	31.43	<3.0	1.1×10^3	7.72×10	4.39×10
Escherichia coli (MPN/ml)	8	22.86	<3.0	2.1×10	2.46	0.96	4	11.43	<3.0	1.1×10^3	3.86×10	3.2×10
Yeast (CFU/ml)	35	100[a]	10^2	4.29×10^4	4.81×10^{3b}	1.28×10^3	19	54.29[a]	$< 10^2$	3.55×10^4	4.96×10^{3b}	1.74×10^3
Mould (CFU/ml)	23	65.71[a]	$< 10^2$	1.2×10^3	2.06×10^{2b}	4.81×10	35	100[a]	10^2	2×10^3	6.06×10^{2b}	9.99×10

[a]Percentages in the same raw with same letters indicate having significance difference (*Chi*-square test).
[b]Aaverages in the same raw with same letters indicate having significance difference (Mann-Whitney *U* test).

Table 2. PCR results for detection of *Stx*1, *Stx*2, *fli*C genes in some *Escherichia coli* isolate.

Serial number of *Escherichia coli* strains	Type of ice cream sample	Colony colour on MacConkey sorbitol agar	Shiga toxin genes		*Escherichia coli* O157:H7 gene
			*Stx*1 gene	*Stx*2 Gene	*fli*C gene
1	Small scale	Pale colony	-ve	+ve	+ve
2	Small scale	Red colony	-ve	-ve	Not tested
3	Small scale	Pale colony	-ve	+ve	+ve
4	Small scale	Red colony	+ve	-ve	Not tested
5	Large scale	Red colony	-ve	-ve	Not tested
6	Large scale	Red colony	-ve	-ve	Not tested
7	Large scale	Red colony	-ve	-ve	Not tested
8	Large scale	Red colony	-ve	-ve	Not tested

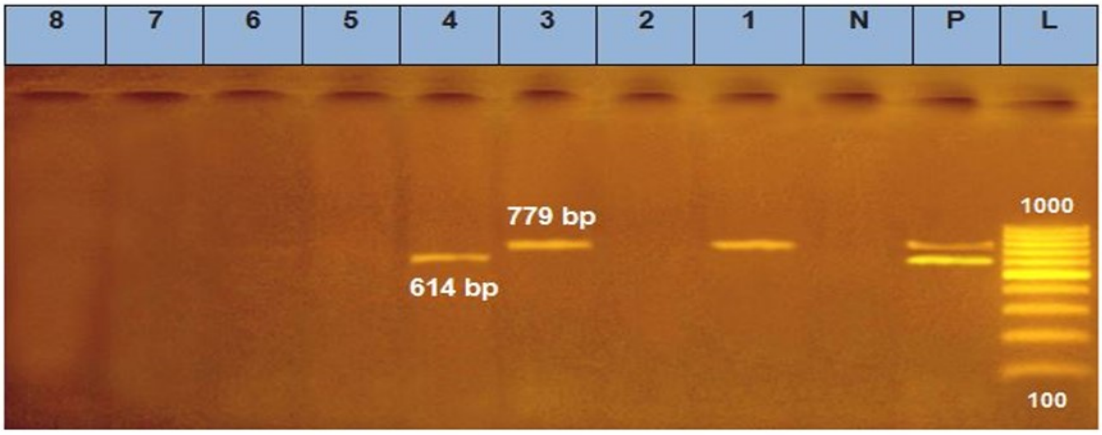

Figure 1: Agarose gel electrophoresis patterns of duplex PCR amplification products for *Escherichia coli Stx*1 and *Stx*2 genes. Lane L, DNA ladder marker (100 bp). Lane P, control positive *Escherichia coli Stx*1 (614 bp) and *Stx*2 (779 bp) genes. Lane N, negative control. Lanes 1 and 3 positive *Escherichia coli* isolates for *Stx*2 gene. Lane 4 positive *Escherichia coli* isolates for *Stx*1 gene. Lanes 2, 5-8, negative *Escherichia coli* isolates for *Stx*1 and *Stx*2 genes.

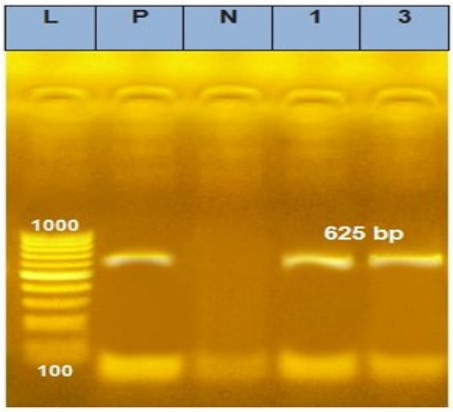

Figure 2: Agarose gel electrophoresis patterns of uniplex PCR amplification products for *Escherichia coli* O157:H7 *fli*C gene. Lane L, DNA ladder marker (100 bp). Lane P, control positive *Escherichia coli* O157:H7 *fli*C gene (625 bp). Lane N, negative control. Lanes 1 and 3 positive *Escherichia coli* O157:H7 isolates for *fli*C gene.

Table 3. Incidences of *Staphylococcus aureus*, CNS and anaerobe in ice cream samples.

Microorganism	Small scale produced ice cream		Large scale produced ice cream	
	Positive samples		Positive samples	
	No./35	%	No./35	%
Staphylococcus aureus	0	0.0	0	0.0
CNS	4	11.43	3	8.57
Anaerobes	28	80[a]	20	57.14[a]

[a]Percentages in the same raw with same letters indicate having significance difference (*Chi*-square test).

Table 4. Acceptability of small and large scale produced ice cream samples in relation to the Egyptian standards (2005/1185-1).

Microbiological parameter	Critical limit	Small scale produced ice cream				Large scale produced ice cream			
		Acceptable		Unacceptable		Acceptable		Unacceptable	
		No./35	%	No./35	%	No./35	%	No./35	%
Total colony count	Not > 15×10^4 cfu/ml	27	77.14	8	22.86	25	71.43	10	28.57
Coliform count	Not > 10 cfu/ml	14	40	21	60[a]	23	65.71	12	34.29[a]
Escherichia coli	absent/ml	27	77.14	8	22.86	31	88.57	4	11.43
Staphylococcus aureus	absent/ml	35	100	0	0.0	35	100	0	0.0

[a]Percentages in the same raw with same letters indicate having significance difference (*Chi*-square test).

DISCUSSION

The illustrated results in Table (1) revealed that, the incidence of counted aerobic bacteria in the examined small scale produced ice cream samples was 100%, with a count ranging from 1.9×10^3 to 3.04×10^5 and with an average count of 9.17×10^4 CFU/ml. The same incidence was reported by **El-Malt et al. (2013)** and **GadAllah et al. (2020)** Lower incidence 84% was found by **Abo El-Makarem (2017**). The incidence of counted aerobic bacteria in the examined large scale produced ice cream samples was 100%, with a count ranging from 1×10^2 to 4.8×10^5 and with an average count of 1.06×10^5 CFU/ml. The same incidence was revealed by **Al-Ansary (2014).** While, **Abo El-Makarem (2017)** found lower incidence of 60%.

The using of statistical analysis between the average count/ml of aerobic bacteria between small and large scale produced ice cream samples there was no significance difference between the two types of ice cream samples (Mann-Whitney $U = 543$ and $P > 0.05$). The heavy aerobic bacterial load in small and large scale produced ice cream samples at this study may be attributed to inferior quality of used ingredients, inefficient or lack of heating process and or post-processing con-

tamination. In addition, the ability of some microorganisms to form biofilm is an issue of concern for persistent of ice cream contamination with various microbial consortia.

The incidence of counted psychrotrophic bacteria in the examined small scale produced ice cream samples was 100%, with a count ranging from 1×10^2 to 1.92×10^5 and with an average count of 4.74×10^4 CFU/ml (Table 1). The same incidence was obtained by **El-Malt et al. (2013) and GadAllah et al. (2020)** while, lower incidence of 66% was revealed by **Sotohy et al. (2022).** Concerning large scale produced ice cream samples, the incidence of counted psychrotrophic bacteria was 74.29% with a count ranging from $< 10^2$ to 2.48×10^5 and with an average count of 3.86×10^4 CFU/ml (Table 1). Higher incidence (100%) was found by **GadAllah et al. (2020).**

The statistical analysis revealed that, there was highly significance difference between incidence of psychrotrophic bacteria in the examined small and large scale produce ice cream samples (χ^2 = 10.33 and $P < 0.01$). Moreover, there was highly significance difference between averages count of both types of ice cream samples (Mann-Whitney $U = 377$ and $P < 0.01$). Interestingly, from statistical analysis it is clear that, small scale ice cream is of inferior quality than large scale produced one based on psychrotrophic results.

From the hygienic point of view, the possible sources of psychrophilic contamination of products include unclean workers' hands, poor water quality for cleaning equipment and utensils, poor hygiene conditions in packaging and storage rooms, and some untreated frozen raw materials such as fruits, nuts, sauces, colors, aromas, were considered to be added to the product. In addition, the presence of psychrophilic bacteria in milk and dairy products can be of great concern as these organisms can grow and multiply during storage even at low temperatures and cause these products to spoil **(Barman et al. 2017).**

The incidence of *Enterococci* in small scale produced ice cream samples was 97.14% with a count ranging from $< 10^2$ to 2.5×10^5 and with an average count of 3.18×10^4 CFU/ml (Table 1). Lower incidences of 62 and 42% were obtained by **El-Malt et al. (2013)** and **Abo El-Makarem (2017),** respectively. For large scale produced ice cream samples, the incidence of counted Enterococci was 42.86% with a count ranging from $< 10^2$ to 4.71×10^4 and with an average count of 2.64×10^3 CFU/ml (Table 1). **El-Malt et al. (2013) and Abo El-Makarem (2017),** respectively obtained lower incidences of 54 and 36%.

Results of the statistical analysis revealed that, there was highly significance difference between incidences of *Enterococci* in small and large scale produced ice cream samples (χ^2 = 24.56 and $P < 0.01$). In addition, there was highly significance difference between averages count of both type of ice cream samples (Mann-Whitney $U = 124$ and $P < 0.01$). From the statistical results, the large scale produce ice cream samples are of superior hygienic quality than small scale produced ones based on the result of *Enterococci* count. This is due to the fact that in the case of production on a large scale, human intervention is less than in case of production on a small scale, which limits the chances of pollution and/or due to use of ingredients of better hygienic quality in the large scale production than that of small scale produced ones.

The results in Table (1) showed that, the incidence of coliform organisms in small scale produced ice cream samples was 65.71% with a count ranging from < 3.0 to 1.1×10^3 and with an average count of 3.3×10^2 MPN/ml. Higher incidence of 100% was reported by **Jannat et al. (2016) and Sotohy et al. (2022).**

On the other hand, lower result of 55.9% was revealed by **Abou-Elkhair et al. (2014).** The incidence of coliform in large scale produce ice cream samples was 45.71% with a count ranging from < 3.0 to 1.1×10^3 and with an average count of 2.18×10^2 MPN/ml (Table 1). Relatively similar result of 42.5% was found by **Vica et al. (2010)** in Alba County, Romania.

The statistical results analysis revealed that, there was no significance difference between incidences of coliform in small and large scale produced ice cream samples (χ^2 = 2.837 and $P > 0.05$). In addition, there was no significance difference between averages count of both type of ice cream samples (Mann-Whitney $U = 473$ and $P > 0.05$).

The presence of coliform in small and large scale produce ice cream samples in this study indicated that bad hygienic measures adopted during production.

Faecal coliform organisms were found in small scale produce ice cream samples in percentage of 31.43% with a count ranging from < 3.0 to 2.4×10^2 and with an average count of 1.79×10 MPN/ml (Table 1). Higher incidence of 100% was recorded by **Sotohy et al. (2022)** while lower incidence (28.57%) was revealed by **Afshin and Saeid (2011).**

Concerning large scale ice cream samples the incidence of faecal coliform was 31.43% with a count ranging from < 3.0 to 1.1×10^3 and with an average count of 7.72×10 MPN/ml (Table 1). Lower incidence of 2.9% was observed by **GadAllah et al. (2020).**

Interestingly, there was no significance difference between incidences of faecal coliform in small and large scale produced ice cream samples. In addition, there was no significance difference between averages count of both type of ice cream samples (Mann-Whitney $U = 608.5$ and $P > 0.05$). The presence of faecal coliform in the ice cream samples can be attributed to the use of animal manure for fertilization of agriculture lands in some Egyptian villages, which contaminates the fruit when it lands on these lands, therefore using these contaminated fruit in ice cream production give final products contaminated with faecal coliform. Furthermore, inadequate heat treatments during ice cream production exaggerate its contamination with various microbial types including faecal coliforms. In addition, unclean workers hands especially after using latrines play a role in ice cream contamination with faecal coliform.

The incidence of *Escherichia coli* in small scale produce ice cream samples was 22.86% with a count ranging from < 3.0 to 2.1×10 and with an average count of 2.46 MPN/ml (Table 1). Higher incidences were reported by **El-Malt et al. (2013) and Jannat et al. (2016)** whereas, lower results of 15 and 20% were revealed respectively by **Hosseini-Naveh et al. (2019) and Sotohy et al. (2022).** In large scale produce ice cream samples, the incidence of *Escherichia coli* was 11.43% with a count ranging from < 3.0 to 1.1×10^3 and with an average count of 3.86×10 MPN/ml (Table 1). Higher result was found by **El-Malt et al. (2013)** while relatively low result (16%) was obtained by **Jannat et al. (2016).**

The statistical results analysis revealed that, there was no significance difference between incidences of *Escherichia coli* in small and large scale produced ice cream samples (χ^2 = 1.609 and $P > 0.05$). In addition, there was no significance difference between averages count of both type of ice cream samples (Mann-Whitney $U = 547$ and $P > 0.05$).

Eight strains (4 strains from small scale and 4 strains from large scale ice cream samples) were selected randomly from the isolated 12 strains of *Escherichia coli* organisms that recovered in this study and examined by molecular assay for detection of *Stx*1 and *Stx*2 genes. In addition, two strains from the selected 8 strains were sorbitol negative and gave pale colonies in MacConkey sorbitol agar and examined for detection of specific gene for *Escherichia coli* O157:H7 (*fli*C gene). The illustrated results in Table (2) and Figures (1 and 2) showed that, from the tested eight strains two strains were positive for presence of *Stx*2 genes (Strains numbers 1 and 3) and one strain was positive for the presence of *Stx*1 gene (strain number 4) while the others strains were negative for Shiga toxin genes. Furthermore, strains numbers (one and 3) were positive for the presence *fli*C gene which indicated that, they were *Escherichia coli* O157:H7. According our knowledge, it is the first time to isolate *Escherichia coli* O157:H7 from ice cream samples in Assiut, Egypt. It is

worth mentioning that, the presence of *Escherichia coli* O157:H7 and Shiga toxigenic *Escherichia coli* in small scale produced ice cream samples in this study indicated the potential health hazards, food infection and even human deaths from consumption of such contaminated samples. Therefore, good hygienic measures must be applied during ice cream production. Also, periodic control and examination must be carried out by the health authorities on ice cream shops to provide a healthy and safe product for the consumers.

The incidence of yeast in small scale ice cream samples was 100% with a count ranging from 1×10^2 to 4.29×10^4 and with an average count of 4.81×10^3 CFU/ml (Table 1). Lower result of 85% was recorded by **GadAllah et al. (2020).** For large scale produce ice cream samples the incidence was 54.29%% with a count ranging from $< 10^2$ to 3.55×10^4 and with an average count of 4.96×10^3 CFU/ml. higher result of 91.4 was found by **GadAllah et al. (2020).**

Results of the statistical analysis revealed that, there was highly significance difference between incidences of yeast in small and large scale produced ice cream samples ($\chi^2 = 20.74$ and $P < 0.01$). In addition, there was highly significance difference between averages count of both type of ice cream samples (Mann-Whitney $U = 286.5$ and $P < 0.01$). From the statistical results, the higher incidence of yeast in small scale produce sample than that of large scale one can be attributed to the fact that in small scale production, the ice cream is produced and circulated in open containers that are exposed to contamination through air, flies, and dust, and through the use of contaminated cups.

From Table (1) the incidence of moulds in small scale produced ice cream samples was 65.71% with a count ranging from $< 10^2$ to 1.2×10^3 and with an average count of 2.06×10^2 CFU/ml. Relatively higher result of 70% was observed by **Sotohy et al. (2022).** For large scale produced ice cream, the incidence of moulds was 100% with a count ranging from 1×10^2 to 2×10^3 and with an average count of 6.06×10^2 CFU/ml. Lower result of 8.57% was revealed by **GadAllah et al. (2020).**

The statistical analysis revealed that, there was highly significance difference between incidences of mould in small and large scale produced ice cream samples ($\chi^2 = 14.48$ and $P < 0.01$). In addition, there was highly significance difference between averages count of both type of ice cream samples (Mann-Whitney $U = 288.5$ and $P < 0.01$). From the previous results it is clear that, large scale produced ice cream samples was higher in moulds incidence and count than that in small scale produce ones. This result is contrary to what was expected and therefore requires other studies and further investigations to explain it.

Interestingly, all the small and large scale produced ice cream samples, in this study, were negative for *Staphylococcus aureus* organism (Table 3). This result coincided with **Vica et al. (2010).**

From Table (3) the coagulase negative *Staphylococci* organisms (CNS) were detected in small and large scale produced ice cream in incidences of 11.34 and 8.57% for the two type of ice cream samples, respectively with no significance difference ($\chi^2 = 0.1587$ and $P = 0.6903$).

From the public health point of view, importance of coagulase negative *Staphylococci* (CNS) have been implicated in infective endocarditis, bacterial eye infections, neonatal septiceamia, Enterotoxins production and prosthetic joint infections **(Patel et al, 2000; Trampuz and Zimmerli 2005 Ghelbi et al. 2008 Veras et al. 2008 and Ogbolu et al. 2011).**

Anaerobes detected in small and large scale produced ice cream samples in incidences of 80 and 57.14%, respectively (Table 3). In addition, there was significance difference between the incidences of anaerobes in small and large scale produced ice cream samples ($\chi^2 = 4.242$ and $P < 0.05$). Presence of anaerobic spore-formers in ice cream samples in this study could be attributed to inefficient heat-treatment process or due to the added contaminated flavouring materials during the product

production. Moreover, anaerobes could affect consumers' health via food poisoning arising from the production of toxins.

Comparison of the average count of aerobic plate count with the limits proposed by the E.S. (**Egyptian Standards, 2005),** 22.86 and 28.57% of the small and large scale produced ice cream samples, respectively, failed to comply with the limits (Table 4). In addition, there was no significance difference between the acceptability percent in both type of ice cream (χ^2 = 0.2991 and $P > 0.05$). Concerning the results of coliform count, 60 and 34.29% of the small and large scale produced ice cream samples, respectively, failed to comply with the limit of the Egyptian Standards (Table 4). Furthermore, there was significance difference between the acceptability percent in both type of ice cream (χ^2 = 4.644 and $P < 0.05$). On the other hand, according to the presence of *Escherichia coli* organism, 22.86 and 11.43% of the small and large scale produced ice cream samples, respectively, failed to comply with the limit of the Egyptian Standards and with no significance difference (χ^2 = 1.609 and $P > 0.05$). While, both types of ice cream samples complied with the Egyptian Standards based on absence of *Staphylococcus aureus* organism.

CONCLUSSION

This study revealed that small and large scale produced ice cream, available in retailer ice cream shops in Assiut city, are largely contaminated with various microorganisms. Most of the examined small scale produced ice cream samples failed to comply with the limits of Egyptian Standards. There were significant differences in the microbiological quality between small and large scale produced ice cream samples, in terms of incidence rates and average count of psychrotrophs, *Enterococci*, anaerobs, yeasts and moulds. Ice cream samples could thus be a source of some pathogens, as *Escherichia coli* O157:H7 and Shiga toxins. Therefore, improvements in the ice cream processing and filling operations are highly required. Good hygienic control measures must be applied in ice cream producing factories and shops in addition to the use of ingredients of good bacteriological quality before heat treatment.

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