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Microbiological and physicochemical quality of refreshments marketed in the municipalities of Barra Mansa and Volta Redonda, state of Rio de Janeiro

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Abstract

Commercially sold refreshments are stored in juice dispensers that, if improperly cleaned, will be a means of contamination, therefore making the consumer vulnerable to foodborne diseases. Assessment of the microbiological quality of food provides information that enable to evaluate, in terms of processing, storage and distribution, the useful life of foodsand risks to public health. Given the current consumption of fruit refreshments and the assumed severity of contaminated food ingestion, this study aimed to evaluate the microbiological quality of juices stored in dispensers and marketed in the cities of Barra Mansa and Volta Redonda-RJ. We collected seven samples of refreshments, five of cashew juice and two of orange juice, in 250 ml sterile packages, from commercial establishments such as coffee shops and bakeries. For microbiological analyzes, we investigated for the presence of *Salmonella* sp. and fecal coliforms; and for the physicochemical analyzes, determinations of titratable acidity and pH were performed. Two of the seven samples were not in conformity with the norms established by the current legislation regarding the presence of coliforms at 45º C or thermotolerant. The results of *Salmonella* sp. Analyses indicated that all samples were in accordance with the applicable legal standards, all samples (100%) of cashew juice analyzed for titratable acidity were in accordance with the law, but, this was not observed for the orange juice sample (50%). It was concluded that 29% (2) of the analyzed samples were unsuitable for consumption and may pose a health risk to consumers.

Key words: Food Contamination. Coliforms. Beverages. Food Handling.

Introduction

According to Moretto,[1] juices are beverages produced by appropriate technological processes by means of the squeezing or extraction of ripe fruits, and are composed of sugars, acids, mineral salts, vitamins and pigments. Cavalcanti et al.[2] add that juice drinks consist of an aqueous "mixture" of various volatile and unstable organic components, responsible for the product's flavor and aroma.

The composition of fruit juices varies according to the fruit species, maturation stage, climate, and crop conditions.[3] Despite their varied composition, the main components found are water, carbohydrates, proteins, lipids, organic acids, vitamins and minerals. More than 80% of the fruits are composed of water, and the moisture content varies during the day as a function of temperature. Following water, carbohydrates are the most abundant components and may range from 2% to 4% in the fruits tissue.[4] Other key aspect is timely harvesting, i.e., at the proper stage of maturity, once it has a direct impact on the product's life and final quality. Thus, fruits harvested when overripe may present a soft texture and bland taste, and fruits harvested early or late are more susceptible to physiological disorders.

The percentage of fruit juice in the beverage is what differentiates juice, nectar, and refreshment. Juices are 100% made up of *in natura* fruit and do not contain preservatives, food dyes and artificial sweeteners. Nectar is composed of 99% to 25% of *in natura* fruit and may contain sweeteners, dyes and preservatives. Refreshments have 24% to 3% of *in natura* fruit, and it is obtained by the dilution of fruit juice with drinking water, with or without sugars addition.[5,6]

Retail sales volume of liquid refreshments has increased, in large part due to the low cost of these drinks, the variety of flavors, and good acceptability. This leads us to a great concern with the consumers' food safety, once refreshments are stored in juice dispensers. If improperly cleaned and sanitized, dispensers may become a means of contamination, making the consumer vulnerable to foodborne diseases (FBD).

FBDs are one of the most common health problems and are classified as "infections" and "intoxication" (poisoning). Infections result from the consumption of foods containing live microorganisms, which grow inside the body. Intoxications are caused by the intake of food containing toxins, even if the microorganism had been eliminated. The consumption of a food containing toxin causes the illness, the most common symptoms being vomits and diarrhea and, depending on the individual and health, may cause death.[7]

Aspects such as hygiene at the points of sale, the water used in the preparation of the foods and for cleaning the utensils, the form of preservation and protection against vectors, are key requirements and must be considered to prevent the proliferation of microorganisms.[8]

The concept of quality foods, from the consumers' point of view, corresponds to the satisfaction of characteristics such as taste, aroma, appearance, packaging, price, and availability. The outbreak of food poisoning occurs because consumers rarely realizes the presence of hazardous contaminants in the food.[9] Today, establishments involved in the marketing of foods must comply with the rules contained in Resolutions no. 216/2004, which sets forth hygienic-sanitary criteria for foods production, and no. 12/2001, which sets forth Microbiological Sanitary Standards for Foods and the criteria for conclusions and interpretation of the results of microbiological analysis of foods for human consumption. [10,11]

The sanitary inspections can check for risk factors to the occurrence of FBDs, and among them we can cite: failure in foods refrigeration; improper conservation of raw foods and finished preparations; inadequate handlers' practices, such as poor personal hygiene; contaminated raw material; improper cleaning of equipment and utensils; inadequate storage; use of unreliable drinking water – in effect, anything that can lead to improper foods handling, making them susceptible to contamination.[12]

Given the above, the present work aimed to assess the microbiological and physicochemical quality of samples of refreshments kept in dispensers and marketed in Barra Mansa and Volta Redonda, state of Rio de Janeiro.

Methodology

Samples collection

Seven samples of refreshments sold in seven establishments located in the municipalities of Barra Mansa and Volta Redonda, state of Rio de Janeiro, were collected. The samples consisted of five samples of cashew refreshment and two of orange, collected in 250-ml sterile bottles. Later, they were stored in isothermal boxes containing ice and sent to the Laboratory of Microbiology and Biochemistry of the University Center of Volta Redonda (UniFOA), for microbiological and physicochemical analyses.

Microbiological analyses

For count of coliforms at 35ºC and thermotolerant, the most probable number (MPN) method was used, employing three series of three tubes. Presumptive test consisted of homogenization of 1ml of each sample in 9ml of peptone water (0.1%). Then, serial dilutions were made in 1%

peptone water. Aliquots of 1ml of the proper dilutions were plated in lauryl tryptose (sulfate) broth and incubated at 35ºC for 24-48 hours. After this period, the positive tubes, which presented gas and turbidity, were transferred to tubes containing brilliant green broth (BG) and incubated at 37ºC for 24-48 hours, and this step consisted of the confirmative test for positive tubes. For the presumptive test, aliquots of culture of positive tubes in BG were transferred to tubes containing a broth of *Escherichia coli* (EC), using a nickel-chrome wire loop and incubated at 45ºC for 24-48 hours. This consisted of the test for determination of MPN of thermotolerant coliforms present in the sample. The results were analyzed according to the dilutions and amount of positive samples of the confirmative test, using the MPN table described by *Bacteriological*.[13]

For testing for *Salmonella* sp, 25 ml of refreshment were taken aseptically from the sample and added to 225 ml of buffer peptone water. The sample was then incubated at 37ºC for 24 hours (pre-enrichment stage). Subsequently, the selective enrichment stage was accomplished, in which 0.1ml of this dilution was transferred to a test tube containing 10 ml of tetrathionate broth, incubated at 37ºC for 24 hours, and 1 ml was transferred to a tube containing Rappaport broth and incubated at 42ºC for 24 hours. From the tubes of previous broths, a portion of each of the pre-enrichment broth was pulled out and inoculated into two Petri plates containing a medium of *Salmonella-Shigella* (SS) and Hecktoen enteric (He) agar. These were incubated at 35ºC for 24 hours. After this period, if typical colonies appeared, the samples would then be sent for confirmatory biochemical testing.[13]

Analyses of pH and acidity were determined twice, following the guidelines described by the Adolfo Lutz Institute.[14] To determine pH, a digital Spencer pH meter, scientific SP3611 model, was used. To determine acidity, the filtrate was titrated with a solution of 0.1 N sodium hydroxide in presence of phenolphthalein, and the result was expressed in grams of citric acid per 100 g of the sample.

Results and Discussion

The results of microbiological analyses of the refreshment samples are described on Table 1.

Table 1. Results of microbiological analyses of refreshment samples. Volta Redonda-RJ, 2014.

Samples	Coliforms at 35ºC and Thermotolerant (45ºC) (MPN/mL)*			Salmonella
	Lauryl	BG**	EC***	
A	2.4 x 10^2	2.4 x 10^2	9.3 x 10	Absence
B	1.5 x 10^2	1.5 x 10^2	9.3 x 10	Absence
C	> 1.1 x 10^3	> 1.1 x 10^3	> 1.1 x 10^3	Absence
D	2.4x 10^2	2.4x 10^2	2.4x 10^2	Absence
E	1.5 x 10^2	1.5 x 10^2	2.3x10	Absence
F	4.3 x 10	4.3 x 10	4.3x10	Absence
G	<3.0	<3.0	<3.0	Absence

* most probable number ** brilliant green broth *** *Escherichia coli* broth

Of seven analyzed samples, two (29% - C; D) were in disagreement with the standards established by current legislation regarding the presence of coliforms at 45ºC or thermotolerant, which establishes as upper limit 10^2 UFC/ml in refreshment samples,[11] thus indicating that the same were improper for consumption (Figure 1).

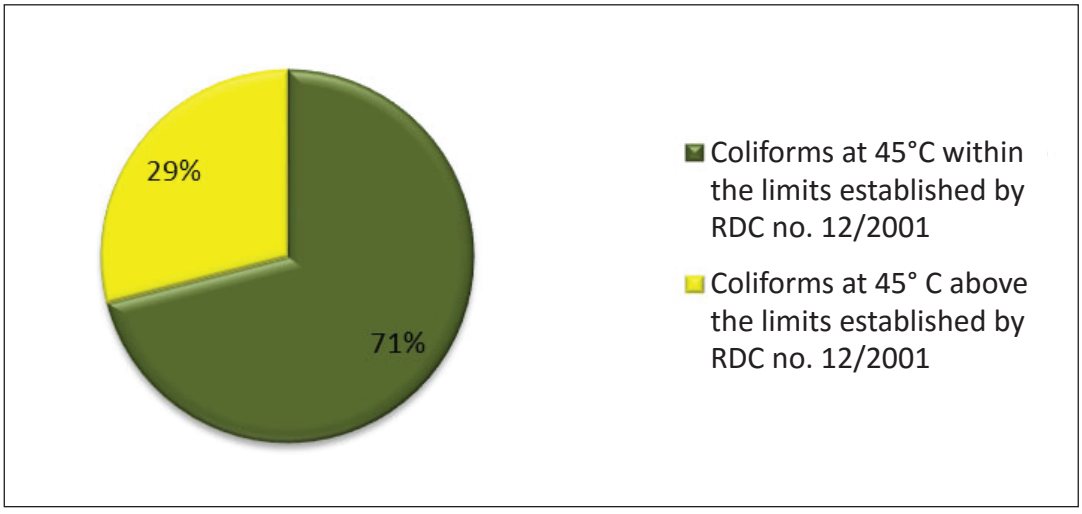

Figure 1. Results of samples with Coliforms at 45ºC from the refreshments collected. Volta Redonda-RJ, 2014

The results corroborate some findings in the literature. Garcia et al.[15] found that of 12 juice samples analyzed, two had coliforms at 45ºC, which exceeds the limit permitted by legislation. Likewise, Silveira et al.[16], in their research for thermotolerant coliforms in orange juice, observed that of five samples analyzed two were improper for consumption. Hoffmann et al.[17] observed that of 19 samples of fresh orange juices analyzed three had thermotolerant coliforms. Oliveira et al.[18] concluded that of 50 samples of *in natura* orange juice, eight were in disagreement with current legislation.

Regarding the analyses for *Salmonella* sp determination, all samples showed negative results. The results presented in this study are in line with those found by Silveira et al.[16] and Pinheiro et al.[19], who observed absence of *Salmonella* sp in their samples. However, Garcia et al.[15] reported that all juice samples analyzed in their research were contaminated by *Salmonella* sp, being, therefore, improper for consumption.

Shinohara et al.[20] reported that *Salmonella* is a bacterium that causes illnesses in humans and animals by the consumption and intake of contaminated foods. The foods most commonly poisoned by *Salmonella* sp are meat, egg, chicken, homemade mayonnaise and vegetables. The incubation period is 12 to 72 hours, and the predominant symptoms are diarrhea, abdominal cramps, fever and vomit.[15] Individuals who are ill or infected with this bacterium contaminate environments, drinking water and foods by their feces. [5] The best way to prevent food contamination is the adoption of good handling practices.[7,21]

According to Kraemer,[22] to ensure safe foods, sanitary and health inspection agents must require the adoption of best practices, such as of fabrication (FBP), agricultural (ABP), and hygiene (HBP).

Standard Operating Procedures (SOPs), as required by Resolution RDC nº 275 of October 21, 2002,[23] are a tool designed to minimize risks and avoid possible causes of Foodborne Diseases (FBD). SOP is a document that describes the work plan, and must be made available to all employees. Food services have four SOPs, directly focused on cleaning and sanitation of water reservoirs, areas, equipment, furniture and utensils, pest control in the establishments, and handlers' hygiene and health.[24,25]

Santos Junior,[26] in researches conducted in the food industry, reports that 50% of the establishments did not have manuals, and among those which had them, most of them were in the hands of the owner or manager only, making it difficult to access these procedures by other employees.

Table 2 presents the results of analyses of pH and acidity of the refreshment samples.

Table 2. Average results from pH and acidity analyses of refreshment samples. Volta Redonda-RJ, 2014.

Samples	pH	Acidity (%)
A	3.70	0.11
B	4.7	0.13
C	4.0	0.15
D	3.49	0.11
E	3.49	0.07
F	3.45	0.29
G	3.47	0.11

The majority (86%) of the samples analyzed for determination of titratable acidity was in conformity with legislation,[27] which prescribes acidity of at least 0.07 g in citric acid/100ml for cashew refreshment. However, the same was not found for sample C, orange refreshment, which did not comply with legislation, which stipulates acidity of at least 0.25 g citric acid/100ml for said refreshment (Figure 2).

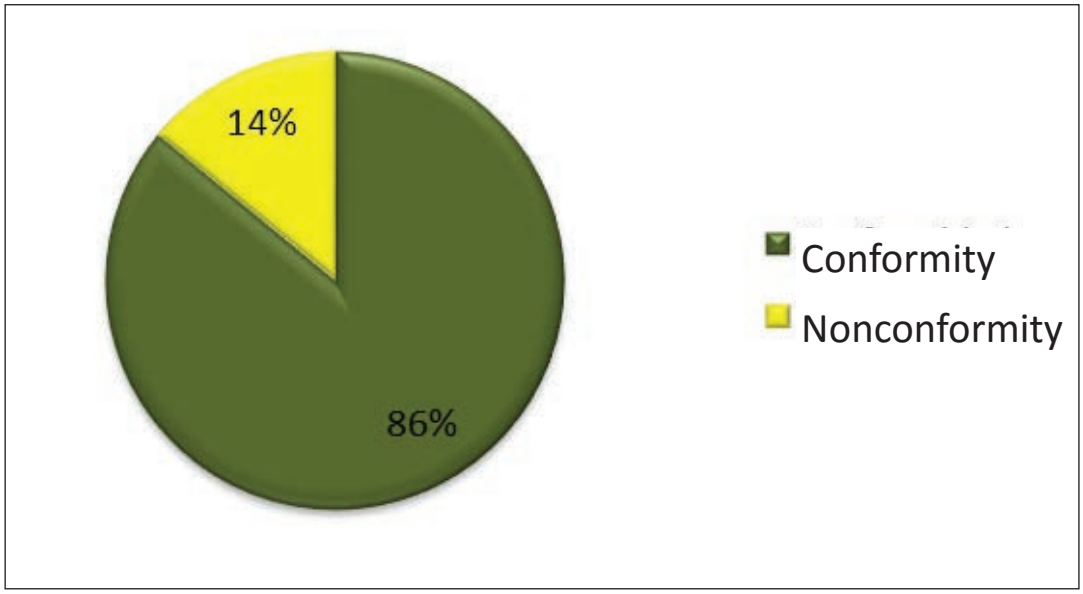

Figure 2. Results for determination of titratable acidity of refreshment samples collected according to Ordinance no. 544/98. Volta Redonda-RJ, 2014.

PH values of the sampled cashew refreshments ranged from 3.47 to 4.70; and of orange refreshment, from 3.45 to 4.00. Similarly to the present study, Pinheiro et al.[19] examined pH and acidity of whole cashew juices, finding values between 3.17 and 4.06 for pH, and 0.45 to 1.26g/100g for acidity.

Although pH is not a parameter required by legislation, it is important to assess it because it is directly related to the quality of the product. According to Forsythe,[28] neutral pH inhibits the proliferation of major foodborne pathogens.

Conclusions

Conclusion is that, despite the inconsistencies observed, such as the presence of coliforms in some samples and inadequate acidity in a sample of orange refreshment, the majority of the samples examined were found appropriate for human consumption regarding the physicochemical and microbiological analyses.

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