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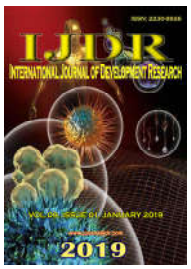

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

MICROBIOLOGICAL QUALITY OF COCONUT WATER AND THE INSTRUMENTS USED FOR THE PERFORATION OF THEFRUIT MARKETED IN THE SÃO LUÍS, MARANHÃO, BRAZIL

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ABSTRACT

Coconut water consumption on the edge of São Luís-MA has had a huge growth, justified by the number of bathers who frequent the beaches during the day or even by the practitioners of physical activities in the period of the night, calling attention to the conditions hygienic-sanitary that such food is marketed. This study aimed to assess the microbiological quality of coconut water and instruments used in the drilling of the fruit. Simulating the takeover of coconut water to the chosen points, a total of 5 samples and using the pour-plate technique was held total count of mesophilic microorganisms and fungi. After the microbiological analysis, note that 20% of the samples analyzed present microorganisms, tools used to puncture the fruit note the presence of 100% of these, corroborating with the questions raised about the hygienic-sanitary procedures in handling food. Since coconut water is presented sterile on the inside of the fruit any microorganisms count confirms the contamination in contact with evil sanitized. In the coconut water samples were found at most values 4, 60 x 10^{2}CFU/mL for fungi; already utensils were detected values of 1, 52 x 10^{4} CFU/mL for mesophilic bacteria and > 250 CFU/mL for fungi in dilutions of up to 10^{-2}. This study proves the absence of good Handling practices, being of interest and responsibility of the competent bodies to invest in health education and monitoring of product sales points, in order to ensure food security of the consumers.

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INTRODUCTION

Dietary habits have changed over the last decades in search of a healthy lifestyle and with this there is a growing appreciation for food of plant origin. Coconut in particular, has become one of these foods, either by the climate of the region, easy access or simply low cost. Such demand causes the number of establishments that commercialize the fruit to proliferate, and often not being handled correctly, incurring risks to consumer health. Food security should be offered to everyone in any situation. A safe food is not always the one that presents its sensorial characteristics unchanged, at sanitary level must be free of pathological agents (SILVA *et al.,* 2012). On the edge of São Luís, there is a vast amount of vendors selling coconut, even more than the place is frequent spot of the practitioners of physical activities.

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The precarious hygienic and sanitary situation raises public health concerns, since non-compliance with sanitary norms may result in so-called Foodborne Diseases (DTA). Studies show that thousands of people are affected every year by the outbreak of contaminated food. Contamination by microorganisms becomes a highlight when it comes to food, since they serve as true indicators for compliance with good handling practices. The consumption of food contaminated by pathogenic microorganisms can lead to an infectious disease, ranging from mild discomfort, intense reactions or even death (SILVA *et al.,* 2012). Although the coconut water is sterile inside the fruit, its composition, rich in(proteins, carbohydrates, minerals and vitamins) provides microbial multiplication, induced by the lack of hygienization of the sharps in direct contact with the liquid. According to Mendes *et al.* (2011), equipment and the like are great vehicles of contamination, especially when it comes to ready-to-eat foods, being extremely important the application and compliance of sanitary measures at points that commercialize the fruit (BRASIL, 2016; *et al.,* 2005).

Microbiological quality control is designed to meet various sanitary parameters, including: cleaning, disinfection, sanitation, conservation, resistance of utensils as well as equipment used during handling resulting in a safe food fit for human consumption. The hygienization of utensils in a correct way is indispensable in food handling, since these are vehicles of direct contamination both by contact with the manipulator and in contact with the atmosphere, benches, sinks among others. The material in which these utensils are made is also extremely important since they can promote the proliferation of microorganisms, especially if they are permeable and rough in extension. Thus, it is necessary to evaluate the microbiological quality in objects used to obtain coconut water as well as its analysis, in order to emphasize the sanitary conditions for the ones that commercialize the fruit (BERTIN, 2009; BRASIL, 2001; SILVA *et al.,* 2010). The total count of mesophilic microorganisms is used as a reliable indicator of compliance with sanitary standards, providing indications of contamination. The high counts of molds and yeasts are directly related to the deterioration of the food (SILVA, BRASIL, 2010). The evaluation of microbiological control and compliance with current sanitary legislation is necessary, especially in places with great public access. The pour-plate method and the application of a questionnaire on Good Handling Practices can generate results to evaluate these criteria (SILVA *et al.,* 2010).

MATERIALS AND METHODS

The research developed is a study of the experimental type, carried out in the period from 10/15/2017 to 11/1/2017. The sanitary determinations were carried out in accordance with the current legislation, Resolutions of the Collegiate Board of Directors (RDC) 216/04, 52/14 and RDC 218/05 of the National Agency of Sanitary Surveillance (ANVISA). Microbiological determinations are in accordance with the methodology recommended by the Brazilian Pharmacopoeia, 5th edition, Volume I and the Manual of Methods of Microbiological Analysis of Food and Water (SILVA *ET AL.,* 2010). For the development of the study, inspections of kiosks and street vendors against sanitary standards were verified according to the questionnaire applied based on the above mentioned resolutions, in order to evaluate compliance with the minimum requirements for food handlers. By means of the sample calculation, $\sqrt{n} + 1$, the quantitative values of the collection sites were determined, where n corresponds to the total amount (19) of points, excluding bars, which market coconut water in natura throughout the stretch of coastline, 7.2 km in the state of Maranhão, Brazil. The calculated sampling corresponds to 5 points to be analyzed. After the determined quantity, the sites to be collected were selected randomly between the beaches of São Marcos and Calhau. Five samples of coconut water, all refrigerated in the same way as consumed, were collected aseptically with approximately 200 mL each, identified with letters A through E, which were placed in a sterile glass vial and then stored in a thermal box containing disposable ice, then with a swab soaked in sterile saline solution (0.1% NaCl) were collected from the samples referring to the surfaces of the utensils used for drilling the fruit. In a second stage a questionnaire was applied with the consent of the street vendors in order to evaluate them regarding good manipulation practices. The questionnaire elaborated contained 14 questions, characterizing the street vendor as to the knowledge about good practices of manipulation, personal hygiene, conservation and hygiene of utensils, among others. The perishable materials were immediately sent to the Laboratory of Microbiology of the Florence Institute of Higher Education. All collected water samples were checked for pH upon receipt. Using a suitably sterilized pipette, 10.0 mL of each sample of collected coconut water was collected, and in each 10.0 mL 90.0 mL of 0.1% soy broth-casein was added with the homogenization, obtaining the dilution 10-1, maintaining the pH of the dilution between 6.0-8.0, and when necessary adjusting the pH with solutions of HCl and 0.1M NaOH, keeping them refrigerated. The swab collected from the thermal box was then withdrawn at a dilution of 10-1, from which 10-2 dilutions were obtained by pipetting 1.0 mL of the above dilution and adding 9.0 mL of sterile saline. Both kept under refrigeration. The respective dilutions were inoculated for analysis of the samples using the pour-plate technique, 1.0 mL of the dilutions were placed in Petri dishes and 15-20 mL of casein soy agar and Sabouraud agar - dextrose maintained at 45-50ºC (in Bath-Maria) and in repeated movements in the form of numeral 8 for about 10 times the homogenization of the whole medium was obtained. Two plates were used for each medium and dilution. Plates containing casein-soy agar at 32.5 ± 2.5 ° C were incubated for 3 days and plates containing Sabouraud-dextrose agar at 22.5 ± 2.5 ° C for 7 days to determine the number of total aerobic microorganisms and molds and yeasts, respectively. Only plaques that presented colonies below 250 (bacteria) and 50 (molds and yeasts) per plaque were considered for results records. The arithmetic mean of the plates of each medium was calculated and the number of CFU per milliliter of the product was calculated according to the Brazilian Pharmacopoeia, 2010, as an example:

Example of calculation:

Dilution	*Colonies by plates*	*CFU/g, ou mL*
1:100	293	2.93×10^4
1:100	100	1.00×10^4
1:1000	41	4.10×10^4
1:1000	12	1.20×10^4

$$\text{Media} = \frac{(2.93+1.00+4.10+1.20)}{4} \times 10^4 = 2.30 \times 10^4$$

RESULTS AND DISCUSSION

According to the application of the checklist, elaborated by the authors that aimed to raise the vendors' knowledge of Good Manipulation Practices, it is noted that 100% (n = 5) of the manipulators do not have knowledge about the practice, to the failure to comply with several hygienic and sanitary parameters (Fig. 1).

Fig. 1. Hygienic sanitary standards of the manipulators

Sanitary standards	Yes (%)	No (%)
They use EPI	0	100
Hand washing	20	80
Personal catering	100	0

Fig. 2. Sanitary standards of the utensils used in the opening of the coconut

Appliance standards	Yes (%)	No(%)
Stored in appropriate place	0	100
Smooth and waterproof surface	0	100
Corrosion resistant	80	20
Resistant to cleaning	80	20

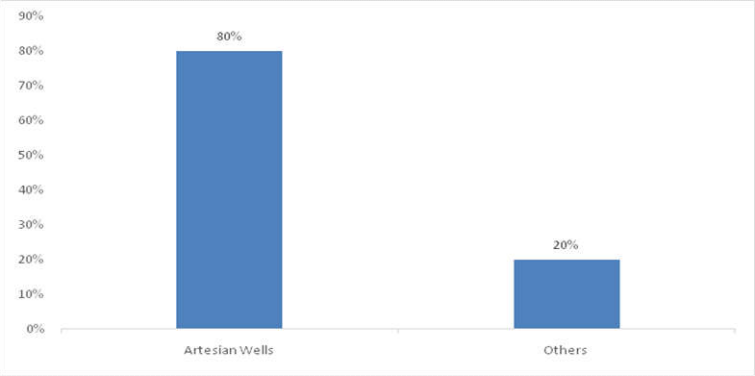

Fig. 3. Obtaining water used for fruit manipulation and hygiene of utensils

Fig. 4. Counts in Casoy agar and effectively used in the calculation of the result. UFC / mL = Colony Forming Units per milliliter

Coconut water (Casoy) - Bacteria			
Sample	**Number of colonies on dilution plates**		**CFU/mL count**
	10^{-1}	10^{-2}	
A	< 10 CFU/ mL	-	< 10 CFU/ mL
B	< 10 CFU/ mL	-	< 10 CFU/ mL
C	< 10 CFU/ mL	-	< 10 CFU/ mL
D	< 10 CFU/ mL	-	< 10 CFU/ mL
E	< 10 CFU/ mL	-	< 10 CFU/ mL
Coconut water (Casoy)- Fungi			
A	65-27	-	4.60 x 10^2CFU/ mL
B	< 10 CFU/ mL	-	< 10 CFU/ mL
C	< 10 CFU/ mL	-	< 10 CFU/ mL
D	< 10 CFU/ mL	-	< 10 CFU/ mL
E	< 10 CFU/ mL	-	< 10 CFU/ mL

Fig. 5. Counts on Sabouraud agar effectively used in the calculation of the result.

Coconut water (Sabouraud) - Bacteria			
Sample	**Number of colonies on dilution plates**		**CFU/mL count**
	10^{-1}	10^{-2}	
A	< 10 CFU/ mL	-	< 10 CFU/ mL
B	< 10 CFU/ mL	-	< 10 CFU/ mL
C	< 10 CFU/ mL	-	< 10 CFU/ mL
D	<10 CFU/ mL	-	< 10 CFU/ mL
E	< 10 CFU/ mL	-	< 10 CFU/ mL
Coconut water (Sabouraud) - Fungi			
A	< 10 CFU/ mL	-	< 10 CFU/ mL
B	< 10 CFU/ mL	-	< 10 CFU/ mL
C	< 10 CFU/ mL	-	< 10 CFU/ mL
D	< 10 CFU/ mL	-	< 10 CFU/ mL
E	< 10 CFU/ mL	-	< 10 CFU/ mL

Fig. 6. Counts in Casoy agar and effectively used in the calculation of the result.

***Swab* of utensils (Casoy) - Bacteria**			
Sample	**Number of colonies on dilution plates**		**CFU/mL count**
	10^{-1}	10^{-2}	
A	31-22	-	2.65 x 10^2 CFU/ mL
B	> 250 CFU	> 250 CFU	> 250 CFU/ mL
C	118-158	25-26	1.97 x 10^3CFU/ mL
D	16-1	< 10 CFU/ mL	8.50 x 10^1CFU/ mL
E	43-57	< 10 CFU/ mL	5.00 x 10^2CFU/ mL
***Swab* of utensils (Casoy) – Fungi**			
A	168-150	5-6	1.07 x 10^3CFU/ mL
B	21-25	-	2.30 x 10^2CFU/ mL
C	27-15	< 10 CFU/ mL	2.10 x 10^2CFU/ mL
D	< 10 CFU/ mL	< 10 CFU/ mL	< 10 CFU/ mL
E	< 10 CFU/ mL	-	< 10 CFU/ mL

Fig. 7. Counts on Sabouraud agar effectively used in the calculation of the result.

***Swab*of utensils (Sabouraud) – Bacteria**			
Sample	Number of colonies on dilution plates		CFU/mL count
	10^{-1}	10^{-2}	
A	> 250 CFU	200-104	1.52 x 10^4CFU/ mL
B	> 250 CFU	> 250 CFU	> 250 CFU/ mL
C	< 10 CFU/ mL	-	< 10 CFU/ mL
D	< 10 CFU/ mL	-	< 10 CFU/ mL
E	10	-	7.00 x 10^1CFU/ mL
Swab of utensils (Sabouraud) – Fungi			
A	18-33	2-5	4.30 x 10^2CFU/ mL
B	< 10 CFU/ mL	-	< 10 CFU/ mL
C	> 250 CFU	37-24	3.05 x 10^3CFU/ mL
D	< 10 CFU	-	< 10 CFU/ mL
E	35-42	Aus.	3.86 x 10^3CFU/ mL

Non-antisepsis of the hands prior to handling is a worrying factor as these are a potential vehicle of contamination for both the fruit and for manipulated utensils, especially when using EPI is not common practice among sellers. The conditions of conservation and contamination of the utensils used in the opening of the coconut also call attention, mainly the way they are exposed in counters, sinks, on wet cloth, propitiating even more the proliferation of microorganisms (Fig. 2). Some vendors based only on hygiene tend to reduce the chances of contamination by preparing sanitizing solutions (hypochlorite) without adequate proportions to the dilution, which can lead to a chemical contamination of the food, adding a danger to the health of the consumer. Nunes *et al.* (2017) report that in 2012 in the city of Ribeirão Preto-São Paulo a case was recorded for food infection involving 9 people including 1 death, after the results of the analyzes the same symptomatology is noticed in third parties without even consuming the suspicious food, however there was the ingestion of a third food handled under the same conditions, suggesting contamination in sinks, countertops and utensils in common. The supply of water used at these points of sale is also another factor that attracts a great deal of attention, a large part of this supply comes from artesian wells, calling into question the drinking water standard, which according to Ministry of Health ordinance 2,914 / 11 be submitted to physical, chemical or combination of these processes in order to meet permitted values as a parameter of water quality for human consumption and comply with the microbiological standard (Fig.3). Samples of coconut water received by the laboratory shortly after collection had pH in the range of 5.0-5.5, which according to the Brazilian Agricultural Research Corporation(EMBRAPA), *fresh* green coconut water has a pH in range from 4.8 to 5.2. The small increase in some samples (pH 5.5) may be directly related to the fruit's maturation process when the production of acids (ascorbic acid and indole acetic acid) decreases. It is difficult for coconut water to undergo sudden changes in pH, such situations can be caused by the increase of microbial activity.
The microbiological analyzes follow the standards established by the American Public Health Association (APHA), indispensable in the evaluation of Good Handling Practices.

The results of the microbiological analyzes carried out with coconut water are shown in Figures 4 and 5, showing a low count and in the great majority absence of the microbial growth, which can be justified by the short time of contact with the utensil, -organisms in these or even the medium of the fruit with a not very propitious pH since mesophilic microorganisms have as optimal pH values close to the neutrality 6.5-7.5 as opposed to coconut water with a pH of 5.0 - 5.5, in contrast justifies the growth of molds and yeasts that are quite resistant to adverse conditions of pH being very little affected in the range of 3.0 - 8.0, according to Alcântara and collaborators (2012) the presence of these micro-organisms does not make the product unfit for human consumption, but it becomes a danger to consumer health when in high populations. In the analysis of coconut water, we can observe a low population of mesophilic microorganisms or, in the great majority, absence, although the Brazilian legislation does not establish microbial limits for mesophiles in foods, the samples analyzed had the limits established by the APHA, of 1.4 x 10^3CFU / mL, so all samples meet the requirements. Importantly, the presence of mesophilic the coconut water **A** is originatedutensils used in the extraction liquid or those residues carreiam of the whole surface to the inner part, since it was not observed any cleaning process in order to reduce the superficial contamination, as evidenced by Carvalho *et al.* (2012), inferring in his studythat the main sources of contamination were the surface of the stands / trolleys where the coconuts are placed for the extraction of water and the instruments used in the cutting. To Fig.4, sample **A**cultivated in the Casoy culture medium showed a higher count when compared to the others, corroborating with the high fungal count of the instrument referring to the same sample sown in Sabouraud (Fig.7).

Conclusion

In the samples of coconut water were found values of maximum 4.60 x 102CFU / mL for fungi; already in the utensils were detected values of 1.52 x 104CFU / mL for mesophilic bacteria and> 250 CFU / mL for fungi in dilutions of up to 10-2. Although the total count of aerobic mesophiles in plaques is not an indicator of food safety, it is not directly related to the presence of pathogens or toxins, it is very useful in the evaluation of quality, where high populations of these microorganisms indicate deficiencies in sanitization or failures in manipulation, being the gateway for pathogens.Once the presence of mesophilic microorganisms in a sample of coconut water has been detected, as well as in the great majority of sharps, it is evident the non-compliance with the Good Practices of Manipulation incurring a health risk to the consumer.The study should not beconsidered as masterful, but should stimulate further research, with more sensitive techniques and with the competent authorities to awaken a monitoring in the units of commercialization of the fruit.

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