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Research Article

# Microbial quality and chemical composition of traditional ice cream collected from Kermanshah province, Iran

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## Abstract

The aim of the present study was to investigate microbial quality and chemical composition of sold ice cream in retail markets of different parts of Kermanshah city, west of Iran. A total 120 samples of traditional ice cream collected from different local markets (north, south, east, west and centre of city). Out of total 120 samples, 93 ice cream samples (77.5%) exceeded standard value of total bacterial count, which is $5\times10^5$ CFU/g. In case of total *Enterobacteriacea* count, 81 samples (67.5%) crossed the standard value which is 100 CFU/g. Similarly, 27 samples (22.5%) and 57 samples (47.5%) were found crossing the standard value of Staphylococcal count (100 CFU/g) and *E. coil* (0 CFU/g), respectively. The mean values of the main components of ice cream were 9.18±1.36% fat, 2.67±1.01% protein, 33.28±1.89 total solids (%), 0.64±0.01% ash and 9.56±2.29% sucrose. Our findings indicate that traditional ice creams produced in different parts of Kermanshah city has poor microbiological quality. It is imperative that bacteriological standards be enforced in order to prevent traditional ice cream-borne diseases.

**Keywords:** Traditional ice cream; bacterial contamination; chemical composition; Kermanshah; Iran

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## Introduction

In recent years, ice cream, a frozen milk based product, is one of the most popular milk based-products of dairy industry among children and adults especially during summer season throughout worldwide (ME-Elahi et al., 2002). In Iran, it is produced both in package (cups, cones and cartons) and in 'open' containers at the retail outlets or artisanal ice cream, which is distributed manually in scoops or cones across the counter. In general, this type of ice cream is manufactured in small-scale production unit, which do not follow completely a standard procedure for production of ice cream (Ghasemi et al., 2009; Hazhir et al., 2005). The microbial quality of retail outlets or artisanal ice cream significantly depends on extrinsic factors that include the post production proper handling of the product as well as sanitary storage frozen conditions (Warke et al., 2000; Pooran et al., 2012).

Ice cream can be considered an excellent medium for growth of numerous pathogens such as *Listeria monocytogenes*, *Yersinia enterocolitica*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp. due to its nutrient content (proteins, lipids, carbohydrates, minerals and vitamins), almost neutral pH (pH 6-7) and long storage period (Pietranera

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et al., 2003; Pooran et al., 2012). Pasteurization, freezing and hardening steps are the most important steps of the ice cream production that can eliminate most of these pathogens. However, due to lack of efficient frozen storage chain and also improper sanitation of equipment and environment during processing and handling, there is chance of microbial contamination after pasteurization of ice cream. As well as, any of contaminated ingredients such as eggs and eggs products stabilizer, scoop water, milk, cream, colouring material, flavours, fruits, nuts, sweetening agents can account for the various specific species of bacteria (Pietranera et al., 2003; Ahmed et al., 2009; Movassagh et al., 2011).

Several studies from different countries such as India (Warke et al., 2000), Republic of Korea (Jo et al., 2007), Trinidad and Tobago (Pooran et al., 2012), Costa Rica (Windrantz et al., 2000), Nepal (Joshi et al., 2004) and Turkey (Kanbakan et al., 2004) reported contamination of ice cream and identification revealed presence of pathogenic or fecal indicator bacteria that included *S. aureus*, *E. coli*, *Klebsiella*, *Enterobacter*, *L. monocytogenes*, *Y. enterocolitica*, *B. cereus*, and *Salmonella* spp. To date, the microbial contamination of ice cream has been studied in some parts of Iran (Ghasemi et al., 2009; Hazhir et al., 2005; Dabbagh Moghaddam et al., 2010; Movassagh et al., 2011; Rahimi et al., 2011). However, no data is available on the incidence of the most important food-borne bacteria such as *S. aureus*, *E. coli*, *L. monocytogenes*, *Y. enterocolitica*, *B. cereus* and *Salmonella* spp. and chemical composition in traditional ice cream collected from the different parts of Kermanshah province, west of Iran. Hence, the aim of the present study was to investigate microbial contamination (enumeration of Total bacteria count, *Enterobacteriaceae* and Staphilococcal count and identification of *S. aureus, E. coli, L. monocytogenes, Y. enterocolitica, B. cereus and Salmonella spp.*) and chemical composition of sold ice cream in retail markets of different parts of Kermanshah city. The results of the current research will help to understand precisely the microbial quality of ice cream and whether the product conform to the specifications prescribed by the Iranian public health regulatory authority.

## Materials and Methods

In this study, a total 120 samples of traditional ice cream collected randomly from five different large local markets of Kermanshah province, west of Iran (north, south, east, west and centre of city). The samples were collected in an ice box and immediately taken to the laboratory where they were kept in deep freezer at -20°C, until they were examined for the bacteriological quality. Samples were kept at 4°C for 10 min until the microbiological analyses. Ten ml of each ice cream sample were diluted with 90 ml 0.1% peptone and plated on to Plate Count Agar (PCA; Merck). The plates were incubated at 30˚C for 48 hours. Colonies were enumerated and total bacteria (colony forming unites) yield from each sample calculate. *Enterobacteriaceae* count was determined by the pour plate technique with an overlay using Violet Red Bile Glucose (VRBG) agar incubated at 37°C for 24h. Large purple colonies with the purple zone were counted as *Enterobacteriaceae* (Djenane et al., 2012). For detection of pathogenic bacteria, MacConkey Agar (coliforms), Eosin Methylene Blue Agar (EMB) (*Escherichia coli*), Baird-Parker Agar and Manitol salt Agar (*S. aureus*), Bacillus cereus selective Agar (*B. cereus*), Palcam Listeria Selective Agar (*Listeria*) were used (Wouafo et al., 1998). Salmonella were identified using ISO Standard 6579 method. In this method, firstly, the ice cream sample was thawed at 25°C, then, 9g of each sample was transferred to a sterile bag mixer containing 90ml sterile peptone water (Merck). After homogenization for 2min, the sample was incubated at 37°C for 24h. Then, 0.1ml portion of the incubated peptone water was added to 10ml Rappaport Vasiliadis broth (RV; Merck) medium and another 1ml portion of this medium (incubated peptone water) was added to 10ml Selenite Cystine broth (SC; Merck). RV broth was incubated for 24h at 41.5°C. As well as, SC broth was incubated for 24h at 37°C. After incubation, from the broths one loopful was subcultured on Brilliant green Phenol Red Lactose Agar (BPLS) (BPLS; Merck) and Bismuth Sulphite agar (BSA; Merck). The media were incubated at 37°C for 24h (for BPLS) and 48h (for BSA). Then, suspected colonies were transferred onto Salmonella-Shigella agar (SS; Merck) plates. After incubation of plates at 37°C for 24h, two or more typical Salmonella colonies were suspended in to biochemical identification media including Triple Sugar Iron agar (TSI; Merck), Simmons Citrate agar (Merck), Voges Proskauer (VP; Merck), Methyl Red (MR; Merck), Sulfide-Indole-Motility (SIM; Merck) and Urea agar (Merck). This bacterium causes a reddening of the slant and acidifies the depths of the TSI tube. The biochemical reaction of Salmonella in SIM, VP and Urea agar is negative. As well as, this bacterium is Simmons Citrate positive (Jamshidi et al., 2010). Fat content was assessed by Gerber method, total solids and ash contents were determined according to the modified method of AOAC (1998), the protein content was determined by Kjeldahl method (AOAC, 1998) and total sugars by Lane and Eynon micrometric method of AOAC (1998).

### Statistical analysis

Data were verified and analyzed using SPSS for WINDOWS statistical software (version 16.0; SPSS

Inc, Chicago). Chi-square ($x^2$) Fishers exact test was employed to statistically evaluate the data.

## Results and Discussion

Based on the result of Table 1, it was found that out total 120 samples, 93 ice cream samples (77.5%) exceeded standard value of total mesophilic aerobic count, which is $5\times10^5$ CFU/g (Anonymous, 1992). In case of total *Enterobacteriacea* count, 81 samples (67.5%) crossed the standard value which is 100 CFU/g. Similarly, 27 samples (22.5%) and 57 samples (47.5%) were found crossing the standard value of Staphylococcal count (100 CFU/g) and *E. coil* (0 CFU/g), respectively. However, none of the samples were found to be contaminated with *L. monocytogenes, Y. enterocolitica, B. cereus* and *Salmonella* spp. (Table 2). The average counts for total mesophilic aerobic bacteria, *Enterobacteriacea* and *Staphylococcus* were found to be $4.8\times10^6$, $1.7\times10^3$ and $1.2\times10^2$ CFU/g respectively. Our result also indicated that *Enterobacteriacea* count was significantly high in north and south compared to the other localities. Similarly, Staphylococcus was also significantly low in west compared to the other locations. From the results obtained in the study, it was evident that more strict regulatory limitations and legislations must be used to reduce bacterial load of traditional ice cream in Kermanshah province. Moreover, good manufacturing practices as well as distribution and retail storage practices for ensuring microbiological safety of ice cream must was be applied. The high total bacterial count, Enterobacteriacea count and Staphylococcal count recorded in the present study agreed with those reported by other authors (Warke et al., 2000; Kanbakan et al., 2004; Ghasemi et al., 2009). It is noteworthy that *Staphylococcus spp* can enter into milk and various dairy products from food handlers either suffering from an acute pyogenic infection or being in a state of healthy carriers harbouring the organisms in nose or throat (ME-Elahi et al., 2002). Our results on the total bacterial and *Enterobacteriacea* count were higher than those of other findings in Iran (Javadi et al., 2011) and Turkey (Wilson et al., 1997). The use of non-potable water and the disrespect to the hygienic rules during the production can be main reasons of microbial contamination (Wouafo et al., 1998). High percentage of *Enterobacteriacea* family in the present study could be attributed to long time storage of ice cream mix under inappropriate environment before freezing (Pietranera et al., 2003).

Ice cream samples from west part of the city had significantly less ($P<0.05$) Staphylococcal count compared to those obtained from any other area. As shown in Table 1, significantly high total bacterial count was found in the north and south of the city. Staphylococcal count was significantly low in west compared to the other parts of the city. The low level of microbial contamination of ice cream collected from west part compared to other areas could be attributed to high sanitation and appropriate hygiene behaviour of ice cream handlers. However, this study shows that the overall microbial quality of traditional ice cream samples being sold in Kermanshah is poor.

Based on our finding, Vanilla flavoured ice cream was the most contaminated type of ice cream compared to the other three flavours (Table 3). For Vanilla ice cream, the average TBC was $9.00\times10^6$ CFU/g, whereas for chocolate, strawberry and saffron the bacterial count was $8.02\times10^6$, $2.97\times10^6$ and $1.25\times10^6$ CFU/g respectively. The results of the present study agreed with those reported by other authors (Maifreni et al., 1999; Pooran et al., 2012). One of the most important reasons of high contamination of traditional ice cream is ingredients used in the preparation of ice cream that are potential sources of contamination (Reij et al., 2004). *Enterobacteriacea* and Staphylococcal count was high in chocolate (100 and 46.66% respectively) and lowest in saffron (40% and 3.33% respectively) as shown in Table 3. Similarly, the results also indicated that with decreasing temperature, the number of bacterial count also decreases (Table 4). High storage temperature of ice cream, especially those sold in open containers lead to multiplication of bacteria (El-Sharef et al., 2005). Warke et al. (2000) reported that during 10 days of frozen storage, *L. monocytogenes* counts grew to >1 log and 1 log cycle at 8-10ºC and 2-4ºC respectively.

The mean chemical components of ice cream samples collected from Kermanshah city, Iran are presented in Table 5.

Moreover, Table 6 shows the chemical composition of different flavours. There was no significant difference in chemical composition of

**Table 1: The bacteriological status of the traditional ice cream samples (CFU/g)**

| | | Total bacterial count ($\times10^6$) | | *Enterobacteriacea* count ($\times10^3$) | | Staphylococcal count ($\times10^2$) | |
|---|---|---|---|---|---|---|---|
| Area | No | SV* | Average | SV* | Average | SV* | Average |
| North | 24 | $5\times10^5$ | $8.57^a$ | $1\times10^2$ | $2.20^a$ | $1\times10^2$ | $2.20^a$ |
| South | 24 | | $9.75^a$ | | $2.97^a$ | | $1.26^a$ |
| East | 24 | | $1.87^b$ | | $1.01^a$ | | $1.25^a$ |
| West | 24 | | $1.20^b$ | | $1.11^a$ | | $0.25^b$ |
| Centre | 24 | | $2.92^b$ | | $1.33^a$ | | $1.25^a$ |

*Standard Value (Anonymous, 1992); Different superscripts in a column differ significantly ($P<0.05$)

**Table 2: Comparison of the bacteriological status of the traditional ice cream samples (CFU/g) with the standard value**

| No. of sample | TBC | *Enterobacteriacea* | *S. aureus* | *E. coli* | *Salmonella* | *Listeria* | *Bacillus* | *Y. entrocolitica* |
|---|---|---|---|---|---|---|---|---|
| samples exceeded standard (n) | 93 | 81 | 27 | 57 | 0 | 0 | 0 | 0 |
| Samples exceeded standard (%) | 77.5 | 67.5 | 22.5 | 47.5 | 0 | 0 | 0 | 0 |

TBC: Total Bacterial Count.

**Table 3:** Frequency and counts of bacteria in ice cream by popular flavours (CFU/g)

| Flavour | No | Total bacterial count | | *Enterobacteriacea* | | Staphylococcal count | |
|---|---|---|---|---|---|---|---|
| | | No. exceeded standard | Average ($\times10^{6}$) | No. exceeded standard | Average ($\times10^{3}$) | No. exceeded standard | Average ($\times10^{2}$) |
| Vanilla | 30 | 29(96.66%) | $9.00^{a}$ | 19(63.33%) | $2.00^{a}$ | 14(46.66%) | $3.10^{a}$ |
| Chocolate | 30 | 28(96.66%) | $8.02^{a}$ | 30(100%) | $3.07^{a}$ | 9(30%) | $1.20^{a}$ |
| Strawberry | 30 | 20(66.66%) | $2.97^{b}$ | 20(66.66%) | $1.57^{a}$ | 3(10%) | $2.20^{a}$ |
| Saffron | 30 | 16(53.33%) | $1.25^{b}$ | 12(40%) | $1.20^{a}$ | 1(3.33%) | $0.27^{b}$ |

Different superscripts in a column differ significantly (P<0.05)

**Table 4:** Microbial load of ice cream by temperature of product on purchase

| Range of temperature °C | No | Average | | |
|---|---|---|---|---|
| | | Total bacterial count ($\times10^{6}$) | *Enterobacteriacea* ($\times10^{3}$) | Staphylococcal count ($\times10^{2}$) |
| < -5.21 | 24 | $8.31^{a}$ | $3.99^{a}$ | $4.45^{a}$ |
| -5.22 to -6.3 | 24 | $7.75^{a}$ | $1.99^{b}$ | $2.25^{b}$ |
| -6.4 to -7.45 | 24 | $6.62^{a}$ | $1.09^{b}$ | $1.49^{b}$ |
| -7.46 to -8.7 | 24 | $5.67^{a}$ | $1.08^{b}$ | $1.21^{b}$ |
| < -8.8 | 24 | $2.92^{b}$ | $1.00^{b}$ | $0.27^{c}$ |

Different superscripts in a column differ significantly (P<0.05)

**Table 5:** Average chemical composition of ice cream samples collected from Kermanshah city, Iran

| | Fat (%) | Protein (%) | Total solids (%) | Ash (%) | Sucrose (%) |
|---|---|---|---|---|---|
| Samples | 9.18±1.36 | 2.67±1.01 | 33.28±1.89 | 0.64±0.01 | 9.56±2.29 |
| Standard value* | 5-9.5 | - | 30-33.5 | - | 9-13 |

* Standard Value (Anonymous, 1992)

**Table 6:** Effect of type of flavour on chemical composition of ice cream

| Composition | Vanilla | Chocolate | Strawberry | Saffron |
|---|---|---|---|---|
| Fat (%) | $6.01\pm2.56^{a}$ | $6.37\pm3.45^{a}$ | $6.89\pm4.41^{a}$ | $6.14\pm3.12^{a}$ |
| Protein (%) | $2.34\pm1.10^{a}$ | $2.45\pm0.89^{a}$ | $2.89\pm1.71^{a}$ | $2.89\pm0.97^{a}$ |
| Total solids (%) | $34.62\pm2.89^{a}$ | $36.89\pm4.67^{a}$ | $32.75\pm2.89^{a}$ | $32.25\pm3.66^{a}$ |
| Ash (%) | $0.47\pm0.25^{a}$ | $0.52\pm0.34^{a}$ | $0.55\pm0.34^{a}$ | $0.46\pm0.26^{a}$ |
| Sucrose (%) | $9.17\pm1.21^{a}$ | $8.98\pm1.87^{a}$ | $9.15\pm1.87^{a}$ | $9.21\pm1.03^{a}$ |

Same superscripts in a column do not differ significantly (P>0.05).

different flavours. Generally, these levels are in accordance with other authors (El Owni et al., 2009; El-Sharef et al., 2005). Moreover, the chemical composition of the ice cream falls within the standard values indicating that the microbial contamination may probably due to unhygienic conditions.

## Conclusion

Our findings indicate that traditional ice cream collected from different parts of Kermanshah city represent a significant vehicle for human pathogenic bacteria. Moreover, the present study shows the sub-standard microbiological quality of open/artisanal ice cream and encourages the consumers to shift to the packed/industrial ice cream with consistently good quality.

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