Microbiological evaluation of locally produced ice cream in Baquba city .Iraq

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Abstract. This study aimed to evaluate the bacteriological quality of ice cream made locally and seller's hands in different sites of Baquba city. The samples include 70 randomly collected ice cream and 25 sample taken from seller's hands. All samples were free from salmonella and shigella also the absence of mold and yeast was concluded. The distribution of isolated bacteria from ice cream samples was as follow : %47, %34, %28, %26, %9 for Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, Streptococcus faecalis and Bacillus cereus while in seller's hands were as %44, %24, %24, %24, %20, %8, %8 for the respectively Escherichia coli, Bacillus cereus, Staphylococcus aureus, Proteus spp., Streptococcus faecalis, Citrobacter spp. and Enterobacter aerogenes. Generally the pathogenic bacteria as Escherichia coli isolated in highest number in both ice cream and seller's hands The range of bacterial total count for ice cream samples was between $3.3 \times 10^5$ and $5.2 \times 10^5$ cfu/g. The present study showed that the microbiological quality of the ice cream samples not fit the standard value. The results of this study encourage the necessity of providing hygienic conditions in the process , preparing, package and marketing to minimize the contamination of such a most consumed dairy product like ice cream.

Keywords: locally Ice cream, pathogenic bacteria , total bacterial count , Baquba .

Introduction:

Ice cream is a delicious nutritionally enriched frozen dairy product made by pasteurized milk. It is a popular dessert which consumed during summer in all age groups especially for children [1,10]. It contains a variety of ingredients in addition to milk such as cream and sugar and other flavor enriching ingredients. Any of these may contribute microorganisms and affect the quality of the product [3,4] Ice cream may sold in packages or in open containers. Its microbial quality has always been crucially important in public health. [3,5] The growth and multiplication of containing microorganisms are possible during production, storage, handling, transportation and marketing of these products. Primary contamination sources include water, raw milk and secondary contamination sources include flavoring agents, utensils and handling of the ice creams. [1,4,6] Many psychrotroph and psychrotolerant microorganisms like Listeria monocytogenes, Staphylococcus aureus, Bacilllus, Salmonella, Shigella, Streptococcus, Pseudomonas, Campylobacter, Brucella sp. And coliform bacteria are generally present in this kind of product, coliform may be identified as a major Post-pasteurization microbial contaminant. Some contaminant organisms may become harmful as they might be responsible for cholera, typhoid, bacillary dysentery [4,5,7]
Psychrotrophic bacteria usually account for more than 90% of the total microbial population in cooled raw milk. Generally, psychrotrophic microorganisms can cause spoilage of milk and dairy product because they are able to produce extracellular or intracellular thermostable enzymes (proteases, lipases and phospholipases). Bacteriological quality of ice cream reflects hygienic practice during production and is an indication of food safety [1,10].

In Iraq, quality control measures and proper standard hygiene procures are not followed strictly, because of lack such studies related with evaluation of such popular products in Baquba city, the present work was conducted to:

a) Assess the quality of the ice cream shops and personal hygiene of the workers and asilers
b) Estimate the total viable bacterial count of the ice cream sample.
c) Identify the presence of pathogenic organisms in the samples.

**Materials and Methods**

**Sampling :**

This study included 95 samples (70 ice cream and 25 swaps from sellers hands) collected randomly from shops of 5 areas in baquba city, the samples were collected depended on the crowdsness and number of local shops of ice cream in the area. (Old market, New Baquba, Al mualeemeen, AL Tahir and 7 Nissan ) for the presence of certain pathogenic microorganisms. The study was carried out over a period of 3 months starting on June to September 2018. The samples are collected in ice-bags and brought to the laboratory of Baquba Technical Institute, Middle Technical University (MTU) and kept at refrigeration temperature till further processing for performing microbiological tests.

**Bacteriological evaluation :**

Isolates we identified using standard procedures. The microbial profile of ice-cream was studied by mixing 1 gram of ice cream sample after melting in a sterile petridishes in 99 ml of phosphate buffer saline (PBS) pH 7.2. The mixture was shaken thoroughly to mix it well and allowed to stand for 10 minutes. Appropriate inocula and swab from sellers hand were used to obtain Standard Plate Count at 37°C as well as counts of indicator organisms like coliform and staphylococci. The same media were used for the swaps taken from seller's hands [6,11]. Streak and spread plate techniques were performed for bacterial isolation. Plate Count Agar (PCA), MacConkey, SS (Salmonella-Shigella) and TCBS (Thiosulfate Citrate bile Salts) agar were used and all of them were prepared according to instructions provided by the manufacturer company. MacConkey agar was used for gram negative bacterial isolation. SS agar, selective and differential medium, was used to isolate Salmonella and Shigella. TCBS agar is an extremely selective media for isolating Vibrio spp. The bacterial isolates were collected and maintained in Nutrient slant agar. Gram staining, KIA (Kligler’s Iron Agar), MIU (Motility Indole Urea) tests were carried out according to the standard procedures for the biochemical properties of the isolates (manual of ICDDRB) appeared on MacConkey, TCBS (Thio-sulfate Citrate bile Salts) and SS (Salmonella-Shigella) media. Catalase and oxidase tests were done also. [1,13,14]

**Mycological evaluation**

Wight 25g of ice cream samples in a sterile 225 ml jar and blend at high speed for 1 minute serial dilution will be obtained (10⁻², 10⁻³ and 10⁻⁴) transfer the mixture to the surface of potato dextrose Agar Sabouraud dextrose agar (SDA) with chloramphenicol. The same culture media used for taken swabs sample then incubated in the plates for 5 days at 25°C. Count and calculate the number of Molds and yeast [6,15].
Bacterial total count:

Serial Dilution prepared mixing 1ml of each ice-cream sample with 9ml of peptone water to give a dilution of 1:10, from this dilution, a serial dilution of up to 10-3 was carried. The prepared samples of concentration range 10-1 and 10-2 were used for inoculation. 1ml of each of this dilution was inoculated on nutrient agar, McConkey agar and Salmonella Shigella agar respectively in duplicate. At the end of incubation, the number of colonies was counted. Average of duplicate plates were counted and recorded as the numbers of colony forming unit (cfu/ ml) of each ice-cream sample. The bacteria plate counts per ml were recorded using:

\[
\frac{R}{V \cdot R}
\]

where:
- \( n \) = Number of colonies
- \( R \) = Dilution factor
- \( V \) = Value of the particular dilution being put on the medium. [16]

Result and Discussion:

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. [8,17]

Information included in Table-1 show the distribution of sample collected in the area depending on enlargement of area and shops. Major of samples are from new Baquba (19) ice cream and (6) seller’s hands while less samples (14) ice cream and (4) seller’s hands were collected from 7 Nissan area.

| Areas         | Ice cream samples | Seller's hands samples | Percentage of sample |
|---------------|-------------------|------------------------|----------------------|
| 7 Nissan      | 14                | 4                      | 19                   |
| AL Tahrir     | 18                | 3                      | 22                   |
| Old market    | 14                | 7                      | 22                   |
| New Baquba    | 19                | 6                      | 16                   |
| Al mualemen   | 14                | 5                      | 20                   |

Total = 95 sample  %100

Primary source of microbial contamination of ice cream include water and raw milk whereas secondary source include flavoring agents, utensils and handling. Although freezing and hardening steps in production can estimate most of the microbial hazards, [18,19].

| Pathogenic samples        | Number | Percentage of sample |
|---------------------------|--------|----------------------|
| *Escherichia coli*        | 33     | 47                   |
| *Staphylococcus aureus*   | 24     | 34                   |
| *Enterobacter aerogenes*  | 20     | 28                   |
| *Streptococcus faecalis*  | 18     | 26                   |
| *Bacillus cereus*         | 6      | 9                    |
| Yeast                     | 0      | 0                    |
| Molds                     | 0      | 0                    |
The results in table (2) show the microbial evaluation regarding with ice cream sample and the percentage of contaminated samples with isolates. *E.coli* was found at a level of 47% and compose the highest percentage in contaminated ice cream samples while *B.cereus* recorded as 6%. *S.aureus* as a second high level as 34%. Non of of examined samples were contaminated with yeast nor molds. These results coincided with finding of Ahmed *et al* who isolate seven species of bacteria, the highest frequency of isolation was *E.coli* (100%) and the least belong to salmonella spp (15%) while our samples were clear from it [4]. According to BIS 1998, the maximum acceptable count of organisms is $2.3 \times 10^5 \text{cfu/g}$ and no coliform bacteria present in the ice cream production [1,20].

Our results also agree with senthikumaran *et al* regarding *E.coli* and *S.aureus* as it is number of normal flora of human skin. The presence of *E.coli* indicated fecal contamination of ice cream, suggesting possible risk of infection [16,21]. Finding of *B.cerus* agree with yusuf *et al* . who isolate *E.coli* also *B.cerus* in ice cream samples from Banchi. Nigeria and with Azadina *et al*. in his isolation of the other sp. of bacteria [5,16,22].

### Table 3- Microbial evaluation of swabs of seller hands samples .

| Pathogenic samples         | Number | Percentage of sample |
|---------------------------|--------|----------------------|
| *Escherichia coli*        | 11     | 44                   |
| *Bacillus cereus*         | 6      | 24                   |
| *Staphylococcus aureus*   | 6      | 24                   |
| *Proteus spp.*            | 6      | 24                   |
| *Streptococcus faecalis*  | 2      | 8                    |
| *Citrobacter spp.*        | 2      | 8                    |
| *Enterobacter aerogenes*   | 5      | 20                   |
| Yeast                     | 0      | 0                    |
| Molds                     | 0      | 0                    |

Regarding with the swap sample of seller’s hands table (3) record the results of microbial evaluation while included reporting of *E.coli* isolates in higher number (44%) then *S.aureus*, *B.cereus* and *S.fecalis* as (24%) from studied samples. The *proteus spp.* and . *E.aerogenosa* (20%) (8%) while the samples were free from yeast and molds. According to results of contamination of seller’s hands, it agree with the finding of El khair *et al* . who indicate the contamination of the workers hands by different levels of bacteria in which these microorganisms may transport to ice cream [18]. The presence of Gram negative organisms such as *E.coli* indicate that there are possible source contamination in post pasteurization period like selling and transporting the product this result is similar to the findings of researchers from Dhaka [1].

### Table 4-Mean of pathogenic bacterial count from both ice cream samples.

| Sample   | Minimum cfu/ml | Maximum cfu/ml | Mean average |
|----------|----------------|----------------|--------------|
| Ice cream| $3.3 \times 10^5$ | $5.2 \times 10^5$ | $4.25 \times 10^5$ |

The total viable count in ice cream samples of 5 different areas of Baquba city per gram reported in range from $3.3 \times 10^5$ to $5.2 \times 10^5$ and as mean count of $4.25 \times 10^5$(CFU/g), this results was close to another study in which the value was about $2.2 \times 10^5$ to $8.2 \times 10^4$ cfu/g in the most samples of ice cream in Gilgit town [4]. Our results are in coordination with Bahareem *et al*, 2007 who showed that
the total bacterial count was \( \geq 1.5 \times 10^5 \) cfu/g in 26 (32.5%) samples [23].
In other study regarding to E. coli contamination, international guidelines for the microbiological quality of processed and ready to eat foods consider unsatisfactory for human consumption those containing \( \geq 10^4 \) CFU/g of coliforms or \( \geq 10^2 \) CFU/g of E. coli.[18]

Our results were close to the findings of Azadain et al., in which he found that the total bacterial count was in range \( 3.6 \times 10^5 – 4.1 \times 10^5 \) and with Mathews et.al who report counts above the microbiological standards for ice cream [5,24].

This study indicated the necessity of periodical controls should be conducted in producing such a wide consumed production like ice cream also made aware of hygien parameters of locally produced ice cream using bare hands in preparing the products may be a possible source of contamination. This study encourage consume of the packed industrial ice cream having good hygien quality.

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