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Analysis of the microbial quality of commercialized tropical fruit ice cream in Belo Horizonte, Brazil

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Abstract

Ice cream is a popular food product and can be produced from dairy bases or water with the addition of fresh fruit and other ingredients. However, poor hygiene, unprocessed fruit and the absence of thermal treatment can contribute to the microbial content of the ice cream. This study aimed to determine the densities of the microorganisms that are indicators of the hygienic sanitary quality, as required by the Brazilian legislation. A total of 51 samples of tropical fruit ice creams were collected in Minas Gerais, Brazil. A questionnaire to evaluate Good Manufacturing Practices was completed, which allowed us to characterize handling conditions. Thermotolerant coliform and the high counts of psychrotrophic microorganisms suggest unsatisfactory sanitary quality. One sample was positive for the presence of coagulase-positive Staphylococcus. High counts of molds and yeasts were observed. The results suggest the need for the better control of sanitary conditions during the manufacturing of these products.

Keywords: ice cream, foodsafety, microbiological quality

1. Introduction

The high temperatures encountered in Brazil during the majority of the year result in ice cream being a popular and highly consumed food product among Brazilians. The ice cream market in Brazil earns over R$ 2 billion per year and is comprised of approximately 10,000 manufacturers, of which 90% are small or micro producers. The annual consumption is 1,209 million liters/year [1]. Brazil has emerged as a major market for trade in tropical fruit ice creams because the addition of fresh fruit or fruit pulp favors the palatability and acceptance of this food product.

Although ice cream is stored frozen, the high content of nutrients such as lactose and proteins and the neutral pH of ice cream make it an excellent growth medium for microorganisms, some of which may cause disease outbreaks [2-4]. The relatively simple manufacturing procedure, combined with a lack of obligation to register the product, has resulted in a proliferation of factories characterized by deficient sanitary-hygienic practices, which are reflected in the quality of the final product [4,5]. In the manufacture

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of homemade ice cream, heat treatment to control the microbial population is not performed or occurs inappropriately. This lack of treatment becomes a public health problem because this procedure is obligatory for meeting sanitary standards [6]. The time at which the ice cream is sold also presents potential contamination risks. Poor hygiene, unprocessed fruit and the absence of thermal treatment can contribute to the microbial content of the ice cream. The quality of ice cream is directly related to the origin of the various ingredients used for their manufacture and to the procedures used during the handling and storage of the food product [5,7].

According to the AgênciaNacional de VigilânciaSanitária – ANVISA, RDC 12 [8], the tolerance values of microorganisms in samples of ice cream must be less than 100 most probable number of coliforms per mL (MPN/mL) at 45 °C, with an absence of salmonellae in a 25-mL volume. Beyond the microorganisms specified by the legislation, an investigation for *Staphylococcus* spp. is also important to determine contamination during handling. In addition, the presence of high yeast and psychrotrophic bacteria counts in the food can add information on the hygienic conditions of the products. The low pH in ice creams is a factor that can promote the development of yeasts, and many yeast species isolated from foods and beverages have been reported as spoiling microorganisms or as opportunistic pathogens [9,10] and may represent risk for the consumers.

The aim of this study was to assess of microorganisms that are indicators of the hygienic-sanitary quality present in samples of tropical fruit ice cream soft five different flavors in a Brazilian city. In addition, the production conditions of the ice cream according to Good Manufacturing Practices were analyzed using a questionnaire.

2. Materials and Methods

Fifty-one ice cream samples made with pieces of fruit or tropical fruit pulp (12 pineapple, 11 açaí, five guava, nine mango and 14 passion fruit) from commercial establishments (seven with industrial production and seven with artisanal production) were collected in Belo Horizonte (Minas Gerais, Brazil) from July 2010 to January 2011. The samples were collected into disposable plastic cups that were provided by the establishment. All samples were then immediately transferred to sterile flasks, transported to the laboratory in isothermal packaging and processed within 24 hours.

At the time of collection, a questionnaire was also completed based on RDC No. 267 of 25 September 2003, established by the ANVISA to examine the sanitary conditions of ice cream [6]. The questionnaire contained a checklist to assess the facilities in the immediate area of the ice cream product, as well as the equipment, furniture, utensils and food handlers. The establishment was classified as adequate or inadequate depending on whether the sanitary conditions met the parameters established by the legislation.

The microbial tests followed the American Public Health Association (APHA) guidelines [11]. These tests included the following: counting both the total and thermotolerant coliforms using the multiple tube method; detection of *Salmonella* spp. with Selenite-Cystine broth (Acumedia, USA) and Rappaport-Vassiliadis broth (Acumedia, USA) for the selective enrichment stage, as well as *Salmonella-Shigella*Agar (Himedia, India) and Hecktoen-Enteric Agar (Himedia, India) for the selective plate method; and yeast counts with malt extract–yeast extract agar (0.3% malt extract, 0.3% yeast extract, 0.5% peptone, 1% glucose, 2% agar, 0.02% chloramphenicol). The psychrotrophic bacterial counts were performed using the spread-plate technique in Plate Count Agar (Himedia, India). Isolation and counting of *Staphylococcus* spp. were performed on Baird-Parker medium (Acumedia, USA) enriched with an egg yolk and potassium tellurite emulsion. *Staphylococcus* spp. were subjected to Gram staining and to assays for the presence of catalase, coagulase and thermonuclease and for furazolidone sensitivity. Colonies of *Salmonella* spp. and *Escherichia coli* were identified with a modified version of Rugai medium [12]. The pH measurements were determined using a T-1000 pH-meter (Teknal, Brazil). Analyses were performed on triplicate samples.

3. Results and Discussion

Indicator microorganisms of hygienic-sanitary quality. Fifty-one samples of tropical fruit ice cream were analyzed, of which 36 samples (70.6%) had

total coliforms, and 22 samples (43.1%) were also positive for coliforms at 45 °C (thermotolerant) (Table 1). Five samples had fecal coliforms counts above that permitted by RDC Nº 12 of ANVISA (8), which establishes a limit 5x10 MPN/ml of coliforms at 45 °C in ice cream. The presence of *E. coli* was confirmed by biochemical tests on a sample of guava ice cream (sample 37) and was most likely due to fecal contamination.

The results of fecal coliforms suggesting deficiencies in the sanitary conditions of the ice cream establishment. According to Schreiner [15], the Brazilian food legislation is considered to be more permissive compared to the legislation of other countries. Kanbakan et al. [16] reported several factors that can contribute to high coliform count in ice creams, including a lack of hand cleaning, the same person handling the ice cream and collecting the money, open cones and unclean cloths for cleaning the scoops. Diogo et al. [17] found enterobacteria in 100% of the water samples collected from the scoops. High counts of total coliforms suggest failures either in food handling and hygienic practices or in the equipment used during the preparation of the ice cream. Kanbakan et al. [16] identified the presence of total and fecal coliforms in ice cream in Denizli, Turkey. These authors reported that this contamination resulted from the poor quality procedures of the handlers and an inefficient cooling process, emphasizing the importance of food handlers as contaminants. All these factors could contribute to the contamination found in the ice cream collected in the present work.

Table 1.Enumeration of total and thermotolerant coliforms, yeasts, psychrotrophic bacteria and staphylococci in tropical fruit ice cream sold in Belo Horizonte, state of Minas Gerais, Brazil.

N°	Sample	Total Coliforms (MPN/mL)	Thermotolerant Coliforms (MPN/mL)	*Staphylococcus* spp. (CFU/mL)	*Salmonella* spp. (Presence/Absent)	Psychrotrophics bacteria (CFU/mL)	Yeasts and molds (CFU/mL)	pH
1	S.3 [(i)]	150	43	3.3 x 10	Absent	4.60 x 10^5	3.3 x 10	6.03
2	S.5 [(a)]	3	<3	1 x 10^2	Absent	4.83 x 10^5	4.7 x 10^3	4.25
3	S.10 [(a)]	93	<3	1.66 x 10^4	Absent	> 3 x 10^4	1.14 x 10^4	5.69
4	S.14 [(i)]	240	3	< 100	Absent	1.12 x 10^5	2.43 x 10^3	5.41
5	S.15 [(i)]	21	<3	6.7 x 10	Absent	5.00 x 10^5	6.66 x 10^2	4.98
6	S.19 [(a)]	240	<3	1.16 x 10^3	Absent	1.38 x 10^5	2.11 x 10^4	6.03
7	S.22 [(i)]	≥2400	<3	< 100	Absent	7.58 x 10^6	6.75 x 10^3	4.89
8	S.26 [(i)]	150	43	2.67 x 10^2	Absent	1.43 x 10^5	5.99 x 10^2	5.86
9	S.33 [(i)]	≥2400	39	3.4 x 10^3	Absent	> 3 x 10^4	4.33 x 10^3	4.95
10	S.37 [(i)]	240	93 [(c)]	7.2 x 10^2	Absent	7.23 x 10^5	5.53 x 10^4	5.13
11	S.42 [(a)]	150	15	6.33 x 10^2	Absent	1.46 x 10^5	4.33 x 10^3	5.57
12	S.46 [(i)]	≥2400	1100 [(c)]	6.7 x 10^2	Absent	> 3 x 10^4	1.26 x 10^4	4.78
13	S.4 [(i)]	<3	<3	< 100	Absent	<100	2.31 x 10^2	4.04
14	S.12 [(a)]	23	<3	3.33 x 10^2	Absent	3.93 x 10^5	1.86 x 10^3	5.40
15	S.17 [(i)]	23	<3	1 x 10^2	Absent	3.3 x 10	1.06 x 10^3	4.65
16	S.24 [(i)]	240	<3	< 100	Absent	3.66 x 10^2	1.09 x 10^3	4.13
17	S.28 [(i)]	<3	<3	<100	Absent	> 3 x 10^4	2.66 x 10^2	3.45
18	S.31 [(i)]	1100	15	2.06 x 10^3	Absent	8.6 x 10^4	5.86 x 10^3	4.68
19	S.35 [(i)]	240	21	< 100	Absent	3.17 x 10^5	4.09 x 10^3	4.87
20	S.40 [(a)]	150	7	3.3 x 10	Absent	8.26 x 10^5	3.36 x 10^3	6.03
21	S.44 [(i)]	150	21	6.7 x 10	Absent	2.3 x 10^5	1.23 x 10^3	4.67

Table 1 – Continuation...

N°	Sample	Total Coliforms (MPN/mL)	Thermotolerant Coliforms (MPN/mL)	*Staphylococcus* spp. (CFU/mL)	*Salmonella* spp. (Presence/Absent)	Psychrotrophics bacteria (CFU/mL)	Yeasts and molds (CFU/mL)	pH
22	S.48 (a)	240	<3	5.53×10^4	Absent	8.86×10^4	5.03×10^4	5.56
23	S.51 (a)	≥2400	<3	3.9×10^4	Absent	6.84×10^5	4.9×10^4	6.29
24	S.8 (a)	9	<3	3.3×10	Absent	1.16×10^5	1.99×10^4	4.21
25	S.32 (i)	460	460 (c)	5.3×10^3	Absent	8.16×10^4	2.29×10^3	4.99
26	S.36 (i)	15	4	< 100	Absent	$> 3 \times 10^4$	2.19×10^3	4.48
27	S.41 (a)	460	4	7.67×10^2	Absent	2.63×10^5	4.99×10^3	5.49
28	S.45 (i)	460	15	6.7×10	Absent	7.7×10^4	1.29×10^3	4.45
29	S.2 (i)	210	75 (c)	1×10^2	Absent	2.66×10^2	2.31×10^2	5.71
30	S.7 (a)	<3	<3	6.7×10	Absent	5.03×10^5	4.26×10^3	4.34
31	S.13 (a)	<3	<3	< 100	Absent	<100	1.13×10^3	4.60
32	S.16 (i)	<3	<3	4.33×10^2	Absent	<100	1.69×10^3	4.88
33	S.20 (a)	<3	<3	< 100	Absent	6.96×10^2	1.28×10^4	4.50
34	S.23 (i)	1100	4	< 100	Absent	5.73×10	1.36×10^3	4.30
35	S.27 (i)	240	<3	2.67×10^2	Absent	1.37×10^4	3.66×10^2	5.29
36	S.38 (i)	23	4	1.5×10^3	Absent	2.33×10^5	1.19×10^3	4.08
37	S.47 (a)	≥2400	9	9.06×10^4	Absent	2.54×105	4.69×10^4	4.43
38	S.1 (i)	<3	<3	6.7×10	Absent	1.66×10^2	1.33×10^3	4.43
39	S.6 (a)	<3	<3	6.7×10	Absent	<100	7.3×10^2	3.56
40	S.9 (a)	<3	<3	< 100	Absent	2.33×10^2	1.26×10^3	3.86
41	S.11 (a)	<3	<3	2.76×10^4	Absent	<100	1.66×10^2	4.35
42	S.18 (i)	<3	<3	1×102	Absent	<100	1.66×10^2	3.98
43	S.21 (a)	<3	<3	3.3×10	Absent	6.00×10^2	1.04×10^4	3.32
44	S.25 (i)	93	15	< 100	Absent	2.70×10^3	5.39×10^3	3.76
45	S.29 (i)	460	4	< 100	Absent	<100	<100	4.09
46	S.30 (i)	<3	<3	6×10^2	Absent	$>3 \times 10^4$	4.33×10^3	4.22
47	S.34 (i)	<3	<3	4.33×10^2	Absent	$> 3 \times 10^4$	8.76×10^3	4.27
48	S.39 (a)	43	<3	2×10^2	Absent	4×10^2	1.53×10^3	4.12
49	S.43 (i)	≥2400	1100 (c)	< 100	Absent	5.43×10^3	3.33×10^3	4.26
50	S.49 (a)	<3	<3	< 100	Absent	2.47×10^5	3.86×10^3	3.11
51	S.50 (a)	240	<3	<100	Absent	3.63×10^3	2.32×10^4	4.28

(a) artisanal ice cream; (i) industrial ice cream; (c) counts above those permitted by RDC n°12/2001.
*** Note: Sample number 1 to 12: pineapple ice cream. 13-23: açai ice cream. 24-28: guava sorbet. 29-37: mango ice cream and 38-51: passion fruit ice cream.**

The presence of *E. coli*, (found in one sample), may indicate contamination during post-processing and deficiencies in sanitation during preparation and/or the storage of the product, which are often caused by poor hygiene of the handlers [2]. El-Sharef et al. [18] found *E. coli* O157: H7, which causes hemorrhagic colitis and hemolytic uremic syndrome, in two of 160 analyzed samples of ice cream in Tripoli, Libya. Vigil et al. [19] studied desserts in Guadalajara, Mexico, and Texas, USA, and their results showed that four of the six samples that contained ice cream were positive for *E. coli*.

The *Staphylococcus* spp. counts ranged from 3.3 x 10^1 to 9.06 x 10^4 CFU/mL among the 35 samples

(68.6%) with positive results. Ten samples of ice cream had counts of *Staphylococcus* spp. over 10^3 CFU/ml (Table 1). Only one sample of açaí ice cream showed the presence of coagulase-positive staphylococci, which are potential enterotoxigenic microorganisms.

Despite the presence of a potentially enterotoxigenic isolate of *Staphylococcus* spp. in a sample of ice cream, the assessment for the presence of coagulase-positive staphylococci is only required by legislation in milk-based ice creams (5 x 10^2 CFU/mL). Importantly, although most isolates of *Staphylococcus* spp. found in this study were coagulase-negative strains, several studies suggest that enterotoxin production by these bacteria is possible in favorable situations [20-23]. Because *Staphylococcus* species are frequently present on the hands, the skin and in the upper respiratory tract of humans, these contaminants most likely originate with the food handlers.

Farias et al. [5] reported a high coagulase-positive *Staphylococcus* count in 50% of the 60 analyzed samples of ice cream in Rio de Janeiro, Brazil. In addition, a high coagulase-positive *S. aureus* count (10^5-10^6 CFU/mL) was found in 50% of the samples, indicating precarious hygienic-sanitary practices by the handlers, deficient sanitization of the utensils, inadequate control of the pasteurization process and the use of substandard raw materials. Ice cream is an ideal medium for the production of enterotoxins, due to its high nutritional value and the presence of starch and protein that promotes microbial growth that can lead to outbreaks of food poisoning [4]. Wilson et al. [24] did not find *S. aureus* in any of the 91 ice cream samples analyzed in England.

Psychrotrophic microorganisms were found in 44 samples of ice cream, and though their growth is slow at 7 °C, the presence of such microorganisms can point to inadequate manipulation during the processing and storage of the ice cream. None of the ice cream samples analyzed in this study had *Salmonella* spp., a result also found in other studies [5, 7, 13].

The total counts of psychrotrophic microorganisms, yeasts and molds are not required by Brazilian legislation. However, high counts of these micro-organisms were found in most samples (Table 1). Presence of psychrotrophic microorganisms in the high counts can serve as indicators of possible failure in the thermal processing of food [7]. The presence of yeasts may be attributed to several factors, including their ability to grow at lower temperatures and assimilate organic acids, their lipolytic and proteolytic activity, and their resistance to low-water-activity environments and to cleaning and sanitizing compounds [25]. These types of food contamination from surviving yeasts and fungi often have their origin in the fruit, which may have been poorly cleaned prior to addition to the food product [25]. The high counts of microorganisms observed in the samples, mainly yeasts, may contribute to the deterioration of ice cream, causing a decrease in pH and hence an acidification of the product.

Yeast and molds in tropical ice cream had counts ranging from 3.3 x 10^1 to 5.53 x 10^4 CFU/mL in 50 samples in the present study. The pH of most of the ice cream samples in this study was in the acidic range (Table 1) from pH 3.11 to 6.29. This finding may explain the presence of molds and yeasts because these microorganisms can be acidophilic or acid-tolerant [14].

Questionnaire for good manufacturing practices. A questionnaire to observe good manufacturing practices was completed at the time of sample collection and allowed the characterization of aspects related to the physical area outside of the ice cream parlor, the exposure area involved in the sale of the product and the relationship of the ice cream vendor and handler (Figure 1).

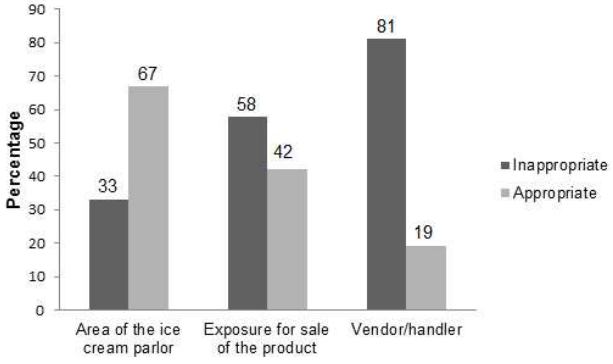

Figure 1. Evaluation of Good Manufacturing Practices by questionnaire from the commercial establishments that were studied.

Sixty-seven percent of the areas where the ice creams were sold had floors, ceilings and walls and were in a good, clean condition. In relation to the exposure area involved in sale of the product, inadequacies were observed in 57.7% of the ice cream parlors with respect to the conservation of ice cream equipment (including refrigerators and freezers) and the lack of thermometers in visible places. In addition, the water container where the ice cream serving spoons were stored were dirty and had a low frequency of exchange. Most ice cream parlors did meet the criteria for the presence of sinks and trash cans near the area of ice cream manipulation. Of the three parameters examined, the vendor/handler parameter had the highest rate of inadequacy, with 81% of the evaluated establishments not meeting the minimum criteria.

The results of the questionnaire demonstrating whether these assessed parameters were adequate based on RDC No. 267 of ANVISA [6]. Although most of the analyzed ice cream samples were considered adequate for areas where the ice creams were sold, 33% had irregularities in this parameter. Milikita [13] conducted a survey of sanitary conditions of ice cream processing factories in the metropolitan region of Curitiba, Brazil, through visual inspection and evaluation of the physical space, equipment, machines, tools and manipulators. The author found inadequacies in 67.5% of the companies analyzed. In the present study, only the outside of the ice cream parlors was examined, although the external conditions of the establishment may reflect the mode of preparation and storage of foods that are sold.

The conservation of ice cream equipment showed irregularities, including the lack of thermometers in visible places (Figure 1). Milikita [13] analyzed the internal areas of an ice cream factory and recorded that 82.5% of them were inadequate for the presence of conservation equipment with thermometers.

The professionals were not wearing protective caps or appropriate uniforms. Many had poor hygiene (dirty nails, wearing of earrings, rings and bracelets) and manipulated money after serving ice cream to the customer without first correctly sanitizing their hands.

The use of self-service practices may also facilitate the microbiological contamination of the final product. The vendor/handler parameter showed the largest discrepancy among the parameters analyzed in the ice cream parlors. The main non-conformities observed may result in the loss of microbiological quality of the ice cream sold under these conditions and cause outbreaks of foodborne diseases. Mitchell et al. [26] suggested that staff training and supervision are the primary interventions that should be used to promote food safety in food services.

4. Conclusion

The results of this study suggest that the ice cream produced by the ice cream parlors studied, although free of *Salmonella* spp., may represent a potential risk to the consumers of these products because the products show the presence of microorganism indicators of poor hygienic and sanitary quality. In addition, inadequate sanitation can encourage the growth of pathogenic microorganisms when in a favorable situation, such as the lack of an ideal storage temperature, which can be verified by a questionnaire of Good Manufacturing Practices. The contamination that can affect the ice cream may have originated in poorly sanitized equipment and utensils exposed to contaminated water or through the inadequate or insufficient hygiene of the handlers because most of these ice cream parlors practice a self-service system. An analysis of these results suggests that the use of Good manufacturing practices, through the promotion of proper training and qualification of staff, may be strategies that such establishments should adopt. A reformulation of space both inside and outside ice cream parlors to try to minimize the source of microbiological contamination is also suggested.

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Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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