Original Article

Journal of Food Safety and Hygiene

Journal homepage: http://jfsh.tums.ac.ir

Evaluation of bacteriological quality and safety of sugarcane juice locally processed and vended in Dar es Salaam City, Tanzania

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ARTICLE INFO

Article history:
Received 21 Oct. 2023
Received in revised form
23 Dec. 2023
Accepted 28 Dec. 2023

Keywords:
Bacteriological quality;
Safety;
Sugarcane juice;
Hygienic practices;
Street vendor

ABSTRACT

The prevalence of foodborne illness linked to the intake of freshly squeezed juices sold by street vendors is on the rise, despite the widespread use of these beverages by millions of people in developing nations. Hence, a study was undertaken to evaluate the microbiological standard and safety of locally processed and street-vended sugarcane juices in Dar es Salaam to ascertain their present condition. A total of 60 samples of sugarcane juice were gathered and examined. Street vendors involved in the sugarcane juice business were interviewed followed by physical-chemical and microbiological laboratory analysis. The pH of unpasteurized sugarcane juice was 4.8 and 4.9 for iced and raw, respectively while the pH for pasteurized and pasteurized juice in which citric acid was added were receptively, 4.3 and 3.1. The average level of titratable acidity was 0.083%. The Soluble solids (°Brix) of unpasteurized raw, iced and pasteurized sugarcane juice ranged from 12.2-22.1, 2.4-13.8 and 14.1-15.8. The total plate counts (TPC) of unpasteurized sugarcane juice showed a mean of 5.592 and 5.64 log cfu/mL for raw and iced sugarcane juice, respectively. About 90% of samples were above TBS and Codex recommended maximum limits of 3.7 to 4 log cfu/mL or 5×10^3-10^4 cfu/mL. Unpasteurized raw and iced sugarcane juice were contaminated with 1.79 and 2.10 log cfu/mL of *E. coli* while no typical *Salmonella spp.* was detected in all 60 samples. The study concluded that the microbiological quality and overall handling practices associated with unpasteurized sugarcane juice sold in Dar es Salaam City were substandard.

Citation: Issa-Zacharia A, Rwabunywenge SP. **Evaluation of bacteriological quality and safety of sugarcane juice locally processed and vended in Dar es Salaam City, Tanzania.** J food safe & hyg 2023; 9 (4): 282- 298. DOI: 10.18502/jfsh.v9i4.15003

1. Introduction

Within the framework of the worldwide food system, the street food vending industry is regarded as a significant economic venture in emerging nations, offering a wide range of employment prospects for the local labour force (1,2). The street food sector provides substantial employment opportunities, frequently for those with limited training and experience (3). Urban locations in developing countries primarily offer fresh juices as part of their street food choices (4,5). Juices sold on the street have difficulties with food safety because they are typically made in unhygienic conditions. Consumption

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of these drinks is requently linked to microbiological risks that pose major health risks (6).

Sugarcane (*Saccharum officinarum)*, is a perennial plant belonging to the *Poaceae* family, cultivated primarily for its stem (cane), which is primarily utilized for the production of sucrose. Sugarcane is one of the major industrial crops worldwide (7,8) and it is a healthful and delicious beverage made by extracting juice from squeezed sugarcane and serving it cold (9). Sugarcane is a significant staple and cash crop that is widely consumed in Tanzania. Sugar cane juice has many health benefits, it provides 40 Kcal/100 ml of energy (10) and it also contains vitamins A, C, B, and numerous other health-supportive compounds (11). Sugarcane juice is offered for sale in all public spaces, including parks, bus stops, and bustling market areas, in many tropical nations. People have fostered a fascination with freshly concocted juices owing to their inherent freshness and lack of preservatives in contrast to commercially processed juices. The consumption of freshly extracted sugarcane juices has risen in Dar es Salaam City, Tanzania, mostly due to the abundance of sugarcane and its associated health benefits (12).

Despite the numerous advantages, sugarcane juice is consumed fresh, and unpasteurized after being extracted. Studies reveal that numerous instances of foodborne illnesses have been documented as a result of consuming unpasteurized and contaminated juices (13). Poor handling, lack of pasteurization, and unhygienic conditions lead to potentially dangerous hazards that pose risks to consumers (14). Numerous cases of food-related illnesses, including cholera and diarrhoea, have been reported across the nation (15). Therefore, sugar cane juice sold by vendors and ice added to it for cooling can pose health hazards. Spoilage microorganisms are the primary reason for the chemical, physical, and sensory deterioration of sugar cane juice (16). There have been reports of salmonella outbreaks involving unpasteurized fruit juices all around the globe (17,18). In Tanzania, diarrhoea and cholera were reported by Penrose and Hawking (19). In the year 2013, a study conducted in Morogoro, Tanzania on other fruit juices revealed that 94% of juice samples were contaminated with *E. coli* (3). Inadequate data on the present status of *E. coli* contamination made it difficult to determine the extent to which unpasteurized sugarcane juice intake has contributed to these diseases (3). The objective of this study was to evaluate the microbiological quality and safety of sugarcane juices to understand the current status of contamination and provide clear information that can protect consumers of sugarcane juice.

2. Materials and Methods

2.1. Study area and sampling

This study was conducted in Dar es Salaam City, Tanzania. Due to its proximity to the equator, the city experiences hot, muggy weather from December through March, with January being the hottest month. Since Dar-es-Salaam is Tanzania's most populous and biggest cosmopolitan commercial city, it was specially chosen as the study region in which sugarcane juice is highly consumed. Sixty (60) samples of freshly made sugarcane juice were randomly chosen from street vendors based on availability in different locations in the city. They were identified from markets, along roadsides/streets and town restaurants in Dar es Salaam city. The sample size was determined using the formula for an unknown population (48).

DOI: 10.18502/jfsh.v9i4.15003

$$n = Z^2.SD^2/e^2$$

Where: n = size of the sample, z = standard variant at 95% confidence level (1.96), SD = the standard deviation of a population, and for the case of this study it will be taken at 19% and e = acceptable error which will be taken at 5% (0.05).

2.2. Study design

A cross-sectional research approach was employed to gather socio-demographic and laboratory data. The study locations/streets were deliberately chosen based on the substantial number of raw sugarcane juice vendors, as well as a random selection of sugarcane juice vendors that were included in the study. The selection of sugarcane juice vendors for the study was based on their willingness to participate, availability and capacity to provide the required information. The investigators employed structured questionnaires to gather data from the sugar cane juice vendors regarding their socio-demographic characteristics. Additionally, a checklist for observations was created by the Codex Recommended General Principles (CAC/RCP 1-1969, Rev.4-2003) of food hygiene (20) for the locations where food is prepared, the washing procedures, the general cleanliness of the vendor and their premises, waste management, and general maintenance of the juices. Finally, samples were collected for laboratory microbiological analysis. The study population was divided into three distinct groups for evaluation: Street sugarcane juice vendors identified along roadsides/streets in the Dar Es Salaam city (TSV), Town restaurant vendor (TRV), and one Factory sugarcane juice producer (FSP) involved in the study for comparison purposes. Samples were collected in different periods from restaurants and streets.

2.3. Sample collection

A total of 60 unpasteurized samples 30 iced sugar cane juice (with the addition of ice blocks) and 30 raw (fresh without any added sugar) were directly collected from the TS and TRV storage containers. Sugarcane juice samples (250 ml) were transferred into a sterile glass bottle and kept in a cool box with ice packs. In addition, pasteurized samples collected from FSP (SMEs in Goba village) were included in the study for comparison purposes. In this case 20 pasteurized sugar cane juice samples (at 80^{o}C for 10 min) and 20 pasteurized at (80^{o}C for 10 min + Citric acid (40 mg/L sugarcane) were taken for laboratory analysis of physicochemical and microbiological analysis.

2.4. Physicochemical analysis of Sugarcane juice

The physicochemical parameters analyzed in the sugarcane juice consisted of total soluble solids (TSS: ºBrix), pH, and total titratable acidity (TTA). The measurement of total soluble solids (ºBrix) was conducted using an RFM 860 refractometer (Bellingham and Stanley Ltd., based in London, UK). Before measuring the ºBrix of the samples, the refractometer was calibrated using distilled water at 0°Brix and a sucrose solution at 30ºBrix and the readings of the samples were subsequently recorded in terms of ºBrix. The pH of the samples was determined using a Mettler Toledo digital pH meter (N.V. Mettler-Toledo S.A, Belgium) that was initially calibrated with standard buffer solutions of pH 7.0 and pH 4.0. The titratable acidity of the sugarcane juice samples (expressed as % citric acid) was determined using the recommended method by (21). The Sodium hydroxide

(NaOH) solution was calibrated to a concentration of 0.1 Normality (N). The acidity of the sample was measured using the formula:

$$\%\ \text{TTA (w/w)} = \frac{\text{Vol. Titrant (ml)} \times \text{N (Titrant)} \times (0.064)}{\text{Sample weight (g)}} \times 100$$

2.5. Microbiological analysis

Sugarcane juice samples for microbiological analysis were collected immediately after the socio-demographic survey. Depending on availability, sugarcane juice samples were collected from a storage container with an ice block of which 250 ml was collected aseptically in sterile bottles, which was termed an "iced sugarcane juice" sample. Another sample was collected directly at the point of extraction from the roller machine outlet without being mixed with ice blocks or anything this was termed a "raw sugarcane juice" sample filled in a sterile bottle. A third sample was collected from the SME factory (SFP) comparison study between a pasteurized sugarcane juice sample and pasteurized sugarcane juice in which Citric acid was added. All samples were marked for identification and immediately stored in a cool box with ice packs and transported directly to TBS Food Laboratory at Ubungo, Dar es Salaam for analysis. Laboratory analysis was done to determine bacterial contamination which involved analysis for Total Plate Counts (TPC), *E. coli*, and *Salmonella*.

2.5.1. Determination of the total plate count

A volume of 25 ml of the sugarcane juice sample was transferred into a glass bottle containing 225 ml of sterilized pre-enrichment buffered Peptone Water (0.1% BPW). The mixture was then well mixed using frequent agitation for 1 min. Serial ten-fold dilutions were prepared from 10^{-1} to 10^{-6} in 0.1% BPW; duplicate pour plates were prepared using 1 ml from each dilution and mixed with 20-25 ml tempered (44-47^{o}C) Plate Count Agar (OXOID® Ltd., Basingstoke, U.K.). The plates were incubated aerobically at 30±1^{o}C for 72±3 h. The number of colony-forming units was determined by counting colonies on at least two dilution plates using a colony counter. Two successive plates with 15-300 colonies were selected for recording. The countable colonies were transformed into the weighted mean colony forming units per milliliter (cfu/mL) using a formula;

$$bacterial\ count = \frac{number\ of\ colonies \times reciprocal\ of\ dilution\ factor}{inocilum\ size(ml)}$$

2.5.2. Determination of *Escherichia coli* in sugarcane juice

The colony-count technique at 44^{o}C on a solid medium (T.B.X) containing a chromogenic ingredient for detection of the enzyme beta-glucuronidase was applied. Serial dilutions were carried out in tenfold from 10^{-1} to 10^{-4}. One ml from each dilution was pour-plated in duplicate and two replicates were prepared for each dilution and gently mixed with about 12-15 ml of the sterilized T.B.X agar (44-47^{o}C) in sterile Petri dish plates. The plates were allowed to cool and solidify on the flat surface of the lamina floor. The petri dishes were inverted and incubated for an initial period of 4 h at 37^{o}C and then raised the incubation temperature to 44^{o}C for 24 h. Three controls were involved and the procedure was done parallel as per sample (positive, negative and blank). Positive *Escherichia coli* (ATCC8739) was employed to assess the effectiveness of the media, negative control was (*Staphylococcus aureus* ATCC 6538) to assess the selectiveness of the media and blank control was (TBX media) to assess

DOI: 10.18502/jfsh.v9i4.15003

sterility of the media and preparation environment. Following incubation, the colonies were enumerated on plates using a colony counter. The number of Colony Forming Units (cfu/mL) was determined based on at least two critical dilutions, where two successive plates containing 15 to 300 colonies were considered. The countable colonies from two conservative plates were converted into cfu/mL of sugarcane juice.

2.5.3. Determination of *Salmonella spp.* in sugarcane juice

The determination of *Salmonella* was achieved by first subjecting the sample to pre-enrichment in a non-selective medium (BPW). This was followed by enrichment in two different media: Rappaport Vassiliadis medium with soya (RVS) broth and Muller-Kauffmann tetrathionate/novobiocin (MKTTn) broth (OXOID® Ltd., Basingstoke, U.K). Subsequently, the two samples were differently plated onto Xylose lysine deoxycholate (XLD) agar (OXOID® Ltd., Basingstoke, U.K.) The agar plates were then incubated at a temperature of 37°C for an additional 24 h. After incubation, the cultures were examined for typical colonies of *Salmonella spp.* Typical colonies of *Salmonella spp.* grown on Xylose lysine deoxycholate (XLD) agar have a black center and light transparent zone of reddish color due to a change of phenol red indicator (5). *Salmonella spp.* confirmation was achieved using biochemical assays utilizing Triple Sugar Iron (TSI) (OXOID® Ltd., Basingstoke, U.K.), inoculation on urea agar (OXOID® Ltd., Basingstoke, UK), and L-Lysine decarboxylation medium (OXOID® Ltd., Basingstoke, UK).

2.6. Statistical data analysis

Data were analyzed by using Statistical Packages for Social Science (IDM SPSS Version 20). Descriptive statistics was used to compute the frequencies and percentages of questionnaire data. Bacteria counts were normalized by log transformation. Analysis of Variance (One-way ANOVA) was used to compute the mean, standard deviation and range of laboratory data and compared the significance at $p<0.05$. Results were expressed as mean±SD and presented in tabular and graphic forms.

3.Results

3.1. Demographic characteristics of sugarcane juice vendors

Demographic characteristics, including Gender, educational level, duration of vending sugarcane juice and vending type are presented in Table 1. Males (96.7 %), constituted most of the respondents compared to females (3.3 %). The majority (51.7%) of sugarcane juice vendors had received primary school education, while the remaining 48.3% had received secondary school education. There was no sugarcane juice vendor with a college education. The majority of the sugarcane juice vendors (63.3%) had less experience in vending duration of less than 1 year. However, fewer vendors (10%) had long experience of more than 3 years in sugarcane juice vending. Values are expressed as frequencies and percent distribution of vendors for gender, education level, duration of vending, and type of vending.

Table 1. Demographic characteristics of sugarcane juice vendors (N = 60)

Parameter	Category	Frequency (%)
Gender	Male	58(96.7)
	Female	2(3.3)
Educational level	Primary	31(51.7)
	Secondary	29(48.3)
Duration of vending sugarcane juice	Less than 1 year	38(63.3)
	1-2 year	16(26.7)
	3-5 years	6(10)
Vending type	Street	40(66.7)
	Restaurant	20(33.3)

3.2. Sugarcane juice preparation and handling practices

Sugarcane juice was produced by using roller machinery and other simple equipment including knives, plastic buckets, cool boxes, plastic cups, glass cups, re-used plastic bottles, mags, plastic funnel as shown in Fig. 1. cold facilities are commonly bucket with ice.

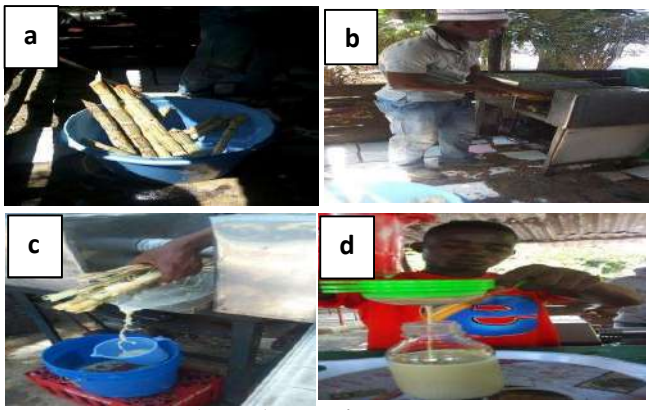

Figure 1. Steps in the production of sugarcane juice in Dar Es Salaam City

Key: (a) Chopped stems at 100 cm (b) Insert stems into the roller machine (c) Press to obtain juice (d) Filtration to obtain fresh sugarcane juice.

Assessed parameters were examined by the structured questionnaire and results are presented in Table 2a. None of the juice providers reported engaging in post-preparation pasteurization of the sugarcane juice. Furthermore, all vendors (100%) were not adding water to sugarcane juice instead ice blocks were commonly added. Lemon was commonly added to sugarcane juice to improve the sensory properties of the sugarcane juice, it was done by all vendors assessed (100%). The majority of the vendors used buckets with ice as cold storage facilities (75%) which is not effective for intended use. Only 5% of vendors use hot water and soap to clean utensils and roller machines which indicates poor washing methods. Most of the vendors (76.7%) did not know the quality of the ice used, since they are obtained from their local suppliers while only 23.4% declared that water used for ice was boiled at home. It was observed that sugarcane juice was prepared in an unhygienic environment that was justified by poor washing methods in which 45% of vendors were observed washing utensils using cold water without detergents. Hand washing equipment and disinfectant were not observed in the majority of the vendors 81.7% (Table 2b) leading to poor hygiene. The majority of vendors (78.3%) had pests on their property, which encourages the presence of flies and bees, sources of germs, in their establishments. Additionally, it was noted that the buckets of juice and the roller machine were not protected from sources of contamination in the vast majority (71.7%) of the sugarcane juice evaluated.

DOI: 10.18502/jfsh.v9i4.15003

Table 2a. Sugarcane juice preparation and handling practice

Assessed parameter	Category	Frequency (%)
Cold storage containers	Icebox	12(20)
	Deep freezer	3(5)
	Bucket with ice	45(75)
Cleaning service utensils	Cold water and soap	33(55)
	Cold water alone	23(38.3)
	Hot water and soap	3(5)
	Hot water alone	1(1.7)
Roller machine cleanliness	washing with cold water	27(45)
	Washing with cold water and soap	22(36.7)
	Washing with hot water	8(13.3)
	Washing with hot water and soap	3(5)
Is ice used in sugarcane juice?	Yes	60(100)
Is the roller machine covered after squeezing	Yes	39(65)
	No	21(35)
Is water used to make ice treated/boiled?	Yes	14(23.3)
	No	46(76.7)
Is water added to sugarcane juice?	No	60(100)
Addition of lemon or ginger in sugarcane juice	Yes	60(100)
Sugarcane juice pasteurization done	No	60(100)
Serving utensils	Glass	39(65)
	plastic cups	15(25)
	disposable plates	6(10)
Waste disposal	in plastic bags	49(81.7)
	dust bin	11(18.3)
Medical check-up	Yes	24(40)
	No	36(60)

Table 2b. Sugarcane juice preparation and handling practices

Assessed parameter	Category	Frequency (%)
Does preparation set min contaminat	Moderately	11(18.4)
	Poorly	49(81.6)
Does the washing process min contamination?	Moderately	33(55)
	Poorly	27(45)
General cleanliness of the handler	Good	42(70)
	Poorly	18(30)
The vendor has a working uniform	Fully	5(8.3)
	Partial	23(38.3)
	No uniform	32(53.3)
Hand washing equipment and disinfectant present	Yes	11(18.3)
	No	49(81.7)
Waste receiving receptacle present	Yes	31(51.7)
	No	29(48.3)
Presence of pests	Yes	47(78.3)
	No	13(21.7)
Is the juice protected from source of contamination	Yes	17(28.3)
	No	43(71.7)
Is the storage facility effective for the intended purpose?	Yes	15(25)
	No	45(75)

3.3. Physical-chemical properties of sugarcane juice

The current study determined the physical-chemical characteristics of the sugarcane juice (Brix, pH, and Acidity) to know the quality status of the sugarcane juice vended and to relate with the microbial status. The physical-chemical characteristics of the sugarcane juice are presented in Table 4. There was a statistically significance difference ($p<0.05$) in Brix between all juice types. The Soluble solids (°Brix) of unpasteurized raw, iced and pasteurized sugarcane juice ranged from 12.2-22.1, 2.4-13.8 and 14.1-15.8, respectively. The majority (72%) of the iced sugarcane juice exhibited brix values that fell below the required criteria outlined in CODEX STAN 247: 2005 for fruit juices and nectars (26). Pasteurized sugarcane juice showed higher values of brix than unpasteurized (Table 4) sugarcane juice vended.

DOI: 10.18502/jfsh.v9i4.15003

Values are mean±standard deviation of duplicate samples with sample size n=20. Means with different (superscripts) on the same row are significantly different at $p<0.05$. Raw=Fresh sample without any treatment, Iced=sugarcane juice with added ice blocks, Pasteurized±Citric acid =Pasteurized sample with addition of Citric acid. TS = Soluble solids (°Brix), TA = Titratable acidity (% citric acid). Values in blacks are ranges of the parameter. The pH of unpasteurized sugarcane juice was determined and found to be 4.8 and 4.9 for iced and raw sugarcane juice while the pH for pasteurized and pasteurized juice in which citric acid was added were 4.3 and 3.1 receptively (Table 4).

3.4. Microbial quality of sugarcane juice

The microbiological quality of sugarcane juice was assessed in terms of total plate count (TPC), *E. coli* and *Salmonella spp.* and results are presented in Table 5. The sugarcane juice samples analyzed were found to be contaminated with higher levels of TPC and *E. coli*.

3.4.1. Total Plate Count

The TPC of unpasteurized sugarcane juice showed a mean of 5.59±0.2 and 5.64±0.2 log cfu/mL for raw and iced sugarcane juice, respectively. The TPC results showed a prevalence of 90%, in which 54 of the 60 samples analyzed were above the recommended maximum limits of 5×10^3-10^4 cfu/mL (35). There was no statistical significance ($p>0.05$) between iced and raw sugarcane juice but the level of contamination in sugarcane juice with ice blocks (iced) tended to be higher than in raw sugar cane juice. The results of the pasteurized sample showed significantly different ($p<0.05$), compared to unpasteurized (iced and raw) in TPC levels (Table 5).

3.4.2. *Escherichia coli* and *Salmonella spp.* contamination in sugar cane juice

Juices can transmit pathogenic microorganisms which can be incorporated in different ways such as raw materials used in juice preparation, hygiene of the preparation environment, fruit quality, equipment and as well as the juice manufacturer (39). The *Escherichia coli* contamination in unpasteurized sugarcane juice was determined and is presented in Table 5. The average counts of *Escherichia coli* in unpasteurized raw and iced sugarcane juice were 1.79 and 2.10 log cfu/mL. The level of contamination of *Escherichia coli* in unpasteurized raw juice showed no significant difference ($p>0.05$) from iced sugarcane juice, however, the level of *Escherichia coli* in iced sugarcane juice tended to be higher. The results of *Salmonella spp.* detection indicated that no typical *Salmonella* was detected in all 60 samples (Table 5).

Table 3. Physical-chemical characteristics of sugarcane juice

Physical parameter	Unpasteurized		Pasteurized	
	Iced	Raw	Pasteurized	Pasteurized+Citric acid
TS (°Brix)	9.73+3.5[a]	18.22+2.2[b]	14.9±0.7[c]	15.1±0.7[d]
	(2.4-13.8)	(12.2-22.1)	(14.1-15.8)	(13.1-16.1)
pH	4.8+0.2[a]	4.9+0.2[b]	4.3±0.3[a]	3.1±0.2[c]
	(3.68-5.48)	3.60-5.89	(3.99-4.9)	(3.0-3.2)
TA	0.08+0.04[a]	0.08+0.04[a]	0.11+0.03[a]	0.57+0.03[b]
	(0.019-0.178)	(0.03-0.12)	0.05-0.178)	(0.51-0.62)

Table 4. Microbiological level of sugarcane juice (log cfu/mL)

	Unpasteurized (log cfu/mL)		Pasteurized (log cfu/mL)		Recommend (log cfu/mL) TZS 585:2003	Prevalence in UPSJ (%)
	Iced	Raw	Pasteurized	Pasteurized +Citric acid		
TPC	5.64+0.2[a]	5.59+0.2[a]	3.45+0.3[b]	2.18+0.1[c]	3.5-4.0	90.0%
E. coli	2.10+0.7[a]	1.79+0.8[a]	X	X	Absent	96.6%
Salmonella	X	X	X	X	Absent	0%

The mean difference is significant at the 0.05 level. Values are mean±standard deviation of duplicate samples (log cfu/mL). Means with different superscripts on the same row are significantly different at $p<0.05$. TPC = Total bacterial count, X = Not detected, Raw=Fresh sample without any treatment, Iced=sugarcane juice with added ice blocks, Pasteurized+Citric acid = Pasteurized sample with addition of 40 mg/L Citric acid, UPSJ = Un Pasteurized Sugarcane Juice

DOI: 10.18502/jfsh.v9i4.15003

4. Discussion

The present study was conducted to assess the quality, safety, and handling practices of sugarcane juices sold along the streets and restaurants in Dar Es Salaam City. The investigations revealed that the sugarcane juice was contaminated and had been prepared under unsanitary conditions.

4.1. Sugarcane juice preparation and handling practices

Sugarcane juice was produced by using roller machinery and other simple equipment including knives, plastic buckets, cool boxes, plastic cups, glass cups, re-used plastic bottles, mags, and plastic funnel. The results of the findings indicated that the sugarcane juice was contaminated and was prepared in unhygienic conditions. The findings were further supported by the laboratory results on TPC and *Escherichia coli* which were significantly high in sugarcane juice above the recommended limits. The contamination observed could be due to several factors including unhygienic transportation. Sugarcane is transported from the upcountry and is delivered to Dar Es Salaam markets by trucks with soil deposited on the stems. The production of quality and safe sugarcane juice depends on the Good Hygienic Practices (GHP) of the vendor and the environment for handling the production of the sugarcane juice. The findings of this study indicate that the socio-economic and demographic data revealed that a significant proportion (96.2%) of the vendors selling sugarcane juice were young males. Nevertheless, Abdalla and Suliman (22) stated that the majority of street food vendors were females. The reason for this may lie in the inherent characteristics of the sugarcane juice industry. The vendors' general methods in terms of handling, preparation, and vending were found to be substandard. The process of preparing street sugarcane juice was shown to render the juice more susceptible to contamination. Poor hygiene includes; poor preparation methods, poor washing process, poor handling of the equipment and disinfectants, presence of pests, poor storage facilities and sugarcane juice was not protected from source of contamination.

Furthermore, the current study results suggested that the factors observed contributing to a poor hygienic environment include; inadequate sanitary conditions of the sugarcane itself (sugarcane comes with soil deposits), they were not washed before squeezing just chopped, the water used for ice blocks making are not treated (the quality of the ice block used), the roller machine (press) left uncovered, the equipment and utensils are washed with plain cold water, water used by vendors to wash their hands. The quality of water used to wash the roller machine (press) was poor coupled with a low of level of compliance with the general principle of food hygiene as documented in Tanzania Bureau Standards (TBS) and Codex Alimentarius food safety standards for sugarcane juice. A study on the quality and safety of street sugarcane juice in Noida City India found similar agreement and reported that contamination was mainly due to improper washing by workers, improper personal hygiene, and the absence of good manufacturing practices (23). The majority of the vendors did not know the quality of the ice used, as they are getting from their local suppliers and there was no information (quality certificate) about the quality of ice used. Fewer of the vendors treat the water by boiling it at home. In this study, the iced sugarcane juice was found to have higher contamination than fresh (raw) one.

Furthermore, there was no washing of the sugarcane stem before squeezing instead chopping only was done. The nature of sugarcane is coming with soil deposited and no washing was done, these could be the factors for the higher microbial load found in sugarcane juice. In the majority of the vendors, the roller machine (press) was left uncovered before and after squeezing and before starting the next pressing, this could contribute to contamination by dust and flies and lead to higher TPC found in this study. It is recommended that a place of food preparation should be kept clean at all times and should be far from any source of contamination (20). The use of plane/cold water for washing utensils and roller machines instead of using hot water could contribute to contamination. Inadequate cooling facilities, such as the usage of ice-filled buckets by most vendors, fail to offer sufficient and durable chilling, while also creating favorable circumstances for microbial proliferation. Several foodborne diseases are associated with the consumption of foods that were previously exposed to pathogenic microbes (24). This scenario is most likely the result of a lack of information regarding hygiene practices and insufficient procedures to ensure food safety. A study in Pakistan reported that higher TVC in the sugarcane juice samples was due to the uneducated food handler and inadequate food safety measures and management (25).

4.2. Physical-chemical properties of sugarcane juice

The present study aimed to assess the physical-chemical properties of sugarcane juice, namely its Brix value, pH level, and acidity, in order to evaluate the quality of the juice being sold and to establish any correlation with its microbiological status. The study found that pasteurized sugarcane juice had a relatively low average pH. Additionally, it was observed that the pasteurized sugarcane juice with the addition of 40 mg of citric acid per liter had the lowest average pH values, the results are consistent with Yusof, Shian (27). According to study findings, fruit juices containing more than 1.2% acid are characterized as sour (28), while juices with less than 7°Brix are described as weak and watery (31). Fortunately, most sugarcane studied in the ongoing investigations were not classified as weak and watery. Similar findings were reported by Corazza, and Rodrigues (29). The Brix of raw sugarcane juice was 18.2±2.2. Similar findings were reported by (30) in which the brix was found to range between 14 to 22. Iced sugarcane juice had an average brix of 9.7, which is very low compared to the recommended. Nevertheless, according to FAO, juices with less than 7°Brix are considered weak and watery (31). Low values of °Brix of iced sugarcane juice observed in this study may be due to over-dilution of the sugarcane juice by the addition of a large amount of ice blocks. The reason might be due to the lack of a standardized amount of ice block added, the amount of ice added dilutes the juice and reduces the brix. The ice used for diluting the sugarcane juice is also an important source of microbial contamination.

The pH of raw sugarcane juice was found to have a mean value of 4.9±0.3 and Iced sugarcane juice had a mean pH of 4.8+0.2. Similar findings were reported by Corazza, Rodrigues (29) and dos Santos Sobrinho, da Silva (30). The results showed a statistically significant difference ($p<0.05$) in pH between unpasteurized and pasteurized sugarcane juice. The lower pH of pasteurized sugarcane juice as compared to

DOI: 10.18502/jfsh.v9i4.15003

unpasteurized sugarcane juice adds value in inhibition of microbial contamination of the juice, while on the other hand, a higher pH of unpasteurized sugarcane juice can support the growth of microbes. The pH of sugarcane juice was further reduced to an average of 3.1 by adding citric acid to pasteurized sugarcane juice. Kunitake and Ditchfield (32) have suggested that high-quality sugarcane juice with good storage stability in refrigeration can be achieved by treating the juice with heat (72°C for 15 s) before adding lemon (3 ml/100 ml) as a source of citric acid (an antioxidant). The impact of pH on preventing microbial contamination has also been studied by Yeneneh and Maitra (33) and Sunday and Crim (34). The acidity of unpasteurized sugarcane juice was found to have a mean value of 0.084±0.04 for iced while that of raw was 0.083±0.04. The results go along with those of Yusof and Shian (27) showing sugarcane juice to have low acidity. Fruit juices containing an acid concentration of approximately 1.2% are characterized by a sour taste (31), fortunately, the acidity of a majority of the sugarcane juices in the current study was within recommendations.

4.3. Microbial quality of sugarcane juice

An evaluation was conducted to determine the microbiological quality of the sugarcane juice in terms of the presence of total plate count (TPC), presence of *E. coli*, and *Salmonella spp*. and the analysis revealed that the samples were contaminated with high levels of TPC and *E. coli*. The TPC results showed a prevalence of 90% and the samples analyzed were above the recommended maximum limits of 5×10^3-10^4 cfu/mL (35). Similar findings were reported by Yasir Abbas Shah and Mbustafa (25) in Pakistan but were higher than the findings reported by Oliveira, Seixas (36) in Brazil in which more than 90% of sugarcane juice samples were above recommended. There was no statistical significance ($p>0.05$) between iced and raw sugarcane juice but the level of contamination in sugarcane juice with ice blocks (iced) tended to be higher than in raw sugar cane juice. The variation in TPC of both types may be due to the quality of the ice block added to the sugarcane juice. From the current study, the juice handling practices covered by the questionnaire showed that 46 (76.7%) of the sugarcane juice vendors were not treating water used to make ice, or due to non-adherence to hygienic measures during the preparation and processing of sugarcane juices during (5,37).

The overall high level of TPC reflects the inadequate hygienic status of the sugarcane itself, and the results suggested that sugarcane juice could be contaminated due to factors such as; the use of unboiled water for making ice, the roller machine press left uncovered, no washing of the sugarcane stem before squeezing, use of un-treated cold water for washing utensils instead of using hot water (5,36). These findings presumably indicate the insufficient hygienic condition of both the sugarcane, the equipment, and the water used by handlers for handwashing (5,38). The primary variables that contribute to foodborne illnesses in most countries are the contamination of food from raw ingredients, the presence of diseased workers, insufficiently cleaned equipment, and improper handling of time and temperature (37).

Juices can transmit pathogenic microorganisms which can be incorporated in different ways such as raw materials used in juice preparation, hygiene of the preparation environment, fruit quality, equipment and as well as the juice manufacturer (39). The level of *Escherichia coli* contamination in juice observed in the

current study was lower than those reported by Simforian (40) in mixed fruit juices and mango juice. The findings of the present investigation reveal that *Salmonella spp*. was not discovered in any of the 60 samples. Oliveira and Seixas (36) reported similar findings in their study, where *Salmonella spp.* was not detected in any of the samples. Similar results were also reported by Simforian (40) in other juices. Nevertheless, multiple instances of *Salmonella* outbreaks associated with fruit juices, particularly unpasteurized varieties, have been documented in various locations worldwide (17,18). The raw material inherently harbors a substantial microbial population in its stems, roots, and leaves, exhibiting considerable variability (41) and can contribute to the higher level of contamination in unpasteurized juices. Contamination of sugarcane juice with *Escherichia coli* in the current study was at the prevalence of 96.6% of all the samples. As to the Tanzania standards (TZS 585:2003), ready-to-drink beverages must not contain any *E. coli* bacteria (42). Unpasteurized sugarcane juice in the current study contained substantial *E. coli* and it was absent in pasteurized juices in which 40 mg/L citric acid was added (Table 5). The Tanzania national food safety guidelines, as outlined by TBS and the Codex Alimentarius Commission (35,43), prohibit the consumption of food that contains potentially harmful pathogenic microorganisms such as *E. coli.* The increased levels of *E. coli* contamination indicate either direct fecal contamination or contamination from the environment (44). Almost all unpasteurized sugarcane juice showed the presence of *E. coli* (96.6% prevalence) which could be explained by poor hygienic conditions, and inadequate or no washing of the sugarcane stem to remove the soil. According to the reports, street food vendors generally have inadequate local infrastructure, lack sanitary facilities, receive insufficient training on food hygiene, have poor sanitation, and possess minimal understanding of personal hygiene. These factors have resulted in countless foodborne problems (45). In the current study, the main source of *E. coli* contamination might be due to the unleashing of the sugarcane stem to remove the soil deposit on the sugarcane before squeezing. Another reason could be the use of contaminated water for making ice blocks. Outbreaks have been reported in different parts of the world due to the contaminated ice (46). Ice added for cooling purposes of juice is sometimes contaminated with pathogens due to the contaminated water source or poor hygiene in its handling and transportation (46). Sugarcane juice sold by vendors and ice added to it for cooling can therefore pose serious health hazards. It can be generalized that, all these are attributed to the absence of good manufacturing practices (47).

5. Conclusion

The quality, safety and handling practices of sugarcane juices vended along streets and restaurants in Dar Es Salaam City were assessed. Juice handling practices, preparation, premises and personal hygiene were poor. The findings indicated that the sugarcane juice was contaminated and was prepared in unhygienic conditions. The pH of most unpasteurized sugarcane juice samples was higher and supported bacterial growth while pasteurized sugarcane juice in which citric acid was added was recorded lower and inhibited *E. coli* and *Salmonella spp.* growth. The acidity of a majority of fresh (raw) sugarcane juice samples was

DOI: 10.18502/jfsh.v9i4.15003

low and within the recommended level and iced sugarcane juice samples had low TSS (Brix) beyond recommended limits. The majority (90%) of unpasteurized sugarcane juice samples showed higher levels of TPC above the recommended maximum level by the regulatory authority (TBS and Codex Alimentarius Commission). Faecal *E. coli* was detected in almost all samples (~97%) and most cases were above the recommended limit for consumption. The prevalence observed in the current study indicates that most of the raw unpasteurized sugarcane juices vended in Dare Es Salaam streets were of questionable microbial quality and posed a threat to human consumption. An immediate need for monitoring and education for the vendors to improve sugarcane juice quality and safety of sugarcane juice is required by the regulatory authorities.

Conflict of Interest

The authors declare no conflicts of interest relevant to this article.

Acknowledgment

The authors wish to acknowledge the Tanzania Bureau of Standards (TBS) for laboratory analysis support.

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