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Microbiological analysis of self-service chocolate ice cream in Sinop-MT, Brazil

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Abstract

Ice cream is a frozen dessert that was created in China by mixing snow, fruit and fruit juice. This product is consumed in warm climate regions, being refreshing, pleasant to taste and with variable nutritional composition. In direct sales to consumers, or in the self-service model, the ice cream is exposed to inadequate sanitary conditions derived from contamination by the consumers' saliva and other secretions. This study aimed to perform microbiological analysis of chocolate ice cream sold in self-service ice cream shops in Sinop-MT, Brazil. Samples from five shops were collected at random and soon after analysis was performed in duplicate. The microbiological testing resulted positive for nine samples of *Salmonella* sp. The presence of total coliform and thermotolerant bacteria and *E. coli* was confirmed in all samples. However, no sample tested positive for mold or yeast. In general, the samples showed unsatisfactory conditions, offering health risk for consumers. Therefore, actions should be taken to control and prevent contamination.

Key words: Ice Cream. Total Coliforms. Fecal Coliforms. Psychrotrophic Fungi. Sanitary Profiles.

Introduction

The creation of frozen desserts began by mixing snow, fruit and fruit juice. The origin of this culinary practice probably dates back to ancient China. Chilled desserts were introduced in Europe at the late thirteenth century, but only the aristocrats consumed this delicacy, which was spread over the world during the major colonization periods.[1]

Ice cream stands out among frozen desserts and is a food product primarily made of a mixture of fats and proteins, with or without addition of other ingredients, most of them substances that are frozen and need to remain frozen to be preserved. Therefore, especial care is necessary in order to keep the product frozen or partially frozen during storage, transportation and delivery to final consumers.[2]

Ice creams are relatively safe products due to the pasteurizing process, but are not free from contamination afterwards. This food is largely consumed in hot climate regions, because it is refreshing, pleasant tasting and with variable nutritional composition. When added with milk, this product becomes more susceptible to contamination by diverse microorganisms due to the fermentation of lactose, improper handling and storing operations and direct contact with the customer in self-service shops.[3]

In the direct selling model, or self-service, ice cream is exposed to unsafe sanitary conditions, such as the contact with air and the water used to keep scoops, and to contamination by saliva and other secretions from consumers. Thus, ice cream manufacturers should not assume that only the pasteurizing and freezing processes are sufficient to prevent contamination and proliferation of pathogenic microorganisms.[4]

Most consumers do not associate ice cream with the risks of microbial contamination, assuming that the low temperature makes it safe for consumption. However, freezing does not inhibit the presence of pathogenic microorganisms resistant to low temperatures, called psychrotrophic bacteria, which can be ingested by consumers. [5]

Several groups of microorganisms are capable of surviving in ice cream, where they found optimal conditions for growth. As a consequence of the demand of this business, which serves all and any public, it is necessary to warn people of the risks that are related to food consumption. Therefore, other microbial groups involved in food contamination, such as total and fecal coliforms, may also be present in the ice cream.[6]

Among the flavors available, chocolate ice cream is very popular and largely consumed, at all ages, mainly by children. Therefore, the quality of this food is of key importance to ensure that it is safe for consumption, as well as to protect the health of population in general. [7]

Thus, this work had the aim of conducting microbiological testing of cholocate-based ice cream sold in self-service shops in Sinop-MT, for the presence of total coliforms, thermotolerant bacteria, *Salmonella* sp., *Escherichia coli*, yeasts and fungi.

Methodology

Ten samples of chocolate ice cream were collected at random from five self-service ice cream shops located in the city of Sinop-MT in May 2014. The samples were collected into sterile flasks and stored in thermal boxes to be transported to the UNIC microbiology laboratory, where testing was carried out soon after. The analyses were made in duplicate.

The Most Probable Number (MPN) method was used to estimate counts of total coliforms and thermolerant microorganisms. The MPN method is a way to estimate the number of microorganisms present in the sample. It is based on the statistical probability of the presence of a certain number of microorganisms in the sample when the quantity or sum of positive results occur by inoculating aliquots of this sample into a series of tubes containing a liquid culture medium suitable for their growth.[8]

Thus, 25g of each sample were weighed aseptically and then transferred to an Erlenmeyer flask containing 225 ml of saline (10^{-1}), and subsequently homogenized. For the presumptive test of total coliforms, decimal dilutions from the 10^{-1} dilution were prepared by pipetting 0.1, 1 and 10ml of the sample into a series of three tubes containing 9.0 ml of lactose broth double concentration and inverted Durham tubes, incubated at 35ºC for 24 to 48 hours. After this period, the tubes showing turbidity and gas production in their interior were considered positive. For the confirmatory testing of total coliforms, a loop portion from each positive tube containing lactose broth was inoculated into other tubes containing brilliant green bile broth (BGBB) and incubated under a temperature of 35ºC for 48 hours. The results of positive tubes were expressed in the MPN table of total coliforms per gram of the ice cream sample.[9]

To confirm the presence of thermotolerant bacteria, the solutions were transferred from the BGBB tubes that indicated the presence of total coliforms into tubes containing EC broth, and incubated at 45ºC for 48 hours in water bath. For *E.coli* count, the tubes with EC broth showing gas production were transferred and seeded onto agar plates containing eosin methylene blue (EMB), incubated at 35ºC for 24 horas, for observation of growth, or not, of *E.coli* colonies.

In the testing for *Salmonella* sp., each sample containing BGBB and showing gas production was inoculated into *Salmonella Shigella* (SS) agar and incubated for 24 hours at 35ºC.

For yeasts and molds, 25g of the ice cream were diluted in a 225-ml physiological solution, resulting in the first 10^{-1} dilution. Then, 1ml was transferred to a tube containing 9ml of physiological solution, resulting in the 10^{-2} solution, and then 1 ml was removed to the second tube 10^{-3}. Aliquots of 0.1 ml of the 10^{-2} and 10^{-3} ice cream dilutions were seeded and spread on the surface with the aid of a Drigalsky strap in Petri dishes containing *sabouraud* agar added with choraphenicol. The solution was incubated under a temperature of 10±3ºC during 14 and 21 days, for determination of psychrotrophic fungi.[5]

Results and discussion

Counts of *Salmonella* sp., total and thermotolerant coliforms, *E.coli*, molds and yeasts present in chocolate ice cream samples are described in Table 1.

Table 1. Results of microbiological testing of chocolate ice cream samples collected in self-service shops in Sinop-MT, May 2014.

Sample	*Salmonella* sp.	Total coliforms (NMP/g)	Thermotolerant coliforms (NMP/g)	Escherichia coli *	Yeasts and molds
1A	+	1100	460	+	-
1B	+	460	240	+	-
2A	+	150	43	+	-
2B	+	28	3.6	+	-
3A	+	64	43	+	-
3B	+	75	38	+	-
4A	+	43	15	+	-
4B	-	15	9.2	+	-
5A	+	240	120	+	-
5B	+	210	160	+	-

* Confirmatory testing; (+) Positive; (-) Negative.

In Table 1, the results obtained for *Salmonella* sp. showed that colorless colonies, which are non-lactose fermenters, grew in nine of ten samples in SS medium, i.e., there was *Salmonella* sp. present in 25g of ice cream. According to the resolution RDC no. 12/01, which sets out the sanitary microbiological standards for foods and that the bacterium *Salmonella* sp. must be absent, the samples did not meet the standards of the National Health Surveillance Agency (ANVISA).[2]

In the testing for *Salmonella*, the present work had a result similar to the research conducted by Oliveira et al.,[10] who assessed the microbiological quality of ice creams sold in major supermarkets in Maceió-AL and found the presence of *Salmonella* sp. in all samples tested. This was also observed by Silveira et al.,[11] in their physicochemical and microbiological testing of *tapioca* ice creams, which resulted positive for *Salmonella* sp. in 75% of the samples. According to Hoffmann et al.,[12] after investigating the hygienic-sanitary quality of ice cream in the city of São José do Rio Preto-SP, 75% of the samples tested positive for *Salmonella* sp.

Salmonella sp. are gram-negative, nonsporulating, pathogenic and lactose- non-fermenting bacteria. Its natural reservoir is the intestine of man and other animals, mainly birds. In general, flies and cockroaches are animals that carry and spread this bacteria.[13]

Food diseases, among them salmonellosis, result from the ingestion of food contaminated by feces. Another disease, among the most serious ones caused by *Salmonella*, is typhoid fever, caused by *S. Typhimurium*, and humans are the only host animal. Usually, dissemination of the infection is interpersonal and through water and foods contaminated with human feces. This disease has the longest period of incubation, produces fever, vomiting, diarrhea and septicemia. The paratyphoid or enteric fever, which is milder than the typhoid one, may develop into septicemia and often to gastroenteritis, fever and vomiting. *Salmonella* sp. can contaminate a wide variety of foods that have high moisture content, carbohydrates, beef, pork and chicken meat and eggs, and especially proteins, such as milk and other dairy products used in ice cream preparation.[14] Thus, the conditions of the ice creams analyzed indicate that the product is potentially capable of causing food poisoning and, therefore, is improper for consumption.

Regarding total coliforms, all samples tested positive. The RDC no. 12/2001[15] does not set standard values for counting of total coliforms, but high counts may indicate poor personal hygiene, from handling to final consumer.[16] With respect to the results for thermotolerant coliforms, samples 2A, 2B, 3A, 3B, 4A, and 4B were within the limit established by legislation. However, samples 1A, 1B, 5A and 5B obtained values above the microbiological limit of tolerance of 50 MPN per gram for indicative sample. All samples tested positive for *E. coli* when seeded onto EMB agar, showing bright-green colonies, typical for *E. coli*.

E. coli, member of the family *Enterobacteriaceae,* are enterobacteria that belong to the thermotolerant coliform group. They usually live in human intestine and in other animals that have hot blood, and some strains are pathogenic in children and adults when present in other parts of the human body, such as the urinary tract or meninges, and may cause diseases, besides poisoning. *E. coli* counting is used as the key indicator of fecal contamination, determining the hygienic-sanitary conditions of foods. Foods contamination occurs especially by contact of feces with food products, so it can occur when foods from animal source are processed, because of poor hygiene of food handlers, and, especially, poor hygiene of consumers.[3,17]

When examining the counts for total coliforms, nine of ten samples are in unsatisfactory conditions for direct consumption, among them sample 1A (1100 MPN/g). Thus, it is safe to state that in the samples with high counts of total coliforms, raw material was contaminated; cleaning and sanitation of the place where foods are prepared were improper; there was contamination after processing.[18]

The data of this study were compared with those reported in a study by Rizzo-Benato,[19] who tested for fecal and thermotolerant coliforms in 12 samples of ice cream and the milk used in the production of the product. In said study, the presence of fecal coliform in the vanilla ice cream was within the standards required by law, except for the chocolate ice cream, showing that such results were expected due to the high contamination of the main raw material, i.e., the milk used in the preparation of the vanilla ice cream. Thus, one can infer that, in the samples examined in the present work, contamination was caused by the raw material used.

Numerous studies examined the hygienic-sanitary quality of ice creams in the recent decades and encountered fecal coliforms counts exceeding the upper limit established by law in the majority of the samples tested. Thus, recurrent contamination in this kind of food has been demonstrated, which is an alert to the relevant institutions to regulate and inspect the production process in order to prevent risks to human health.[20-22]

There was no growth of yeasts and molds in all ice creams samples tested, but it should be noted that the growth of molds and yeasts in this study occurred at low temperature. So, we can state that there was no growth of psychrotrophic fungi. The legislation does not sets out limits for fungi and yeast counts, even though they constitute a large group of microorganisms, mostly soilborne or airborne, which present risks to human health.

The importance of determining the presence of molds and yeasts in the samples is due to the fact that fungal mycotoxins can be major liver cancer agents. However, the ice cream storing temperature is not conducive to fungi growing, and usually these mycotoxins do not appear directly in the ice cream, which may be carried to the ice cream by elements contained in the garnish or other products available for self-serve consumption of ice creams.[3]

Psychrotrophic bacteria are microorganisms that grow well in foods under refrigeration at 0 to 7ºC in seven to ten days, and some groups of fungi are capable of proliferating in frozen foods, with low water activity, high acidity or packaging conditions that inhibit microbial growth.[9] Diogo et al.[13] and Silveira et al.[11] found in most of the ice cream tested the presence of mesophilic fungi. So, there were no reports of ice cream studies that aimed to psychrotrophic testing. Thus, a comparison with other studies was not possible, because the present study investigated for fungi that grow in low temperatures.

Foods contamination can occur by numerous factors, primarily during the food preparation, due to poor hygiene of handlers, equipment, utensils, environment, transport and inadequate storage conditions. In addition, after the stage of acquisition, preparation and processing, the foods continue to be exposed to contamination in the places where they are consumed, whether in supermarkets, restaurants or homes.[18]

Consumers of self-serve systems have contributed to increase contamination of the foods because they are in direct contact with the foods exposed on the serving counter.[3,23]

Research carried out by Medeiros et al.[24] investigated the most common risk behaviors of consumers in self-service restaurants and found poor consumers' habits, such as not washing the hands before serving, which indicates lack of habit or awareness of the great importance of this practice. Handwashing removes dirty and reduces the amount of bacteria present in the skin. By running the hands through the hair, the hair may carry a large amount of bacteria, fungi and parasites. Contamination may also occur when other parts of the body, accessories and clothing contaminated with microorganisms present in the air and dust are in contact with the foods; when talking, coughing or sneezing over uncovered foods on the serving counters; when leaving serving spoons fallen inside the foods – all these are factors that contribute to ice cream contamination, which may occur from raw material to the product acquisition.

Final considerations

All samples tested positive for total coliforms and thermotolerant coliforms and confirmed by the presence of *E. coli*. Nine samples showed colonies of *Salmonella* sp. and none had the presence of psychrotrophic fungi. Five of the samples tested for thermotolerant coliforms exceeded the limit prescribed by the RDC no. 12/01[15], which deals with frozen foodstuffs.

In the general context of this research, the samples did not present satisfactory microbiological quality and might pose risks to the consumers' health. So, it has been demonstrated the need to avoid the direct exposure of ice creams in the self-service model, as well as the adoption of best production practices, training of handlers and owners, controlling hygiene and the quality of raw material in ice cream production.

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