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Microbiological and physicochemical quality assessment of fresh orange juices

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Abstract
Economic instability in Brazil has led to the growth of informal commerce, particularly street vending of orange juice. However, the lack of regulation and adequate sanitary conditions poses public health risks. This study aimed to evaluate the microbiological and physicochemical quality of fresh orange juices sold by street vendors in Goiânia, Goiás, Brazil. This observational cross-sectional study, conducted between August and November 2023, involved the collection of 60 fresh, non-pasteurized orange juice samples, which were analyzed based on the guidelines outlined in RDC No. 331/2019 and NI No. 60/2019. The assessment revealed significant non-compliance with regulatory standards. Elevated levels of thermotolerant coliforms (46.67%), molds and yeasts (40%), and Salmonella spp. (21.67%) indicate inadequate hygiene and handling practices. Additionally, failures in density (56.66%), °Brix (45%), ascorbic acid content (51.66%), and suspended solids (55%) compromise the juices' sensory, nutritional, and safety standards. These deficiencies result from poor-quality raw materials, adulteration, and improper processing. Stricter oversight, the implementation of good manufacturing practices, and food handler education are necessary to ensure compliance with safety regulations and protect consumers.

Keywords: Quality, orange juice, street vendors, Goiânia, Goiás, Brazil

Introduction

The growing economic instability in Brazil has posed significant challenges to the population, directly affecting the subsistence conditions of many individuals, particularly within the most vulnerable classes [1]. In response, the search for viable survival alternatives has intensified, with informal commerce, particularly street vending, becoming a key source of income for many workers. However, this type of commerce faces substantial challenges, such as a lack of regulation and inspection, raising concerns about the quality and safety of the products sold [2].

The exponential growth of street vending has contributed to income generation, but it has also raised concerns regarding the safety of the products offered, especially in the food sector. The lack of guarantees regarding the quality and origin of the products sold, particularly fresh orange juices, has become a growing concern [3]. These juices are popular due to their low production cost and widespread acceptance, making them a common option in the informal market and an economic opportunity for vendors [4].

However, juices sale without proper regulation and quality control poses risks to public health. While oranges are widely recognized for their nutritional benefits and appeal as a fresh product, fresh orange juices sold informally lack guarantees regarding their physicochemical and microbiological quality, exposing consumers to potential contamination [5]. Additionally, the lack of proper infrastructure for storage and preparation, combined with poor hygienic and sanitary conditions, facilitates the occurrence of foodborne diseases, making it a significant concern for public health authorities [6].

The street vending environment, often characterized by hot temperatures and constant exposure to environmental agents, fosters the development of pathogenic microorganisms that can compromise food safety [7]. Improper food handling and the lack of temperature control, crucial for maintaining the quality of the juice, increase the risk of cross-contamination. The use of non-hygienic utensils and exposure to the intense heat of the streets contribute to the heightened risk, potentially leading to outbreaks of foodborne illnesses [8].

Therefore, it is urgent to implement measures to ensure the physicochemical and microbiological quality of fresh orange juices sold informally, in order to protect public health [9]. Ensuring adequate hygienic and sanitary conditions and raising awareness among vendors about the importance of safe food handling are essential steps to mitigate health risks and promote safer and more responsible business practices [10]. In this regard, this study aimed at assessing the microbiological and physicochemical quality of fresh orange juices sold by street vendors in Goiânia, Goiás, Brazil.

Methodology

This observational cross-sectional study employed both qualitative and quantitative approaches and was conducted between August and November 2023. Sixty samples of fresh, non-pasteurized orange juice, bottled and sold by street vendors in the central region of Goiânia, Goiás, Brazil, were collected.

Specimens were purchased directly from the vendors, stored in polystyrene coolers, and transported to the Physical Chemistry Laboratory at Centro Universitário Alves Faria (UNIALFA). Subsequently, they were transferred to the Microbiology Laboratory at the State University of Goiás (UEG - Itumbiara Campus) for analysis. According to vendors, all samples were refrigerated, freshly prepared, and within their expiration dates.

The laboratory analyses were performed in accordance with the guidelines established by the Collegiate Board Resolution (RDC) No. 331 [11] and Normative Instruction (NI) No. 60 [12], both issued on December 23, 2019. Microbiological evaluations included the enumeration of molds and yeasts (Colony Forming Units - CFU), determination of the most probable number (MPN) of thermotolerant coliforms, and detection of *Salmonella spp.*, following methodologies described by Salfinger and Tortorello (2015) [13]. The physicochemical analyses assessed parameters such as density at 20°C, soluble solids content (°Brix), total sugars (%), acidity as citric acid, °Brix/acidity ratio, ascorbic acid (vitamin C) content (mg/%), and suspended solids (%), according to protocols outlined by the Adolfo Lutz Institute (2008) [14].

Results were presented as absolute and relative frequencies, means and standard deviations. Statistical analyses were conducted using GraphPad Prism software version 5.01 for Windows. Differences between groups were evaluated using the chi-square test, with statistical significance set at $P<0.05$.

Results and Discussion

The analysis of microbiological quality revealed significant non-compliance with the standards established by RDC No. 331/2019 [11] and NI No. 60/2019 [12] for ready-to-drink beverages. The results highlight major sanitary challenges and potential public health risks (Table 1). Regarding molds and yeasts, 40% (n=24) of the analyzed samples exhibited counts exceeding 10^3 CFU/mL, significantly surpassing the tolerable limit for fresh juices without added preservatives ($P<0.05$). Such elevated levels can compromise product stability, promote early spoilage, and, in some cases, trigger allergic reactions or gastrointestinal irritations in susceptible individuals [3,4]. Similarly, approximately 46.67% (n=28) of the samples had concentrations exceeding 10 MPN/mL of thermotolerant coliforms, surpassing the acceptable microbiological limit ($P<0.05$). This contamination indicates inadequate hygiene conditions during the handling or storage of the products [6]. Moreover, the presence of thermotolerant coliforms above the permitted level represents a robust indicator of fecal contamination and potential dissemination of enteropathogenic pathogens, emphasizing the need for sanitary interventions in the production chain [7].

Table 1: Microbiological quality assessment of fresh orange juices sold by street vendors in Goiânia, Goiás, Brazil, between August and November 2023. *$P<0.05$, chi-square test.

Microorganisms *	*n*	%
Molds and Yeasts		
adequate samples (< 10^3 CFU/mL)	36	60
inadequate samples (> 10^3 CFU/mL)	24	40
Thermotolerant coliforms		
adequate samples (< 10 MPN /mL)	32	53.33
inadequate samples (> 10 MPN /mL)	28	46.67
Salmonella spp.		
adequate samples (absence in 25mL)	47	78.33
inadequate samples (presence in 25mL)	13	21.67

Regarding *Salmonella spp.*, the presence of this pathogen was detected in 21.67% (n=13) of the samples, contradicting the absence requirement established by national regulations. Although 78.33% (n=47) of the samples complied, detecting *Salmonella* in any quantity constitutes a critical violation of food safety standards, as it is associated with severe gastrointestinal diseases, potentially progressing to systemic conditions in at-risk groups such as children, the elderly, and immunocompromised individuals [15]. These findings highlight structural and operational deficiencies in hygiene and handling practices during the preparation and commercialization of orange juices by street vendors. Furthermore, results suggest the need for increased oversight by health authorities and educational initiatives aimed at food handlers to ensure compliance with current regulations. Exposing consumers to products with inadequate microbiological parameters may lead to foodborne illness outbreaks (FBOs), directly impacting public health and overburdening local healthcare services.

The physicochemical quality parameters also revealed significant failures in the production and handling of orange juices sold by street vendors in Goiânia (Table 2). Concerning density at 20°C, 56.66% (n=34) of the samples showed values incompatible with those expected for fresh orange juices [11, 12]. This alteration may be attributed to water addition, a practice that reduces not only nutrient concentration but also the product's energy value. Inadequate density compromises juice authenticity and may mask other fraudulent practices [7].

Table 2: Physicochemical quality assessment of fresh orange juices sold by street vendors in Goiânia, Goiás, Brazil, between August and November 2023. **P*<0.05, chi-square test.

Parameters *	*N inadequate samples*	%
Density at 20°C	34	56.66
Soluble solids content (°Brix)	27	45
Total sugars (%)	3	5
Acidity (% citric acid)	5	8.33
°Brix/acidity ratio	23	38.33
Ascorbic acid (mg/%)	31	51.66
Suspended solids (%)	33	55

Regarding soluble solids content (°Brix), 45% (n=27) of the samples had values below the recommended standard. This inadequacy suggests the use of under-ripe fruits or juice dilution, resulting in products with lower sweetness and reduced concentrations of bioactive compounds. Reduced soluble solids directly impact the product's sensory quality, negatively affecting consumer acceptance. Total sugar content (%) was deemed inadequate in 5% (n=3) of the samples. Although this percentage is relatively low, significantly reduced values may compromise sensory experience and indicate adulteration. Inadequate sugar levels may also reflect poor-quality raw materials or alterations during product processing [16].

Acidity (% citric acid) was inadequate in 8.33% (n=5) of the analyzed samples. Values outside the ideal range may result from using unripe fruits or early fermentation processes. Excessive acidity, when frequently consumed, may cause gastrointestinal discomfort, especially in individuals predisposed to conditions such as gastritis or gastroesophageal reflux [17]. The °Brix-to-acidity ratio revealed that 38.33% (n=23) of the samples exhibited significant imbalance (P<0.05). This ratio is an important indicator of the harmony between the sweet and acidic flavors of the juice. Inadequacy in this parameter compromises sensory acceptance and suggests flaws in fruit selection or adulterations during processing [18].

Ascorbic acid levels (mg/%) – an essential parameter for nutritional evaluation – were below the recommended level in 51.66% (n=31) of the samples. This reduction may be attributed to degradation during storage or the use of fruits with low vitamin content. Ascorbic acid, being a potent antioxidant, plays a crucial role in maintaining immune health and preventing diseases. A deficiency in this compound compromises the product's nutritional potential [19]. Lastly, 55% (n=33) of the samples exhibited inadequate percentages of suspended solids, evidencing issues with juice consistency and homogeneity. This parameter is directly related to the product's appearance and texture, essential for ensuring good consumer acceptance. Changes in suspended solids may indicate inadequacies in processing, such as insufficient centrifugation or improper mixing [20].

This study's results revealed concerning discrepancies regarding the standards established by Brazilian legislation [11, 12]. These findings align with, but also differ from, previous studies. It was identified that 46.67% of the samples were contaminated with thermotolerant coliforms. This percentage exceeds the 5.7% reported by Ruschel *et al.* (2001) [7] in a study conducted in Porto Alegre. The discrepancy may be attributed to geographic and cultural differences, including handling practices and environmental exposure of the product. In this study, the samples were collected under conditions more prone to contamination, such as locations with high urban traffic and hot climates, which favor bacterial growth. The lack of adequate refrigeration and the use of contaminated utensils, as observed on-site, further reinforce this scenario.

In contrast, Almeida *et al.* (2003) [18] reported acceptable levels of fecal coliforms, suggesting better control in their samples. This difference may be explained by the implementation of superior sanitary practices at the study location, which were not observed among the street vendors evaluated here. The presence of thermotolerant coliforms at such a high proportion is alarming and reflects inadequacies in the sanitation of utensils and fruits used in preparation. Figueredo *et al.* (2009) [21], while studying snack bars in Goiânia, identified 100% microbiological inadequacy for total coliforms, reinforcing that the issue seems linked to the general environment of food handling in the region. Direct comparison suggests that street vendors, often less regulated, are even more exposed to failures in sanitary controls.

The presence of *Salmonella spp.* in 21.67% of the samples in this study is significantly higher compared to the findings of Almeida *et al.* (2003) [18], who did not detect this pathogen in their analyses. This difference could be related to inadequate storage of the fruits used or cross-contamination with infected surfaces and utensils, a recurring issue in unsupervised environments. Additionally, the methodology used by Almeida *et al.* (2003) [18] may have had limitations in detecting this microorganism, whereas the present study employed more sensitive and specific techniques for microbiological identification. Direct contact with *Salmonella spp.* poses a severe public health risk, being associated with outbreaks of foodborne diseases (FBD) [22]. Literature, as reported by Oliveira *et al.* (2006) [4], highlights orange juice as an ideal medium for microbial growth due to its high sugar and carbohydrate content, corroborating this product's vulnerability to pathogens.

The contamination levels of molds and yeasts in this study (40%) are comparable to the 44.23% reported by Ruschel *et al.* (2001) [7]. However, variations in results may be justified by regional and methodological factors. Both studies indicate that inadequate sanitation of fruits and utensils is the main cause of the proliferation of these contaminants, which thrive in environments with high sugar concentrations and poor storage conditions.

Regarding density, soluble solids (°Brix), total sugar content, acidity, and ascorbic acid, this study identified deficiencies in a considerable number of samples, partially aligning with the findings of Brito *et al.* (2005) [23]. These authors reported that 86.66% of natural juice samples showed inadequacies in physicochemical parameters, while 40% of industrialized samples also failed to meet standards. Here, although the proportion is lower, the reduction in ascorbic acid levels in 51.66% of the samples suggests a significant loss of nutritional value, which may be attributed to the use of deteriorated fruits or inadequate processing. Sugai *et al.* (2002) [16] demonstrated that methods such as pasteurization at 87°C preserve physicochemical parameters and extend the shelf life of juices, which could be applied to street vendors with adapted technologies. The absence of such practices among the vendors analyzed may explain the greater degradation of nutritional components observed in this study.

Figueredo *et al.* (2009) [21] identified 63.3% sanitary inadequacies in snack bars in Goiânia, while the present study revealed similar issues among street vendors, but in greater proportion, indicating a direct relationship between the type of establishment and quality standards. While fixed snack bars may be subject to stricter inspection, street vendors often operate under precarious and less regulated conditions, contributing to the higher levels of microbiological and physicochemical inadequacies found here.

Overall, the findings underscore the urgency of implementing public policies emphasizing the training of food handlers and rigorous inspection of the natural juice trade. Interventions such as those suggested by Oliveira *et al.* (2006) [4] and Sugai *et al.* (2002) [16], including training in Good Manufacturing Practices and the application of preservation technologies, such as pasteurization, are crucial to mitigating the risks associated with consuming orange juice sold by street vendors. Furthermore, comparison with previous data indicates that sanitary issues are endemic in food establishments, requiring integrated approaches that consider environmental conditions and handler education to reduce contamination rates and improve the physicochemical quality of the final product.

Conclusion

The microbiological and physicochemical analysis revealed serious regulatory non-compliance, posing public health risks. Prominent levels of thermotolerant coliforms (46.67%), molds, yeasts (40%), and *Salmonella spp.* (21.67%) reflect inadequate hygiene and handling. Additionally, failures in density (56.66%), °Brix (45%), ascorbic acid (51.66%), and suspended solids (55%) compromise the juices' sensory, nutritional, and safety standards. These deficiencies result from poor-quality raw materials, adulteration, and improper processing. Also, stricter oversight, the implementation of good manufacturing practices, and food handler education are necessary to ensure compliance with safety regulations and protect consumers.

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Declaration of interest statement

Authors report no potential conflicts of interest.

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