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Microbial Quality of Industrially Processed Sachet-Packed Fruit Drinks Consumed Mostly by School Children in Nigeria

P. T. Fowoyo[1*] and H. Amadi[2]

[1]Microbiology Department, Baze University, Abuja, Nigeria.
[2]Microbiology Department, Salem University, Abuja, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Fruit drinks are often packed as accompaniments with school children's lunch packs in Nigeria. In recent times, reports from consumers stated that a lot of these drinks when opened up and poured into cups before drinking, fungal mass was present and this has queried the safety of consumption of these drinks. The aim of this study was to determine whether commercially packed fruit drinks consumed majorly by school children were microbially contaminated. Twenty (20) samples of sachet packed fruit drinks comprising of 4 different flavours precisely orange, pineapple, apple and multivitamin flavours were analyzed for their microbial quality. The total bacterial and fungal counts in the samples examined did not exceed the regulatory microbiological criteria for fruit drinks. *Lactobacillus*, *Bacillus*, *Staphylococcus aureus*, *Rhizopus*, *Aspergillus* and *Penicillium* species were isolated from the samples. *Lactobacillus* poses no health risk to the consumer however, the incidence of *Bacillus* and *Staphylococcus aureus* in the drink samples is quite worrisome as they have been implicated as potential pathogens. The fungal species isolated are of public health concern especially as some have been implicated as mycotoxin producers. The presence of these organisms in the drinks may be attributed to indigenous microflora of fruits or concentrates used, poor hygienic practices during production and low pH of the drinks. It is therefore necessary that

**Corresponding author: Email: patience.fowoyo@bazeuniversity.edu.ng;*

fruit drink manufacturers adhere strictly to microbiological quality standards and specifications during production especially for foods to be consumed by children with developing immune systems.

Keywords: Sachet packaging; processed fruit drinks; food safety; microbial contamination; school aged children.

1. INTRODUCTION

Fruits are important in human diets due to their contribution of vital nutrients, most especially vitamin C. Although, they are very low in fats and proteins but high in sugar as they contain large amount of glucose, fructose, and sucrose. According to [1], fruit drink means the unfermented but fermentable liquid obtained from a named fruit which can be taken as fresh fruit drink, frozen, canned or made from concentrates. Fruit drinks can be prepared by mechanically squeezing or macerating fruit or vegetable flesh without the application of heat or solvents [2]. The drink may be concentrated and later reconstituted with water suitable for maintaining the essential composition and quality factor of the drink. Most fruit drinks contain sufficient nutrients that could support microbial growth. Several factors encourage, prevent, or limit the growth of microorganisms in drinks. The most important factors are water activity (a_w), low pH, hygienic practice and storage temperature and concentration of preservative. The most likely mechanisms by which fruit drinks become contaminated with pathogenic microorganisms are through direct contact with animal or human faeces, or indirect contact with contaminated water, soil, processing equipment, or infected food handlers [3]. Specific spoilage microbes can grow even in products produced under good manufacturing practices. In case of production failures, less specialized opportunistic species are often involved, as they are more common in the production environment. New ingredients or new applications of established ingredients could introduce new spoilage species and growth factors in beverages, thereby expanding the spoilage microbe range beyond the well-known species.

Therefore, if drinks are contaminated and consumed, the probability of illness exists, especially in young children, the elderly, and the immune compromised [4]. Processed fruit drinks are gradually becoming the drink of choice for school children. In the last few years, consumers have reported occurrences of mass of microbial growth in processed, sachet-packed fruit drink consumed mostly by school children. The consumption of contaminated processed fruit drinks by school aged children could present a public health risk thus there is the need to assess the microbial quality and safety of popularly consumed processed fruit drinks by school children. The primary aim of this research work is to determine the microbial quality of various commercially processed and branded fruit drink consumed by most school children.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Sachet-packaged, industrially processed and branded fruit drink samples were collected from various retail outlets in Lokoja, Kaduna, Abuja, Akure and Enugu towns. The constituents of the fruit drinks was a mix of fruit concentrates and water, and packed into sachets or pouch made from ultra-thin laminated foil. A total of twenty (20) samples comprising 4 different flavors precisely orange, pineapple, apple and multivitamin flavors were analyzed. Triplicate batches of each of the brands were purchased randomly. The samples were kept refrigerated at 4^0C in the laboratory before analysis.

2.2 Determination of Physicochemical Parameters

2.2.1 Determination of pH

A pH meter (HI 2211) was used in determining the pH of the fruit drink samples.The electrode was first standardized using astandard buffer solution of pH 4 and pH 7. The electrode was then rinsed with distilled water and immersed into the samples. The sample (25ml) was put into a beaker and the pH was checked and recorded.

2.2.2 Determination of titratable acidity

This was determined as described by [5]. Each of the samples (10ml) was pipetted into a conical flask to which 25 ml of distilled water and three drops of 1% phenolphthalein were added. Two hundred milliliters (200 ml) of 0.1 M NaOH was

measured into a burette and was titrated against the sample in the conical flask until a pink colouration was observed and the corresponding burette reading wastaken.

TTA was calculated using this formula:

$$\text{TTA}(\%) = \frac{Titre\ x\ blank\ x\ normality\ of\ base\ x\ ml\ equivalent\ of\ citric\ acid}{Weight\ of\ sample} \times 100$$

ml equivalent of citric acid (meq) = 0.06404

2.3 Isolation and Enumeration of Bacteria and Fungi

The samples were serially diluted. Aliquots (1 ml) of the second dilution (10^{-2}) of each of the samples was inoculated onto nutrient agar (NA) and potato dextrose agar (PDA) using the pour plate technique, this was done in triplicates. The NA plates were incubated at 37^0C for 18 - 24 h. The colonies that developed on the plates were enumerated. For fungi, the potato dextrose agar (containing 0.1% streptomycin) was incubated at 25^0C for 3 to 5 days.

2.3.1 Identification of bacterial isolates

The colonial and cellular characteristics of the bacterial isolates was examined and used for identification. The colonial characteristics observed the morphology or features of the organisms such as colour, shape, opacity and margin on the agar plate. The cellular characteristics were determined through Gram staining, spore staining, catalase test, coagulase test, sugar fermentation test, starch hydrolysis, citrate utilization, oxidase test, gelatin hydrolysis test and urease test.

2.3.2 Wet mount preparation of fungal isolates

The slides were prepared using methylene blue dye. The dye was dropped into the center of the clean glass slide made to stand on the staining rack. Fungal hyphae were aseptically removed from the sub-cultured plate with a wire loop and teased apart on the stain. The slide preparation was carefully covered with a cover slip to avoid air bubbles. Blotting paper was used to remove excess stain coming through the edge of the cover slip; slides of each colony were made and observed under the low power objective(x10) and high power objective(x40) lens of the Phase contrast microscope. For the purpose of identification, the characteristics and type of spore as well, as mycelia forms of the different fungal isolates were recorded [6].

3. RESULTS

In Table 1, apple drink samples had pH values ranging from 4.20-4.30 and titratable acidity (TTA) values in the range of 0.46% – 1.12%. pH values of orange drinks varied from 2.50 - 4.30 with TTA value of 0.030% in all orange drink samples examined except for the sample collected from Abuja that had a TTA value of 0.032%. The pH of the pineapple drink samples ranged between 3.36 – 3.86 and had TTA of 0.032%. The pH of the multivitamin drink samples had a pH range of 3.40 – 3.44 with titratable acidity in the range of 0.028% – 0.032%. Orange and apple drinks had the highest acidity values with the lowest acidity values indicated in the multivitamin drink samples.

Among the four varieties of fruit drinks examined in this study, bacterial count of 3.0 x 10^2 cfu/ml which is the highest was from pineapple and multivitamin drinks from Akure and Kaduna There was no bacterial growth in apple and orange drink samples from Abuja as shown in Table 2. Apple and orange drinks had the highest fungal count of 1.0 x 10^3 cfu/ml and 8.0 x 10^2cfu/ml respectively as depicted in Table 3.

Lactobacillus species were isolated from orange, apple and multivitamin fruit drink samples. *Staphylococcus aureus* occurred in orange and pineapple fruit drinks. *Bacillus* species were isolated from apple fruit drinks as shown in Table 4.

Table 1. Physicochemical analysis of different varieties of processed fruit drink samples

Fruit drink Types	Lokoja		Kaduna		Abuja		Akure		Enugu	
	pH	TTA (%)	pH	TTA (%)	pH	TTA (%)	pH	TTA (%)	pH	TTA (%)
Apple drink	4.30	1.05	4.20	0.46	4.25	0.81	4.28	1.12	4.27	0.78
Orange drink	2.86	0.030	4.30	0.030	3.50	0.032	2.50	0.030	3.36	0.030
Pineapple drink	3.86	0.032	3.36	0.032	3.36	0.032	3.74	0.032	3.63	0.032
Multivitamin drink	3.41	0.028	3.40	0.032	3.43	0.028	3.44	0.028	3.43	0.032

Table 2. Viable total bacterial count in fruit drink samples

Fruit drink types	Lokoja (cfu/ml)	Kaduna (cfu/ml)	Abuja (cfu/ml)	Akure (cfu/ml)	Enugu (cfu/ml)
Apple drink	1.0×10^2	1.0×10^2	NG	1.0×10^2	1.0×10^2
Orange drink	1.0×10^2	1.0×10^2	NG	2.0×10^2	1.0×10^2
Pineapple drink	2.0×10^2	1.0×10^2	1.0×10^2	3.0×10^2	2.0×10^2
Multivitamin drink	2.0×10^2	3.0×10^2	1.0×10^2	3.0×10^2	2.0×10^2

Key: NG -No growth

Table 3. Viable total fungal count in fruit drink samples

Fruit drink Types	Lokoja (cfu/ml)	Kaduna (cfu/ml)	Abuja (cfu/ml)	Akure (cfu/ml)	Enugu (cfu/ml)
Apple drink	8.0×10^2	1.0×10^3	6.0×10^2	6.0×10^2	9.0×10^2
Orange drink	8.0×10^2	6.0×10^2	7.0×10^2	5.0×10^2	6.0×10^2
Pineapple drink	5.0×10^2	2.0×10^2	1.0×10^2	1.0×10^2	2.0×10^2
Multivitamin drink	2.0×10^2	3.0×10^2	4.0×10^2	3.0×10^2	2.0×10^2

Key: NG - No growth

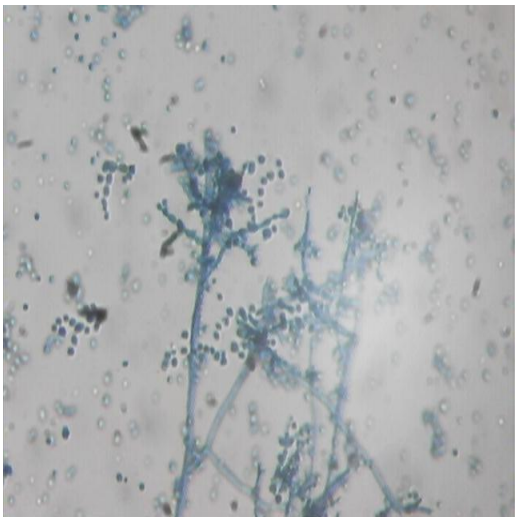

Fig. 1. Image of *Penicillium* sp. using phase contrast microscopy

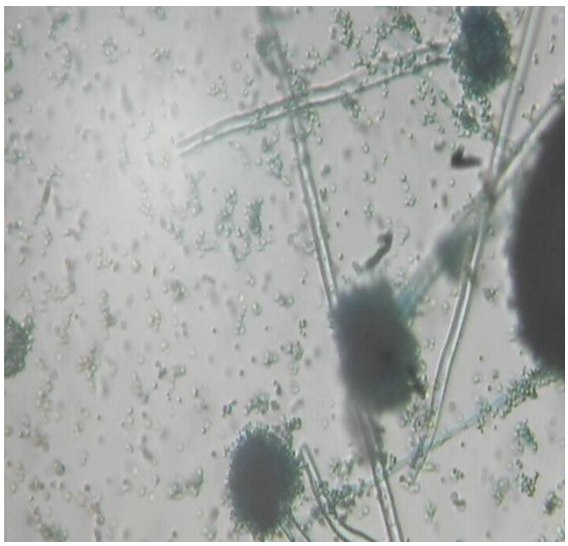

Fig. 2. Image of *Aspergillus* sp. using phase contrast microscopy at magnification of x400

Table 4. Morphology & biochemical characteristics of bacterial isolates

Bacterial Isolates	Color	form	Elevation	Margin	Light intensity	Cell Arrangement	Catalase	Coagulase	Oxidase	Urea	Starch Hydrolysis	Citrate utilization	Gelatin liquefaction	Grams staining	Spore staining	Glucose	Sucrose	Maltose	Fructose	Lactose	Suspected organism
Bacterial isolate from Apple drink	cream	round	Flat	Entire	opaque	Rod in Chains	+	–	+	–	+	+	–	+	-	A	A	AG	A	A	*Lactobacillus* sp.
Bacterial isolate from orange drink	white	Round	raised	Even egde	opaque	Cocci in clusters	+	+	-	-	+	-	+	+	-	A	-	-	-	+	*Staphylococcus aureus*
Bacterial isolate from Pineapple drink	cream	Round	raised	Even edges	opaque	Cocci in clusters	+	+	-	-	-	-	+	+	-	A	-	-	+	+	*Staphylococcus aureus*
Bacterial isolate from Multivitamin drink	cream	round	Flat	Entire	opaque	Rod in Chains	+	–	+	–	+	+	–	+	-	A	A	AG	A	A	*Lactobacillus* sp.
Bacterial isolate from Apple drink	opaque	round	Flat	Irregular edges	opaque	rods	+	–	-	-	+	+	+	+	+	A	-	-	A	-	*Bacillus sp.*
Bacterial isolate from orange drink	cream	round	Flat	Entire	opaque	Rod in Chains	+	–	+	–	+	+	–	+	-	A	A	AG	A	A	*Lactobacillus* sp.

Table 5. Colonial and microscopiccharacteristics of fungalisolates

Fungal Isolates	Description	Suspected organism
O1	White colony spreading all over the culture plate has spores enclosed in the sporangium with a non septate hyphae	*Rhizopus* sp.
P2	Black colony with a whitish outer layer, conidiophores and a septate hyphae	*Aspergillus* sp.
M1	Green colony with awhitish outer layer, with conidiophores and septate hyphae	*Penicillium* sp.

Key: O1: From orange drink samples P1: From pineapple drink samples M1: From multivitamin fruit drink samples

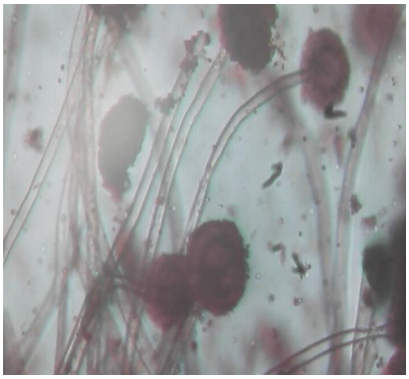

Fig. 3. Image of *Rhizobium* sp. using phase contrast microscopy at magnification of x400

Thecolonial and microscopicfeaturesoffungi isolatedfrom the fruit drinksare described in Table 5. Figs. 1-3 are images of the fungal isolates taken with a phase contrast microscope andthe imagesaided further identification of the isolates.

4. DISCUSSION

From the results, all the drink samples examined were acidic. The pH of the fruit drink samples ranged between 2.50– 4.30. This low pH favors the growth of acid tolerant bacteria like *Lactobacillus* species and may have encouraged the growth and proliferation of fungi [7]. A low pHwill favorthe spoilage of the samples and shorten the storage stability of the products. pH influences the type of microorganisms that will grow and survive in the drink and invariably the stability of the drink [8]. Low pH tends to allow acid tolerant pathogenic bacteria such as *Salmonella* sp., *S. aureus*, *E. coli*, and *Listeria monocytogenes* to survive in fruit drinks [9]. It was suggested that pathogenic organisms that developed in drinks were able to withstand high acidity achieved through employing adaptive mechanisms involving both active and passive homeostasis, and production of enzymes to regulate internal pH [7]. The apple drinksexamined had the highesttotal titratable acidity whichinvarably meantthey were highly acidicand this could have been responsible for the high fungal growth in the drink.

[10] had reported on somefactors responsible for contamination of fruit drinks. Improper washing of fruits adds these bacteriato drinks leading to contamination. In addition, thelack of application of basic safety rules by fruit processors contribute to theaugmentation of the microbial loads. These include theuse of improperly sterilized extractors, homogenizers and other equipment used in the process line, unavailability of treated running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust [11].

The total bacterial counts from the drink samples were not above the acceptable limit for human consumption. According to [12], the acceptable limit of bacteria load in drinks for human consumption should not exceed 10^6cfu/ml. The presence of *Staphylococcus aureus* in the samples may be reflective of poor physical hygiene practices by the food handlers. *Bacillus* species may have been from contaminating soil particles adhering to the fruits after harvesting and possibly not properly cleaned before thedrink was extracted.

The presence of fungal contaminants in the products could be a reflection of the quality of the raw materials, processing types ofequipment, environment, packaging materialsand personnels in the production process [13]. The incidence of

Aspergillus, *Penicillium* and *Rhizopus* shows poor quality processing ofthe products. These fungihave been reported to produce potent mycotoxinsresponsible for various mycotoxicosis in humans [14,15].

5. CONCLUSION

This study identified *Bacillus* sp., *Staphylococcus aureus* which could portend some health risk since they have been implicated as potential pathogens. *Lactobacillus* sp. poses no threat to human health due to its probiotic properties.The fungi isolated from the drinks may pose a health threat to humansbecause of their capacity to produce mycotoxins. It is recommended that good manufacturing practices (GMP) be put in place and also the National food regulators should ensure random and regular quality checks on both local and imported fruit drinks to ascertain they adhere to specified guidelines to protect the health of children especially since they are the major consumers of these drinks and are prone to becoming sick due to their developing immunity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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