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Sanitary-higienic conditions of Italian style and pasta (artisanal and industrial) ice cream marketed in the northwestern region of Rio Grande do Sul, Brazil

Juliana Raquel da Silva Damer[1]
Viviane Garcia[2]
Aldoir Almeida Gusmão[3]
Terimar Ruoso Moresco[4]

[1] Universidade Federal de Santa Maria, Departamento de Análises Clínicas e Toxicológicas, Programa de Pós-graduação em Ciências Farmacêuticas. Santa Maria-RS, Brasil.

[2] Universidade Federal de Santa Maria, Laboratório de Biologia. Santa Maria-RS, Brasil.

[3] Universidade Federal de Santa Maria, Hospital Universitário de Santa Maria. Santa Maria-RS, Brasil.

[4] Universidade Federal de Santa Maria, Departamento de Ciências da Saúde. Santa Maria-RS, Brasil.

Correspondence
Juliana Raquel da Silva Damer
E-mail: juliana _ damer@yahoo.com.br

Abstract

This article aimed to evaluate the microbiological quality of ice cream sold in Northwest Rio Grande do Sul, Brazil. The samples were submitted to analysis the following: estimates of Most Probable Number (MPN/g) of total coliforms, fecal coliforms, *Escherichia coli*, the presence of *Salmonella* spp., counting colony forming units (CFU/g) of *Staphyococcus* coagulase positive, mesophiles and total aerobic psychrotrophs, besides the investigation into the morphotinctorial characteristics of colonies from the psychrotrophs count. Samples of 21 ice cream and gelato types were analyzed, of which seven Italian style gelatos, seven artisanal ice creams and seven industrialized ice creams, from different locations. Fecal coliforms above the values established by legislation were found in 28.6% of both types of ice cream. *E. coli* was found in 42.8% and 14.3% of artisanal and industrialized ice cream, respectively. *Salmonella* spp. and *Staphylococcus* coagulase positive were not detected. All Italian style gelato samples showed high counts of mesophiles, differing significantly from the industrialized ice cream, but not the artisanal ice cream. High psychrotroph counts were also found in Italian style gelato, differing from both ice cream types. Gram-negative rods predominated among the psychrotrophs. The results indicate possible poor hygiene during production and/or storage of the ice creams and gelato. Thus, increased oversight is necessary at the selling and production points of ice cream, mainly through training and the Good Practices. Special attention should be given to the artisanal ice cream and Italian gelato, as manufacturers do not follow a standard production process.

Key words: Ice Cream. Foods. Bacteria. Food Microbiology. Food Handling.

Introduction

Ice cream is an emulsion of fat and proteins or a mixture of water, sugar and other ingredients that are then subjected to freezing. They are considered complete, high-energy foods. When milk is used in its preparation, ice cream is comprised of 10% fat, 20% total solids, vitamins A, B, C, D, E, K and the minerals calcium and phospor.[1] It also contains 12-17% carbohydrates, 55-65% water and 0.2-0.5% emulsifiers and stabilizers.[2] Being an innovative matrix as well as being well accepted by the population, research has been conducted to incorporate live microorganisms (probiotics) in appropriate dosages to benefit health, particularly for the gastrointestinal micro flora; probiotics such as *Lactobacillus* species, which contribute to improving the nutritional and functional characteristics.[1,3,4]

To maintain the characteristics and avoid product degradation, the National Health Surveillance Agency (ANVISA) recommends that ice cream produced with milk and eggs, should be obligatorily pasteurized.[5] However, this process is not carried out for homemade ice creams, and even though pasteurized, sterilized or powdered milk is used, contamination may occur after cooking, turning this food into a potential disseminator of pathogens.[6,7] The pasteurization of milk is a mild heat treatment and doesn't restore poor quality raw material,[8] which is directly proportional to the microbiological quality of the final product.[9]

Under Brazilian law, ice creams fall in the group of edible ices and should be partially or completely frozen to ensure its stability during transport and storage.[10] Freezing food is intended to extend the shelf life, but some microorganisms can develop even in products stored at temperatures below 0ºC.[11] For Kanbakan et al.,[12] the cooling method of ice cream, when it's inefficient, leads to an increased microorganism count in the final product in relation to raw materials.

Microbial contamination of food can occur directly, via contaminated raw materials, or indirectly by means of the manipulator, especially when there are poor hygiene conditions.[6] Microorganisms present in healthy skin, nose, hands, and animal or human feces or skin wounds are commonly found in spoiled foods or in those causing poisoning.[11] Kanbakan et al.[12] detected coliforms in the hands of workers in ice cream sales and in the delivery phase of the final product to the consumer.

Cases of diseases related to the consumption of ice cream contaminated with microorganisms or their toxins have been reported, particularly those caused by *Salmonella* spp., *Staphylococcus aureus*, forming toxins, *Shigella* spp. and enteropathogenic strains of *Escherichia coli*.[6,11]

The World Health Organization (WHO) states that foodborne diseases with gastrointestinal symptoms are a public health problem worldwide and result from the ingestion of food contaminated with microorganisms in the production or consumption stage, and may result from environmental contamination of water, soil and air.[13]

To minimize or even eliminate these problems, food industries have implemented the System of Hazard Analysis and Critical Control Points (HACCP). By using this system, the ice cream industry was able to reduce the total mesophiles and coliform counts by 20 to 35%, demonstrating that hygienic control and the control of pasteurizing and freezing processes are essential to obtain a good quality product.[14]

Although the failures that may occur during production are important, the highest infection rates occur through improper handling of the product in selling points. Incorrect hand hygiene of food handlers and the water in which the spoon to serve ice cream is kept represent the main sources of contamination.[12]

According to Kokkinakis et al.,[14] in work that evaluates the entire manufacturing process, the Good Manufacturing Practices and Good Hygienic Practices are associated with the absence of *S. aureus* and total coliforms as well as *E. coli*. In this context, this study aimed to investigate the sanitary quality of different types of ice creams as a final product, marketed in Northwest Rio Grande do Sul, Brazil.

Methodology

During the months of December 2012 to July 2013 21 ice cream samples were collected, seven Italian style gelatos, seven artisanal ice creams and seven industrialized ice creams, all from different retailers. After being acquired as if for consumption, the samples were immediately placed in cool boxes and transported to the Microbiology Laboratory of the Federal University of Santa Maria, Palmeira das Missões, Rio Grande do Sul.

Estimates of the most probable number (MPN/g) of total coliforms, fecal coliforms and *Escherichia coli* as well as the tests for the presence of *Salmonella* spp. in 25g and the counts in colony forming units (CFU/g) of *Staphylococcus* coagulase positive, total aerobic mesophiles and psychrotrophs, followed the methodology described by the American Public Health Association.[15] Morphotinctorial characterictics of psychrotrophic microorganisms from five colonies of different macroscopic aspects of each sample were determined using the Gram method.

The values of the counts of microorganisms found in different types of ice creams were transformed to log base 10. The frequency of the parametric variables are presented as mean ± standard deviation (SD) and were analyzed using 1-way ANOVA. Nonparametric data were analyzed using Kruskal-Wallis and are presented as median and percentiles. Tukey's test was used for comparing the groups. In all analysis, a significance level of $p < 0.05$ was considered. The tests were conducted by the type *italianoware 11.0® SigmaPlot* (Systat type italianoware, Inc. SigmaPlot for Windows).

Results

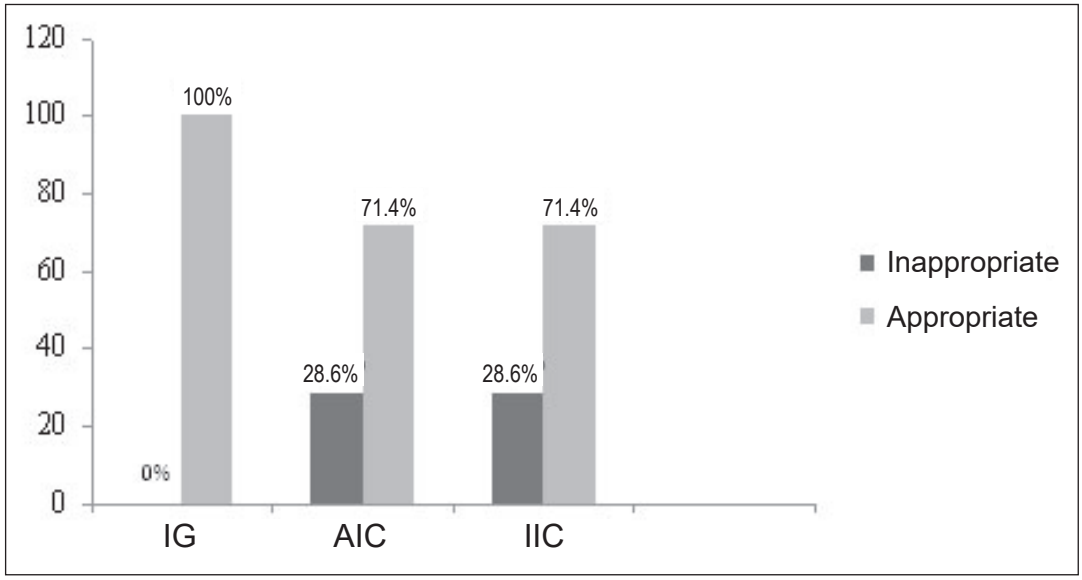
Table 1 presents the results of the microbiological analysis in the three kinds of ice cream marketed in Northwest Rio Grande do Sul.

Table 1. Results of the microbiological analysis in ice creams marketed in Northwest Rio Grande do Sul, Brazil, in 2013.

Microrganism	IG (n=7)	AIC (n=7)	IIC (n=7)	Total (n=21)
Mesophiles (CFU/g)	2,1 x 10^4 –2,5 x 10^6	1,8 x 10^4 – 3,1 x 10^6	<10,0 –2,4 x 10^5	<10,0 - 3,1 x 10^6
Psychrotrophs (CFU/g)	2,7 x 10^4 -1,0 x 10^7	<10,0 – 8,0 x 10^6	<10,0 –5,8 10^5	<10,0 – 1,0 x 10^7
Coliforms 35ºC (MPN/g)	<3,0 – 460	3,6 –>1.100	<3,0 – 1.100	<3,0 –>1.100
Coliforms 45ºC (MPN/g)	<3,0	<3,0 – 460	<3,0 – 1.100	<3,0 – 1.100
Escherichia coli (MPN/g)	<3,0	<3,0 – 15	<3,0 – 93	<3,0 – 93
Salmonella spp. (presence/ absence)	Absence	Absence	Absence	Absence
Staphylococcus coagulase positive (CFU/g)	<100	<100	<100	<100

IG: Italian Gelato
AIC: Artisanal Ice Cream
IIC: Industrialized Ice Cream

As for the estimates of total coliforms, 54.2%, 71.4% and 71.4% of the Italian gelato, the artisanal ice scream and industrialized ice cream were contaminated with such bacteria, respectively; 28.6% of both ice creams showed fecal coliforms greater than 50 MPN/g. The Italian style gelato presented estimates below this value for these microorganisms. The presence of *E. coli* was detected in 42.8% and 14.3% of the samples of artisanal and industrialized ice cream, respectively (Figure 1).



IG: Italian Gelato
AIC: Artisanal Ice Cream
IIC: Industrialized Ice Cream

Figure 1. Percentage of ice creams suitable and unsuitable for human consumption in accordance with microbiological standards established by the RDC 12 of 2001[14]. Northwest Rio Grande do Sul, Brazil, in 2013.

Salmonella spp. and *Staphylococcus* coagulase positive were not detected in the analyzed samples.

The mesophilic count in Italian style gelato did not differ significantly from the one in artisanal ice cream, but differed from the one in industrialized ice cream. The contamination in artisanal ice cream was higher than that obtained in industrial ice cream, but the difference wasn't significant (Table 2).

Table 2. Count of mesophiles and psychrotrophic bacteria in ice cream marketed in Northwest Rio Grande do Sul, Brazil, 2013

	Type of Ice Cream		
	IG	AIC	IIC
Mesophiles [1,3] (log CFU/g) average ± SD	5,40±0,78^{A}	4,91±0,88^{AB}	3,57±1,59^{B}
Psychrotrophs [2,3] (log CFU/g) median (P25-P75)	Italian (median)	Artisanal (median)	Industrialized (median)
	6,15^{C}(5,13-6,54)	1,00^{DE}(1,00-3,99)	1,00^{E}(1,00-3,77)

IG: Italian Gelato

AIC: Artisanal Ice Cream

IIC: Industrialized Ice Cream

[1]Values in the lines, followed by unequal letters differ by Tukey's test (P = 0.02).
[2]Valores on the lines followed by unequal letters differ by Tukey's test (P = 0.07).
[3] Logarithms in the number of colony forms units/g of ice cream.

For the samples of the ice creams, 57% of artisanal and 71.4% of industrialized ice cream showed no growth of total aerobic psychrotrophs (Table 1). The Italian type gelato showed higher scores for these microorganisms, differing statistically from both ice creams (Table 2).

Using Gram staining, the morphotinctorial characteristics of the psychrotrophic aerobic microorganisms can be observed in figure 2.

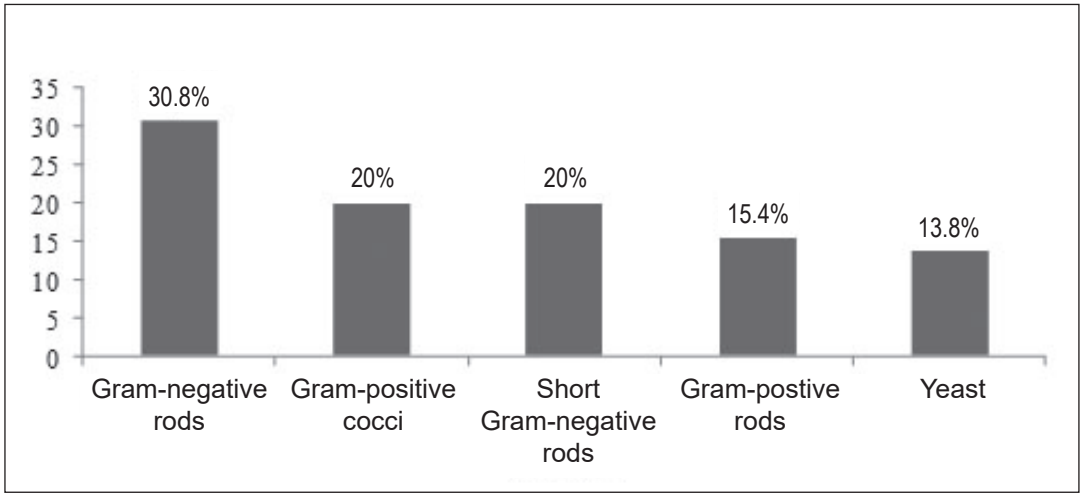


Figure 2. Morphotinctorial characteristics of psychrotrophic microorganisms in the samples of the three types of ice cream, n = 65 smears. Northwest Rio Grande do Sul, Brazil, in 2013.

Discussion

According to the Collegiate Board Resolution (RDC) No. 12,[16] edible ices can be consumed safely when the estimates for fecal coliforms are less than 50 MPN/g, the count of *Staphylococcus* positive coagulase is less than 500 CFU/g and there is no pathogen *Salmonella* spp. in 25g.

The estimates of total coliform obtained in the three types of ice creams analyzed revealed that more than half of the samples were contaminated, regardless of the type of ice cream. Estimates or counts from this group of bacteria in food are not required under Brazilian law; however, the research is important as it indicates recontamination during the different production steps.[17]

Both the ice creams (artisanal and industrialized) were highly contaminated with fecal coliforms, with 28.6% of the samples from both groups unfit for human consumption, as the amounts exceeded the allowed limit recommended by Brazilian law.[16]

The Italian style gelatos all presented fecal coliforms estimates that were within the standards established by Brazilian legislation.[16] However, compliance with the rules does not mean absence of other potentially pathogenic microorganisms or deterioration processes, because even within the standards of consumption, these samples showed the highest levels of contamination by mesophiles and psychrotrophic microorganisms.

The species *E. coli* was detected in both soft ice creams; among them the industrialized ice cream had the highest values of MPN/g. But the artisanal ice cream had the highest number of contaminated samples. The presence of these bacteria is connected to hygienic failures at some stage prior to consumption, as it is present in the intestinal tract of warm-blooded animals. Importantly, some strains of *E. coli* cause gastrointestinal diseases with high severity.[6]

Schrijver et al.[7] reported that *E. coli* strains verotoxin producers were capable of causing severe diarrhea and hemolytic uremic syndrome in children who had ingested ice cream. The authors suggested that possible cross-contamination occurred during handling, after the pasteurization of milk, as identical strains were also isolated from feces of calves and farm straw.

In a similar study conducted in Turkey, packaged ice creams were not contaminated by coliform or *E. coli*, differing from the non-packaged ice cream, which showed 50.1% and 63.6% outside the local standards established for such microorganisms, respectively.[18]

Similar results were obtained in Egypt,[19] where 55%, 43.7% and 57% of the ice creams were analyzed with unacceptable values for total coliforms, fecal coliforms and *E. coli*, respectively. Lower infection rates were obtained in Turkey by Yaman et al.[20] and by Rizzo-Benato & Gallo[21] in Brazil.

In our work, none of the ice cream samples showed contamination with the pathogen *Salmonella* spp., which is responsible for outbreaks of severe poisoning by eating contaminated food of animal origin, mainly meat products, eggs, poultry and milk,[22] including ice cream. [23,24,25] Thus, all samples were suitable for consumption according to Brazilian law.[16] This result corroborates those obtained by Pazianotti et al.[26] and Pooran et al.,[27] but differs from those presented by Yaman et al.,[20] Queiroz et al.,[2] and Ambily & Beena, [28] who all identified *Salmonella* spp. In 6.8%, 75% and 3.7% of the analyzed ice cream samples, respectively. Rose et al.[9] found this pathogen in the raw material and the industrial ice cream, besides finding problems in the Good Manufacturing Practices, confirming that the flaws during processing and in the choice of raw materials are reflected directly on the quality of the final product.

None of the samples showed contamination with *Staphylococcus* positive coagulase, all of which are in accordance with the standards established by RDC Nº 12[16] in this regard. Among these bacteria, *S. aureus* is the most important at a clinical level, as it is associated with foodborne illnesses due to the production of enterotoxins which cause food poisoning outbreaks, especially through the consumption of milk and contaminated milk products.[11] It is present in the nasal cavity, throat and skin, and can often be found in milk sample.[6] The fact that this bacterium wasn't detected in the analyzed ice creams may be related to the correct pasteurization process associated with good quality milk. This result corroborates those obtained by Vica et al.[29] but differs from those obtained by El-Sharef et al.[30] and Ambily & Beena.[28]

Although the artisanal ice creams were more contaminated with mesophiles, they didn't differ significantly from the industrialized ice creams. This may have occurred because the storage temperature prevented the growth of most microorganisms. Pooran et al.[27] found significant differences between the industrialized and artisanal ice creams, for in the latter the mesophilic counts were always higher. El-Sharef et al.[30] also obtained high scores, ranging from 10^2 to 10^8 for open ice creams and from 10^1 to 10^8 for packaged ice creams. Azadnia et al.[31] found high averages for mesophilic counts (10^6), of which 88% of ice cream were presented outside the standards set by local laws. Movassagh et al.[32] found similar results in ice cream marketed in Iran.

The Italian style gelatos were the most contaminated with psychrotrophs, differing statistically from both types of ice creams. This may be due to the differences in storage temperatures, because the ice creams are stored at a temperature of -18 °C and Italian gelato at around 0 °C, allowing these microorganisms to develop.

Even with an efficient pasteurization process, ice cream, being a product derived from milk, is a good culture medium, thanks to the high nutritional value, the almost neutral pH and the duration of the storage period. Pasteurization doesn't completely eliminate contaminating microorganisms, allowing the survival of heat-resistant non-spore-forming bacteria and of the spores of most of the bacteria, only reducing the number of microorganisms to an acceptable level. If there is any deviation at the maturation stage, with a rise in temperature above 5 °C, exceeding the critical limits of time/temperature, there may be a proliferation of pathogenic microorganisms surviving the pasteurization phase. Good practices after processing, including the transportation and display for sale, are also important.[33]

The analysis of the morphotinctorial characteristics of the total aerobic psychrotrophs using Gram staining showed great diversity in the microorganisms. Gram-negative rods were predominant, which are the main psychrotrophic causes for the deterioration of milk and milk products for the production of proteases and lipases.[6] The most common gram-positive cocci that cause deterioration of processed dairy products are the *Micrococcus* and *Streptococcus*, as they are microorganisms that are resistant to high temperatures and could possibly survive pasteurization.[11]

Gram-positive rods, particularly the forming of spores are important spoilage of thermally processed dairy products. Representatives of this group are the *Bacillus* spp., which form spores that survive heating.[6]

Regarding gram-positive short rods, the genus *Listeria*, main representative, is often associated with dairy products, as well as other acid-lactic microorganisms, getting more attention because of outbreaks caused by *Listeria monocytogenes*.[11] Degenhardt & Silva, [34] when searching for the presence of *Listeria* in ice cream sold in self-service ice cream shops, obtained a positive result in 25.44% of the samples, of which 9.8% from the species *L. monocytogenes*. Pooran et al.[27] and Ambily & Beena[28] didn't detect this bacterium in any of the samples.

Yeasts were also found in Gram staining of psychrotrophs, suggesting that its control is necessary for ice cream, since some species have the ability to grow and deteriorate foods with high concentrations of sugar.[6]

Conclusions

The results of this study demonstrated that there is need for greater oversight by the competent bodies regarding the hygienic and sanitary practices. This need is related to the presence of fecal coliforms, above the amount allowed by Brazilian law and *E. coli* microorganism belonging to the intestinal tract of warm-blooded animals in the ice cream.

Further studies are required to determine at what critical point a failure in hygiene occurs as well as to detect if contamination occurs during the production process or the point of sale.

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