

## Microbiological Quality of Ice-Creams Produced in Alba County, Romania

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### Abstract

The microbiological quality of 40 ice cream samples of various flavours produced by one large-scale manufacturer in Alba County, Romania, was evaluated. Total number of mesophyll bacteria (cfu/g), total coliforms, *E. coli* and presence of *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes* were performed on all samples. Total bacterial count (TCB) ranged to  $3.0 \times 10^4$  cfu/g and coliform bacteria to 60/g. The level of bacterial contamination found in this study reflects the unhygienic conditions prevalent in manufacturing and storage of ice creams. In the mean time it was confirmed that the overall risk associated with the consumption of ice-cream is low. The experimental study results suggest that ice cream processing factory must adopt quality guarantee systems, such as good manufacturing practices, hazard analysis and critical control points system. Grape seed oil obtained by extraction with petroleum ether can be used for technical purposes only.

**Keywords:** ice-cream, microbiology, food safety, quality

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### 1. Introduction

Ice cream is a popular frozen food consumed particularly in summer as well as throughout all year. It continues to presents a dominant interest for a large segment of population [1]. The ice cream is a polydisperse colloidal system, whose characteristics are given by milk, by-products and others ingredients added. The ingredients of ice cream may be various mixtures of milk, cream, evaporated or condensed milk, dried milk, coloring materials, flavors, fruits, nuts, sweetening agents, eggs and eggs products, stabilizers. Any of these may develop microorganisms and affect the quality of the product as judged by its bacterial load or its content of various specific species of bacteria [2,3].

The term ice cream is used to distinguish different varieties of desserts, which are consumed frozen or semi-frozen. Ice cream is a frozen cream made up of an oil-in-water emulsion with more than 55% weight of water and ice.

Carbohydrate solutions give the sensation of sweetness and, in the mean time controls the frozen and melting characteristics of ice cream. Ice cream mix properties depend primarily on lipids and proteins quantities. The small quantities of emulsifier and hydrocolloid act as stabilizers of oil-in-water emulsions. For flavoring, natural flavors are used (from real fruit, cocoa, coffee, ginger, vanilla). All this allow great variations in ice cream flavor, taste and way of structure formation.

As food, ice cream is a very nourishing product, with a great energy value, due to its reach content in carbohydrates, lipids and proteins. It contains vitamins (especially vitamin A and B) and mineral salts (calcium and phosphorus salts) also.

The microbiological quality of ice cream can be low, as it is a good growth-medium for microbes due to its nutrients (lactose, proteins, etc) and to its almost neutral pH of 6–7 [4].

There are numerous reports on human pathogens incidence in ice cream. *Listeria monocytogenes* [5], *Salmonella* spp. [6], *Escherichia coli* [7], *Staphylococcus aureus* [8], *Yersinia enterocolitica* [9], *Bacillus cereus* [10], *Listeria monocytogenes*, *Brucella* spp [11], *E. coli* O157 : H7 [12], etc. *Shigella* spp., *Pseudomonas* spp., *Streptococcus* spp., *Campylobacter* spp., are generally present in ice-cream [13].

Time-dependent heating during the ice cream production reduces largely the vegetative forms of the microorganisms. On the other hand, spore bearing microorganisms may well pose risks through consumption of this kind of milk products. Furthermore, the presence of pathogens in ice cream samples is mostly due to tools and equipments, water, workers, environment, packaging materials and contaminations during the transportation and distribution of ice cream [14, 15].

This study aimed to investigate the microbiological quality of several ice cream samples with various flavours produced in Alba County from November 2007 to February 2008. The experimental results indicate the necessity for a more steady microbial examination of ice creams, since ice cream is particularly consumed by children of vulnerable age groups.

## 2. Materials and method

In order to determine the hygienic status of the ice cream, 40 samples with different flavours were analyzed.

*Total number of mesophyll bacteria (cfu/g), coliform bacteria, E. coli and Staphylococcus aureus*

10g of sample have been homogenized with 90 ml of SFP (physiological serum with peptons) resulting decimal dilutions at  $10^{-1}$  until  $10^{-5}$ .

Commercial microbial identification systems named Petrifilmes were used. The specific dehydrated culture medium is fitted on a adhesive base and wrapped in foil. Liquid sample inoculation, allow the liquid to hydrate the medium. By thermostation, the microorganism growth is possible [16].

Petrifilm Aerobic count plate is used to determine total aerobic bacteria populations. Only three steps are required: 1. Inoculate and spread plate with one ml of sample. 2. Incubate at 30°C for 48 hours. A red indicator dye in the plate colors all colonies red. 3. Count the red colonies.

Petrifilm E. coli/Coliform count plate is a ready-made culture medium system for the enumeration of *E.coli* and coliform bacteria. Petrifilm E.coli / Coliform Count Plates contain violet red bile (VRB) lactose nutrients, cold water gelling agent, triphenyl tetrazolium chloride (TTC), an indicator that colours bacterial colonies red, and BCIG, an indicator that colours *E.coli* blue. Incubation is made at 37°C for 24-48 hours.

Petrifilm Staph Express count system consists of a Petrifilm Staph Express count plate and a Petrifilm Staph Express disk. The Petrifilm Staph Express count plate is a sample-ready culture medium system. The chromogenic, modified Baird-Parker medium in the count plate is selective and differential for *Staphylococcus aureus*. *Staphylococcus aureus* appears as red-violet colonies on the count plate. The Petrifilm Staph Express disk is designed for the detection of desoxyribonuclease (DNase) reactions specific for *Staphylococcus aureus* isolated on the Petrifilm Staph Express count plate; it contains toluidine blue-O that facilitates the visualization of the DNase reactions.

*Salmonella genus determination* [17]

25 g of each sample were placed (embedded) in 225 mL of buffered peptone water. The contents were incubated at 37°C for 18 hours. From each flask, 1 mL was transferred in Muller Kauffmann Tetrathionate Broth Base (With Novobiocin) and 0.1 mL in Rappaport-Vassiliadis modified Broth Base. The two culture mediums were incubated at 37°C and 42°C for 24 hours. From each culture microorganisms were transferred, with a wire loop or needle, to XLD, Rambach and Chromagar Salmonella Plus and incubated at 37°C for 24 hours. The characteristic bacterial growths obtained were subjected to biochemical tests on the following broths: TSI, Lysine Decarboxylase Broth,

Christensen agar, Klarck medium for Voges – Proskauer reaction and Tryptophan broth.

#### *Listeria monocytogenes* determination [18]

25 g of each sample were placed (embedded) in 225 mL of Half-Fraser Broth - FRASER ½ (pre-enrichment). The contents were incubated at 30°C for 24 hours. From each flask, 0.1 mL was transferred in Fraser Broth - FRASER 1 (enrichment) and incubated at 37°C for 48 hours. Using a wire loop or needle microorganisms were transferred to ALOA and Oxford agar and incubated at 37°C for 48 hours. The characteristic bacterial growths obtained were subjected to the following tests: catalysis test, Gram's method, microscopic examination, dispersion survey in TSYEB, hemolysis test, carbohydrates use (ramnose and xylose), CAMP test.

### 3. Results and Discussion

The experimental data obtained from the microbiological analisis of ice cream samples in this study are presented in figure 1 and 3.

As shown in figure 1, the aerob mesophile bacteria – TAB (figure 2) ranged between  $1.5 \times 10^2$  (sample 7) and  $3.0 \times 10^4$  (sample 33) cfu/g. The samples 2 to 6, 8, 12, 14, 15, 20, 27, 33 and 34 have a microbial growth, but the ice cream is considered having unacceptable hygienic quality when the TAB exceeds  $10^5$  cfu/g which is the legal limit.

These findings support the results of other reserchers who were suggested that use fresh ice creams contained not more than 100,000 cfu/ml of total bacterial count (TBC) per ml [19-23].

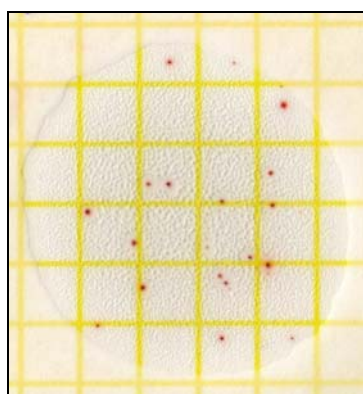


Figure 2. Positive reaction - the aerob mesophile bacteria (TAB)

As regards the coliforms bacteria presents in ice-cream (figure 4), only 23 samples (57,5%) from

the current study present a lower number of coliform bacteria (total coliforms) (<10 cfu/g). 3 samples (7,5%) contain 10 cfu/g and the rest of 14 samples (35%) as it is show in figure 3 presents a high microbiological growth (higher then 10 and up to 60 bacteria/g).

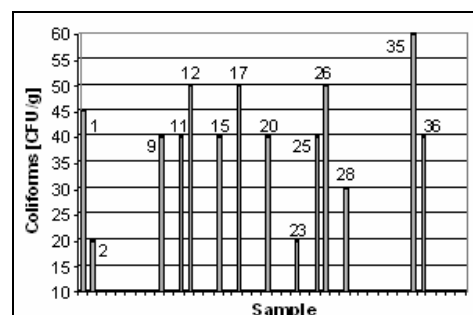


Figure 3. Presence of coliforms in ice cream samples

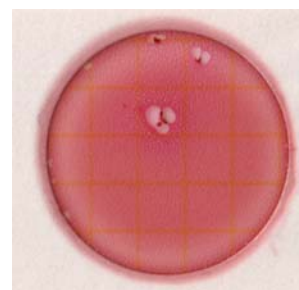


Figure 4. Positive reaction - coliforms bacteria

The results suggest negligence such as poor sanitation during the preparation and/or storage of these products. These include the observed dirty premises and utensils used, the use of bare hands in preparing the products (personal communication with the handlers). All 40 samples analyzed have negative results as regarding the presences of *Salmonella* species microorganism (*Salmonella* spp./25 g absent), *Listeria monocytogenes* (*List m.*/25 g abs), *Escherichia coli* (*E. coli*/g <10) and *Staphylococcus aureus* (Staf. cp./g <10). *E. coli* is an indicator for fecal contamination and denotes the possibility of enteric pathogens presence. The presence of *salmonella* spp. in ice cream may possibly be due to either fresh eggs or egg powder used in the ice cream production. The presence of *Salmonella* spp. may pose a great risk for public health since *Salmonella* occurrence from ice cream have been reported previously [24-26].

*L. monocytogenes* has not been isolated in ice-cream samples. It reflected that good environmental hygiene in production of ice-cream was maintained.

Figure 5 outlines the microbiological results of the 40 samples examined.

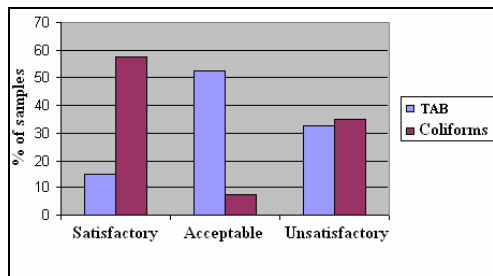


Figure 5. Microbiological status results of ice-cream samples regarding TAB and coliform

All tested ice cream samples prove that there is no risk for human health because the presence of pathogenic germs (*Salmonella* spp., *Listeria monocytogenes*, *E. coli* and *Staphylococcus aureus*) was not detected.

Anyway, figure 5 shows that some samples (approximal 35 %) are not completely safe for use due to the positive values of TAB and Coliform.

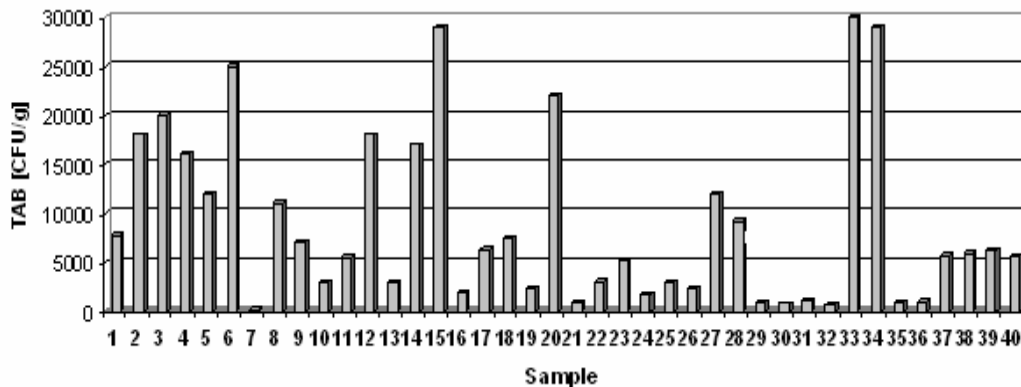


Figure 1. The presence of total aerobic mesophile bacteria in ice cream samples

#### 4. Conclusion

The preservation by freezing of ice cream products assure the microbiological stability and reduce the risk of contamination but it is not enough to guarantee for the safety of the final product. The microbiological analysis results confirm the necessity of an adequate processing of ice-cream and optimal storage condition.

The study showed that the overall risk associated with the consumption of ice-cream is low.

Based on the microbial analyses, the ice-cream products fabricate in Alba County, Romania are considered safe for consumption.

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