

Microbiological Quality of Freshly Squeezed Sugar-cane Juices Vended in Dar es Salaam, Tanzania

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Twenty fresh sugarcane juice samples were randomly bought from street vendors from 20 localities in Dar es Salaam city. Each sample was subjected to identification of microbial contaminants and microbiological assays. All samples were clear and odorless with pH ranging from 3.6 to 4.8. Most of the sugarcane juices harbored microorganisms beyond acceptable limits. Bacterial counts ranged from 1.44×10^5 to 6.0×10^5 cfu/ml and fungal counts from 1.36×10^5 to 2.64×10^5 cfu/ml, exceeding the specified limits by 10 to 100 folds. A total of 25 bacterial and 23 fungal (15 yeasts and 8 molds) isolates were found. Predominantly isolated bacteria were *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* while isolated fungi were *Candida albicans* and *Aspergillus flavus*. Over 60% of bacterial contaminants were fecal coliforms, an indication of poor sanitary and unhygienic conditions of vendors/production sites. The microbiological quality of sugarcane juices vended in Dar es Salaam streets was thus questionable.

Key words: Microbiological quality, sugarcane juice, microbial contaminants

INTRODUCTION

Sugarcane, *Saccharum officinarum*, is a perennial grassy plant in the family Poaceae grown for its stem (cane) which is principally used to produce sucrose. Sugarcane juice is a nutritious tasty drink extracted from squeezed sugarcane and served chilled. It is also rich in vitamins, mineral salts, simple sugars and organic acids that are assimilated by human beings [1]. Nowadays sugarcane juice has become part of the daily diet in most communities partly due to its thirst quenching ability in tropical hot weathers [2]. However, because of favorable pH, high water and sugar contents as well as suitable temperature; sugarcane juice tends to deteriorate rapidly even under refrigeration [2].

Currently, there is an increase of sugarcane juices producers/vendors [1-3]. Occasionally, during the production process, hygienic conditions are not well observed. Raw materials (sugarcane) are inadequately cleaned, and the squeezing process is carried out without using protective gear, which may compromise the microbiological quality of the juices. Such poor sanitary conditions during processing may also accelerate the

physicochemical changes affecting its composition and pH leading to microbial proliferation. An excessive number of microorganisms in juices can lead to food-borne infections. Such infections may not only interfere with the diagnosis of other infectious microbial diseases but contribute to high morbidity and mortality in vulnerable individuals to food-related diseases [4, 5].

Hence, this study aimed to assess the microbiological quality of the sugarcane juices available in the Dar es Salaam market. It also intended to alert food safety authorities to the need to impose more stringent measures on availability of ready-to-eat street-sold foodstuffs such as juices, which may contribute to food-borne disease outbreaks [6-8].

METHODOLOGY

Study area

Freshly prepared sugarcane juice samples were randomly bought from 20 different localities from street vendors, along bus stops, sports playing grounds and other public buildings/settings such as schools and colleges

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within Dar es Salaam City. The city is situated near the equator, experiencing hot humid weather from December through March, the hottest month being January. Dar-es-Salaam was specifically selected as the study area because it is the most populous and largest cosmopolitan commercial city in Tanzania. Thus the city has a comparatively large number of both sugarcane juice producers/vendors and consumers [7, 9].

Sample collection and physicochemical properties assessment

A total of 20 different sugarcane juice samples were aseptically collected using sterile capped bottles, deposited in a cool box and transported to the Pharmaceutical Microbiology Laboratory. The samples were processed within 150 minutes of collection. Visual inspection for color, turbidity and pH of each sample was done. In the analysis of microbiological quality of collected samples, standard laboratory methods and media were employed as per the World Health Organization and Tanzania Bureau of Standards guidelines (TBS) [10-11].

Qualitative microbiological assessment of sugarcane juice

Aseptic measures were observed to avoid potential contamination of the samples by

using clean gloved hands and inoculation of each sample into sterile broths and on agar plates. Two 100 μ L aliquots of each sample were separately inoculated onto a sterile 9 cm diameter agar plate and another into the universal bottle-containing broth and then aerobically incubated at the requisite time-temperature combinations. Nutrient agar and broth (NA/B) were used for detection of bacterial contaminants while Sabouraud's dextrose agar and broth (SDA/B)–(Oxoid, Hampshire, UK) were used for assessment of yeasts/fungal contamination. To detect total coliforms, violet red bile agar was used while the presence of fecal coliforms was detected using Eosin methylene blue agar. Contamination with *Staphylococcus* species was tested for using Mannitol salt agar (Himedia, India). For the detection of viable but non-culturable bacteria, 1 ml of sugarcane juice was introduced into 9 ml of alkaline peptone water and selenite cysteine broth (SCB) for enrichment followed by inoculation onto Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar (Himedia, India). Salmonella-Shigella agar (SSA) was employed in the detection of *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. [12-13]. Normal saline was the negative control. Colonies from each sample were compared to allowable microbial limits (safe ranges) for fresh juices or beverages as per food safety recommendations as indicated in **Table 1** below.

Table 1: Recommendations on microbial quality for fresh juices or beverages [11]

Type of Microbes	Maximum limit (count)	Method of test
Total viable count per ml	10	TZS 118:2007
Yeast and Mould cfu/ml	Shall be absent	TZS 131:2006
<i>E. coli</i> MPN/ml	Shall be absent	TZS 731:2007
<i>Salmonella</i> per 25 ml	Shall be absent	TZS 122:2007

Quantitative microbiological assessment of sugarcane juices

Total aerobic bacterial counts (TABC) and total yeast and mold counts (TYMC) were estimated for enumeration of microbial contaminants in sugarcane juice samples. An aliquot of 1 ml was drawn from each sample, homogenized in normal saline and serially diluted to a final ratio of 1:100,000 (10^{-5}). Two 0.1 ml aliquots were drawn and separately spread-plated into freshly prepared NA and SDA plates, and incubated at 37 °C for 24-72

hours. After incubation, with the help of a magnifying lens, each plate was visually inspected for the presence of microbial growth/colonies and total viable microbial counts were recorded as colony forming unit per millimeter (cfu/mL).

The recorded findings were compared with the recommended microbial standards for ready-to-eat foodstuffs (5.0×10^4 cfu/ml and 1.0×10^3 cfu/ml for TABC and TYMC, respectively, and for coliform bacteria 1.0×10^{-3} cfu/ml) [14-16].

Identification of microbial contaminants

Aliquots of 5 μ l of fresh microorganisms broth ensuing from isolated single colonies on agar plate were streaked on several selective and differential media, namely, NA/B, MacConkey agar, SDA/B and SSA for microbial identification through colony morphology, Gram staining as well as biochemical and physiological tests [17, 18].

Statistical data analysis

Each of the above procedures was conducted in duplicate and performed twice for statistical purpose and consistency of results. Therefore, numerical data are expressed as mean. A computer software SPSS version 20 (Chicago, IL) was used for the analysis of variance for cfu/mL of microbial contaminants found in sugarcane juices. ANOVA was employed to determine associations among relevant variables. Differences of means of cfu/ml among the assayed juice samples against the established microbial limits were done by the t-test, and the differences were considered statistically significant when $p < 0.05$.

RESULTS

Microbiological quality of sugarcane juices

A total of 20 sugarcane juice samples were collected. Out of these, 15 (75%) were microbiologically contaminated with either bacterial and/or fungal (yeasts and molds) contaminants that exceeded acceptable limits by 100 and 1000 folds for some bacterial and fungal contaminants respectively ($p < 0.05$). The most abundant microbial contaminants were due to bacterial isolates ranging from 2.44×10^5 cfu/ml to 3.20×10^5 cfu/ml (**Table 2**). ANOVA (Eta squared = 0.058) revealed a mild association between localities where juice samples were collected and total aerobic microbial counts (TABC and TYMC).

Physicochemical properties observation of the samples revealed clear and odorless sugarcane juices, with pH ranging from 3.6 to 4.8 with a mean of 4.1. The highest TABC was observed in samples of pH between 4.4 and 4.7 while for samples with the highest TYMC, the pH was between 3.6 and 3.9 as shown in **Table 2**.

Table 2: Total microbial aerobic counts from sugarcane juice samples

Microbes	pH	Number of samples	¹ cfu/ml \pm std	Maximum ¹ (cfu/ml)
Bacteria	3.6 - 3.9	6	2.72 \pm 0.36	3.20
	4.0 - 4.3	3	2.78 \pm 0.16	2.96
	4.4 - 4.7	5	3.18 \pm 1.77	6.00
	>4.8	1	2.44 \pm 0	2.44
Yeasts	3.6 - 3.9	7	1.78 \pm 0.38	2.40
	4.0 - 4.3	3	1.75 \pm 0.42	2.16
	4.4 - 4.7	4	1.71 \pm 0.06	1.80
	>4.8	1	1.48 \pm 0	1.48
Molds	3.6 - 3.9	6	1.80 \pm 0.41	2.56
	4.0 - 4.3	1	1.65 \pm 0.32	2.62
	4.4 - 4.7	1	1.87 \pm 0.11	1.35

Note: ¹x = cfu/ml $\times 10^5$

From 15 sugarcane juice samples, a total of 25 bacterial, and 23 fungal (15 yeasts and 8 molds) isolates were found. Forty percent (n=8) of tested juice samples were contaminated with mold isolates. The isolated microorganisms were classified into 5 bacterial species, 3 yeast species, and 2 mold species on the basis of phenotypic characteristics. The most predominantly isolated microorganisms were bacteria (n=25;

52.1%) followed by yeasts (n=15; 31.3%) as shown in **Tables 3-4**.

About 65% (n=15) of fungal contaminants were yeasts. Of 15 isolated yeasts, the predominant species was *Candida albicans* (n=10; 66.7%), followed by *Saccharomyces* species (n=3; 20%). Of 8 mold contaminants, *Aspergillus flavus* isolates were predominant (n=5; 62.5%) as indicated in (**Table 3**).

Table 3: Fungal contaminants isolated from sugarcane juices

Fungal contaminants	Isolates (%)
¹ <i>C. albicans</i>	10 (43.5)
¹ <i>C. krusei</i>	2 (8.7)
<i>Saccharomyces</i> spp	3 (13.1)
<i>A. flavus</i>	5 (21.7)
<i>Fusarium</i> spp	3 (13.1)

Key: ¹ and ² stand for yeast and mold species respectively

Of the 25 bacterial contaminants, the most frequently isolated were enteric bacteria such *E. coli* (n=9; 36%), *P. aeruginosa* (n=5; 20%) and *K. pneumonia* (n=3; 12%). About 8% of the bacterial isolates were *Salmonella typhi* (n=2). *Staphylococcus aureus* was the only Gram-positive bacterial species found in 6 (30%) sugarcane juice samples as shown in **Table 4**.

Table 4: Bacterial contaminants isolated from sugarcane juices

Bacterial contaminants	Isolates (%)
<i>P. aeruginosa</i>	5 (20.0)
<i>K. pneumonia</i>	3 (12.0)
<i>S. aureus</i>	6 (24.0)
<i>E. coli</i>	9 (36.0)
<i>Salmonella</i> spp.	2 (8.0)

DISCUSSION

In the present study, the frequencies of isolation of bacterial contaminants were comparable to that of fungal contaminants (yeasts and molds), which could be attributed to favorable pH and sugar content in sugarcane juices [21]. This may result from the presence of microorganisms on the sugarcane that are introduced into the juices during processing [22, 23]. The fact that microbial counts exceeded the recommended limits in most of the juice samples is confirmatory of potential contamination. Likewise, the presence of fecal coliforms such in over 60% of juice samples is a health concern [24]. Enteropathogens can survive on the hands for several hours [25] implying that recovery of fecal coliforms from sugarcane juices is indicative of fecal

contamination [24, 26]. This points to non-adherence to hygienic measures during the processing of sugarcane juices [7].

Presence of microbial contaminants in sugarcane juices can be attributed to several factors including improper handling and processing of raw materials, use of untreated or contaminated water for various purposes, use of dirty processing equipment and contaminated juice collecting vessels. Keeping sugarcane juices in open wide-mouth vessels at ambient temperature is another factor that can lead to the microbial count increase. Consequently, poor personal hygiene and sanitary conditions are key aspects that greatly facilitate the transmission of pathogens through juices to human [27].

Evidence shows that TABC or/and TYMC are directly related to health risks either from epidemiological studies or from correlation with the occurrence of foodborne pathogens [28]. Specific strains of microbial species that form part of TABC or/and TYMC microbiota may cause infections in certain vulnerable people especially the immunocompromised [29, 30]. For that matter, the national food safety guidelines as stipulated by TBS and the Codex Alimentarius Commission [10-11] prohibit the consumption of foodstuffs containing potentially pathogenic microorganisms such as *E. coli*, *P. aeruginosa* and *K. pneumoniae*. These microorganisms are the main causes of several community-acquired foodborne infections [31-33]. Such infections may interfere with the diagnosis of potential infectious microbial diseases and increase morbidity and mortality in a vulnerable population [4, 5].

Most of the microbial juice contaminants are responsible for several microbial-related infections such as urinary tract infections (UTI), infections in the gall bladder and infection of the middle ear [34]. *Escherichia coli*, *K. pneumonia*, *P. aeruginosa* and *S. aureus* are associated with health care facility acquired UTI and may cause bacteremia [35, 36]. Apart from being the leading cause of UTI, *E. coli* among other diseases conditions, also causes diarrhea, pyogenic infections and septicemia, which sometimes can be fatal [37, 38]. The presence of *Pseudomonas* species from freshly prepared sugarcane juices is of

concern. Whether the bacteria species present in the samples are pathogenic or not, the risk of contamination and health menace to consumers cannot be underrated. *Pseudomonas*, *Staphylococcus* and *E. coli* have been attributed to enterotoxins. Food poisoning from these microorganisms may lead to severe and fatal illnesses with the elderly and infants most affected [34]. *Staphylococcus aureus* is part of the common human flora, and in most cases was isolated together with other bacteria. The bacteria are found in the nasopharyngeal tract and on the skin [39]. When collecting the samples, it was observed that during the preparation of sugarcane, or picking the ice, vendors use their bare hands and converse during the preparation possibly causing contamination through droplets [7].

Similarly, the presence of *K. pneumoniae* is not unusual, because it is part of the human flora and associated with opportunistic infections in some vulnerable individuals [39-40]. Nonetheless, the bacterium is a member of fecal coliforms and its presence in juice samples raises questions on proper handling of the sugarcane and the squeezed juices [15, 41]. *Salmonella* has caused foodborne illnesses globally and it has been a rising threat to fresh produce [42]. The microorganism may be introduced in juices by using untreated water for dilution or as ice cubes [7]. Consumption of microbiologically unsafe foodstuffs such as sugarcane juices may contribute to outbreaks of community-acquired foodborne-infections like those caused by *Salmonella* species [42].

About 75% of the sugarcane juices were contaminated with yeasts, namely, *C. albicans*, *C. krusei* and *Saccharomyces* spp. [43]. Most of the yeasts are normal flora that can only become opportunistic pathogens in immunocompromised individuals [44-46]. *Candida* species is the fourth leading cause of bloodstream infections in hospitalized patients [46]. Recently, positive cases of fungemia due to yeast strains of *Saccharomyces cerevisiae* in healthy individuals have been reported [47-48]. Also, virulent factors attributed to potential pathological conditions have been identified [48]. Dominant molds recorded in fresh juices belong to *Aspergillus flavus*, *Penicillium* species, *Cladosporium* species, *Fusarium* species and *Aspergillus niger* [22].

They tend to alter the juice's taste and viscosity through the production of mycelia [49]. In the present study, the most frequently encountered molds were *Aspergillus flavus* and *Fusarium* species. *Aspergillus flavus* produces common allergens and may cause opportunistic invasive infections [50]. A number of them produce health threatening mycotoxins such as ochratoxin that causes kidney diseases [51]. *Fusarium* is phytopathogenic involved in food contamination and production of toxins that have a negative impact on the health of consumers hence its importance for the food industry and public health [52].

The isolated microbial contaminants may indirectly get access into the human body by ingesting contaminated stuff such as juices [53]. Recognizing the direct interactions of humans with food chain may assist in the prevention of infectious disease and the spread of antimicrobial resistant microorganisms [54].

CONCLUSION & RECOMMENDATIONS

Microbiological observations revealed that sugarcane juices sold in Dar es Salaam streets were of questionable microbial quality. Most of the assayed sugarcane juice samples had microbial contaminants above the acceptable limits. A total of 25 bacterial, 15 yeasts and 8 mold contaminants were detected. Of these, *Escherichia coli*, *Candida albicans* and *Aspergillus flavus* were the most frequently isolated bacteria, yeasts, and molds respectively. Of the isolated bacteria, 68% were fecal coliforms, an indication of poor sanitary and unhygienic conditions of vendors/production sites. We call upon the regulatory authorities to re-enforce measures on microbiological food safety in processing of ready-to-eat foodstuffs like sugarcane juices.

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