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ORIGINAL ARTICLE

MICROBIOLOGICAL QUALITY OF SUGARCANE JUICE SOLD IN GOIÂNIA, GOIÁS, AND RISKS TO PUBLIC HEALTH

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ABSTRACT

Sugarcane juice has a pleasant flavor, is easy to process, and is well-accepted by the general population. However, microbiological quality is not always assured, which can result in risks for consumption. The objective of this study was to evaluate the microbiological quality of sugarcane juice obtained from commercial establishments in the city of Goiânia. Sugarcane juice samples were collected from 21 locations between February and July 2018. The analysis was performed using the multiple tube technique, and quantification by the most probable number (MPN) of microorganisms present in each sample. The microbiological analysis investigated total and thermotolerant coliforms, in addition to Salmonella, as recommended by current legislation. Total coliforms were found in 100% of the samples, with a count >1600 MPN/mL in 57.1%. For thermotolerant coliforms, 100% of the samples were positive, with 33.3% being above the legal limit and 19.1% being positive for *Escherichia coli*. Among the 21 samples evaluated, one was positive for *Salmonella* ser. Typhimurium. The quantity and quality of microorganisms isolated in the sugarcane juice samples sold in the city of Goiânia indicate the need to improve the hygienic and sanitary quality of the establishments. There is an urgent need to establish training courses for traders in this sector to provide guidance on the correct way to process, store, and transport food and raw materials, which will guarantee the quality of the food and protect the health of the consumers.

KEY WORDS: Most probable number; multiple tubes; coliforms; Salmonella.

INTRODUCTION

In today's world, people's lifestyles, lack of time, and convenience are factors that contribute to meals being eaten in commercial establishments. However, this practice has led to an increase in the number of outbreaks of

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foodborne diseases (Froder et al., 2021), and concerns about food safety are growing (Souza et al., 2015). Sugarcane juice, or "garapa" is a popular Brazilian energy drink, rich in sugars, minerals, and vitamins A, B1, and B2, retaining sugarcane's natural nutrients (Hentschel, 2009).

Sugarcane juice has a high energy value and is widely consumed by the population, especially in tropical and subtropical regions of the country, particularly in the summer. The lack of information from handlers and the precarious hygiene and sanitation of the places where it is sold contributes to this food's microbiological and parasitic contamination (Pina et al., 2018). Limited information is available on the safety of popular fresh juices sold on the street, which can cause diseases when prepared improperly, despite the health benefits of consuming fresh fruit juices (Issa-Zacharia & Rwabunywenge, 2023). In addition to food, water is also an important source of contamination, which can result in Waterborne and Foodborne Diseases (WTD).

Over 250 types of Diseases Transmitted by Hydric and Alimentary Means (DTHA) are known worldwide, caused by bacteria and their toxins, viruses, opportunistic intestinal parasites, or chemical substances (Brasil a, 2024). According to the Centers for Disease Control and Prevention (CDC), each year, 48 million people become ill due to DTHA, 128,000 are hospitalized and 3,000 die (CDC, 2018).

According to data from the Ministry of Health in Brazil, between 2007 and 2020, 662 DTHA outbreaks were reported per year, involving 156,691 patients (average of 17 patients/outbreak), 22,205 hospitalized, and 152 deaths (Brasil b, 2024).

In a study carried out in Brazil in 2019, between 2000 and 2018, 13,163 DTHA outbreaks were reported to the Health Surveillance Secretariat, with an estimated exposure of 2,429,220 individuals, 247,570 patients, and 195 deaths. Water was the vehicle most frequently associated with the outbreaks, followed by mixed foods, multiple foods, and eggs (Finger et al., 2019).

Another study was carried out in Brazil comparing data before (2018/2019) and during (2020/2021) the COVID-19 pandemic; from 2018 to 2021, 2,206 cases of DTAH were recorded. The fatality rate was 0.5% in both periods. The incidence rate was 6.48 per 100,000 inhabitants in the first period and 3.92 per 100,000 inhabitants in the second period evaluated (Nepomuceno et al., 2024).

The DTHA can be considered an outbreak when two or more people or one unusual case or isolate cases present similar clinical symptoms after ingesting food and/or water from the same source (Brasil, 2010; Correia et al., 2024), and its severity will depend on the microorganism present and the immunological condition of the affected person, especially in the age group of children under five years old, immunocompromised individuals and the

elderly. Among the important bacteria in this type of disease are *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp., *Bacillus cereus*, and *Clostridium perfringens* (Brasil, 2010). Thus, the present study aimed to evaluate the hygienic-sanitary quality of sugarcane juice sold by street vendors in the city of Goiânia, State of Goiás, under current Brazilian legislation.

MATERIAL AND METHODS

Study area and samples

The city of Goiânia is divided into seven regions: North, Northwest, South, Southeast, Center, East, and West. Samples were collected from three different neighborhoods in each region, totaling 21 sugarcane juice samples. The neighborhoods were chosen by drawing lots. A questionnaire was administered to the salesperson at all points of sale, and the collections were carried out between February and July 2018.

Sample collection

Sterilized Erlenmeyer flasks with a capacity of 300 mL were used to collect sugarcane juice samples. Two hundred mL of sugarcane juice were collected from each establishment, and the samples were transported in an isothermal container to the Laboratory of Anaerobes, Phenotyping and Molecular Biology of the Tropical Pathology and Public Health Institute of the Federal University of Goias (LAFEBIM/IPTSP/UFG), where they were processed.

Sample processing and analysis

The methodology used to quantify coliforms and detect *Salmonella* in food was based on the Compendium of Methods Microbiological Examination of Foods -APHA/American Public Health Association (APHA, 2001).

For coliform testing, a 25 mL aliquot of the sample was transferred to an Erlenmeyer flask containing 225 mL of sterile peptone water (dilution 10^{-1}). From this dilution, serial dilutions were made up to 10^{-3} with peptone water.

To determine the most probable number (MPN/mL) of total and thermotolerant coliforms, 1 mL aliquot of each dilution was transferred to a series of five tubes containing 9 mL lactose broth with an inverted Duran tube. The tubes were incubated at 35-37 °C for 24 hours, this stage being known as presumptive. From the tubes with positive readings, turbidity, and gas formation, the confirmatory test stage for total coliforms was performed in

brilliant green broth at 35-37 ºC for 24-48 hours. To determine thermotolerant coliforms, the stage was performed in *E. coli*-EC broth, at 45 ºC for 24-48 hours. The MPN/mL values were calculated according to the FUNASA Manual (Brasil, 2014).

To confirm the presence of *E. coli*, a loop of the tubes containing EC broth that presented turbidity with gas production inside the Duran tube was plated on Petri dishes containing eosin methylene blue (EMB) agar. The plates were incubated at 35-37 ºC for 24 hours in aerobic conditions. EMB agar is selective for gram-negative enteric bacteria, and *E. coli* colonies may present a characteristic metallic green reflection due to the rapid fermentation of lactose. The colonies with a metallic appearance were subjected to biochemical tests to confirm the species.

To detect bacteria of the genus *Salmonella*, the pre-enrichment stage was initially performed, where 25 mL of the sample was placed in an Erlenmeyer flask containing 225 mL of lactose broth, which was incubated for 24 hours at 35-37 °C in aerobiosis. After this period, 1 mL of the pre-enriched medium was transferred to a test tube containing 9 mL of tetrathionate broth, which was incubated for 24 hours at 35-37 °C in aerobiosis, the selective enrichment stage. For better selectivity, two drops of 0.1% brilliant green solution and four drops of iodine-potassium iodide solution were added to inhibit the growth of thermotolerant coliforms and bacteria of the genus *Proteus*. Once incubation was complete, a swab was seeded by exhaustion on a plate with *Salmonella-Shigella* (SS) agar, which is selective for these bacterial genera. Colonies with a blackened appearance were subjected to biochemical tests to confirm the genus. Upon confirmation of the genus, samples identified as *Salmonella* by biochemical tests were sent to the National Reference Center for Bacterial Enteroinfections of FIOCRUZ for serology.

The biochemical tests used to identify enterobacteria were glucose fermentation and gas production; lactose fermentation; motility; oxidase; lysine decarboxylation; production of hydrogen sulfide, indole, urease, phenylalanine deaminase, and gelatinase; and citrate utilization (Brasil, 2013).

Statistical Analysis

The results were entered into Microsoft Excel® spreadsheets, where descriptive statistical analysis and the chi-square test were subsequently performed, with a significance level of $p \leq 0.05$.

Ethical aspects

The project was submitted to and approved by the Ethics Committee of the Clinical Hospital of the Federal University of Goias, under nº. 1,545,514.

RESULTS

Among the locations where sugarcane juice was collected, 52.4% (11/21) were fixed sales locations, such as kiosks or stalls, and 47.6% (10/21) were mobile or temporary stands, meaning they lacked a permanent structure and were often improvised. All the sales locations that participated in the project did not fully apply the Technical Regulation of Hygienic-Sanitary Procedures for the Marketing of Vegetable-Based Foods and Beverages (Brasil, 2001) (Table 1).

Table 1. Responses obtained from 21 sugarcane juice sellers who participated in the survey in the city of Goiânia, Goiás.

Quiz Questions	YES	NO
Wore gloves?	42.9% (9/21)	57.1% (12/21)
Wore a cap?	4.8% (1/21)	95.2% (20/21)
Wash your hands before preparing the juice?	66.7% (14/21)	33.3% (7/21)
The point had access to water?	33.3% (7/21)	66.7% (14/21)
The same person handled food and money?	90.0% (19/21)	10.0% (2/21)
Some type of training regarding proper food handling has already been completed?	0.0% (0/21)	100.0% (21/21)
Knew the Technical Regulation of Hygienic-Sanitary Procedures for the Marketing of Vegetable-Based Foods and Beverages?	0.0% (0/21)	100.0% (21/21)

The total coliform group was detected in 100% (21/21) of the analyzed samples, with a count >1,600 MPN/mL in 57.1% (12/21) of the samples. In the confirmatory test of the dilutions for thermotolerant coliforms, 100% (21/21) positivity was found, of which 33.33% (7/21) had results above that permitted by RDC nº. 12 of January 2, 2001 (Brasil, 2001), which is 10^2, and the other 66.67% (14/21) were within the acceptable range. Among the samples positive for thermotolerant coliforms, 19.1% (4/12) were confirmatory for the presence of the species *E. coli*. In the research for bacteria of the genus *Salmonella* spp., one sample was positive (Table 2), identified by serology as *Salmonella* ser. *Typhimurium* (Report nº 247/18-B).

Table 2. Results of total coliforms, thermotolerant coliforms (NMP/mL), presence of *Escherichia coli* and *Salmonella* spp. in 21 samples of sugarcane juice from the city of Goiânia, Goiás.

Sample	Point type	Total Coliforms (MPN/ mL))	Thermotolerant coliforms (MPN/mL)	Presence of *Escherichia coli*	Presence of *Salmonella* spp.
1	Fixed	>1600	>1600	Absent	Absent
2	Fixed	>1600	<1.8	Absent	Absent
3	Fixed	>1600	<1.8	Absent	Absent
4	Fixed	>1600	<1.8	Absent	Absent
5	Not fixed	>1600	>1600	Present	Absent
6	Fixed	>1600	<1.8	Absent	Absent
7	Fixed	>1600	<1.8	Absent	Absent
8	Not fixed	>1600	<1.8	Absent	Absent
9	Not fixed	>1600	<1.8	Absent	Absent
10	Fixed	350	<1.8	Absent	Present
11	Not fixed	1600	31	Absent	Absent
12	Not fixed	>1600	430	Present	Absent
13	Not fixed	24	21	Present	Absent
14	Not fixed	9.3	<1.8	Absent	Absent
15	Fixed	920	31	Absent	Absent
16	Fixed	430	130	Absent	Absent
17	Not fixed	>1600	>1600	Absent	Absent
18	Not fixed	23	23	Absent	Absent
19	Not fixed	23	23	Absent	Absent
20	Not fixed	>1600	>1600	Present	Absent
21	Fixed	350	350	Absent	Absent

When comparing the presence of thermotolerant coliforms with the responses obtained from the questionnaire, no significant difference was found between the locations evaluated and the microbiological aspects ($p > 0.05$) (Table 3).

Table 3. Statistical analysis of the presence of thermotolerant coliforms in sugarcane juice and the responses provided by handlers during the preparation of sugarcane juice in the city of Goiânia, Goiás.

Presence of Thermotolerant Coliforms	Wore gloves		Use of cap		Wash your hands before preparing		Access to water		Handling food and money		Fixed Point	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Above	2	5	0	7	4	3	1	6	7	0	3	4
Within	7	7	1	13	10	4	6	8	12	2	7	7
X^2 Test	p= 0.349		p= 0.468		p= 0.513		p= 0.190		p= 0.293		p= 0.757	

However, when comparing the presence of *E. coli* in the sugarcane juice with the responses obtained from the questionnaire, a statistically significant difference ($p < 0.05$) was found for hand washing before preparing the sugarcane juice and whether the sales point was fixed or mobile. Among the 14 individuals who practiced hand washing, 64.30% (9/14) used only water, 28.57% (4/14) used soap, and 7.14% (1/14) used 70% alcohol (Table 4). At the locations where *E. coli* was detected, in one instance, hygiene was performed using only water, and in three instances, no hand hygiene was performed.

Table 4. Statistical analysis of the presence of *Escherichia coli* in sugarcane juice and the responses provided by handlers when preparing sugarcane juice in the city of Goiânia, Goiás.

Presence of *Escherichia coli*	Glove use		Use of cap		Wash your hands before preparing		Access to water		Handling food and money		Fixed Point	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Yes	0	4	0	4	1	3	0	4	4	0	0	4
No	9	8	1	16	13	4	7	10	15	2	10	7
X^2 Test	p= 0.054		p= 0.619		p= 0.049		p= 0.116		p= 0.471		p= 0.034	

DISCUSSION

A high count of total coliforms was detected in the analyzed sugarcane juice samples, indicating contamination from the environment or the intestinal microbiota of animals or humans. According to the presence of this type of bacteria in food, it may indicate unsatisfactory microbiological quality, proving the hygienic-sanitary deficiency in the process of obtaining sugarcane juice (Silva et al., 2010; Anjos et al., 2024). According to Santos et al. (2021), sugarcane juice exhibits high carbohydrate levels, facilitating the growth of pathogenic or spoilage microorganisms. Its shelf life is influenced by both spoilage activity and oxidative processes, potentially causing visual alterations and product rejection.

In the present study, it was observed that in the 21 points visited, 47.6% (10/21) of the locations were fixed. Therefore, they had a better infrastructure to organize the trade. However, it was noted that, although these locations had health licenses, the hygienic-sanitary procedures required by law for food commercialization were not being followed. This may justify the similarity in the high contamination levels in all samples, regardless of the type of establishment. According to Sprenger et al. (2016), the low microbiological quality of sugarcane juices is due to failures from sugarcane harvesting to the production and storage of sugarcane juice. These breaches of hygiene protocol include the use of dirty raw materials, use of unknown water sources, lack of personal hygiene and poor hygiene habits among producers, handling of money without subsequent hand hygiene, lack of hygiene of utensils used throughout the production process, inadequate packaging of the product, exposure of raw materials and products to large temperature variations and lack of protection of sales locations against physical and biological hazards.

66.7% (14/21) of the surveyed establishments did not have access to water at the point of sale, requiring them to bring water from their homes and store it in barrels. Water is an important means of food contamination by pathogenic microorganisms, which can be transferred to food and result in DTHA. There are over 250 types of DTHA globally, caused by bacteria and their toxins, viruses, opportunistic intestinal parasites, or chemical substances (GDF, 2023). According to RDC nº. 218, issued by ANVISA on July 29, 2005, the water used in establishments must be potable and in sufficient quantity to meet preparation needs. Similarly, the ice used in food preparation must be made with potable water and stored in a sanitized container with a lid to prevent contamination (Brasil, 2005). However, during sample collection, it was observed that many water storage locations were unsuitable or did not comply with ANVISA's requirements.

Another source of food contamination is the hands of food handlers, which can serve as vehicles for transmitting microorganisms due to inadequate hygiene, and long nails that harbor dirt. In this study, it was observed that in

90% (19/21) of the establishments, the merchant himself prepared the sugarcane juice, handled money, and managed utensils, sugarcane bagasse, and garbage generated at the place of business. The inadequate conditions observed in this study were also reported by other authors, who found that vendors handled money and food without sanitizing their hands. The lack of information on proper hand sanitization can lead to foodborne illnesses, compromising food quality and consumer safety (Ponath et al., 2016; Sprenger et al., 2016).

In the four establishments where *E. coli* was detected in this study, the handlers did not use gloves or hats, had no access to a water source, handled both food and money, did not have a fixed location, and, of the four, only one washed their hands before preparing the sugarcane juice, despite using only water for this procedure. The fact that the establishment was not fixed and that the handler lacked access to a bathroom may have contributed to the contamination, along with poor hand hygiene before preparing the sugarcane juice.

According to Resolution nº. 216 of September 15, 2004, food handlers must wash their hands thoroughly upon arriving at work, before and after handling food, after any interruption in work, after touching contaminated materials, after using the toilet, and whenever necessary. Hands must be cleaned with soap and water, in addition to antisepsis with a chemical substance that has an antiseptic effect (Brasil, 2004). In the present study, only one food handler reported using 70% alcohol for hand antisepsis, while the others did not follow this practice.

In the present study, *Salmonella* ser. Typhimurium was detected in one sample, which demonstrates the need to monitor the quality of this type of food. The legislation recommends the total absence of bacteria of the genus *Salmonella* spp*.,* as this bacterium causes serious foodborne infections since most serotypes are pathogenic for consumers (Brasil, 2001). According to the Ministry of Agriculture and Livestock (Brasil, 2023), the genus *Salmonella* includes two species: *S. enterica*, with 2,610 serovars, and *S. bongori* with 23 serovars. The species *S. enterica* is subdivided into six subspecies: *S. enterica*, *S. arizonae*, *S. diarizonae*, *S. houtenae*, and *S. indica*. Among these subspecies, *S. enterica enterica* is the most important for human and animal health, as it includes the serovars Gallinarum (Gallinarum and Pullorum biovars), Enteritidis, and Typhimurium, the latter two having a major public health impact. *Salmonella* can cause food poisoning, severe infections, and even death. It can be present in the intestinal tract of birds, reptiles, and occasionally insects. Therefore, the raw materials used to prepare sugarcane juice have numerous sources of contamination, highlighting the need for careful handling and proper storage of sugarcane until it is used, as noted by Sprenger et al. (2016).

Implementing stringent hygiene and quality control protocols across all production stages can successfully mitigate bacterial contamination in

sugarcane juice, including potentially pathogenic microorganisms (Santos et al., 2021). The quantity and quality of microorganisms isolated from sugarcane juice samples in Goiânia suggest suboptimal hygiene and sanitary practices, emphasizing enhanced quality control measures to safeguard consumer health.

There is an urgent need for training courses for traders in this sector to guide the correct way to process, store, and transport food and raw materials under current legislation, as 100% of those interviewed stated they were unaware of current legislation and had not received any prior training on proper food handling. All of these actions are essential to raise awareness among sellers of fresh products, ensuring food quality and protecting consumer health.

CONFLICT OF INTEREST

There is no conflict of interest involved.

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