Microbial Quality and Antimicrobial Residue of Local and Industrial Processed Fruit Juice Sold in Tamale, Ghana

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Abstract

Fruits are essential part of human nutrition that provides numerous health benefits. When processed into juice, they are either packaged and stored or consumed immediately. There are reports on contraction of foodborne illness due to consumption of these natural beverages possibly from contamination by pathogenic microbes. Also, concerns of antimicrobial resistance due to antimicrobial residues in fruit juices has been raised. Thus, this study assessed the microbial quality of fruit juice by determining the microbial load of *E. coli* and *Salmonella* spp., and the presence of antimicrobial residues in locally processed fruit juices (n = 25) and industrially (n = 3) processed fruit juices sold in Tamale, Ghana. Spread plate technique was adopted for isolation and enumeration of bacteria. Whereas the presence of antimicrobial residue was determined using the Premi® test kit. Neither *E. coli* nor *Salmonella* spp. is recorded in all three industrially processed juice samples. However, the locally processed juice samples recorded 88% *E. coli* and 40% *Salmonella* spp. with microbial load above the acceptable limits. Antimicrobial residues were absent in all 28 samples analyzed. The incidence of high microbial load found in the locally processed fruit juice is of concern to avert possible foodborne illness linked with the consumption of fruit juices within Tamale.

Introduction

Fruits are essential component of a healthy diet. They contain a variety of phytonutrients which has been linked with numerous health benefits including enhancing the immune system (Clemens et al., 2015).

They may be consumed in their raw form or processed into juice. The juice may either be consumed immediately or further processed and packaged locally or by fruit juice companies to extend the shelve life.

Freshly prepared fruit juice as being considered healthy, may not be entirely true due to contamination by microbes (Raybaudi -Massilia *et al*., 2009). Commonly found microorganisms in street juice include, *Pseudomonas* spp., *Staphylococcus* spp., *Vibrio cholerae*, *Escherichia coli*, *Salmonella typhi* (Sharma et al., 2020). Factors that may contribute to microbial contamination of fruit can arise from several factors, ranging from contamination of fruits from the farm, packaging, transportation, and handling (Food and Drug Authority (FDA), 2001).

When the fruits to be used for the juice are not handled properly, the contamination can be transferred to the juice. During the juice production process contamination can be due to unhygienic environment, food flies, airborne dust, extended preservation without proper pasteurization and refrigeration (Salomão, 2018; Tasnim et al., 2010; Sahu *et al*., 2014), and contaminated source of water especially for locally made ready to drink and packaged fruit juice.

Aside microbial contamination, fruit juices can be contaminated with metals (Anastácio et al., 2018; Fathabad et al., 2018), residues from pesticides (Savini et al., 2019) and many other contaminants harmful to human health. Antimicrobial residues have been found to be another contaminant that has raised concerns in the food value chain especially food of animal origin (Bogialli & Di Corcia, 2009). In plant food, antimicrobial residue such as oxytetracycline has been reported in vegetables like cabbage (He et al., 2018) and applied on some fruits such as apple for instance, streptomycin is given to apple trees during blooming (bloom) to suppress fire blight in countries where it is permissible (Expert Panel on Antibiotic Resistance, 2005). When juice is made from such fruits, the juice maybe contaminated with antimicrobial residues. The continuous consumption of antimicrobial residue contaminated foods may lead to the emergence of antibiotic resistance pathogens which can affect humans. This is especially important because identical wide host-range plasmids harbouring antibiotic resistance genes have been discovered in plant and human pathogens (Palmer et al., 1997; Sundin & Wang, 2008; Yau et al., 2010). Also, a recent study in India, recorded 80% of bacterial strains isolated from orange juice sample to be drug resistant (Jain and Yadav, 2015). However, these antimicrobials (antibiotics) are only required for the control of plant bacterial diseases, for example, pear and apple fire blight and peach bacterial spot (Stockwell & Duffy, 2012). Antimicrobial residue (antibiotics) may be introduced into fruit through the water used for irrigation or manure applied on the farm. According to Kumar et al., (2005), organic crops are likely to be contaminated with antibiotics because of exposure to antibiotic residues found in manure. Furthermore, Amarasiri et al., (2020) reported that antimicrobial residues, antimicrobial-resistant bacteria, and antimicrobial resistant genes (ARGs) can all be widely distributed in water. In Ghana, antimicrobial residue contamination is typically found in animal and food products such as meat and milk, vegetables, honey and uptake of antibiotics from irrigation water (Aning et al., 2007; Azanu et al., 2016; Azanu *et al.*, 2018; Nzeh et al., 2020). Research on microbial quality conducted in the Northern region, Tamale, are mainly on beef, ready-to -eat vegetable salad and raw milk (Abakari et al., 2018; Adzitey et al, 2010; 2020). Report by Saba and Gonzalez-Zorn (2012) point out that microbial quality in fruit was one that was lost among all other publications in Tamale but happens to pose risk to consumers since it is utilized without heating and are also not hygienically handled properly. Thus, this study was conducted to determine the microbial load in locally and industrially processed fruit juice and to determine if any, the presence of antimicrobial residues in these fruit juice.

Materials And Method

Study area

Fruit juice samples were obtained in Tamale, the capital city of the northern region of Ghana.

Sample collection

A total of 28 fruit juice samples were collected and divided into two categories: locally processed fruit juice which were obtained from catering services sold from dispensers (watermelon, pineapple, tamarinda, mango, and pineapple ginger and pineapple sugarcane) and industrially processed fruit juice which were obtained from shelves of supermarkets, stores, and mini marts.

Microbiological Analysis

Microbial analysis of all fruit juice samples was carried out in the Microbiology section of the Spanish Laboratory complex of the University for Development Studies. To determine the microbiological quality of the fruit juice samples, the indicators of sanitary quality (*Escherichia coli)* and sulphite-reducing *Salmonella spp.* were analysed.

Samples were analysed under aseptic condition. To culture for *E. coli* and *Salmonella* spp., sample were spread on MacConkey media (MCA; HiMedia, Mumbai, India) and *Salmonella – shigella* (SS) agar (SS; HiMedia, Mumbai, India) respectively. Subculturing for identification was carried-out on nutrient agar (Nutrient Agar; HiMedia, Mumbai, India).

Briefly, as outlined by Mahale *et al*., (2008), 10 ml fruit juice sample were pipetted and homogenized with 90 ml of 0.1 % peptone water. Serial dilutions to the second (2^{nd}) level that is, 10^{-1} and 10^{-2}, is carried out for each juice sample using 0.1 % peptone water as diluents. Following serial dilution, 100 µl of 10^{-2} diluted sample was inoculated on MacConkey media and *Salmonella – shigella* (SS). For *E. coli,* each sample was evenly distributed on the plate using sterilized glass beads and the inoculated MacConkey agar plates were incubated at 37 °C for 24-48 hours inverted. After incubation, colonies of pink-colored bacteria were counted to assess the colony forming units per millilitre (CFU/ml), then sub-cultured to obtain pure colonies for a confirmatory test of the suspected *E. coli*.

With regards to *Salmonella* spp., following spread plate method of inoculation on samples on SS agar, the inoculated plates were incubated at 37 °C for 24-48 hrs inverted. The colourless colonies with black centre among the colonies were enumerated to assess the colony forming units per millilitre (CFU/ml), then sub-cultured to obtain pure colonies for a confirmatory test of the suspected *Salmonella* spp.

Identification and Confirmation of Microbial Isolates

To obtain pure cultures, presumptive isolates of the microorganisms under research were streaked on nutrient agar (Nutrient Agar; HiMedia, Mumbai, India) and incubated at 37°C for 18-24 hours. Biochemical assays such as Gram stain, catalase test, citrate test, and oxidase test were performed on distinct pure colonies. These tests were carried out in order to confirm and further identify the isolates.

Antimicrobial residue detection

undefinedAntimicrobial residues detection was performed on all 28 samples using a PremiTest kit (R-Biopharm AG, Germany) as described by Nzeh *et al*., (2020). Briefly, 100 µL of each sample was pipetted into the ampoules labelled with the sample codes. The ampoules containing the samples were pre-incubated at room temperature for about 20 minutes before being gently inverted to distribute the sample. The remaining samples in the ampoules were carefully removed by filling and emptying ampoules with deionized water. This is followed by inversion on tissue paper to drain any remaining water. The test ampoules were then wrapped in aluminium foil provided by the manufacturer and incubated in a water bath at 64°C until the control changed colour (Nzeh *et al*., 2020).

Result

Occurrence of bacteria isolates

Salmonella spp. and *Escherichia coli* were detected in some fruit juice sampled for this study. Out of the 25 locally processed fruit juice, 10 (40%) recorded the presence of *Salmonella* spp whilst 22 (88%), out of the 25 samples recorded the presence of *E. coli* (Table 1). There was no *E. coli* or *Salmonella* detected in the industrially processed fruit juice.

Table 1: Occurrence of bacteria isolates in locally made fruit juice samples

BACTERIA	NO. OF SAMPLES (+)	PERCENTAGE	NO. OF SAMPLES (-)	PERCENTAGE	TOTAL
E. coli	22	88%	3	12%	25(100%)
Salmonella	10 40%		15	60%	25(100%)

Microbial load profile of the fruit juice samples

The detectable *E. coli* count ranged from 1.33E+03 to 9.23E+04 which was recorded in the locally processed juice with a mean count of 1.74E+06 (Table 2) with no detectable count in the industrially processed ones.

Salmonella spp. count also ranged from 3.33E+02 to 9.53E+05 in the locally processed juices with a mean count of 9.87E+05.The industrially processed fruit juice had no detectable growth.

Table 2: Microbiological analysis of fresh juice samples

Type	Source	*E. coli*	*Salmonella spp.*
Watermelon	Local	1.87E+04	1.67E+02
Watermelon	Local	9.23E+04	ND
Pineapple	Local	1.79E+04	3.33E+02
Pineapple	Local	5.04E+04	6.67E+01
Pineapple ginger	Local	2.77E+04	4.13E+03
Pineapple-sugarcane	Local	2.67E+03	2.97E+04
Pineapple	Local	3.88E+04	1.00E+03
Watermelon	Local	1.30E+04	ND
Tamarind-ginger	Local	ND	ND
Watermelon	Local	ND	1.22E+04
Pineapple-ginger	Local	1.33E+03	ND
Mango	Local	4.00E+03	ND
Pineapple-ginger	Local	1.21E+05	ND
Pineapple-ginger	Local	3.73E+04	ND
Pineapple	Local	ND	ND
Mango	Local	6.33E+03	ND
Pineapple-ginger	Local	3.37E+04	ND
Pineapple-ginger	Local	1.90E+04	ND
Mango	Local	2.02E+05	ND
Pineapple	Local	8.49E+05	ND
Pineapple	Local	7.00E+04	ND
Mango	Local	2.70E+04	3.33E+02
Watermelon-ginger	Local	6.43E+04	9.35E+05
Pineapple-ginger	Local	2.53E+04	3.67E+03
Pineapple-ginger	Local	2.27E+04	ND
Average		1.74E+06	9.87E+05

*ND = Not Detected

Antimicrobial residue profiling

There were no antimicrobial residues detected in all the 28 samples, both locally and industrially processed juice samples tested negative.

Discussion

Microbial quality

Despite the potential benefits derived from fruit juice consumption, safety and quality of these juices have become of concern in both industrially processed and locally produced juices. Fruits are prone to contamination during harvesting and transportation. Also, during fruits juices processing, additional contamination may occur since they move through a series of handling and preparation before reaching the consumer. Hence, fruit juices have recently been identified as "emerging vehicles" for foodborne illnesses caused by bacterial pathogens (Dewanti-Hariyadi, 2013; Lima Tribst *et al.*, 2009).

Results from the current study showed that all the industrially processed fruit juice samples were devoid of both *E. coli* and Salmonella spp. unlike the locally processed fruit juice that recorded microbial load for both microorganisms. The two bacterial genera enumerated has been identified as the leading cause of foodborne diseases (Hendriksen *et al.*, 2011; Fratamico & Smith, 2006). The results of the industrially processed samples are consistent with Addo *et al.*, (2008) that recorded an absence of *Salmonella* and any other coliform in imported fruit juice samples and similar to a report by Rahman *et al.*, (2011) where total viable bacterial count was found to be greater in most fresh juices than in commercially packed juices. It was attributed to the fact that these juices are pasteurised and well packaged which limits *E. coli* and *Salmonella* spp. contamination. Also, the industrially produce fruit juices might have been processed under sanitary conditions and with clean water adhere to standards of regulatory institution. For the locally processed samples, *E. coli* was detected in 22(88%) samples but absent in only 3(12%) of the samples. Also, *Salmonella* was present in 10(40%) samples but absent in 15(60%) of the samples. Per the study, the data indicates that, locally produced fruit juices in Tamale are contaminated with *E. coli* (88%) followed by *Salmonella* (40%). These findings contradict Addo *et al.*, (2008) where all freshly prepared fruit juice samples tested negative for *E. coli*. However, it is in line with Jesús *et al.*, (2021) who recorded the presence of *E. coli* and *Salmonella* in 85% of fresh orange juice samples as well as Wedajo & Kadire, (2019) where 81.25% of all fresh juice samples contained *E. coli* and 62.5% contained *Salmonella*.

The microbial load for *E. coli* ranged from 1.3×10^4 cfu/ml - 9.23×10^4 cfu/ml and that of *Salmonella* was also within the range 1.0×10^3 cfu/ml - 9.35×10^5 cfu/ml (Refer to Table 2). The values obtained show that the levels were above the acceptable limits of 1×10^2 cfu/ml specified by the Ghana Standard Authority – GS724: 2003 and GS168: 2005 (GSA, 2003; GSA 2005).

The microorganisms found in this study were previously found in fruit by Eni *et al.*, (2010) in his research in Nigeria and in commercially packed and fresh fruit juices in Dhaka by Rahman *et al.*, (2011). The variation in the microbial load of these ready-to-drink fruit juices could imply how fruits are handled by specific vendors/processors and has a substantial impact on the level of microbial contamination (Eni *et al*., 2010). Pollutants from soil, irrigation water, the environment during transit, washing/rinsing water, or processor handling could all be factors in the bacteria found in this study (Ofor *et al*., 2009).

The presence of both *E. coli* and *Salmonella* could also be attributed to poor hygienic conditions and as well as source of water used for the fruit juice preparation and probably the lack of pasteurisation of the locally processed fruit juices. Artés & Allende, (2014) affirmed that one of the most common causes of fruit juice contamination is lack of pasteurization. Also, pathogens can be elevated in prepared juices due to lack of understanding about safe fruit juice production as well as contamination sources (Tasnim *et al*., 2010). Interestingly, it worth nothing that although sample 5 and 9, were obtained from the same vendor, peharps under same processing conditions, Sample 9, Tamarinda-ginger was devoid of both microbes. This perhaps could be attributed to possible antibacterial property of the mix. This is in agreement with Paz *et al.,* (2015) where the widest antibacterial spectrum was found in tamarind pulp extracts, which inhibited the proliferation of all microbes tested, both Gram positive and Gram negative.

Antimicrobial Residues

To enhance crop yields, the agricultural business (including crop cultivation and livestock production) faces hurdles. As a result, the use of antibiotics in livestock and antimicrobials on some disease-causing bacteria in fruit trees, are now common. For example, in countries where it is legal, streptomycin is applied to apple trees during bloom to prevent fire blight, with 0–4 applications per season based on disease forecast (Expert Panel on Antibiotic Resistance, 2005).

In this study, antimicrobial residues were absent in all 28 samples examined. Antibiotic residues have been linked to the usage of antimicrobial residue-contaminated manure in a number of studies (Phares *et al*., 2020; Quaik *et al*., 2020). According to Kumar *et al*., (2005), organic crops are likely to be infected with antibiotics due to exposure to antibiotic residues found in manure. Taylor and Reeder (2020) discovered no evidence of antibiotic use on crop plants in Africa, including Ghana.

According to the Phares *et al*., (2020) study, 84.2% of poultry and cattle farmers obtain antibiotics without a veterinarian's prescription, which could lead to antibiotic abuse that enters the food supply chain. Farmers may have unknowingly introduced antibiotics into the environment by using poultry droppings and dung on agricultural areas, especially when the antibiotic withdrawal time is not followed, and this can make its way into the food value chain. The recent investigation found no antimicrobial residues in any of the 28 fruit juices, which could be because the manure used on the farmland where the fruits were grown is likely to be antimicrobial residue-free, or that the antibiotic withdrawal periods were followed. Perhaps the farmers did not use any manure on their farm. Another possibility is the water used for irrigation may have not been polluted with antimicrobial residues. Since, antimicrobial residues from antibiotics can seep into water bodies when exposed to the environment, according to Amarasiri *et al*. (2020) wether from veterinary or other sources.

Conclusion

Majority of the locally processed fruit juice was contaminated with *E. coli* with a few contaminated with *Salmonella* whilst the industrially processed juice was devoid of contamination with respect to both microorganisms. Also, the microbial load for both *E. coli* and *Salmonella* were above the recommended limit of 1×10^2 cfu/ml set by Ghana Standard – GS724: 2003 and GS168: 2005. Antimicrobial residues were absent in all fruit juice samples for both industrially and locally processed juices. However, there is the need for surveillance on fruits, vegetables and across the food chain.

Although fruit juices boost the immune system of consumers and more, but they can also harm humans if they are infected with foodborne pathogens. To avoid outbreaks of food-borne disease, the quality of the fruit juices should be monitored through regular surveillance.

Abbreviations

***E. coli*:** *Escherichia coli*

FDA: Food and Drug Authority

GSA: Ghana Standard Authority

MCA: MacConkey Agar

ND: Not Detected

S-S: *Salmonella-Shigella* Agar

Declarations

Availability of data and materials

The data generated during the study is available upon request from the corresponding author.

Competing interests

The authors declare no competing interest

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Authors' contributions

FIJ and AM: contributed equally to the work; participated in the design, Sample collection, Sample/data analysis and Writing-first draft, EGA and JN; were involved in coordination, sample/data analysis and Validation, LQ and OAD; Conceptualization of project, Supervision, Methodology, Validation, Writing – review & editing of manuscript.

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