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INTERFACES CIENTÍFICAS

MICROBIOLOGICAL ANALYSIS OF COCONUT WATER *COCOS NUCIFERA* L. SOLD BY STREET VENDORS IN SOBRAL/CE, BRAZIL

ANÁLISE MICROBIOLÓGICA DA ÁGUA DE COCO *COCOS NUCIFERA* L. COMERCIALIZADA POR VENDEDORES AMBULANTES EM SOBRAL/CE, BRASIL

ANÁLISIS MICROBIOLÓGICO DEL AGUA DE COCO *COCOS NUCIFERA* L. VENDIDA POR VENDEDORES AMBULANTES EN SOBRAL/CE, BRASIL

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ABSTRACT

Green coconut water is rich in nutrients and widely consumed in the world. However, the consumption of coconut water out of good practices for food production treat consumer health. The aim of this work was to evaluate the microbiological quality of coconut water sold by vendors street in Sobral - CE. Samples of fresh coconut water were collected bimonthly from four different points (named A, B, C and D) during 12 months (n=24). Samples were characterized by quantification of total coliform (TC), thermotolerant coliform (TTC) and mesophilic aerobic bacteria (MAB). The Most Probable Number (MPN) and Pour Plate techniques were applied to quantification of TC and TTC, and mesophilic aerobic bacteria (MAB), respectively. All samples presented contamination by TC, TTC and MAB. TC-MPN ranged from $4.97x10^4$ MPN/mL to $1.18x10^5$ MPN/mL, and TTC-MPN ranged from $8.39x10^2$ MPN/mL to $1.28x10^3$ to MPN/mL. Samples C and D presented contamination by *Escherichia coli*. The number of MAB ranged from $3.12x10^4$ CFU/mL to $6.73x10^4$ CFU/mL. TTC number are above of the permitted values according to National Health Surveillance Agency (ANVISA), being considered as unsuitable for human consumption. Then, it is necessary to adopt preventive measures and good hygiene practices in coconut water commercialization for improvement of the microbiological quality of the product and prevent risks to consumers health.

KEYWORDS

Food Microbiology; Microbial contamination; Coliforms; Hygienic-sanitary.

RESUMO

A água de coco verde é rica em nutrientes e amplamente consumida no mundo. No entanto, o consumo de água de coco fora das boas práticas de produção de alimentos prejudica a saúde do consumidor. O objetivo deste trabalho foi avaliar a qualidade microbiológica da água de coco comercializada por camelôs de Sobral - Ceará. Amostras de água de coco fresca foram coletadas bimestralmente em quatro pontos diferentes (denominados A, B, C e D) durante 12 meses (n=24). As amostras foram caracterizadas pela quantificação de coliformes totais (CT), coliformes termotolerantes (TTC) e bactérias aeróbicas mesófilas (MAB). As técnicas de Número Mais Provável (NMP) e *Pour Plate* foram aplicadas para quantificação de TC e TTC, e bactérias aeróbicas mesófilas (MAB), respectivamente. Todas as amostras apresentaram contaminação por TC, TTC e MAB. TC-MPN variou de $4,97x10^4$ MPN/mL a $1,18x10^5$ MPN/mL e TTC-MPN variou de $8,39x10^2$ MPN/mL a $1,28x10^3$ a MPN/mL. As amostras C e D apresentaram contaminação por *Escherichia coli*. O número de MAB variou de $3,12x10^4$ UFC/mL a $6,73x10^4$ UFC/mL. Os números TTC estão acima dos valores permitidos pela Agência Nacional de Vigilância Sanitária (ANVISA), sendo considerados impróprios para consumo humano. Assim, faz-se necessária a adoção de medidas preventivas e boas práticas de higiene na comercialização da água de coco para melhoria da qualidade microbiológica do produto e prevenção de riscos à saúde do consumidor.

PALAVRAS-CHAVE

Microbiologia Alimentar. Contaminação Microbiana. Coliformes. Higiênico-Sanitária.

RESUMEN

El agua de coco verde es rica en nutrientes y se consume ampliamente en el mundo. Sin embargo, el consumo de agua de coco por buenas prácticas de producción de alimentos perjudica la salud del consumidor. El objetivo de este trabajo fue evaluar la calidad microbiológica del agua de coco comercializada por camellos de Sobral - Ceará. Se recolectaron muestras de agua de coco fresca cada dos meses en cuatro puntos diferentes (denominados A, B, C y D) durante 12 meses (n=24). Las muestras se caracterizaron mediante la cuantificación de coliformes totales (CT), coliformes termotolerantes (TTC) y bacterias aerobias mesófilas (MAB). Se aplicaron las técnicas de Número Más Provable (MPN) y Pour Plate para la cuantificación de TC y TTC, y bacterias aerobias mesófilas (MAB), respectivamente. Todas las muestras presentaron contaminación por TC, TTC y MAB. TC-MPN varió de 4,97x104 MPN/mL a 1,18x105 MPN/mL y TTC-MPN varió de 8,39x102 MPN/mL a 1,28x103 a MPN/mL. Las muestras C y D presentaron contaminación por Escherichia coli. El número de MAB varió de 3,12x104 UFC/mL a 6,73x104 UFC/mL. Las cifras de TTC están por encima de dos valores permitidos

por la Agencia Nacional de Vigilancia Sanitaria (ANVISA), considerándose no apto para el consumo humano. Asimismo, sería necesario adoptar medidas preventivas y buenas prácticas de higiene en la comercialización del agua de coco para mejorar la calidad microbiológica del producto y prevenir riesgos a la salud del consumidor.

PALABRAS CLAVE

Microbiología de los alimentos. Contaminación microbiana. coliformes. Higiénico-sanitario.

1 INTRODUCTION

Cocos nucifera L. is a plant native to Southeast Asia and was widely introduced throughout the coastal territory of Brazil (Silva *et al.*, 2021b), being recognized as one of the most useful plants in the world, and its leaves, root, stem, inflorescence and fruit (coconut) are used for food, nutritional, artisanal, agroindustrial and medicinal purposes (Silva *et al.*, 2017). Green coconut has a hollow compartment containing a white pulp and a liquid (i.e. coconut water) rich in sugars, vitamins, proteins and minerals. Due the chemical composition, pleasant taste and low caloric value, coconut water is indicated for replacement of electrolytes lost during conditions of dehydration or physical wear (Costa *et al.*, 2005; Jesus *et al.*, 2018).

Coconut has been grown in over 90 countries and represents an expanding market. The Brazilian coconut market has grown significantly in recent years, being mainly grown on the coast of Northeast Brazil, that accounting for about 80% of all national production (SEBRAE, 2016). The low cost and ease of handling of coconut *in natura* also stimulates its marketing in outside locations, like sidewalks and street (Santos *et al.*, 2013), which increase the risk of microbial contamination.

Although being sterile inside of the healthy fruit, coconut water can be contaminated by microorganisms during the extraction process, transport and/or storage (Santos *et al.*, 2018). In addition, poor of equipment and utensils hygiene, and nutritional changes caused by chemical contaminants influence the low quality of the product (Silva *et al.*, 2017; Silva *et al.*, 2018). Another worrying factor is the quality of the ice used for conservation and which is deposited inside thermal reservoirs (Froehlich, 2015). The coconut water sold in external locations and by street vendors may ignore good food preparation, handling and storage practices. Factors such as the lack of septic measures when using instruments for drilling, cutting and packaging coconut water can lead to microbial contamination, threatening the health of consumers (Jesus *et al.*, 2018).

Foods are considered an important vehicle for the transmission of microorganisms and diseases that can cause food outbreaks. The main microorganisms that cause infections and/or toxinfections food are *Escherichia coli*, *Campylobacter* spp., *Salmonella* spp., and *Shigella* spp. (Franz *et al.*, 2018). Thermotolerant coliforms, that include *E. coli*, are considered microbiological indicators of food qual-

ity, and their presence indicate contamination of fecal origin. In addition, the presence of the genre *Salmonella* indicates contamination and high risk of food poisoning (Dias *et al.*, 2015). Then, microbiological hazards are a serious concern for food safety and human health.

Considering the high prevalence of diseases related to the consumption of contaminated food, is very important the microbiological monitoring and control of these products. Therefore, microbiological management of food safety aims to minimize the risk of foodborne diseases, and preventive measures are essential throughout the production chain, and ensuring adequate hygienic-sanitary quality, avoiding microbial contamination (Lima *et al.*, 2015). Given the above, the objective of this study was to determine the microbiological quality of coconut water sold by street vendors in Sobral-Ceara through the quantification of total and thermotolerants coliforms, and mesophilic aerobes bacteria.

2 METHODS

2.1 SAMPLING

Samples of fresh green coconut water (200mL each) were obtained from four street vendors (A, B, C and D) in the center of Sobral, Ceará State. The collections were carried out bimonthly in the period from 2021 to 2022, totaling 24 samples. After collection, the samples were kept in their original packaging, sealed and without any possibility of external contamination. Afterwards, the samples were placed in isothermal boxes containing sanitized ice sheets in order not to compromise the microbiological quality of the samples and transported to the Microbiology Laboratory of the Universidade Estadual Vale do Acaraú – UVA, where they were subjected to microbiological analysis.

2.2 MICROBIOLOGICAL TECHNIQUES

All microbiological analyzes were performed according to the Compendium of Methods for the Microbiological Examination of Foods of the American Public Health Association (Downes; Ito, 2001) and the Manual of Methods for Microbiological Analysis of Foods (Silva *et al.*, 2010).

2.3 PRESUMPTIVE TEST

The presumptive test consists of analyzing the presence of lactose fermenting microorganisms, such as those belonging to the coliform group, using Lactose Broth as a culture medium. Samples were serially diluted to magnitude 10^{-3} using sterile saline (0.85%) in a 1:9 ratio (sample/saline). Aliquots of 1mL of each dilution were inoculated in quintuplicate tubes with Lactose Broth and inverted *Durham* tubes that were placed in a bacteriological oven at 36°C for 24 hours. After the incubation period, the Lactose Broth tubes that showed gas production with bubble formation in the *Durham* tubes and/or turbidity of the medium, were considered positive.

2.4 COUNT OF TOTAL AND THERMOTOLERANT COLIFORMS

The quantification of Total Coliforms (TC) and Thermotolerant Coliforms (CTT) was performed using the Most Probable Number (MPN) method through the multiple tube technique. For the quantification of total coliforms, aliquots of each tube of Lactose Broth positive in the presumptive test were removed, with the aid of a platinum loop, and inoculated in tubes with 5mL of Brilliant Green Bile Broth (BVB) and inverted Durham tubes. Then, the inoculated tubes were placed in a bacteriological oven at 36°C for 48 hours.

For the quantification of thermotolerant coliforms, aliquots of positive Lactose Broth tubes were inoculated in tubes with 5mL of *Escherichia coli* Broth (EC) and inverted Durham tubes. Finally, the inoculated tubes were placed in a water bath at 45ºC for 48 hours. Tubes that showed bubble formation inside the *Durham* tube and/or cloudy medium were considered positive. The determination of the Most Probable Number for CT and CTT was performed by consulting the standard NMP table, taking into account the number of positive tubes observed for each sample in each dilution.

2.5 IDENTIFICATION AND ISOLATION OF *ESCHERICHIA COLI*

From the EC tubes that showed a positive result, aliquots were removed and seeded in Petri dishes with Eosin Blue Methylene Agar (BEM) that were incubated in a bacteriological oven at 36ºC for 24 hours. After the incubation period, the colonies that showed a bright green color, positive characteristics for *E. coli*, were isolated in Tryptic Soy Agar (TSA) medium and later incubated in a bacteriological oven at 36°C for 24 hours. Then the bacterial colonies were submitted to the IMVIC biochemical test to identify bacteria belonging to the Enterobacteriaceae family by consulting the table of biochemical tests to differentiate bacterial species (Koneman *et al.*, 2008).

2.6 STANDARD PLATE COUNT (POUR PLATE)

For the quantification of mesophilic aerobic bacteria, the standard plate count was used, performed using the Pour Plate technique. For this test, 1mL of the 10^{-1}, 10^{-2} and 10^{-3} dilutions of each sample was inoculated into 15mL of Plate Count Agar (PCA) culture medium, which were poured into sterile Petri dishes and homogenized in gentle movements in a circular motion. of eight. Then, the plates were left to rest until the total solidification of the culture medium, incubated in a bacteriological oven at 36ºC for 24 hours and, finally, the Colony Forming Units (CFU) were counted with the aid of a colony counter. The analyzes were performed in duplicate and the results expressed in CFU/mL. For plate duplicates that had counts between 25 and 250 CFU, the simple arithmetic mean value of each duplicate was multiplied by the respective dilution value.

2.7 STATISTICAL ANALYSIS

The data obtained from the NMP for CT, CTT and MAB were subjected to analysis of average comparison by the Tukey test at 5% probability, using the SAS statistical package.

3 RESULTS AND DISCUSSION

The microbiological results for the coconut water samples were interpreted according to Resolution RDC No. 12, of January 02, 2001, of the Ministry of Health, which establishes a tolerance limit for coconut water of up to 100 MPN/mL of thermotolerant coliforms. With regard to total coliforms, the current Brazilian legislation does not establish a limit for any type of food, and its classification as satisfactory/unsatisfactory was based on the standard used for thermotolerant coliforms, allowed in up to 100 MPN/mL. The presence of *E. coli* was confirmed by carrying out biochemical tests, showing a positive result in some samples of coconut water. The presence of mesophilic aerobic bacteria was also verified in all analyzed samples.

For the determination of total coliforms in the present study, it was demonstrated that 100% of the samples (24/24) were contaminated by this group of bacteria. Total coliform MPN ranged from $4.97x10^4$ MPN/mL to $1.18x10^5$ MPN/mL, with no statistical difference (0.05> p) between the studied samples. Regarding the months in which the samples were collected, the values ranged from $3.15x10^4$ MPN/mL to $1.27x10^5$ MPN/mL, also showing no statistical difference (p>0.05). (Table 1). Although the Brazilian legislation does not establish a standard limit of total coliforms in food, we used as a reference value the same adopted for thermotolerant coliforms, therefore, it was verified that all samples presented contaminations above the established standards, since the the level of contamination found in the analyzed samples was greater than 100 NMP/mL, being classified as unsatisfactory for human consumption.

Total coliforms are one of the main groups of bacteria used as parameters in microbiological research on the occurrence of pathogenic microorganisms in food and beverages. Since this group of bacteria is considered an important indicator of hygienic-sanitary conditions. As evidenced, the low microbiological quality of the analyzed samples presented by the high number of total coliforms indicate deficient hygienic-sanitary practices at the place of sale, of the machinery and utensils used for the commercialization, opening and manipulation of the coconut, as well as the non-adoption of the good practices by the handler for handling the product (Valiatti *et al.*, 2017; Fernandes *et al.*, 2019; Silva *et al.*, 2021a).

Table 1 – Most Probable Number (MPN) of Total Coliforms (TC), Thermotolerant Coliform (TTC), and counting of mesophilic aerobic bacteria (MAB) in samples of coconut water sold bay street vendors in Sobral - Ceará

Collect	Coliforms (MPN/mL)		Mesophilic Aerobic Bacteria (UFC/mL)
	Total	Thermotolerant	
Score			
A	$6.04x10^4$a	$8.39x10^2$a	$6.73x10^4$a
B	$4.97x10^4$a	$9.42x10^2$a	$3.12x10^4$a
C	$1.18x10^5$a	$8.86x10^2$a	$5.32x10^4$a
D	$6.35x10^4$a	$1.28x10^3$a	$6.35x10^4$a
p-value	0.0557	0.6104	0.0919

Collect	Coliforms (MPN/mL)		Mesophilic Aerobic Bacteria (UFC/mL)
	Total	Thermotolerant	
Period			
May	$3.15x10^{4}$a	5.65x10b	$2.00x10^{4}$b
July	$1.27x10^{5}$a	$1.28x10^{3}$ab	$5.48x10^{4}$ab
September	$5.44x10^{4}$a	$1.66x10^{3a}$	$5.82x10^{4}$ab
November	$4.23x10^{4}$a	$1.01x10^{3}$ab	$8.10x10^{3}$b
January	$9.07x10^{4}$a	$9.92x10^{2}$ab	$9.07x10^{4a}$
March	$9.07x10^{4}$a	$9.17x10^{2}$ab	$9.10x10^{4}$a
p-value	0.0459	0.0472	0.0008

Source: Research data.

Regarding this group of microorganisms, Silva *et al*. (2017), found 62.5% (5 samples) of coconut water sold in the municipality of Aracaju, Sergipe, contaminated by total coliforms, suggesting failure in the application of good practices for the bottling and commercialization of coconut water. However, Jesus *et al*. (2018) found these microorganisms in 100% of the coconut water samples analyzed at points of sale in the municipality of Ouro Preto do Oeste, Rondônia. Results presented by Silva *et al*. (2021a), who, when analyzing the microbiological quality of coconut water commercialized in Sinop, Mato Grosso, the authors observed that of the 18 samples evaluated, all (100%) presented contamination by total coliforms, being nine samples (50%) with enumeration greater than $1.1x10^{3}$NMP/mL.

As for thermotolerant coliforms, 100% of the samples (24/24) showed MPN values ranging from $8.39x10^{2}$ MPN/mL to $1.28x10^{3}$ MPN/mL with no statistical difference ($p>0.05$) between the four points analyzed, therefore, considered unfit for consumption in accordance with current Brazilian legislation, which recommends tolerable values of thermotolerant coliforms in food products, such as coconut water, maximum permitted tolerance of up to 100 MPN/mL (Brasil, 2001) (Table 1).

For the months of collection, there was a significant difference ($p<0.05$) in relation to the presence of microorganisms, with values of 5.65x10 MPN/mL in May and $1.66x10^{3}$ MPN/mL in September, this factor may have been determined by the rain. There was rainfall of 98.6 mm in May, while in September 0.0 mm (FUNCEME, 2022). The collection was carried out close to rainy days, with the presence of rain the temperature is lower, reducing the microbial proliferation.

The group of thermotolerant coliforms are gram-negative bacteria, indicators of fecal contamination, in the form of bacilli, oxidase-negative, characterized by the activity of the enzyme-galactosidase, being able to grow in media containing surfactants and ferment lactose at temperatures of 44- 45 °C, with production of acid, gas and aldehyde. Therefore, the detection of these enterobacteria in the coconut water samples studied, shows a sign of fecal contamination, indicating that there was, in the product or during the process, direct or indirect contact with human feces

or warm-blooded animals, characterizing a deficiency in the manipulation or poor hygiene of the raw material, therefore presenting epidemiological importance as a cause of diseases in humans (Moura *et al.*, 2007; Silva *et al.*, 2021a; Silva *et al.*, 2022).

Studies carried out by Dias *et al.* (2015) in Vitória da Conquista, BA and by Lima; Silva (2019) in the Federal District corroborate this finding, so that the results of their work point to the contamination of coconut water by coliforms thermotolerant. The results found differ from similar works such as the one described by Souza; Souza (2019) who evaluated the microbiological quality of 09 samples of green coconut water sold at kiosks in Praça do Coco on the edge of the city of Macapá, Amapá, and observed that only 11.11% (1/9) of the coconut water samples was unfit for consumption, since it had a value greater than $1x10^2$ MPN/mL. In this perspective, the scientific literature contains several studies with similar results, whose levels of thermotolerant coliforms are within the limits established by legislation (Dias *et al.*, 2022).

The number of mesophilic aerobic bacteria showed values ranging from $2.0x10^2$ CFU/mL to $9.4x10^4$ CFU/mL (Table 1). The identification of this group of microorganisms in samples of coconut water does not make it inappropriate for human consumption. However, when present in high numbers in foods, these agents can cause deterioration and/or reduced shelf life. Although, not being mandatory analyzes by legislation, mesophilic aerobic microorganisms were determined for the evaluation of the hygienic-sanitary conditions of the analyzed samples (Fortuna; Fortuna, 2008; Silva *et al.*, 2009).

Thus, the results found showed that (100%) of the samples presented high values of mesophilic aerobic bacteria, signaling the use of contaminated materials for coconut cutting, as well as inadequate processing and inefficient storage in terms of time and temperature performed by street vendors. Observing the results presupposes that street vendors are unaware of proper coconut handling and sanitation practices. It is also important to note that the vast majority of carts do not have a potable water system for cleaning the equipment and utensils used, as well as the personal hygiene of the seller, resulting in product contamination.

Based on these data, it appears that effective hygienic-sanitary control measures must be taken immediately to reduce this contamination, as the presence of these microorganisms reveals the need for interventions due to the potential for multiplication when transferred to coconut water, which could endanger the health of the consumer.

Regarding the months of collection, there was a significant difference ($p<0.05$) for the presence of mesophilic aerobic bacteria, between January $9.07x10^4$ CFU/mL, march $9.10x10^4$ CFU/mL, may $2.00x10^4$ CFU/mL and November $8.10x10^3$ CFU /mL. This difference may have been defined by the amount of rain in this period, which was: 102.8 mm, 165.7 mm, 98.6 mm, and 15.5 mm to January, march, May and November, respectively (FUNCEME, 2022). Aerobic mesophilic bacteria grow at a room temperature of 25 to 45°C, with an ideal temperature change of 30°C (Tortora *et al.*, 2012). In the months of January and march, when more rainfall was observed, the temperature was close to 30°C, thus increasing the proliferation of these microorganisms.

Costa *et al.* (2005), in a work on the microbiological evaluation of samples of coconut water preserved by different processes, showed counts of mesophilic aerobic microorganisms below 10 CFU/mL. Showing good hygienic-sanitary conditions in the various stages of processing, adequate clea-

ning and sanitizing operations of equipment and utensils. On the other hand, Fortuna; Fortuna, 2008, when evaluating the microbiological and hygienic-sanitary quality of coconut water sold in street carts in the municipality of Teixeira de Freitas (BA), found that of the 32 samples of coconut water analyzed, 25 (78.1%) there was growth of mesophilic aerobic bacteria.

Of the twenty-four samples analyzed, 133 bacterial strains were identified, all belonging to the Enterobacteriaceae family. This is considered one of the most relevant bacterial families, being present in soil, water and vegetation. Some species are commensal, that is, they are part of the normal microbiota of most animals, including humans. While other species are always associated with human disease, accounting for a third of all cases of bacteremia, over 70% of urinary tract infections and many intestinal infections (Zaniolo *et al*., 2018).

Of the 133 strains isolated from coconut water (100%), 45 (33.8%) refer to *Serratia liquefaciens*, 26 (19.5%) *Klebsiella pneumonia*, 16 (12.0%) *Enterobacter* sp, 10 (7,5%) *Serratia marcesens*, 10 (7.5%) *Yersinia enterocolitica*, 9 (7%) *Proteus mirabllis*, 6 (4.5%) *Hafnia alvei*, 4 (3%) *Escherichia coli*, 4 (3%) *Providencia alcalifaciens* and 3 (2.2%) *Citrobacter freundii*.

The genera *Serratia*, *Enterobacter*, *Hafnia*, *Providencia* and *Proteus* are microorganisms present in nature, being also found in the intestinal flora of man and other warm-blooded animals. However, these pathogens are also opportunistic beings, since, once they find favorable conditions in the host, they are major causes of problems in debilitated patients (Rios *et al*., 2020). Other genera of great clinical importance, such as *Klebsiella*, *Escherichia*, *Yersinia* and *Citrobacter*, are also pathogens harmful to health, as they are etiological agents of great occurrence, being responsible for numerous infections in humans and also in animals, arousing the interest of public health (Albuquerque *et al*., 2020).

5 CONCLUSION

Based on the results obtained from the microbiological analysis of coconut water, it was concluded that all the samples collected showed a high rate of contamination by total and thermotolerant coliforms, in addition to the presence of mesophilic aerobic bacteria, indicating that they are in disagreement with current elegislation. In view of the results presented, it is suggested the need for improvements in the training of street vendors regarding good handling practices that prevent contamination of coconut water, in addition to the importance of active inspection by inspection bodies to improve handling conditions. and selling this product.

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