JOURNAL OF VETERINARY MEDICAL RESEARCH 2017, 24 (1): 299-307

Journal homepage:
http://www.bsu.edu.eg/bsujournals/JVMR.aspx

Online ISSN: 2357-0520 Print ISSN: 2357-0512

Original Research Article

Microbial quality of street-vended ice cream

Abo El-Makarem, H.S.

Department of Food Hygiene, Faculty of Veterinary Medicine, Alexandria University, Egypt

ABSTRAC

Ice cream is a delicious dairy product commonly consumed during summer in all age groups. Due to its composition, it can harbor many potent pathogens. Most ice creams become contaminated with microbes during production, transit, and preservation. Such contaminated food product can be responsible for food borne infections in children, elderly people and immune-suppressed patients. Therefore, the study was conducted to evaluate the microbiological quality of street-vended ice creams sold in different areas of Alexandria city, Egypt. One hundred street vended ice cream samples (50 packed and 50 unpacked) randomly collected samples and analyzed for total bacterial count, *Enterobacteriaceae* count, coliform count, enterococci count and *Staphylococcus aureus*. The results revealed that the mean value of total viable count, Enterobacteriaceae count, Coliform count, Enterococci count and *Staphylococcus aureus* in packed and unpacked ice cream samples were $1.9x10^3±0.3x10^3$, $1.0x10^6±0.8x10^6$; $2.1x10^3±0.8x10^3$, $1.9x10^4±0.8x10^4$; $1.6x10^3±$ $0.6x10^3$, $0.8x10^4±0.6x10^4$; $1.3x10^3±0.05x10^3$, $7.4x10^4±5.5x10^4$ and $9.1x10^2±2.6x10^2$, $0.8x10^4±0.4x10^4$cfu/ml, respectively. *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae* and *Citrobacter* sp. could be isolated and identified from the examined packed and unpacked ice cream samples. Serological identification of *E.coli* showed that the $O_{111:}$ $K_{58:}$ B_4 is the most serotype of *E.coli* isolated from unpacked ice cream samples while $O_{128:}$ $K_{67:}$ B_{12} is the most prevalent *E.coli* serotype isolated from packed ice cream samples. It is recommended to launch awareness programs to minimize the contamination of ice cream products.

ARTICLE INFO

Article history:

Received: 15 October 2016
Accepted: 20 November 2016
Available Online: 27 August 2017

Keywords:

Ice cream, coliform, enterococci, *Staph. aueus*, *E.coli*, serology

* Corresponding author: Husseinsobhy4@yahoo.com

1. Introduction

Ice cream is the major dairy product which is one of the favorite food items in large segments of the population. It is a nutritionally enriched frozen dairy product consumed by all age groups particularly children mostly during summer (Sharif et al., 2005).

Different ingredients are used in ice cream preparation like milk, cream, evaporated or

condensed milk, dried milk, coloring material, flavors, fruits, nuts, sweetening agents, egg products and stabilizers must be of good quality to obtain a high-quality ice cream with the required taste, flavor, viscosity, consistency and appearance (Yaman et al., 2006). Fruits, nuts and syrups are optionally added into ice cream for flavour enrichment. It is sold in packages or in open containers at retail outlets/ice cream parlours, the open variety being distributed manually in scoops, cones, or sundaes across the counter (Caglayanlar et al., 2009). During production, transportation and storage, it may become contaminated with several microorganisms. Microorganisms are transmissible to humans through milk and milk products (Tomislav et al., 2012). The high content of nutrients like lactose of ice cream, proteins and its neutral pH (6-7) make it an excellent growth medium for microbes, some of which may cause serious diseases and outbreaks like cholera, typhoid and bacillary dysentery in human beings (Ahmed et al., 2009).

Ice cream is a vehicle for the transmission of pathogens. The pasteurization, freezing, and hardening steps in production can eliminate most microbiological hazards (Andreasen and Nielsen, 1998).

The microbial counts in ice cream samples may have resulted from inadequate processing, such as initial improper cooling of the hot ice cream mix, which may lead to the multiplication of microorganisms present in ice cream immediately after pasteurization (Ojokoh, 2006). Coliform bacteria most commonly used to ensure food safety including coliform bacteria, faecal coliform bacteria, *E. coli*, total *Enterococcus* species and aerobic plate count (Pierson and Smoot, 2007). Although some of these Coliforms are not pathogenic, their presence indicates possible faecal contamination and the corresponding presence of intestinal pathogens responsible for a variety of diseases (Cakir et al., 2002). The majority of *E. coli* strains do not constitute a serious health hazard, but some serotypes can cause food poisoning and alimentary intoxications. The most dangerous among them are enterohaemorrhagic *E. coli* strains, especially serotype O157: H7. *E. coli* O157: H7 consider has major concern in dairy industries and to public health because of its ability to cause severe illness, such as haemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Reuben et al., 2013). *Enterococcus* species are commensal microorganisms that colonize in the gastrointestinal tract of humans and animals and are able to survive adverse environments, such as 6.5% NaCl, pH of 9-6, high heat as well as being able to grow and survive under other harsh environmental conditions, like those found in various soils, surface water, raw plants and animal products (Johnston and Jaykus, 2004).

Many psychrophiles and psychrotolerant microorganisms like *Staphylococcus*, *Streptococcus* species and coliform bacteria are generally present in ice cream. *Streptococcus* (group-D) (*Strept. faecalis* and *Strept. facium*) and coliforms are very important in food science because the presence of this agent in recent years considered as the best index of faecal contamination (Benson, 1994).

Bacteriological quality of ice cream reflects hygienic practice during production and is an indication of food safety (Ambily and Beena, 2012). Hence, the present study was taken up to determine the bacteriological quality of street-vendor ice cream marketed in Alexandria city and to assess the potential of these frozen products to pose the risk to public health.

2. Materials and methods

2.1. Collection of samples

A total of 100 ice cream samples (100 g each) were collected and examined during July and August, 2016 in Alexandria city, Egypt. Samples were transported to the laboratory in a cool box and stored at -20º C prior to examination.

2.2. Preparation of samples

The preparation of the samples was carried out in according with the method of (Warke et al., 2000). Ice creams samples was kept in water bath at 45°C , after thawing and when complete liquefaction from the top is so opened that the sterile pipette could be introduced for collection of samples. A quantity of about 10 ml of liquid ice cream was pipetted out from different depths and transferred into a sterile glass bottle fitted with a screw capped stopper.

2.3. Microbiological evaluation of ice cream

One ml of previously prepared and thoroughly mixed ice cream samples was pipetted out aseptically and transferred into a sterile test tube and plugged with cotton contained 9 ml of diluent to give a 1:10 dilution v/v. The choice of dilution for the preparation of samples was sterile 0.1% peptone water at pH 6.8-7.0. Further decimal dilutions as required were prepared according to the standard

method given by (APHA, 2001). Samples of ice cream are tested for total bacterial count, *Enterobacteriaceae*, coliform count, enterococci and *Staphylococcus aureus* count.

- Total viable bacterial count on standard plate count agar 37°C for 24 h according to BAM (2001).
- *Enterobacteriaceae* count on Violet Red Bile Glucose Agar (VRBGA) (Oxoid), incubated at 37°C for 48 h according to APHA (1992).
- The total coliform count in the ice cream was conducted according to the method described by APHA (2001). Typical colonies were picked up and identified as *Escherichia coli* by standard biochemical reactions (characteristic greenish metallic color on EMB), and confirmed with the indole, methyl-red, Voges-Proskauer, and citrate utilization (IMVIC) tests (Collee et al., 1996).
- Serological identification of *E. coli* isolates: It was carried out in the Clinical Microbiology Unite, Central Health Laboratories of Ministry of Health, Egypt.
- Homogenized samples were inoculated onto (KAA agar) Kanamycin-esculin-azide agar (Merck, Darmstadt, Germany) incubated at 37°C for 72 h.
- Enumeration of *Staphylococcus aureus*: It was counted using Baird Parker RPF medium after incubation at 35°C for 24- 48 h (Oxoid, 2006), for identification of *S. aureus*, typical and atypical presumptive staphylococci species colonies were examined by Gram stain, coagulase, catalase, and latex agglutination test (Oxoid, FT0203) .

3. Results

Table 1. Statistical analytical of microbiological examination for examined packed ice cream

Count	Packed ice cream (n=50)				
	Positive samples		Minimum	Maximum	Mean ± SEM
	No.	%			
Total bacterial count	32	60	1.0×10^3	3.0×10^3	$1.9 \times 10^3 \pm 0.3 \times 10^3$
Enterobacteriaceae	13	26	1.5×10^2	5.3×10^3	$2.1 \times 10^3 \pm 0.8 \times 10^3$
Coliforms	11	22	1.2×10^2	3.9×10^3	$1.6 \times 10^3 \pm 0.6 \times 10^3$
Enterococci	18	36	1.2×10^3	1.5×10^3	$1.3 \times 10^3 \pm 0.05 \times 10^3$
Staph. aureus	14	28	1.2×10^2	1.8×10^3	$9.1 \times 10^2 \pm 2.6 \times 10^2$

Table 2. Statistical analytical of microbiological examination for examined unpacked ice cream samples.

Count	Unpacked ice cream (n=50)				
	Positive samples		Minimum	Maximum	Mean ± SEM
	No.	%			
Total bacterial count	42	84	1.5×10^4	5.3×10^6	$1.0x10^6 \pm 0.8x10^6$
Enterobacteriaceae	24	48	1.5×10^3	5.3×10^4	$1.9x10^4 \pm 0.8x10^4$
Coliforms	23	46	6.0×10^2	3.9×10^4	$0.8x10^4 \pm 0.6x10^4$
Enterococci	21	42	3.0×10^3	3.5×10^5	$7.4x10^4 \pm 5.5x10^4$
Staph. aureus	14	28	1.2×10^2	1.8×10^4	$0.8x10^4 \pm 0.4x10^4$

Table 3. Comparison between the results obtained from microbiological examination of examined packed and unpacked ice cream and the Egyptian Standards (2005).

Count	Egyptian standards (2005)	Packed ice cream (n=50)		Unpacked ice cream (n=50)	
		Within permissible limit	Failed to conform ES	Within permissible limit	Failed to conform ES
Total bacterial count	<150000 cfu /g	100%	0	60%	40%
Coliform count	<10 cfu /g	78%	22%	54%	46%
Staph. aureus count	absent in 1 g	72%	28%	72%	28%

Table 4. Frequency distribution of enteric bacteria isolated from packed and unpacked examined ice cream samples.

Species	Unpacked ice cream		Packed ice cream	
	Frequency	%	Frequency	%
Citrobacter diversus	3	6.39	3	6.25
Citrobacter freundii	3	6.39	5	10.4
Edwardsiella tarda	2	4.25	3	6.25
Enterobacter agglomerans	1	2.13	3	6.25
Enterobacter cloacae	2	4.25	8	16.7
Escherichia coli	23	48.96	13	27.0
Klebsiella ozaenae	2	4.25	2	4.2
Proteus mirabilis	2	4.25	4	8.3
Providencia alcalifaciens	2	4.25	2	4.2
Providencia rettgeri	1	2.13	2	4.2
Serratia liquefaciens	2	4.25	3	6.25
Serratia marcescens	4	8.50	-	-
Total	47	100	48	100

Table 5. Frequency distribution of *E. coli* serotypes isolated from positive examined samples.

Serotypes	Unpacked ice cream		Packed ice cream	
	No.	%	No.	%
$O_{55} : K_{59} : B_5$	3	13.04	2	15.38
$O_{127} : K_{63} : B_8$	2	8.69	1	7.69
$O_{111} : K_{58} : B_4$	4	17.39	-	-
$O_{125} : K_{70} : B_{15}$	3	13.04	1	7.69
$O_{126} : K_{71} : B_{16}$	3	13.04	2	15.38
$O_{26} : K_{60} : B_6$	5	21.73	1	7.69
$O_{119} : K_{69} : B_{14}$	-	-	1	7.69
$O_{128} : K_{67} : B_{12}$	2	8.69	3	23.07
$O_{114} : K_{90} : B$	1	3.34	2	15.38
Total	23	100	13	100

4. Discussion

It has been found that the mean values of total bacterial count, Enterobacteriaceae, Coliform count, Enterococci and *Staphylococcus aureus* count of packed ice cream samples were $1.9x10^3 \pm 0.3x10^3$, $2.1x10^3 \pm 0.8x10^3$, $1.6x10^3 \pm 0.6x10^3$, $1.3x10^3 \pm 0.05x10^3$ and $9.1x10^2 \pm 2.6x10^2$ cfu/ml, with incidence rates of 60, 26, 22, 36 and 28%, respectively (Table 1). Moreover, the mean values of total viable bacterial count, Enterobacteriaceae, coliform count, enterococci and *Staphylococcus aureus* count in unpacked ice cream samples were $1.0x10^6 \pm 0.8x10^6$, $1.9x10^4 \pm 0.8x10^4$, $0.8x10^4 \pm 0.6x10^4$, $7.4x10^4 \pm 5.5x10^4$ and $0.8x10^4 \pm 0.4x10^4$ cfu/ml with the incidence rate 84, 48, 46, 42 and 28 % respectively (Table 2).

According to the permissible limits of (Egyptian Standard, 2005) which stated that total viable count of ice cream samples not more than 150000 cfu/ml,

all packed ice cream samples within the permissible limit of Egyptian standards and 60% of unpacked ice cream samples comply with the standard and 4000% not comply with the Egyptian standard (Table 3).

In present work, it was indicated that ice creams sold in small portions from bulk containers, exposed to the open air, have a high microbial load, indicating the low hygienic quality of the products. These high counts may return to high load of initial microflora of raw milk and the other ingredients and their quality, the environment, insufficient heat treatment and poor personal hygiene. It has been previously stated that the production of ice cream on a small scale rather than industrially is an important factor associated with the contamination of ice cream (Bostan and Akin, 2002; Kanbakan et al., 2004).

The incidence of *Enterobacteriaceae* count (48%) in unpacked ice cream samples was similar to those given by Erol et al. (1998) who reported that the incidence of *Enterobacteriaceae* in ice cream samples was (53%). The presence of *Enterobacteriaceae* in food products resulted from faecal contamination. Therefore, in this study, the microbiological quality of ice cream samples seemed to be low due to insufficient heat treatment, unhygienic handling of the ice creams before and during storage (Jay, 1996).

The existence of *Enterobacteriaceae*, coliforms and *E. coli* in examined ice cream samples might indicate the lack of a good manufacturing practice during the production which has an important role in gastrointestinal diseases therefore, implementing regulatory measures like good manufacturing practices, hygienic distribution and retail storage practices important for microbiological safety of ice cream sold in open containers are necessarily. Kanbakan and Con (1999) reported that coliform contamination on the hands of persons in sales department was higher than on the hands of factory workers.

The presence of coliforms in ice cream samples might result from insufficient heat treatment, unhygienic materials or tools used and contaminated water. Much attention has been paid towards *E. coli* because of its importance as an organism of true faecal origin associated with enteric pathogens (Tsen et al., 1998). Inadequate cleaning of the hands, same person selling ice cream and collecting money, open cones and unclean cloth for cleaning the scoops can contribute to high coliform count (Kanbakan et al., 2004).

Pasteurization kill coliform organism so their post pasteurization presence in ice creams refer to faulty heat process during preparation. Meanwhile, contamination may arise from water, bad personal hygiene of the ice cream manufacturer and utensils used for ice cream (Jadhav and Raut, 2014).

Currently, the incidence of coliforms was 46% and 22% in unpacked and packed ice cream samples. Those results agreed with El-Ansary (2015) who reported that the incidence of coliforms in examined ice cream sample was 21% of with a mean value of $4.58x10^3 \pm 1.50x10^3$. On the contrary, a higher incidence of coliforms in ice cream samples (56%) was given by Abou-El Khair et al. (2014).

According to Egyptian Standards (2005) for ice cream which stipulated that the coliform counts must not exceed 10 cells/g, 78% of packed ice cream samples and 54% of unpacked ice cream samples comply with Egyptian standard, It is evident from the results that Coliforms contaminate high percent of street-vendor unpacked ice cream samples this may attributed to poor hygienic measures, carelessness during handling and distribution, poor quality ingredients, ineffective sanitizing method and prolonged storage of the mixture.

Table 1 showed that *Staphylococcus aureus* was found at the level of 28% in both packed and unpacked ice cream. Such finding was higher than the acceptable limits of Egyptian Standard (2005) which stipulated that *Staphylococcus aureus* must be absent in 1 gm ice cream, so 72% of both packed and unpacked ice cream samples comply with Egyptian standard. Our results coincided with those obtained by Nazem et al. (2010) who reported that 27.5% of examined ice cream samples were contaminated with *Staphylococcus aureus*. The later may be present on skin, eyes, throat and intestinal tract. From these sources, the organism finds its way into air and dust, onto clothing, and in other places from which it may contaminate foods (Jay, 1996). Although the observed counts of *Staphylococcus aureus* may not be sufficient to cause illness, these counts may reach high levels under temperature abuse condition. These can usually get into ice cream from sources such as soil, dust, contaminated equipment and hands of the persons either during storage or during filling of the vending machine and selling (Mathews et al., 2013). *Staphylococcus aureus* could survive freezing well and increased in

counts in the frozen milk (Hubackora and Rysanek, 2007).

Table 4 revealed that *Citrobacter diversus*, *Citrobacter freundii*, *Edwardsiella tarda*, *Enterobacter agglomerans*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella ozaenae*, *Proteus mirabilis*, *Providencia alcalifaciens*, *Providencia rettgeri*, *Serratia liquefaciens* and *Serratia marcescens* were isolated and identified form packed ice cream samples at a rate of 6.39, 6.39, 4.25, 2.13, 4.25, 48.96, 4.25, 4.25, 4.25, 2.13, 4.25 and 8.50%, respectivley. From unpacked ice cream samples, rates were 6.25, 10.4, 6.25,6.25, 16.7, 27.0, 4.2, 8.3, 4.2, 4.2 and 6.25%, respectively. *Serratia marcescens* failed to be identified in unpacked ice cream sample.

The level of presence of all coliforms groups such as *Escherichia coli*, *Citrobacter* species, *Enterobacter aerogenes* and *Klebsiella* species in foods has been described as an index of bad hygiene (Jay, 1996).

E. coli is an indicator for faecal contamination and potential presence of enteric pathogens. As regards to *E.coli*, it was found in 23 (48.96%) unpacked ice cream samples, and in 13 (27.0%) packed ice cream samples (Table 4). The incidence of *E.coli* in packed ice cream samples agreed with Yaman et al. (2006) who reported that *E. coli* was found in 15 (20%) out of 73 ice cream samples with a count higher than $1.1x10^2$ MPN/g in all positive samples**.** Similarly, Kivanc et al. (1994) and Nazem et al. (2010) reported that the incidence of *E. coli* was 22% and 30% in Turkey and Egypt, respectively. The incidence of *E.coli* in unpacked ice cream samples agreed with Masud (1989) who found that 46% of the samples were contaminated with *E. coli*. On the other hand, a higher incidence of *E.coli* (96%) in an examined ice cream with fruits was reported by Abou-El Khair et al. (2014).

The public health hazard of *E. coli* organisms has been emphasized by several investigators as they cause many cases of gastroenteritis, epidemic diarrhea in infants, as well as food poisoning (Veronozy, 1997). It was revealed that the frequency percentage of *E.coli* serotypes isolated from unpacked ice cream samples O_{55} : K_{59} : B_5, O_{127} : K_{63} : B_8 , O_{111} : K_{58} : B_4 , O_{125} : K_{70} : B_{15} , O_{126} : K_{71} : B_{16} , O_{26} : K_{60} : B_6 , O_{128} : K_{67} : B_{12} and O_{114} : K_{90} : B were 13.04, 8.69, 17.39,13.04, 13.04, 21.73, 8.69 and 3.34% respectively. While from packed ice cream samples O_{55} : K_{59} : B_5, O_{127} : K_{63} : B_8 , O_{125} : K_{70} : B_{15} , O_{126} : K_{71} : B_{16} , O_{26} : K_{60} : B_6 , O_{119} : K_{69} : B_{14}, O_{128} : K_{67} : B_{12} and O_{114} : K_{90} : B were 15.38, 7.69, 7.69, 15.38, 7.69, 7.69, 23.07 and 15.38% respectively (Table 5).

O_{86}:K_{61}, O_{55}:K_{59}, O_{111}:K_{58}, O_{114}:K_{90} are enteropathogenic *E. coli* (EPEC) causing infantile diarrhea which is watery and mucoid without blood. O_{124}:K_{72} is enteroinvasive *E. coli* (EIEC) may result in fever, severe abdominal pain, malaise and profuse watery diarrhea containing mucous, strikes of blood and faecal leucocytes. O_{128}:K_{67} is enterotoxigenic *E. coli* (ETEC) which is responsible for watery diarrhea (traveler's diarrhea) which affects human especially during visiting to warmer countries due to production of heat labile toxin (Cholera like toxin) and heat stable toxin (Diarrheal toxin).

Conclusion

The current study has indicated a bad level of hygiene in the service of openly sold ice cream in Alexandria City, Egypt. It is clear from the previous and current studies that there is a necessity for improving the hygienic status of locally produced ice cream in domestic or catering premises especially in all steps, post-pasteurization and at retail level in Egypt. Further improvements should be include general hygiene conditions of premises, the quality of the raw material prior to process, storage of the products under appropriate conditions to prevent the infections resulting from pathogenic microorganisms, workers and sales people in the point of sell should be regularly checked for sustaining the favorable hygienic status.

References

Abou-El Khair E, Abd Al-Raziq S, Hamdan R, Abdelrahman K, Hashem A (2014). Bacteriological quality of packaged ice-cream in Gaza City, Palestine J. Food Nutr. Sci.; 2(3): 68–73.

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

Ambily R, Beena AK (2012). Bacteriological quality of ice-cream marketed in Thrissur town, Kerala, India. Vet. World 5(12): 738–741.

American Public Health Association, APHA (2001). Compendium of Methods for the Microbiological Examination of Foods Fourth edition. F.P.

Downes and K. Ito (editors), American Public Health Assoc., Washington, D.C.

Andreasen TG, Nielsen H (1998). Ice cream and aerated desserts. In: Early R, editor. Technology of dairy products. 2nd ed. London: Blackie Academic and Professional p. 301–324.

APHA (1992). Standard Methods for Examination of Dairy products. 16th ed., American Public Health Association, Washington DC, USA.

Bacteriological Analytical Manual (BAM) online (2001). US Food and Drug Administration. Center for Safety and Applied Nutrition.

Benson HJ (1994). Microbiological applications: A laboratory manual in general microbiology, 6th ed. WM. C. Brown Company Publishers, Dubuque, Iowa.

Bostan K, Akin B (2002). Endustriyel dondurmalarin mikrobiyolojik kalitesi uzerine bir arastirma. Turk. J. Vet. Anim. Sci., 26: 623–629.

Caglayanlar GE, 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.

Cakir I, Dugan HB, Baspinar E, Keven F, Halkman K (2002). The need for confirmation in Coliform and *E. coli* enumeration in foods. Turk. J. Vet. Anim. Sci., 26: 1049–1053.

Collee JG, Miles RS, Watt B (1996). Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A (eds). Mackie & McCartney practical medical microbiology, 14th ed. London: Churchill Livingstone, p. 131–149.

Collins CH, Lyne PM, Grange JM (1989). Collins and Lyne's Microbiological Methods. London: Butterworth's.

Egyptian Standard (2005). Ice cream. ES. 1185/01, Egyptian organization for Standardization and Quality Control, Ministry of Industry, Cairo, Egypt.

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

Erol I, Kuplulu O, Siriken B, Celik TH (1998). Ankara'daki cesitli pastanelere ait dondurmalarin mikrobiyolojik kalitelerin belirlenmesi. Tr. J. Vet. Anim. Sci., 22: 345–352.

Hubackora M, Rysanek D (2007). Effects of freezing Milk samples on the recovery of Alimentary pathogens and Indicator Microorganisms. Acta Vet. Brno, 76: 301–307.

Jadhav AS, Raut PD (2014). Evaluation of microbiological quality of ice creams marketed in Kolhapur city, Maharashtra, India. Int. J. Curr. Microbiol. Appl. Sci., 3(9): 78–84.

Jay JM (1996). Modern Food Microbiology, 5th ed., Van
Nostrand Reinhold Co. New York. pp 661.

Johnston LM, Jaykus L (2004). Antimicrobial resistance to Enterococcus species isolated from produce. Appl. Environ. Microbiol., 70: 3133–3137.

Kanbakan U, Con AH, Ayar A (2004). Determination of microbiological Contamination sources during ice cream production in Denizli, Turkey. Food Control 15: 463–470.

Kanbakan U, Con AH (1999). Denizli'de uc yillik periyotta tuketime sunulan dondurmalarin bazi mikroorganizmalar acisindan incelenmesi. Ekonomik ve Teknik dergi., Standart. TSE, Aylik yayin organi, Ankara, 445: 89–94.

Kivanc M, Yamac M, Kunduhoglu B (1994). Eskisehir'de halkin tuketimine sunulan dondurmalarin mikrobiyolojik analizi. Gida. 19 (5**):** 317–322.

Masud T (1989). Microbiological quality and public health significance of ice cream. JPMA. J. Pak. Med. Assoc., 39(4): 102–104.

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.

Nazem AM, Amer AA, Soukayna A (2010). Prevalence of some food poisoning microorganisms in some dairy products. Alex. J. Vet. Sci., 30 (1): 1–6.

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

Oxoid (2006). The Oxoid Manual, Budapest: Oxoid Ltd.

Pierson M, Smoot L (2007). Indicator microorganisms and microbiological criteria. In: Doyle MP, Beuchat LR, Montville TJ (eds.). Food Microbiology: Fundamentals and Frontiers, 2 ed. ASM Press, Washington, DC, pp, 78–81.

Reuben CR, Okolocha EC, Bello M, Tanimu H (2013). Occurrence and antibiogram of Escherichia coli O157:H7 in locally fermented milk (Nono) sold under market conditions in Nasarawa state, Nigeria. Int. J. Sci. Res., 2(2): 591–598**.**

Sharif N, Ghanghesh KS, Gnan YAS, Rahouma A (2005). Bacteriological quality of ice cream in Tripoli-Libya. Food Control 17: 637–641.

Tamsut LS, Garcia CE (1989). Microbiological quality of vanilla ice cream manufactured in Caracas, Venezuella. Arch. Latinoam Nutr., 39(1): 46–56.

Tomislav P, Samarzija D, Zamberlin S (2012). Psychrotrophic bacteria and milk and dairy products quality. Mljekarstvo 62 (2): 77–95.

Tsen HY, Lin CK, Chi WR (1998). Development and uses of 16s rRNA gene targeted PCR primer for identification of *E.coli* cells in water. J. Appl. Microbiol., 85: 554–560.

Veronozy CR (1997). Detection of *Escherichia coli* O157 and other Verotoxin producing *E.coli* (VTEC) in Food. J. Appl. Microbial., 82: 537–557.

Warke R, Kamat A, Kamat M, Thomas P (2000). Incidence of pathogenic psychrotrophs in ice creams sold in some retail outlets in Mumbai, India. Food Control 11: 77–83.

Yaman H, Elmali M, Ulukanli Z, Tuzcu M, Genctav K (2006). Microbial quality of ice cream sold openly by retail outlets in Turkey. Revue. Méd. Vét., 157 (10): 457–462.