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Presence of *Candida tropicalis* in commercial 'açaí' (*Euterpe oleracea M*art.) pulps from Rio de Janeiro

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PRESENCE OF *CANDIDA TROPICALIS* IN COMMERCIAL 'AÇAÍ' (*Euterpe oleracea,* Mart) PULPS FROM RIO DE JANEIRO

ABSTRACT

Microbial analysis of fruit pulps is important to guarantee their sanitary quality. Increasing 'açai' pulp consumption has led to its industrialization and its quality is monitored by Brazilian legislation with the objective of reducing the contamination risk in population. This study evaluated the microbiological quality of commercial 'açaí' pulps consumed in Rio de Janeiro city to verify if their sanitary conditions were in compliance with Brazilian food legislation. In this work the analysis of moisture and pH were carried out and for the 30 samples of frozen and pasteurized 'açaí' pulps the following microbiological analysis were performed: determination of the most probable number of total and fecal coliforms, *Salmonella* spp. yeasts and molds count. Most of the samples (70%) presented high moisture and therefore, low total solids content which represents a potential risk of contamination by microorganisms. The microbiological results revealed that the heat treatment eliminated the bacterial growth in pulps. *Candida tropicalis* (46.5%) was found in some samples although below the limit established by legislation. However, it may represent a risk if these 'açai' pulps are offered as part of the diet to hospitalized patients or immunocompromised, suggesting greater rigor in quality control during manufacturing, storage and transport of frozen 'açaí' pulps to ensure food safety.

Keywords: açaí, food safety, molds, yeast, health

PRESENÇA DE *CANDIDA TROPICALIS* EM POLPAS COMERCIAIS DE AÇAÍ (*Euterpe oleracea,* Mart) DO RIO DE JANEIRO

Resumo: A análise microbiológica de polpas de frutas é fundamental para garantir sua qualidade sanitária. O aumento do consumo de açaí estimulou sua industrialização e a qualidade do produto deve ser assegurada e monitorada através de normatização nacional com o objetivo de se reduzir o risco de contaminação do produto entre a população consumidora. Este estudo avaliou a qualidade microbiológica de polpas comerciais de açaí consumidas no município do Rio de Janeiro, RJ para verificar se a qualidade sanitária das polpas estava dentro dos padrões exigidos pela legislação nacional. Foi realizada umidade e análise do pH das marcas selecionadas. Para as 30 amostras de polpas comerciais de açaí pasteurizadas e congeladas as seguintes análises microbiológicas foram realizadas: determinação do número mais provável de coliformes totais e termotolerantes, pesquisa de *Salmonella* spp. e contagem de bolores e leveduras. A maioria das amostras (70%) apresentou umidade elevada e, portanto, baixo conteúdo de sólidos totais, o que pode aumentar o risco de contaminação por microrganismos indesejáveis. Os resultados para a análise microbiológica demonstraram que a pasteurização empregada pelos fabricantes inativou o crescimento bacteriano nas amostras selecionadas. Por outro lado foi observada a presença de *Candida tropicalis* em algumas amostras (46,5%), apesar de estarem abaixo do limite estabelecido por legislação. Contudo,

presença do microrganismo pode representar risco se estas polpas forem consumidas por pacientes hospitalizados ou imunocomprometidos, o que sugere maior rigor no controle de qualidade durante a fabricação, armazenamento e transporte de polpas de açaí congeladas para garantir a segurança alimentar.

Palavras chave: Açaí, segurança alimentar, bolores, leveduras, saúde

Introduction

Microbial analysis of food is considered an important tool to ensure the sanitary quality required by food legislation and is a part of food safety (JASSON et al., 2010). The microbial analysis of fruit juice and pulp is monitored by sanitary authorities and the microbiological criterium for the assessment of these foods is based on standard methods in compliance with food legislation. Standardized methods in Brazil are acknowledged as reference analytical methods for Brazilian official control (BRAZIL, 2001).

These standardized microbiological methods mainly consist on culture methods, which use selective nutritious broth or agar media to grow, isolate or enumerate the microorganisms while suppressing the indigenous background flora of the food under assessment (JASSON et al., 2010).

The Center for Disease Control and Prevention (CDC-USA) estimates that there are 76 million cases of gastrointestinal illnesses resulting in 325.000 hospitalizations and 5000 deaths every year in the United States from foodborne diseases (BISHWA, ANGULO; MELTZER, 2004).

Although the prevalence of food contaminants such as *E. coli*, *Salmonella*, *Listeria*, and *Campylobacter* has declined in the previous decade, the incidence of illnesses has stayed fairly constant in recent years, and 2007 saw a sharp peak of recalls (SCOTT, 2008). Quality losses in fresh-cut fruits and unpasteurized juices may occur as a consequence of microbiological, enzymatic, chemical, or physical changes (ZAND, 2011).

Safety and quality losses in fruit and juice products by microbiological causes should be avoided since they may be hazardous for consumers due to the possible presence of microbial toxins or pathogenic microorganisms, and also because of the economic losses as a result of microbial spoilage (RAYBAUDI-MASSILIA, et al., 2009).

Fruits are used as raw material for manufacturing different food products, among which, fruit pulp, defined by the Ministry of Agriculture, Livestock and Supply (BRAZIL, 2000), as "non-fermented, non-concentrated, undiluted, obtained by crushing the pulp fruit through suitable technological process, with a minimum total solids content from the edible portion of the fruit (BRAZIL, 2000).

Pulps have great acceptance among consumers and are basically used in juices and soft drink preparations (RAYBAUDI-MASSILIA et al., 2009). Thus, a great number of industries produce fruit pulps mainly because these products have characteristics of practicality, not only being purchased for home use, but also for hotels, restaurants, cafes and even hospitals (BUENO et al., 2002). On the other hand fruits are important microhabitats for a large number of fungal organisms, and in particular for the group of yeasts, in view of their high concentration of simple sugars and low pH (LACHANCE; STARMER, 1998).

Because of their chemical and physical properties, fruit juices and its products, generally constitute a rather unique ecosystem due to their composition rich in organic acids, with pH usually between 2.0 and 4.5, with high carbohydrate content and water activity (ZAND, 2011). Thus, microbiologic spoilage of these products can occur due to growth of molds, yeasts or acid-tolerant bacteria. Yeasts are unicellular fungi that form colonies, have chitin cell wall, the cytoplasm membrane with ergosterol and reserve carbohydrates composed by glycogen, and reproduce by budding, which allows them to grow on surfaces, and especially give them greater ability to reproduce and disperse in liquid media (DEAK, 2007).

Occasionally, pathogenic bacteria also survive in the juices for a certain period of time and species of the *Enterobacteriaceae* family, as

E. coli, *E. freudii, Citrobacter* sp. and even other species like *Salmonella* sp. (VANDERZANT; SPLITTSTOESSER, 1992) can be found transiently. Thus, it becomes important to focus on the microbiological risk associated with consumption of this kind of food, since plants have a resident microbiota which guarantees an average count of aerobic bacteria between 10^4 and 10^6. However, the presence of pathogens is considered a potential health risk to consumers (MADDEN, 1992).

Thus, this work aimed to evaluate the microbial quality of commercial açaí pulps consumed in Rio de Janeiro to verify their safety for human consumption and check if the sanitary conditions are in accordance with the microbiological standards established by the Brazilian legislation.

Material and Methods

Samples

Thirty samples of pasteurized and frozen commercial açaí pulps of ten different brands were acquired on different markets in Rio de Janeiro and codified with the letters A to J. The pulp samples were kept frozen until analysis. The microbiological and pH analyses were performed after the samples were defrosted at room temperature (25 ºC) and homogenized.

Total solids, moisture, carbohydrates analyzes

Determination of moisture, pH, carbohydrate and kilocalories was carried out in triplicate, according to Adolfo Lutz Institute (IAL, 2005). The results of the chemical analyses were expressed by dry matter and the energy content in kilocalories. The total solids (TS) were calculated by subtracting the grams of moisture from 100 g of sample according to the method described by the IAL (2005).

pH analysis

The pH was determined by the electrometric method using a DIGIMED® potentiometer, model DMPH-2, calibrated with buffer solutions with pH 4.0 and 7.0 (Merck®).

Colimetry

Coliforms were assayed by the multiple-tube method in lauryl-sulfate tryptose (LST) liquid as medium for the presumptive test, and incubated at 36 ºC for 24 h. An aliquot was taken from the positive tubes and transfered to tubes with EC liquid medium. The EC (*Escherichia coli*) liquid with the microorganisms was incubated at 44.5ºC for 48 h (faecal coliforms). Coliforms were enumerated using the most probable number (MPN) method described in the Normative n.12 defined by the Brazilian food legislation (Brazil, 2000; APHA, 2000; ICMSF, 1980; Hoskins, 1933) and the results expressed as colony-forming units per gram (CFU/g).

Total coliforms

The samples were inoculated in three series of three tubes containing 9 mL of lauryl sulfate tryptose liquid with 1 mL of the dilutions at 10^{-1}, 10^{-2}, 10^{-3}, homogenized and incubated at 35°C for 48 h. The *Hoskins* table was used to calculate the most probable number (MPN) of total coliforms (HOSKINS, 1933).

Fecal coliforms

The multiple-tube technique was applied using EC liquid followed by bacteria inoculation and the tubes were incubated at 45°C in water bath over 48 h. The *Hoskins* table (HOSKINS, 1933) was used to calculate the most probable number (MPN) of faecal coliforms.

Salmonella sp.

To determine the presence of *Salmonella* sp. non selective enrichment liquid (water peptoned solution) was used at 36ºC for 24 h. After this period, 1 mL of the liquid was fractioned in tubes containing selective enrichment liquid with tetrathionate and selenite-cystine and incubated for 24 h at 36ºC. After incubation, this liquid was fractioned in plates containing Agar BG by the *Streak-plate* method at 41ºC for 24h, followed by plate reading as recommended by Normative n.12 (BRAZIL, 2000; ICMSF, 1980; APHA, 2000).

Molds and yeasts

Mold and yeast count was performed by the plating method directly on the surface's dilution 10^{-1} and 10^{-2} in potato dextrose (PD) agar medium added of 0.2 mg/mL of chloramphenicol. Aliquots of 100 µL were seeded on the surface of PD agar and the plates were incubated at 22-25ºC for three to five days. The results were expressed as number of colony-forming units (CFU) per gram of material (CFU/g), according to ICMSF (1980) recommendation. Identification of potentially pathogenic *Candida* species (*C. albicans, C. tropicalis and C. krusei*) was also carried out, using the germinative tube test (TASCHDJIAN, BURCHALL; KOZINN, 1960) and chlamidospore (DALMAU,1929) production for the morphological identification of *C. albicans* and

tests in medium CHROMagar-Candida (Probac, Brazil) for the chromogenic identification of *C. albicans* (green), *C. tropicalis* (cobalt-blue) and *C. krusei* (pink).

Statistical analysis

Data were treated descriptively using mean, standard deviation and percentage mean

(*SigmaStat® 3.5*) to compare the results with standardized microbiological methods for the açaí pulps (BRAZIL, 2000; ICMSF, 1980).The Anova test (Friedman test) was used to evaluate the differences between the nutritional parameters of the samples and to compare the results.

Results and Discussion

The pH values of açaí pulp samples varied from 4.12 to 5.36 in compliance with Brazilian legal requirements that establish pH between 4.0 and 6.2 for fruit pulps (Table 1). The Table 1 also shows the limit values for moisture in 100 g of the commercial açaí pulps samples based in the food legislation (BRAZIL, 2000; IAL, 2005).

In this study all the pulps analyzed not presented significant difference in the centesimal composition (data not shown), but was observed that the most of the samples (70%) presented high moisture content (93.6±0.56%) when compared with C (88.2±0.61%), D (91.5±0.82%) and E (85.9 ±0.36%) açaí pulps (Table 1). Thus the most of the commercial açaí pulps were not in compliance with the Brazilian food legislation, that classifies the açaí pulp according to the addition or not of water and its amount, as special açaí pulp with moisture content below 86% (14% of total solids); regular açaí pulp with 89-86% (11 to 14% of total solids) and popular açaí pulp with 92-89% (8 to 11% of total solids) (BRAZIL, 2000). Fruits can be important microhabitats for a large number of fungal organisms and their nutritional content associated with high moisture, low pH and inadequate transport and storage conditions can promote and increase the risk of proliferation of pathogenic microorganisms.

Table 1 - Moisture ($g.100g^{-1}$ ±SD) and pH of commercial açaí pulps

	Commercial açaí pulps									
Analysis	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Moisture ($g\ 100g^{-1}$)	93.5 (±0.78)	93.0 (±2.91)	88.2 (±0.61)	91.5 (±0.82)	85.9 (±0.36)	93.2 (±0.08)	93.3 (±0.12)	94.6 (±0.17)	93.7 (±1.29)	94.1 (±1.44)
pH	5.36	4.17	4.77	4.51	4.12	4.62	4.89	4.77	4.99	4.68

Commercial açaí pulps (Rio de Janeiro-Brazil) expressed by letters (A-J). Values expressed as mean± SD.

The results of the bacteriological analysis (Table 2) showed absence of *Salmonella* sp. in 25 grams in 100% of the samples analyzed and the results of faecal and total coliform counting in the samples were also below the limit ($<10^2$ MPN/g) required by national food law (APHA, 2000; HOSKINS, 1933). Thus, the açaí pulp samples analyzed were found to be adequate for human consumption, suggesting their innocuity and the efficiency of the thermal treatment for pulp conservation as presented in Table 2. The mycological results for mold and yeast count were also below the limit ($<10^3$ CFU/g) established by the food legislation (BRAZIL, 2000), although 46.5 % of the pulps studied presented *Candida tropicalis* yeast.

The causal agents of microbiological spoilage in fruits and derivatives can be bacteria, although yeasts and molds are the main spoilage agents due to the low pH of most fruits (RAYBAUDI-MASSILIA et al., 2009). It is noteworthy that there are three types of fruit processing: freezing (without heat treatment); pasteurization (non-aseptic filling) and storing under refrigeration and sterilization (aseptic), which allows fruits to be stored at room

temperature (SCOTT, 2008; FARAONI et al., 2008). These pulps are mainly used for juice and nectar production consumed without any heat treatment or preservative addition that could reduce the number of microbial contaminants.

According to the available literature, the methods for food preservation applying high temperatures, such as thermal pasteurization, ensure efficacy to eliminate and inactivate the pathogenic microorganism growth although they may damage the sensorial properties of food (MOSQUEDA-MELGAR, RAYBAUDI-MASSILIA, MARTÍN BELLOSO, 2008; HEPING et al., 2008).

Previous studies also showed that heat treatment of fruit pulp is efficient in controlling microorganism growth since fecal coliforms were not detected. Some Brazilian authors (BUENO et al., 2002; FARAONI et al., 2008; SANTOS, COELHO; CARREIRO, 2008) obtained similar results when detected coliforms in less than 5.0 % of their analyzed samples. In this work the microbial analyses indicated that, from a microbiological point of view, (*Salmonella* sp. and counting of total coliforms and fecal coliforms) the samples were in compliance with food legislation.

The isolation of fungi in açaí has been reported by Brazilian authors (PEREIRA et al., 2006; NYANGA et al., 2007; ROGEZ et al., 1996), including some species with pathogenic potential. Indeed, some yeast are actually part of the microbiota of fruits and vegetables, although the number and population of these organisms vary from one habitat to another depending on environmental and harvest and storage conditions (DEAK, 2007, SANTOS, COELHO; CARREIRO, 2008; PEREIRA et al., 2006).

Yeasts such as *Aurobasidium pullullans, Candida glabrata, Candida parapsilosis, Debaryomyces hansenii, Rhodotorula glutinis, Rhodotorula mucilaginosa and Saccharomyces cerevisie* have already been isolated from fruits and fruit pulp in mycological quality assessments (NYANGA et al., 2007). Some genera of filamentous fungi have been isolated from fruit in natura, including orange, among which *Aspergillus* spp., *Fusarium* spp., *Geotrichum* spp., *Penicillium* spp. and *Rhizopus sp.* (SANTOS, COELHO; CARREIRO, 2008) can be mentioned.

Several factors seem to contribute with fungal contamination of açaí pulp including the açaí fruit itself, since it is a suitable substrate for microorganism growth. Other factors are the surface of the pulp in contact with the air, the fruit small thickness (1 mm), the growth of the açaí palm in tropical areas with very high humidity and temperature, wrong handling and use of poor techniques in some regions, use of plastic containers for fruit collection and transport (ROGEZ, et al., 1996).

Other reasons that favor fungal growth are the carbohydrates content, moisture and the small acidity of the açaí pulp. In this study however, most samples (54.5%) did not present growth of yeasts in PD agar. In the samples where fungical growth was observed (46,5%), the only yeast of medical interest identified was *C. tropicalis,* which exhibited in CHROMagar-*Candida* its characteristic cobalt blue coloration. Fungi may represent a serious danger and risk to human health since they can cause allergic reactions to sensitive persons, and also produce mycotoxins, or toxic metabolites that may in some cases, have carcinogenic properties. Whereas health and nutrition are closely related, this study was performed to evaluate the sanitary-hygienic conditions, of the açaí pulp largely consumed in Rio de Janeiro-Brazil. Spoilage yeasts are classically associated primarily with acid foods, liquid or semi solid, inside which the dispersion of the cells is Spoilage yeasts are classically associated primarily with acid foods, liquid or semi solid, inside which the dispersion of the cells is facilitated, and oxygen availability is reduced. In addition the growth of yeasts in aqueous substrate is typically followed by ethanol and CO_2 production, film formation and flocculation (DEAK, 2007). They can also use organic acids, raising the pH, and produce acetaldehyde, causing characteristic smell of fermentation (ICMSF, 1980). Among the main yeasts isolated from fruits and derivatives, *Candida sp* has been frequently reported (MELO et al., 2007; MOSQUEDA-MELGAR, RAYBAUDI-MASSILIA; MARTÍN-BELLOSO, 2008).

Their clinical importance is due to the fact that some of them are opportunistic pathogens in immune-compromised individuals, presenting mortality rates of 40% (BOFF et al., 2008), and can be transmitted in the hospital diet. *Candida* species of medical interest, *Candida tropicalis* is one of the most commonly found in plant foods (BOFF et al., 2008). This yeast has been considered the second or third most frequent

Table 2 - Microbiological analysis of açaí commercial pulps

Microbial Analyses	Results	Food law
Total coliforms	< 10^2 MPN.g^{-1}	< 10^2 MPN.g^{-1}
Faecal coliforms	< 10^2 MPN.g^{-1}	< 10^2 MPN.g^{-1}
Salmonella sp (25.g^{-1})	absence	absence
Molds and yeasts	< 10^3 CFU.g^{-1}	< 10^3 CFU.g^{-1}

Food law (RDC n.12. 2001). MPN: most probable number; CFU/g 45°C- coliforms count.

etiologic agent in bloodstream infections in immune-compromised patients, notably those with malignant hematological pathologies (COLOMBO; GUIMARÃES, 2003).

The risk of invasive infection by *C. tropicalis* is considerably higher in patients with neutropenia, mucositis and under prolonged antibiotic therapy (KONTOYIANNIS et al., 2001), since such patients are susceptible to an array of opportunistic organisms. The presence of this yeast could represent a risk, if these açaí pulps were included in the diet. In fact, this study corroborates and reinforces the importance of this evaluation, since there are few reports on pathogenic yeast contamination in fruit pulps. In this work we showed that açaí pulp samples were in accordance with pH requirements (from 4.12 to 5.36).

The bacteriological analysis indicated that all the açaí pulp samples analyzed were free of contamination, while the mycological analysis revealed that the mold and yeast populations found were within the limits established by food legislation (BRAZIL, 2000; MELO et al., 2007).The microbiological quality assessment suggested that pulps submitted to heat treatment inactivated bacterial growth. However, further attention must be given to açaí pulps by manufacturers, mainly in order to better control raw materials regarding the selection step and further elimination of deteriorated açaí fruits to prevent fungal growth.

Conclusion

This study showed that the most of commercial açaí pulps samples (70%) were not in compliance with the Brazilian food legislation based on the moisture content. However the presence of *Candida tropicalis* in 46.5% of commercial açaí pulps was observed and although the amount was below the limits established, it should be given more attention to ensure the quality of those products widely consumed in Rio de Janeiro. *C. tropicalis* yeast is an opportunistic pathogen that can represent a risk to hospitalized patients, if these açaí pulps were offered as part of the diet, but the commercial açaí pulps were found to be adequate for consumption by healthy individuals since the analyses showed that were in according with the microbiological standards required by Brazilian food legislation.

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