Journal of Advances in Biology & Biotechnology

23(11): 1-8, 2020; Article no.JABB.63905
ISSN: 2394-1081

Escherichia coli in Fruit Juice Dispensers

Ana Claudia Chesca[1*], Ariana da Silva Costa[1] and Carolina Bragine Mangucci[1]

[1]Uberaba University, 'Av. Nenê Sabino, 1801.CEP 38.055-500, Uberaba, Minas Gerais, Brazil.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2020/v23i1130185
Editor(s):
(1) Dr. Anil Kumar, Devi Ahilya University, India.
Reviewers:
(1) Zainab Adil Ghani Chabuck, University of Babylon, Iraq.
(2) Suresh K. Ghotekar, Savitribai Phule Pune University, India.
(3) Tutut Pujianto, Sekolah Tinggi Ilmu Kesehatan Karya Husada Kediri, Indonesia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63905

Original Research Article

Received 10 October 2020
Accepted 17 December 2020
Published 31 December 2020

ABSTRACT

Aims: The aim of this study was to evaluate the microbiological quality of juices kept in juice dispensers and sold in retail in canteens and snack bars.
Study Design: The study was done with thirty samples of juice, collected in the morning and in the afternoon, obtained at commercial establishments near Uberaba University, whereas two samples were of cashew juice, two samples of tamarind juice, two samples of passion fruit with mango juice, four samples of guava with acerola juice, eight samples of grape juice and twelve samples of passion fruit juice.
Place and Duration of Study: Food Microbiology Laboratory - University of Uberaba.
Methodology: The temperature at the time of collection was measured. A microbiological analysis was performed. The samples that were positive for thermotolerant coliforms, the analysis was continued, reaching the isolation of *Escherichia coli* and its subsequent serological identification.
Results: The results point to a contamination, over current legal standards, of 66.7% for the samples collected in the morning and of 73.34% for the samples collected in the afternoon.
Conclusion: Greater care in good practices is suggested, especially regarding the personal hygiene of handlers, water quality, sanitation and correct temperature control of fruit juice dispensers.

Keywords: Classical enteropathogenic E. coli (EPEC); enteroinvader E. coli (EIEC); juice.

**Corresponding author: E-mail: ana.chesca@uniube.br;*

1. INTRODUCTION

Fruit juices are consumed and appreciated all over the world, not only for their flavor, but also because they are natural sources of carbohydrates, carotenoids, vitamins, minerals and other important components [1].

The growth in consumption of ready-to-drink juices follows the worldwide trend of consuming healthy foods that offer health, practicality, innovation, flavor and pleasure [2].

Ready-to-drink fruit juices must meet the requirements of the Ministry of Agriculture, Livestock and Supply (MAPA) regarding the definition, classification, registration, standardization, labeling and quality requirements and requirements of ANVISA regarding the labeling of packaged foods, the labeling nutrition, portions, complementary nutrition labeling and declaration on the presence of gluten [3-10]. According to current legislation, refreshment is defined as the fruit or vegetable drink, not carbonated, unfermented, obtained by diluting the fruit juice, pulp or plant extract of its origin, giving birth to sugars, in drinking water. The legislation also states that the soft drink should not have its organoleptic characteristics and composition altered by the materials of the containers, the equipment used during the processing and commercialization [4].

According to Lee [11] the manual elaboration of fruit juices has become an inconvenience to the fast pace of life in society. This fast pace of life leads consumers to purchase more "ready-to-eat" products, eat their meals outside the home, or replace their meals with snacks accompanied by refreshments.

The utensils and equipment used for processing can represent potential sources of contamination, as they usually have parts that are difficult to clean where microorganisms will be housed. Uneven surfaces on the equipment also allow microbial infiltration, providing conditions for the development of microorganisms [12].

According to Franco and Landgraf [13], among the most important parameters that determine the quality of a food, undoubtedly are those that define its microbiological characteristics, which allows to evaluate it regarding the conditions of processing, storage, distribution for consumption, useful life and health risks to the population.

This work had as specific objectives to verify the presence of thermotolerant coliforms and their serology, in samples of juices stored in fruit juice dispensers and sold in retail in snack bars.

2. MATERIALS AND METHODS

The work was developed with 30 samples of juice from a commercial establishment installed near the University of Uberaba, 02 samples of cashew juice, 02 of tamarind juice, 02 of passion fruit juice with mango, 04 of guava juice with acerola fruit, 08 of grape juice and 12 of passion fruit juice. The samples were collected, during a period of fifteen days, twice a day, with one collection carried out at around 09:30 am and the other at around 04:30 pm. The temperature at the time of collection was measured. The samples were collected in disposable cups and with lids so that was no contamination during the journey until reaching the analysis site. These samples were sent to the Food Microbiology Laboratory at the University of Uberaba-MG. The samples that were positive for thermotolerant coliforms, the analysis was continued, reaching the isolation of *Escherichia coli* and its subsequent serological identification. In serotyping, polyvalent antisera and their respective monovalent, from Probac® in Brasil, were used for anti-*E. coli* pathogenic to humans (EPEC Polyvalent A: Anti O26, O55, O111, O119; Polyvalent B: Anti O114, O125, O142 e O158; Polyvalent C: Anti O86, O126, O127, O128. EIEC Polyvalent A: Anti O28ac, O29, O136, O144 e O152; Polyvalent B: Anti O112ac, O124, O143, O164, O167). Microbiological analyzes were performed according to methodologies proposed by Vanderzant and Splittstoesser [14] and Silva et al. [15].

3. RESULTS AND DISCUSSION

The inadequate handling is one of the factors responsible for most cases of diseases of microbial origin, along with irregular temperature during preparation and conservation, deficiency in personal hygiene and equipment. The control of these factors is very important, especially in places like restaurants and snack bars. From the results presented in Table 1, there is an oscillation in the temperature control of the fruit juice dispenser and only two samples of juices, collected in the morning, are within the legal recommendation and it is still noticed that the temperatures measured in the afternoon, are higher than in the morning. Brazilian law states

that the temperature of exposure to the sale of chilled foods should be 4.0°C [16].

The resolution RDC nº 12, of January 2, 2001, which approves the technical regulation on microbiological standards for fresh foods, juices and soft drinks, including coconut water, sugarcane juice, berry juice and similar, alone or in mixtures, establishes a maximum coliform limit at 45ºC of 102NMP / mL [17]. Table 2 presents the results of the investigation of thermotolerant coliforms in the samples of juice from fruit juice dispenser and 66.67% of the samples collected in the morning are contaminated and 73.34% of the samples collected in the afternoon are above current legal standards.

These results are similar to those found by Nascimento and Furlanetto [18] who collected 100 samples of orange juice sold in coffee shops and restaurants, in the university city of the University of São Paulo and observed that for total coliforms, 48 samples were revealed with NMP of zero to 3 / 100 mL, in 52 samples this value was 10 or more / 100 mL, and the most frequent result being between 10 and 103 / 100 mL. For fecal coliforms, 92 of the 100 samples examined were developed with NMP from zero to 3 / 100 mL.

Oliveira et al. [19] found results like the findings in the present study, since the values of total and fecal coliforms were above the established by the current legislation in 74% of the 50 samples of fresh orange juice. Santos and Ribeiro [20] found coliforms values above the standards allowed in fresh orange juices marketed in the city of Pelotas-RS in 3% of the 30 samples evaluated.

Anders et al. [21] evaluated the hygienic-sanitary conditions of the salty and juices produced and / or commercialized by the canteens of the Federal University of Goiás and the results, for a total of 39 samples in triplicate, only two samples, one of orange and another of pulp juice had a total coliform count above that established by law.

In order to assess the quality of orange juices sold on public roads in Porto Alegre-RS, Ruschel et al. [22], collected 52 samples and microbiological analyzes showed that 44.23% (n = 23) of the samples were at odds with current standards, with molds and yeasts found at inappropriate levels in all failed samples. Fecal coliforms were found above the permitted amounts in 5.7% (n = 3) of the samples and *Salmonella cholerasuis* was identified in one of the analyzed samples. The physical-chemical analyzes showed 89.47% of the samples were in disagreement with at least one of the analyzed parameters.

Sousa et al. [23] observed the presence of high contamination by fecal and total coliforms in all 12 analyzed samples of berry juice from the Coroado fair, Manaus, AM. Silva et al. [24] found that all of the four samples analyzed after microfiltration were also within the standards required by legislation in organic passion fruit juices processed by microfiltration.

Table 1. Measurement of the temperatures of the juice samples

Collect	Juice samples			
	Collection time (09:30 am)		Collection time (04:30 pm)	
	Sample	Temperature (^{0}C)	Sample	Temperature (^{0}C)
01	Passion fruit	6.0ºC	Passion fruit	6.8ºC
02	Grape	4.8ºC	Grape	4.4ºC
03	Passion fruit	5.4ºC	Passion fruit	6.4ºC
04	Cashew	5.8ºC	Cashew	8.9ºC
05	Passion fruit	6.0ºC	Passion fruit	5.3ºC
06	Passion fruit	5.2ºC	Passion fruit	10.4ºC
07	Guava with acerola fruit	5.2ºC	Guava with acerola fruit	5.4ºC
08	Passion fruit	8.4ºC	Passion fruit	12.4ºC
09	Grape	4.2ºC	Grape	9.4ºC
10	Guava with acerola fruit	5.0ºC	Guava with acerola fruit	6.8ºC
11	Passion fruit with mango	2.6ºC	Passion fruit with mango	5.6ºC
12	Grape	2.6ºC	Grape	10.4ºC
13	Tamarind	4.4ºC	Tamarind	10.2ºC
14	Passion fruit	6.8ºC	Passion fruit	10.6ºC
15	Grape	4.4ºC	Grape	6.8ºC

Source: *Food Microbiology Laboratory-UNIUBE.*

Table 2. Thermotolerant coliforms in juice samples

Collect	Samples Juice			
	Collection time (09:30 am)		Collection time (04:30 pm)	
	Sample	Coliforms at 45^0C (NMP/mL)	Sample	Coliforms at 45^0C (NMP/mL)
01	Passion fruit	>1.1x10^3	Passion fruit	4.6x10^2
02	Grape	2.1x10	Grape	2.8x10
03	Passion fruit	3.6	Passion fruit	<3.0
04	Cashew	4.6x10^2	Cashew	>1.1x10^3
05	Passion fruit	1.5x10	Passion fruit	<3.0
06	Passion fruit	>1.1x10^3	Passion fruit	>1.1x10^3
07	Guava with acerola fruit	>1.1x10^3	Guava with acerola fruit	4.6x10^2
08	Passion fruit	2.3x10	Passion fruit	>1.1x10^3
09	Grape	1.1x10^3	Grape	2.4x10^2
10	Guava with acerola fruit	>1.1x10^3	Guava with acerola fruit	>1.1x10^3
11	Passion fruit with mango	1.1x10^3	Passion fruit with mango	1.1x10^3
12	Grape	4.6x10^2	Grape	>1.1x10^3
13	Tamarind	4.3x10	Tamarind	2.9x10^2
14	Passion fruit	>1.1x10^3	Passion fruit	3.6
15	Grape	4.6x10^2	Grape	>1.1x10^3

Source: *Food Microbiology Laboratory-UNIUBE.*

Brazilian legislation is broad and clear about the definition, classification, registration, standardization, labeling and quality requirements for juices, but it is clear that the soft drinks stored in juice dispensers and intended for sale in retail in coffee shops and canteens, offer risks to the consumer. These soft drinks are prepared at the point of sale, using quality raw materials (frozen fruit pulp, concentrated juices, fresh fruit, solid drinks for refreshments, etc.) and it is at that moment of preparation that the unwanted contamination suffers. These contaminations can be related to the low quality of the water used to dilute the juice, inadequate hygiene of the juice dispensers, lack of personal hygiene of the manipulator, inadequate maintenance of the dispensers' temperature, among others.

Andrade et al. [25], analyzed the sanitary and hygienic quality of the berry juice sold in the city of São Luís, 13 juice samples were collected in 4 neighborhoods of São Luís and fecal coliforms occurred in almost 70% of the samples. The *Enterobacter* genus was found in more than 45% of the samples. The following bacterial species were also detected: *Citrobacter freundii, Citrobacter diversus, Serratia liquefaciens, Klebsiella pneumoniae, Salmonella paratyphi A, Proteus mirabilis, P. vulgaris, P. penneri, Escherichia coli and Morganella morganii*.

In this investigation of the hygienic-sanitary quality of juices from fruit juice dispensers, from the positivity of the samples for thermotolerant coliforms, the isolation was done of *Escherichia coli* and its subsequent serology for classical enteropathogenic *E. coli* (EPEC) and enteroinvader *E. coli* (EIEC). The results are shown in Tables 3 and 4.

E. coli is included both in the group of total coliforms and in that of thermotolerant coliforms. Their natural habitat is not necessarily the intestinal tract of warm-blooded animals, they can also be found in environmental reservoirs, so they can be introduced into food from non-fecal sources. These microorganisms are common in food manufacturing environments and can become part of the resident microbiota especially if the cleaning conditions are inadequate [15]. According to Silva et al. [15], several strains of *E. coli*, coliforms or enterobacteria can grow in refrigerated foods.

Infections caused by *E. coli* can be limited to the surface of the intestinal mucosa or spread to the host's organism. Three clinical syndromes result from infection caused by pathogenic *E. coli*: urinary tract infections, sepsis followed by meningitis and gastroenteritis. The different classes of *E. coli* involved in diarrhea are identified and classified based on their pathogenicity factors. This bacterial genus includes, among others, invasive strains such as enteroinvader *E. coli* (EIEC) and strains associated with watery diarrhea such as classical enteropathogenic *E. coli* (EPEC) [26].

EPEC is an important cause of childhood diarrhea in developing countries and even in developed countries it remains a public health

problem [27-29]. Diarrhea caused by EPEC in general is more severe than that caused by other pathogens, with a death prevalence greater than 30% [30,31].

Table 3. Serology of *E. coli* isolated from juice samples (collect 09:30 am)

Collect	Collection time (09:30 am)				
	Classical enteropathogenic *E. coli* (EPEC)			Enteroinvader *E. coli* (EIEC)	
	A	B	C	A	B
01	-	-	O86 O126, O127, O128	-	O112, O124 O143, O164
02	-	-	O127	-	-
03	-	-	O127	O28, O29, O136, O144, O152	
04	-	-	O86, O126, O127, O128	O28, O29, O136, O144, O152	O124, O124
05	-	O114, O125, O158	O86, O126, O127, O128	O28, O29, O136, O144, O152	O112, O143, O164
06	-	-	-	-	-
07	-	O114, O124, O142, O158	O86, O126, O127, O128	-	-
08	-	-	-	-	-
09	O26, O55 O111, O119	O114, 0125, O142, O158	O86, O126, O127, O128	-	-
10	O26, O55	O142, O158	-	O28, O29, O136, O144	-
11	O26, O111	-	-	-	O112, O124
12	-	-	-	-	-
13	-	-	-	-	-
14	-	-	-	O28	O124
15	O26, O55, O111, O119	O114, O125, O142, O158	O86, O126, O127, O128	O28, O29, O136, O144, O152	O112, O124, O143, O164, O167

Source: *Food Microbiology Laboratory-UNIUBE.*

Table 4. Serology of *E. coli* isolated from juice samples (collect 04:30 pm)

Collect	Collection time (04:30 pm)				
	Classical enteropathogenic *E. coli* (EPEC)			Enteroinvader *E. coli* (EIEC)	
	A	B	C	A	B
16	-	O114, O124, O142, O158	O86, O126, O127, O128	-	O124, O143, O164
17	O26, O55, O111, O119	O114, O125	O86, O126, O127	O28, O29, O136, O144	O112, O124, O143
18	-	O112, O124, O143, O158	O86, O126, O127, O128	-	O112, O124, O143, O158
19	O26, O55, O111, O119	O114, 0125, O158	O86, O126, O127, O128	O28, O136, O144, O152	O112, O124, O143, O164
20	-	-	O86, O126, O127, O128	O28, O29, O136, O144	-
21	-	-	O86, O126, O86 O128	O28, O29, O136, O144, O152	0112, O124, O145, O164, O167
22	-	-	O86, O126 O128	-	O112, O124 O143, O164, O167
23	O26, O55, O119	-	O86, O126, O127, O128	-	-

Collect	Collection time (04:30 pm)				
	Classical enteropathogenic *E. coli* (EPEC)			Enteroinvader *E. coli* (EIEC)	
	A	B	C	A	B
24	O26, O55, O119	O114, O125, O142, O158	O86, O127, O128	O28, O29, O136, O144, O152	O112, O124, O143, O164, O167
25	-	O125, 142, O114	-	-	-
26	O55, O119	O114, O125, O142, O158	O86, O126, O128	-	-
27	-	0125, O142	O126, O128	O29, O136, O144	O112, O124, O164, O167
28	-	-	O86	-	O124, O143, O164, O167
29	O55, O119	-	O86, O128	O136, O144, O152	O124, O143, O164
30	O55, O111	-	O86, O128	-	O124, O143, O164, O167

Source: *Food Microbiology Laboratory-UNIUBE.*

In 1989, the World Health Organization recognized twelve O serogroups designated: O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158. These serogroups include typical and atypical EPEC strains, enteroaggregative *E. coli*, as well as other *E. coli* variants that cause diarrhea [32-35]. Of the different recognized O serogroups, all were detected in the *E. coli* isolates from the different samples of juice from fruit juice dispensers.

4. CONCLUSION

The results point to a contamination, over current legal standards, of 66.7% for the samples collected in the morning and of 73.34% for the samples collected in the afternoon. Based on the results found, greater care in good practices is suggested, especially regarding the personal hygiene of handlers, water quality, sanitation and correct temperature control of fruit juice dispensers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pinheiro AM, Fernandes AG, Fai AEC, Prado GM, Sousa PHM, Maia GA. Chemical, physical-chemical and microbiological evaluation of whole fruit juice: Pineapple, Caju and Passion Fruit. Food Science and Technology, Campinas. 2006;26(1):98-103.
2. De Marchi R. Development of a passion fruit-based drink (*Passiflora edulis* Sims. F. Flavicarpa Deg.) With hydrolytic replacement properties. Dissertation (Master) - Faculty of Pharmaceutical Sciences. Paulista State University Julio de Mesquita Filho, Araraquara. 2001;92.
3. Brazil. Ministry of Health. National Health Surveillance Agency. Ordinance No. 27, of January 13, 1998a. Technical regulation regarding supplementary nutritional information. Official Gazette [of] the Federative Republic of Brazil, DF, Jan 16. 1998.
Available:http://e-legis.anvisa.gov.br/leisref/public/showAct.php?id=97
(Accessed on: 20 Jun 2012)
4. Brazil. Ministry of Agriculture, Livestock and Supply. Ordinance No. 544, of November 16, 1998b. Approves the Technical Regulations for Setting Identity and Quality Standards, for soft drinks, soft drinks, prepared or liquid concentrates for soft drinks or soft drinks, prepared solid for soft drinks, syrup and tea ready for consumption. Official Gazette [of] the Federative Republic of Brazil, DF, November 17; 1998.
Available:http://extranet.agricultura.gov.br/sislegisconsulta/consultarLegislacao.do?operacao=visualizar&id=1012
(Accessed on: 20 Jun 2020)
5. Brazil. Ministry of Agriculture, Livestock and Supply. Decree no. 3,510, of June 16, 2000. Amends provisions of the Regulation approved by Decree no. 2,314, of September 4, 1997, which provides for the

standardization, classification, registration, inspection, production and inspection of drinks. Official Gazette [of] the Federative Republic of Brazil, DF, June 19. 2000. Available:http://extranet.agricultura.gov.br/sislegisconsulta/consultarLegislacao.do?operacao=visualizar&id=1012
(Accessed on: 20 Jun 2020)

6. Brazil. Ministry of Health. National Health Surveillance Agency. Resolution - RDC n.259, of September 20, 2002. Technical regulation for the labeling of packaged foods. Official Gazette [of] the Federative Republic of Brazil, DF, 23 September. 2002.
Available:http://e-legis.anvisa.gov.br/leisref/public/showAct.php?id=1261
(Accessed on: 20 Jun 2020)
7. Brazil. Ministry of Health. National Health Surveillance Agency. Resolution - RDC n.360, of December 23, 2003a. Technical regulation on nutritional labeling of packaged foods. Official Gazette [of] the Federative Republic of Brazil, DF, December 26; 2003.
Available:http://e-legis.anvisa.gov.br/leisref/public/showAct.php?id=9059
(Accessed on: 20 Jun 2020)
8. Brazil. Ministry of Health. National Health Surveillance Agency. Law No. 10,674, of May 16, 2003b. It requires that commercialized food products that inform about the presence of gluten, as a preventive and control measure for celiac disease. Official Gazette [of] the Federative Republic of Brazil, DF, May 19. 2003.
Available:http://e-legis.anvisa.gov.br/leisref/public/showAct.php?id=58 54
(Accessed on: 20 Jun 2020)
9. Brazil. Ministry of Health. National Health Surveillance Agency. Resolution - RDC n.359, of December 23, 2003c. Technical regulation on nutritional labeling of packaged foods. Official Gazette [of] the Federative Republic of Brazil, DF, December 26; 2003.
Available:http://e-legis.anvisa.gov.br/leisref/public/showAct.php?id=58 54
(Accessed on: 20 Jun 2012)
10. Ferrarezi AC. Interpretation of the consumer, evaluation of the purchase intention and the physical-chemical characteristics of the nectar and orange juice ready to drink. 2008. 120 f. Dissertation (Master in Food Science) - Faculty of Pharmaceutical Sciences, Universidade Estadual Paulista Júlio de Mesquita Filho, Araraquara; 2008.
11. Lee HS, Coates GA. Vitamin C in frozen, fresh squeezed, unpasteurized, polyethylene-bottled orange juice: A storage study. Elsevier Food Chemistry. 1999;65(2):165-168.
12. Pilon L. Establishment of the useful life of vegetables minimally processed under modified atmosphere and refrigeration. Dissertation for obtaining a Master's degree in Food Science and Technology, presented to the Higher School of Agriculture “Luiz de Queiroz”, University of São Paulo, Piracicaba; 2003.
13. Franco BDGM, Landgraf M. Food microbiology. São Paulo: Atheneu. 2007; 27-31.
14. Vanderzant C, Splittstoesser DF. Compendium of methods for the microbiological examination of foods. 3. ed. Washington: American Public Health Association. 1999;1219.
15. Silva N, et al. Manual of methods of microbiological analysis of food. 3. ed. São Paulo: Varela. 2007;552.
16. Brazil. Ministry of Health. National Health Surveillance Agency. Ordinance No. 518 of 25 March 2004. Establishes the procedures and responsibilities related to the control and surveillance of the quality of water for human consumption and its standard of drinking water, and other measures. Official Gazette [of] the Federative Republic of Brazil, DF, 26 March; 2004.
Available:http://e-legis.anvisa.gov.br/leisref/public/showAct.php?id=58 54
(Accessed on: 20 Jun 2020)
17. Brazil. Ministry of Health. National Health Surveillance Agency. Resolution - RDC n.12, of January 2, 2001. Technical Regulation on microbiological standards for food. Official Gazette [of] the Federative Republic of Brazil, Brasília, DF, Jan. 2. 2001.
Available:http://www.anvisa.gov.br/legis/portarias/451-97.htm
(Accessed on: 20 Jun 2020)
18. Nascimento D, Furlanetto SMP. Quantitative determination of groups of

bacteria in fresh orange juices. Revista de Saúde Pública, São Paulo. 1981;15(2).
19. Oliveira JC, Setti-Perdigão P, Siqueira KAG, Santos AC, Miguel MAL. Microbiological characteristics of fresh orange juice. Food Science and Technology Magazine, Campinas. 2006; 26(2):241-5.
20. Santos J, Ribeiro GA. Microbiological evaluation of "in natura" orange juices marketed in the city of Pelotas-RS. Food Hygiene Magazine, São Paulo. 2006; 20(138):104-7.
21. Anders T, et al. Hygienic-sanitary quality of food and beverages produced and / or marketed by food and nutrition units, like canteens, on campuses I and II of the Federal University of Goiás in Goiânia. Magazine of the Brazilian Nutrition Association, São Paulo. 2008;21.
22. Ruschel CK, Carvalho HH, Souza RB, Tondo EC. Microbiological and physical-chemical quality of orange juices sold on public roads in Porto Alegre-RS. Food Science and Technology, Campinas. 2001; 219(1):94-97.
23. Sousa MAC, Yuyama LKO, Aguiar JPL, Pantoja L. Açaí juice (*Euterpe oleracea* Mart.): Microbiological evaluation, heat treatment and shelf life. Health Sciences Journal, Manaus. 2006;36(4): 497-502.
24. Silva TT, Modesta RCD, Penha EM, Matta VM, Cabral LMC. Organic passion fruit juice processed by microfiltration. Revista de Pesquisa Agropecuária Brasileira, Brasília. 2005;40(4):419-2.
25. Andrade OMM, et al. Hygienic-sanitary quality of açaí juice sold in 4 neighborhoods of São Luís-MA. Revista Nutrição em Pauta, São Luís. 2007;86:85.
26. Trabulsi LR, Ordonez JG. *Escherichia coli* enteropathogenic. In: Trabulsi LR, Alterthum F. Microbiology. São Paulo: Atheneu. 2004;277-288.
27. Gomes TAT, et al. Prevalence of *Escherichia coli* strains with localized, diffuse, and aggregative adherence to HeLa cells in infants with diarrhea and matched controls. Journal of Clinical Microbiology, United States. 1989;27:266-269.
28. Gomes TAT, et al. Enteropathogens associated with acute diarrheal disease in urban infants in São Paulo, Brazil. The Journal of Infectious Diseases, United States. 1991;64:331 – 337.
29. Nataro JP, Kaper JB. Diarrheogenic *Escherichia coli*. Clinical Microbiology Reviews, United States. 1998;11:142-201.
30. Levine MM, Edelman R. Enteropathogenic *Escherichia coli* of classic serotypes associated with infant diarrhea: Epidemiology and pathogenesis. Epidemiologic Reviews, United States. 1984;6:31-5.
31. Senerwa D. Enteropathogenic *Escherichia coli* serotype 0111: HNT isolated from preterm neonates in Nairobi, Kenya. Journal of Clinical Microbiology, United States. 1989;27:1.307-1.311.
32. Campos LC, et al. *Escherichia coli* serogroup O111 includes several clones of diarrheogenic strains with diferrent virulence properties. Infection and Immunity, United States. 1994;62(3):282-3.288.
33. Rodrigues J, et al. Clonal structure and virulence factors in strains of Escherichia coli of the classic serogroup O55. Infection and Immunity, United States. 1996;64: 2.680-2.686.
34. Scotland SM. Use of gene probes and adhesion tests to characterize *Escherichia coli* belonging to enteropathogenic serogroups isolated in the United Kingdom. Journal of Medical Microbiology, England. 1996;44:438-443.
35. Do Valle GR, Gomes A, Irino K, Trabulsi LR. The traditional enteropathogenic *Escherichia coli* (EPEC) serogroup O125 comprises serotypes which are mainly associated with the category of enteroaggregative *E. coli*. FEMS Microbiology Letters, England. 1997;152: 95-100.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63905