

Microbial Quality of Commercially Packed Fruit Juices in South-East Nigeria

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Abstract: The microbiological quality of commercially packed fruit juices sold in South-East Nigeria were assessed. A total of forty (40) juice samples consisting of orange, apple, pineapple, lemon, and guava flavoured varieties were collected. Samples were screened for total viable count, total coliform count, faecal coliform count, total staphylococcal count and total fungal count using standard microbiological methods. The highest total bacteria load of 4.4×10^5 cfu/ml was observed in sample A (Orange) while the lowest was observed in sample D of Apple variety (1.95×10^4 cfu/ml). The total coliform count ranged from no count in samples A, B, C and D to 9.8×10^1 cfu/ml in sample I (Guava juice). In all the samples, there was no faecal coliform found. The staphylococcal count ranged from no count in samples E, F, I and J to 8.4×10^2 cfu/ml in sample G (lemon juice). The highest total fungal count of 1.6×10^5 cfu/ml was observed in sample I (Guava) and the lowest count was observed to be 1.2×10^3 cfu/ml in an apple juice (sample D). The microorganisms isolated from the samples included *Staphylococcus aureus*, *Bacillus* species, *Enterobacter* species, *Acetobacter* species, *Lactobacillus* species, *Saccharomyces cerevisiae*, *Aspergillus* species, *Rhizopus* species and *Penicillium* species. *Bacillus* species was the most common (70%), followed by *S. aureus* (60%), *Enterobacter* spp., *Lactobacillus* spp. and *Saccharomyces cerevisiae* had the same rate of 50% respectively, and *Acetobacter* spp. and *Penicillium* spp. had the same rate of 40% while the least occurrence was seen in *Aspergillus* spp. and *Rhizopus* spp. with the rate of 30% respectively. The result showed that different bacterial and fungal species occurred within the fruits juices. The materials used for the production of the juice, poor sanitation, extraction, raw material contamination, lack of both proper heat sterilization and adequate quality control during processing of fruit juice could be a contributory factor to the presence of these organisms in the fruit juices. Some of the fungal isolates especially *Penicillium* sp. and *Aspergillus* sp. could result in the production of mycotoxins, which could lead to health hazards for the consumer and their presence in the fruit juices is of public health significance. Therefore, regular monitoring of the quality of commercially available fruit juices for human consumption is recommended to avoid outbreak of food borne illness resulting from the organisms encountered in this study.

INTRODUCTION

Fruit juices are beverages and nutritious drinks with great taste and which are commonly consumed for their refreshing attributes, nutritive values or vitamin content and health benefits (Suaad and Eman, 2008; Nwachukwu and Aniedu, 2013; Rashed *et al.*, 2013). According to Tasnim *et al.* (2010), fruit juices are becoming an important part of the modern diet in many communities. They are nutritious beverages and can play a significant part in a healthy diet because they offer good taste and a variety of nutrients found naturally in fruits. For example, orange juice is rich in vitamin C which is an excellent source of bio-available antioxidant phytochemicals as reported by Franke *et al.* (2005) and significantly improves blood lipid profiles in people affected by hypercholesterolemia (Kurowska *et al.*, 2000; Tasnim *et al.*, 2010). Fruit juices promote detoxification in the human body (Deanna and Jeffrey, 2007). Juices are fat-free, nutrient-dense beverages rich in vitamins, minerals and naturally occurring phytonutrients that contribute to good health (Tasnim *et al.*, 2010).

Fruit juices are available in their natural concentrations or in processed forms. Juice is

prepared by mechanically squeezing fresh fruits or is extracted by water. A whole fruit can be directly squeezed, macerated or crushed so as to produce a considerable amount of pulp or juice or may be extracted by water. The extracted juice could be used in their natural state or could be concentrated by evaporation or freezing and could be preserved by chemical means, bottling, canning or freezing (Frazier and Westhoff, 1998; Oranusi *et al.*, 2012). The constituents of processed juices are mainly water, sugar, preservatives, colour, and fruit pulp. The most commonly used preservatives are benzoic acid, sorbic acid or sulphur dioxide. Natural colours such as anthocyanins and betanin are used. Acid is an essential universal constituent of juice and the most commonly used acid is citric acid (Oranusi *et al.*, 2012). Fruit juices are easy to process and blended with other products (Bate *et al.*, 2001).

Fruit juices contain sufficient nutrients that could encourage the growth of microorganisms. The most important factors which could encourage or limit the growth of microorganisms in juices includes pH, hygienic practice and storage temperature as well as the concentration of the preservative used

(Okigbo and Obire, 2009; Tasnim *et al.*, 2010; Odu and Adeniji, 2013). Several reports of illnesses due to the food borne diseases associated with the consumption of fruit juices at several places around the world have been reported (Mosupye and Holy, 2000; Muinde and Kuria, 2005; Chumber *et al.*, 2007). Moreover, several factors can act as source of microbial contamination of the fruit juices such as use of unhygienic water for dilution, dressing with ice, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust (Tasnim *et al.*, 2010; Babalola *et al.*, 2011; Odu and Adeniji, 2013). Various authors have reported the presence of pathogens, namely, *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus* (Sandeep *et al.*, 2004; Rashed *et al.*, 2013). Changes in pH may also promote the growth of pathogens (Food and Drug Administration [FDA], 2001). Tasnim *et al.*, (2010) asserted that water used for juice preparation can be a major source of microbial contaminants including coliforms, faecal coliforms, faecal streptococci, etc. The quality of fruit juices is strictly maintained in the developed countries and enforced under several laws and regulations. However, in many developing countries, although most of these are government agencies that regulate and control food and drug administration, small and medium entrepreneurs may not adhere strictly to microbiological safety and hygiene (Tasnim *et al.*, 2010) and lack of strict adherence to standard operating procedures (SOP). Thus, the contamination of the juice becomes inevitable which could pose a public health risk. Therefore, this study evaluates the microbial quality of commercially packed fruit juices sold in South-East Nigeria.

MATERIALS AND METHODS

Source of Materials

A total of 40 samples of fruit juices were collected from major cities in South-East, Nigeria. Five (5) varieties of fruit juices (orange, apple, pineapple, lemon, and guava) were chosen based on the consumer demand. Four samples from two different brands each of orange, apple, pineapple,

lemon, and guava flavoured variety were bought. Samples were tested within an hour after procurement.

Determination of Microbial Load

Determination of microbial load of the samples was done based on the method described by Rashed *et al.* (2013). Serial dilutions of samples were made up to 10^{-7} with sterile normal saline. Exactly 0.1ml of each dilution was evenly spread on nutrient agar medium and incubated at 37°C for 24 hours. Plates were screened for the presence of discrete colonies after incubation period and the actual numbers of bacteria were estimated as colony forming unit per ml (cfu/ml). The load of specific microorganisms was determined by plating on selective media. Total coliform count (TCC), faecal coliform count (FCC), total staphylococcal count (TSC) and total fungal count (TFC) were performed in similar manner as described above using MacConkey agar, membrane faecal coliform (mFC) agar, Mannitol Salt Agar (MSA) and Sabroud dextrose agar (SDA) medium, respectively. In each case plating was done in triplicates and counts taken from plates that had less than 300 colonies. Estimation of bacteria load was performed by standard method (International Commission on Microbiology Specification for Food [ICMSF], 1998).

Isolation of Microorganisms from the Fruit Juices

The colonies obtained from the process above were examined closely and distinct colonies were taken and subcultured in fresh sterile medium. The subcultures were incubated at 37°C for 24-48h. The uniformity of the growth is an indication of purity. The pure cultures were characterized and identified as previously described (Fawole and Oso, 1988; Okereke and Kanu, 2004; Holt *et al.*, 1994; Cowan and Steel, 1975; Majali *et al.*, 2016; Althunibat *et al.*, 2016).

RESULTS

Most of the fruit juice samples showed high viable bacterial count. The highest total bacteria load of 4.4×10^5 cfu/ml was observed in the orange juice

Table 1. The Average Microbial Load of the Different Brands of Fruit Juices.

Samples Code/Juice Type	Microbial count (cfu/ml)				
	Total Viable Count (TVC)	Total Coliform Count (TCC)	Faecal Coliform Count (FCC)	Total Staphylococcal Count (TSC)	Total Fungal Count (TFC)
A (Orange)	4.4×10^5	ND	ND	1.0×10^3	1.2×10^3
B (Orange)	1.6×10^5	ND	ND	2.4×10^2	1.5×10^5
C (Apple)	4.64×10^3	ND	ND	1.99×10^2	1.2×10^3
D (Apple)	1.95×10^4	ND	ND	4.54×10^2	2.0×10^3
E (Pineapple)	7.8×10^4	6.5×10^1	ND	ND	3.1×10^4
F (Pineapple)	2.3×10^4	2.1×10^1	ND	ND	4.0×10^4
G (Lemon)	2.3×10^4	4.8×10^1	ND	5.0×10^2	1.0×10^4
H (Lemon)	3.4×10^4	9.4×10^1	ND	8.4×10^2	1.2×10^4
I (Guava)	2.2×10^5	9.8×10^1	ND	ND	1.6×10^5
J (Guava)	1.4×10^5	8.1×10^1	ND	ND	1.2×10^5

ND= None detected.

(sample A) while the lowest was observed in sample D of Apple variety (1.95×10^4 cfu/ml). The total coliform count ranged from no count in samples A, B, C and D to 9.8×10^1 cfu/ml in sample I (Guava juice). In all the samples, there was no faecal coliform found. The staphylococcal count ranged from no count in samples E, F, I and J to 8.4×10^2 cfu/ml in sample G (lemon juice). The highest total fungal count of 1.6×10^5 cfu/ml was observed in sample I (Guava) and the lowest count was observed to be 1.2×10^3 cfu/ml in an apple juice (sample D) (Table 1).

The microorganisms isolated from the samples include *Staphylococcus aureus*, *Bacillus* species, *Enterobacter* species, *Acetobacter* species, *Lactobacillus* species, *Saccharomyces cerevisiae*, *Aspergillus* species, *Rhizopus* species and *Penicillium* species. *Staphylococcus aureus* was present in samples A, B, C, D, G and H, *Bacillus* species was present in all the samples except F and H, *Enterobacter* species was isolated from sample E, F, H, I and J, *Acetobacter* species was present in E, F, I and J while *Lactobacillus* species was only isolated from samples A, C, D, I, and J. *Saccharomyces cerevisiae* was isolated from A, B, D, G and I, *Aspergillus* species was only positive to B, E and J. *Rhizopus* species was isolated from C, F and H while *Penicillium* species was positive to samples B, F, I and J (Table 2). The frequency of occurrence of the isolated microorganisms on the fruit juices under study showed that *Bacillus* species was the most common (70%), followed by *S. aureus* (60%), *Enterobacter* spp., *Lactobacillus* spp. and *Saccharomyces cerevisiae* had the same rate of 50% respectively, and *Acetobacter* spp. and *Penicillium* spp. had the same rate of 40% while the least occurrence was seen in *Aspergillus* spp. and *Rhizopus* spp. with the rate of 30% respectively.

Table 2. Frequency of Occurrence of the Organisms Isolated from the Fruit Juice Samples.

Isolates	NUMBER OF SAMPLES		
	Examined	Positive	Rate (%)
<i>Staphylococcus aureus</i>	40	24	60.00
<i>Bacillus</i> species	40	28	70.00
<i>Enterobacter</i> species	40	20	50.00
<i>Acetobacter</i> species	40	16	40.00
<i>Lactobacillus</i> species,	40	20	50.00
<i>Saccharomyces cerevisiae</i>	40	20	50.00
<i>Aspergillus</i> species	40	12	30.00
<i>Rhizopus</i> species	40	12	30.00
<i>Penicillium</i> species	40	16	40.00

% = percentage

DISCUSSION

Microorganisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts and breaks that occur during growing or harvesting (Durgesh et al., 2008). Contamination from raw materials and equipment, additional

processing conditions, improper handling, prevalence of unhygienic conditions contribute substantially to the entry of bacterial pathogens in juices prepared from these fruits or vegetables (Oliveira et al., 2006; Nicolas et al., 2007; Durgesh et al., 2008; Odu and Adeniji, 2013).

In the present study, the total bacterial count ranged from 4.64×10^3 cfu/ml to 4.4×10^5 cfu/ml for all the fruit samples. These counts were high and above the maximum permitted standard of 1.0×10^5 cfu/ml (Gulf Standard, 2000) except sample C (4.64×10^3 cfu/ml) of the apple variety. This observation is slightly above the counts obtained in the work of Odu and Adeniji (2013) who reported high bacterial load ranging from 3.0×10^2 cfu/ml to 9.0×10^4 cfu/ml in some of the fruits under study and in consistent with the work of Tumane (2011) who reported total bacterial count of juice samples in the range of 1.0×10^5 cfu/ml to 2.0×10^6 cfu/ml in Nagpur, India. Also, this observation could be related to the reports of Al-Jedah et al. (2002) and Durgesh et al. (2008) that most fruits have bacteria load of 1.0×10^5 cfu/cm² on their surfaces and improper washing of fruits add these bacteria to juices leading to contamination. In addition, it could be attributed to lack of simple safety and hygienic rules by vendors such as prolonged preservation without refrigeration which can encourage bacteria growth.

In the present study, the total coliform count ranged from no growth to 9.8×10^1 cfu/ml and no faecal coliform count was recorded in all the fruits juices under study. This observation falls within the acceptable limit of the Gulf Standard (2000) and agreed with the work of Rashed et al. (2013) who reported no coliform and no faecal coliform on some strawberry, apple, orange and mango packed fruit juices studied and the works of Tasnim et al. (2010), Oranusi et al. (2012) and Odu and Adeniji (2013) who recorded similar observation on different fruit juices including pineapple, orange, apple etc. the absence of fecal coliform in all the fruit juices is an indication of product conformity to standard. The presence of the coliform mostly of the *Enterobacter* type in some of the fruit juices has been reported to be natural flora of fruits which may be introduced into the fruit juice if improperly processed (Oranusi et al., 2012). This observation also agreed with the work of Babalola et al. (2011) who reported the presence of *Enterobacter aerogene* in apple, pineapple and orange fruit juices. However, Safe Food Consumption Standard prohibit coliforms in fruit juice (Andres et al., 2004), therefore, samples E, F, G, H, I and J can be considered unfavourable for consumption.

The staphylococcal count ranged from no count (samples D, E, I and J) to 1.0×10^3 cfu/ml in the present study. The values obtained for the

staphylococcal count are within the acceptable limit of the Gulf Standard (2000). Coagulase-positive staphylococci may cause human disease through the production of toxins (Rashed *et al.*, 2013). However, effective levels of toxin formation require a high number of microorganisms (approximately 10^5 - 10^6 microorganisms per ml of food) (International Dairy Federation [IDF], 1994). Few reports have shown the prevalence of staphylococci in fruit juice samples (Ahmed *et al.*, 2009; Tambekar *et al.*, 2009; Babalola *et al.*, 2011; Oranusi *et al.*, 2012; Rashed *et al.*, 2013).

The fungal count in all the products in the present study is of the order 10^3 - 10^5 . This finding is in consistent with the reports of Rahman *et al.* (2011) and Oranusi *et al.* (2012). The low count could be associated to the suppressive effect of preservatives used and the limitations of growth in the absence of air (Parish, 1991). Fungi are common environmental contaminants and the moulds bear resistant spores that easily contaminate surfaces and can resist the juice condition (Parish, 1991). Fungi are the major causes of spoilage of fruits and vegetables (ICMSF, 1998), the presence of aflatoxin and other mycotoxin producing mould contamination on fruit surfaces have been reported to generally end in the juice (Oranusi *et al.* 2012). In the present study, the microorganisms isolated from the samples included *Staphylococcus aureus* (60%), *Bacillus* species (70%), *Enterobacter* species (50%), *Acetobacter* species (40%), *Lactobacillus* species (50%), *Saccharomyces cerevisiae* (50), *Aspergillus* species (30%), *Rhizopus* species (30%) and *Penicillium* species (40%). The result showed that *Bacillus* species is the most common. Similar observation has been recorded in previous studies in Nigeria (Oranusi *et al.*, 2012; Odu and Adeniji, 2013). *S. aureus* are common contaminants often from the food handlers and environment and post process contamination could explain their presence in the juice. The *Bacillus* and moulds are spore bearers and common food contaminants from the environment. The presence of fungi organisms in some of the fruit juices under study could be explained by the fact that the low pH of the juice favours the yeast and mould (Tournas *et al.*, 2006; Oranusi *et al.*, 2012). This also suggests the presence of fermentative organisms (Odu and Adeniji, 2013). This is confirmed by the presence of yeast, *Saccharomyces cerevisiae* which was also isolated in most of the fruit juice samples. However, the presence of *Penicillium* species and *Aspergillus* species in some of the fruit juice samples examined in the present study could result in the production of mycotoxins, which could lead to health hazards for the consumer (Odu and Adeniji, 2013). The mycotoxins produced by *Penicillium* sp. have been reported to cause renal damage/necrosis of the

kidney (Odu and Ameweiye, 2013). *Penicillium* and related genera are present in soils and plant debris from both tropical and Antarctic conditions but tend to dominate spoilage in temperate regions (Doyle, 2007; Adebayo-Tayo *et al.*, 2012; Odu and Ameweiye, 2013). Although they can be useful to humans in producing antibiotics and blue cheese, many species are important spoilage organisms, and some produce potent mycotoxins (patulin, ochratoxin, citreoviridin, penitrem) (Doyle, 2007; Adebayo-Tayo *et al.*, 2012; Odu and Ameweiye, 2013). The presence of microbial contaminants in all the products could be a reflection of the quality of the raw materials, processing equipments, environment, packaging materials and the personnel's in the production process. The presence of these organisms needs to be controlled to prevent spoilage and food borne illness (Mudgil *et al.*, 2004; Oranusi *et al.*, 2007).

CONCLUSION

The present study has shown that the average total bacteria counts of the commercially packed fruit juice samples examined are generally above the maximum allowable limit in foods for consumption (10^3 cfu/g). The coliform count, faecal coliform count and staphylococcal counts fall within the maximum acceptable limits of the Gulf Standard (2000). However, the average ranges obtained for the bacteria indicated a public health concern as they showed counts far above this limit. These high counts suggested heavy bacterial contamination of the fruit juice by handling, processing equipment, environment, packaging materials and the personnel's in the production process. With the number of isolated bacteria and fungi from the different packaged fruit juice sold in South-East Nigeria, it can be concluded that different bacterial and fungal species occurred within the fruits juices under study. The materials used for the production of the juice, poor sanitation, extraction, raw material contamination, lack of both proper heat sterilization and adequate quality control during processing of fruit juice could be a contributory factor to the presence of these organisms in the fruit juices. Some of the fungal isolates especially *Penicillium* sp and *Aspergillus* sp. could result in the production of mycotoxins, which could lead to health hazards for the consumer. Their presence in the fruit juices is of public health significance. Therefore, regular monitoring of the quality of commercially available fruit juices for human consumption is recommended to avoid outbreak of food borne illness resulting from the organisms encountered in this study.

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