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Research Article

Bacteriological Quality, Antibiotic Susceptibility of Isolated Bacteria and Proximate Composition of Different Brands of Packaged Fruit Juice

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ABSTRACT

Juices are drinks expressed from the edible part of fruits or any concentrates of such fruits. Fruit juice makes a good medium for microbial growth. This study was conducted to determine the bacterial load, antibiotic susceptibility of isolated bacteria, and the proximate composition of packaged fruit juices sold in Port Harcourt, Nigeria. A total of forty (40) samples of packaged fruit juice of four (4) different brands were analyzed using the Analytical Observational Cohort design. Results showed that, mean value of total heterotrophic bacterial count was highest in Ribena 1.88±1.04 x 10^3CFU/ml, followed by Capri-sun 0.77±0.67 x 10^3CFU/ml, then Chi-exotic 0.57±0.74 x 10^3CFU/ml, while Happy-hour recorded the least value of 0.49±0.64 x 10^3CFU/ml. There was no statistically significant difference in the total heterotrophic bacterial counts. Isolated and identifies bacteria were ten species of *Bacillus* sensitive to Gentamicin, Erythromycin, Ciprofloxacin and Chloramphenicol, but resistant to Tetracyclin, Trimethoprim, penicillin and Cefoxitin; three *Paenibacillus* sensitive to Gentamicin, Erythromycin and Tetracyclin, but resistant to Ciprofloxacin, Trimethoprim, Chloramphenicol, Penicillin and Cefoxitin; and two *Lysinibacillus* species that were sensitive to Gentamicin, Erythromycin and Cefoxitin, but resistant to Tetracyclin, Ciprofloxacin, Trimethoprim, Chloramphenicol and Penicillin. Multiple antibiotic resistance (MAR) index was greater than 0.2. The ash content of Capri-sun, Chi-exotic, Happy-hour and Ribena were 0.04±0.00, 0.12±0.00, 0.04±0.00 and 0.04±0.00% respectively; Crude protein was 0.22±0.00, 0.19±0.00, 0.08±0.00 and 0.08±0.00% respectively; fat content was 0.10±0.00, 0.11±0.01, 0.10±0.00 and 0.08±0.00 % respectively, while carbohydrate was 12.34±0.23, 12.72±0.04, 12.56±0.06 and 12.43±0.06%, respectively. All brands of fruit juices met Gulf standard for microbial load. However, spore bearing *Bacillus* found in this study is of public health concern. There is therefore the need for thorough quality control measures to control the presence of undesired bacteria in fruit juices.

Keywords: Fruit Juice, bacteriological quality, proximate composition, *Bacillus*, *Lysinibacillus*, antibiotic resistance.

Introduction

Juices are drinks that are extracted or expressed from the edible part of one or more fruits or vegetables or any concentrates of such vegetables or fruits. Fruit juices are consumed as a beverage or used as an ingredient or flavouring in foods or other beverages owing to their refreshing taste, health benefits and nutritive values (Suaads and Hamed, 2008; Nwachukwu and Aniedu, 2013; Rashed *et al*., 2013). Fruit juice may be directly extracted from fruit(s) or obtained from the reconstitution of fruit(s) concentrate. In industrial application, fruit juice drinks must contain at least 20% of the fruit juice.

Water is the predominant component of fruit juice. Fruit juice contain carbohydrate including sucrose, fructose, glucose and sorbitol in varying quantities (Heldman and Lund, 1998), as well as small amount of protein, but no fat and cholesterol. Fruit juice contains no fibre, unless the pulp is included. Fruit juices are rich in minerals, vitamin A and C, but could be fortified with more minerals, vitamins or potassium and may have added sugar (Pao *et al*., 2001).

On the other hand, Juices are fermentable but unfermented product or aqueous liquids extracted or expressed from the edible part of fruit or vegetables or any concentrates of such vegetables or fruits.

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Fruits undergo tremendous chemical changes once separated from the parent plant, until spoilage occurs. Typical changes may show in texture, colour and flavour of processed fruits.

Spoilage of fruit juices generally results from the growth of acid tolerant bacteria, yeasts or moulds that are sensitive to pasteurization (Guillotin *et al*., 2009; Petrisor *et al*., 2010). Poor manufacturing practices and the nutritionally rich components of fruit juices make the product, good medium for microbial growth and vehicle of food borne pathogens (Ketema *et al*., 2008).

In the developed countries, the quality of fruit juices is strictly being maintained under several laws and regulations including set standards for the maximum permissible level of microbes in fruits, fruit juices and related products (Al-jedah and Robinson., 2002). The recommended microbiological standards for the Maximum bacterial load anticipated for Total viable count, Coliform, Faecal coliform, and Staphylococcal count should be 5.0 x 10^3/ml, 10/ml, 0/ml and 100/ml respectively while the Maximum bacterial load permitted should be 1.0 x 10^4/ml, 100cells/ml, 0/ml and 1.0 x 10^3/ml respectively, for any fruit juice consumed. (Gulf Standards., 2007).

In many developing countries including Nigeria, the manufacturers are not much concerned about the safety and hygiene of fruit juices because of lack of enforcement of the law (Tasnim *et al*., 2010). Ingestion of coliform bacteria causes nausea, diarrhoea, vomiting, stomach cramps and other symptoms of food poisoning which keep children out of school and workers away from work, with an attendant cost of medical care and loss of manpower. People of all ages are consuming fruit juice almost every day with higher demand during school days and vended shops in many areas in the locality.

However, their microbial quality is questionable as data concerning the microbiological quality of the packed fruit juices sold in Port Harcourt, is lacking, hence the necessity of this study. This study was carried out to determine the microbial load of the fruit juices, isolate and identify microorganisms found in the juices phenotypically and genotypically, carryout antibiotic susceptibility test on the bacterial isolates and analyse the proximate composition of fruit juices.

Materials and Methods

Study Design and Duration

An Analytic Observational Cohort design was used in this study conducted between May and August 2020, which involved laboratory techniques to detect any coliform and heterotrophic bacteria from packaged fruit juice.

Collection of Samples

Ten (10) samples of 100ml pack of four (4) different brands of packaged fruit juices (Capri-sun, Ribena, Happy-hour and Chi-exotic) were randomly purchased from Mile III Market located at Nkpolu-Oroworukwo Community within Diobu in Port Harcourt Metropolis of Rivers State, Nigeria. Ten (10) samples of 100ml pack of each brand was randomly purchased Monthly for a period of four (4) Months which amounted to a total of forty (40) 100ml packs of fruit juice used for this study. Capri-Sun Orange and Capri-Sun Apple, Ribena Blackcurrant, happy Hour chivita Orange Safari, and Chi-exotic Pineapple and Coconut nectar).

Only samples before their expiry date were purchased and transported immediately to the Department of Microbiology laboratory for analysis. The samples were kept in the refrigerator at 4ºC from time of purchase till microbiological and proximate analyses were carried out.

Enumeration and Isolation of Bacteria

Preliminary investigation during this study conducted by culturing 10 fold serially diluted fruit juice samples on microbiological media did not yield any growth of microbial colonies. Thus, each 100 ml pack of fruit juice were vigorously shaken for thorough mixing, after which, an aliquot of 0.1 ml was collected with the aid of a sterile syringe and needle, and inoculated onto freshly prepared Nutrient agar, MacConkey agar, *Salmonella-Shigella* agar, Mannitol Salt agar and Eosin Methylene blue (EMB) agar, in sterile Petri plates by spread plate methods for total heterotrophic bacterial counts, total Coliform counts, *Salmonella* and *Shigella* counts, *Staphylococca*l counts and *E. coli* counts respectively. Duplicate plates were inoculated for each medium while separate uninoculated plates containing the various media served as control.

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All the cultured plates except the EMB plates were incubated at 37ºC for 24 – 48 hours for colony formation. While the Eosin methylene blue plates were incubated at 44.5ºC for 24 hours. The colonies which developed following incubation were counted manually with the aid of a hand-held magnifying lens and the counts obtained were recorded and calculated as colony forming units (CFU) per millilitre of fruit juice sample.

Discrete colonies were sub-cultured onto freshly prepared nutrient agar medium and incubated at 37ºC for 24 hours. Pure cultures of isolates were separately inoculated into glycerol solution in MacCartney bottles and stored in the refrigerator at - 4ºC till required for further analysis.

Characterization and Identification of Bacterial Isolates

Identification of the pure isolates was based on their colonial and morphological characteristics following macroscopic visualisation, microscopic examination and biochemical tests. Colony of bacteria on the agar media after incubation were studied and characterized based on colonial features such as shape, size, colour, edge, texture and elevation of the colony (Ogunware *et al*., 2020).

References were made to Bergey's manual of determinative Bacteriology (Holt *et al.*, 1994) and Advanced Bacterial Identification Software (ABIS) online tool for identification of bacteria (Holt *et al.*, 1994; Sorescu and Costin., 2021).

Antibiotic Susceptibility Test of Bacterial Isolates

Antimicrobial susceptibility pattern for each of the isolates was done using conventional disc diffusion method by Kirby-Bauer according to Clinical Laboratory Standard Institute guidelines (CLSI, 2021).

After incubation, the zone of inhibition for each antibiotic was measured using a metered ruler placed on the surface of the covered agar plate and values recorded according to the Clinical Laboratory Standard Institute (CLSI, 2021; Douglas and Amuzie, 2019).

Proximate Composition of Juice Sample

Proximate Composition of commercially packed fruit juice was applied to proportion of fat, carbohydrate, protein, fibre, and ash present in fruit juice, as the fruit drinks were made from concentrates (Douglas and Amuzie, 2019; Taiwo and Kemi., 2019). Testing for ash and mineral matter was done using Fume hood method. A comparism method which involved complete separation of the fat solution and comparism with a previously known weight of fat was used in determination of fat. Total available carbohydrate (Manual Clegg Anthrone Method) was used in carbohydrate estimation, while Kjedahl method was used in Crude protein determination.

Data Analysis

Microsoft Office Excel 2013 was used to analyze the data obtained from the measurement of the zones of inhibition of antibiotics. Analysis of variance (ANOVA) was carried out to test for significant difference in the total heterotrophic bacteria count and proximate composition. Descriptive statistics was used to summarize all data obtained.

Results

The total heterotrophic bacteria count for Capri-sun juice ranged from 1.0 x 10^3 to 3.0 x 10^3 CFU/ml, Happy-hour juice ranged from 1.0 x 10^3 to 2.0 x 10^3 CFU/ml, Chi-exotic juice from 2.0 x 10^3 to 4.0 x 10^3 CFU/ml, while the count for Ribena juice ranged from 10.0 x 10^3 to 39.0 x 10^3 CFU/ml.

There were no detectable coliforms, *E. coli*, *Salmonella/Shigella* and *Staphylococci* in all brands of fruit juices studied, as presented in Table 1. The mean heterotrophic bacterial count of Ribena juice was 1.88±1.04 x 10^3 CFU/ml, Capri-sun juice was 0.77±0.67 x 10^3 CFU/ml, Chi-exotic was 0.57±0.74 x 10^3 CFU/ml, while Happy-hour juice had 0.49±0.64 x 10^3CFU/ml. There was no statistically significant difference (P > 0.000) in the total heterotrophic bacterial counts of Capri-sun, Chi-exotic, Happy-hour, and Ribena. The results of the mean bacterial counts of the various juice samples are presented in Table 2.

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Table 1: Bacterial counts (x10^3 CFU/ml) in brands of fruits Juice samples sold in Port Harcourt

Sample No.	Bacterial counts (x10^3 CFU/ml) in Juice samples							
	Total heterotrophic bacteria (THB)				All brands of juice studied			
	Capri-Sun	Happy-Hour	Chi-exotic	Ribena	Total coliform	*E. coli*	*Salmonella/ Shigella*	Staphylococci
1	2	0	0	17	0	0	0	0
2	2	0	0	39	0	0	0	0
3	0	1	2	0	0	0	0	0
4	0	0	3	14	0	0	0	0
5	2	2	0	10	0	0	0	0
6	1	0	4	19	0	0	0	0
7	0	2	0	15	0	0	0	0
8	2	0	2	16	0	0	0	0
9	0	2	0	0	0	0	0	0
10	3	0	0	23	0	0	0	0

Key: THB - Total heterotrophic bacteria, TC – Total coliform, SSC – *Salmonella*/*Shigella*, SC - Staphylococcal count

Table 2: Mean values of bacterial counts (×10^3CFU/ml) of packaged fruit juice sample

Juice Sample	Bacterial count (×10^3CFU/ml) of packaged fruit juice sample				
	Total Heterotrophic Bacteria	Total Coliform	*Escherichia coli*	*Salmonella/ Shigella*	*Staphylococci*
Capri-sun	0.77±0.67	0	0	0	0
Chi-exotic	0.57±0.74	0	0	0	0
Happy Hour	0.49±0.64	0	0	0	0
Ribena	1.88±1.04	0	0	0	0

Twenty-two (22) isolates belonging to three bacterial species; *Bacillus* species, *Paenibacillus* species and *Lysinibacillus* species, were isolated from the fruit juices as presented in Table 3. The distribution of the isolates were; Six (6) bacterial isolates belonging *Paenibacillus septentrionalis, Bacillus smithii, Bacillus mycrides, Bacillus coagulans* and *Bacillus cereus Bacillus aryabhattai* isolated from Capri-sun, Four (4) bacterial belonging to *Bacillus brevis, Bacillus cereus, Paenibacillus cellulositrophicus,* and Bacillus *thyringiensis* isolated from Chi-Exotic juice, Four (4) bacterial isolates to *Bacillus brevis, Bacillus cereus Paenibacillus cellulositrophicus* and *Lysinibacillus fusiformis* isolated from Happy hour juice and Eight (8) bacterial isolates belonging to *Bacillus cereus, Bacillus firmus, Bacillus acidifier, Bacillus simplex, Paenibacillus septentrionalis, Bacillus coagulans Peanibacillus assamensis* and *Lysinibacillus alkalisoli* isolated and identified from Ribena. Ten (10*) Bacillus* species were identified which are *Bacillus cereus, Bacillus brevis, Bacillus smithii, Bacillus mycoides, Bacillus coagulans, Bacillus aryabhattai, Bacillus thuringiensis, Bacillus firmis, Bacillus acidifier* and *Bacillus simplex*. Three (3) *Paenibacillus* species isolated were *Paenibacillus cellulositrophicus, Paenibacillus septentrionalis,* and *Paenibacillus assamensis.* Only two (2*) Lysinibacillus* species were isolated *Lysinibacillus fusiformis* and *Lysinibacillus alkalisoli*. The Percentage occurrence of the bacterial isolates is presented in Table 4.

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Table 3: Cultural and biochemical characteristics of bacteria isolated from some brands of fruit juice

Juice	Gram	Shape	Cat	Oxi	Mot	Cit	Ind	MR	VP	STH	Glu	Lac	Suc	Man	Organisms
Capri-Sun	+	Rod	+	-	+	-	-	-	+	+	A	-	-	-	*P. septentrionalis*
	+	Rod	+	+	+	-	-	-	-	+	A	-	-	-	*Bacillus smithii*
	+	Rod	+	-	-	+	-	+	+	+	A	-	-	-	*Bacillus mycoides*
	+	Rod	+	-	+	-	-	+	-	+	A	-	-	A	*B. coagulans*
	+	Rod	+	-	+	+	-	+	+	+	A	-	A	-	*Bacillus cereus*
	+	Rod	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**	**A**	**+**	**+**	**+**	*B. aryabhattaii*
Chi-exotic	+	Rod	+	+	+	+	-	+	-	-	A	-	-	+	*Bacillus brevis*
	+	Rod	+	-	+	+	-	+	+	+	A	-	A	-	*Bacillus cereus*
	+	Rod	+	+	+	+	-	+	-	+	A	+	A	-	*P. cellulositrophicus*
	+	Rod	+	-	+	+	-	-	+	+	A	-	A	-	*B. thuringiensis*
Happy-hour	+	Rod	+	+	+	+	-	+	-	-	A	-	-	+	*Bacillus brevis*
	+	Rod	+	-	+	+	-	+	+	+	A	-	A	-	*Bacillus cereus*
	+	Rod	+	+	+	+	-	+	-	+	A	-	A	-	*P. cellulositrophicus*
	+	Rod	+	+	+	+	-		-	-	-	-	-	-	*L. fusiformis*
	+	Rod	+	-	+	+	-	+	+	+	A	-	A	-	*Bacillus cereus*
Ribena	+	Rod	+	-	-	+	-	+	-	+	A	-	-	A	*Bacillus firmus*
	+	Rod	+	+	-	-	-	+	-	+	A	-	A	A	*Bacillus acidifier*
	+	Rod	+	-	-	+	-	-	-	-	A	-	+	-	*Bacillus simplex*
	+	Rod	+	-	-	+	-	-	+	+	A	-	-	A	*P. septentrionalis*
	+	Rod	+	-	-	+	-	+	-	+	A	-	-	A	*B. coagulans*
	+	Rod	+	+	-	+	-	-	-	+	A	-	+	-	*P. assamensis*
	+	Rod	+	-	+	+	-	-	-	-	-	+	-	+	*L. alkalisoli*

Keys: Cat – Catalyst, Oxi- oxidase, Mot – Motility, Cit – Citrate test, Ind – Indole test, MR – Methyl Red test, VP – Voges - Proskauer, STH – Starch hydrolysis, Glu – Glucose, Lac – Lactose, Suc – Sucrose, Man – Mannitol, A – Acid, +: Positive, -: Negative.

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Table 4: Percentage occurrence of bacteria isolated from the packaged fruit juices

Juice sample	Occurrence (%) of bacteria isolated from the packaged fruit juices			
	***Bacillus* spp**	***Lysinibacillus* spp**	***Paenibacillus* spp**	**Total**
Capri-sun	83.33	0.00	16.67	100
Chi-Exotic	75.00	0.00	25.00	100
Happy Hour	50.00	25.00	25.00	100
Ribena	62.50	12.50	25.00	100

The result of the Antibiotic Susceptibility Pattern of the bacteria isolated from the different brands of Fruit Juice is as presented in Table 5 The *Bacillus* spp isolated from the fruit juices were sensitive to Gentamicin, Erythromycin, Ciprofloxacin and Chloramphenicol, but resistant to Tetracyclin, Trimethoprim, penicillin and Cefoxitin. The highest percentage for sensitivity of 86.6% was recorded for Gentamicin, whereas Cefoxitin had the least percentage sensitivity of 6.67%. The *Paenibacillus* spp isolated from the fruit juices was sensitive to Gentamicin, Erythromycin and Tetracyclin, but showed resistance to Ciprofloxacin, Trimethoprim, Chloramphenicol, Penicillin and Cefoxitin. The highest percentage of sensitivity of 80%, was displayed in Gentamicin, whereas penicillin was the least sensitive. The *Lysinibacillus* spp isolated from the fruit juices was sensitive to Gentamicin, Erythromycin and Cefoxitin, but showed resistance to Tetracyclin, Ciprofloxacin, Chloramphenicol, Trimethoprim, and Penicillin. Similarly, Gentamicin, Erythromycin and Cefoxitin had the highest percentage sensitivity of 100%, whereas Tetracyclin, Ciprofloxacin and Trimethoprime, recorded the least percentage sensitivity.

The result of the drug resistance pattern of the isolates from the fruit juices is as presented in Table 6. Multidrug resistance to the *Bacillus* spp involved Tetracyclin, Trimethoprim, penicillin and Cefoxitin, multidrug resistance to the *Paenibacillus* spp involved Ciprofloxacin, Trimethoprim, Chloramphenicol, Penicillin and Cefoxitin, while multidrug resistance of the *Lysinibacillus* spp involved Chloramphenicol, Tetracyclin, Ciprofloxacin, Trimethoprim, and Penicillin. The multidrug resistance index (MAR) index of the *Bacillus* spp is 0.5, whereas *Paenibacillus* spp and *Lysinibacillus* spp recorded similar MAR index of 0.6.

Table 5: Percentage Susceptibility Pattern of isolates

Antibiotics (conc.)	***Bacillus* spp**			***Paenibacillus* spp**			***Lysinibacillus* spp**		
	S (%)	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**
Gentamicin (10µ)	13(86.67)	1(6.67)	1(6.67)	4(80.00)	1(20.00)	0(0.00)	2(100.00)	0(0.00)	0(0.00)
Erythromycin (15µ)	12(80.00)	2(13.33)	1(6.67)	3(60.00)	1(20.00)	1(20.00)	2(100.00)	0(0.00)	0(0.00)
Tetracyclin (30µ)	4(26.67)	9(60.00)	2(13.33)	3(60.00)	1(20.00)	1(20.00)	0(0.00)	1(50.00)	1(50.00)
Ciprofloxacin (5µ)	8(53.33)	4(26.67)	3(20.00)	1(20.00)	3(60.00)	1(20.00)	0(0.00)	1(50.00)	1(50.00)
Trimethoprim (5µ)	2(13.33)	11(73.33)	2(13.33)	0(0.00)	4(80.00)	1(20.00)	0(0.00)	1(50.00)	1(50.00)
Chloramphenicol (30µ)	7(46.67)	4(26.67)	4(26.67)	0(0.00)	2(40.00)	3(60.00)	0(0.00)	1(50.00)	1(50.00)
Penicillin (10 units)	2(13.33)	1(6.67)	12(80.00)	0(0.00)	1(20.00)	4(80.00)	0(0.00)	0(0.00)	2(100)
Cefoxitin (30µ)	1(6.67)	4(26.67)	10(66.67)	1(20.00)	3(60.00)	1(20.00)	2(100.00)	0(0.00)	0(0.00)

Key: S= Sensitive, I = Intermediate, R = Resistant

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Table 6: Multiple Antibiotic Resistance Index of Bacterial Isolates from the fruit Juices

Organism	No. of Antibiotics Tested	No. of Antibiotics showing Resistance	Multiple Antibiotic Resistance (MAR) index)
Bacillus spp	8	4	0.5
Paenibacillus spp	8	5	0.6
Lysinibacillus spp	8	5	0.6

*A MAR index > 0.2 means Antibiotic use in high-risk source of contamination.

Results for the proximate analysis of the juice samples are presented in Table 7. The ash content of Capri-sun, Chi-exotic, Happy-hour and Ribena were 0.04±0.00, 0.12±0.00, 0.04±0.00 and 0.04±0.00%, crude protein was 0.22±0.00, 0.19±0.00, 0.08±0.00 and 0.08±0.00%, fat content was 0.10±0.00, 0.11±0.01, 0.10±0.00 and 0.08±0.00%, while carbohydrate was 12.34±0.23, 12.72±0.04, 12.56±0.06 and 12.43±0.06%, respectively. There was no statistically significant difference (P>0.0001) in the values of carbohydrate and fat content. However, there was a significant statistical difference observed in the crude protein and Ash contents (P< 0.0001).

Table 7: Mean values of Proximate Composition (%) of packaged fruit juices

	Proximate composition (%) of packaged fruit juices			
Juice sample	**Ash**	**Carbohydrate**	**Crude protein**	**Fat**
Capri-sun	0.04 ± 0.00^{b}	12.34 ± 0.23^{a}	0.22 ± 0.00^{a}	0.10 ± 0.00^{a}
Chi exotic	0.12 ± 0.00^{a}	12.72 ± 0.04^{a}	0.19 ± 0.00^{b}	0.11 ± 0.01^{a}
Happy hour	0.04 ± 0.00^{b}	12.56 ± 0.06^{a}	0.08 ± 0.00^{c}	0.10 ± 0.00^{a}
Ribena	0.04 ± 0.00^{b}	12.43 ± 0.06^{a}	0.08 ± 0.00^{c}	0.08 ± 0.00^{a}
P value	<0.0001	0.0001	<0.0001	0.0001

Discussion

This present study has revealed the Bacteria load of different packaged fruit juices sold in Port Harcourt, Nigeria. The highest mean heterotrophic bacterial counts were detected in the Ribena juice, followed by Capri-sun,Chi-exotic while the least was detected in the Happy-hour juice. There was no statistically significant difference in the total heterotrophic bacterial counts of all fruit juices. *Bacillus cereus* was isolated from all brands of juice sampled in this study. Similar organisms; *Bacillus cereus, Bacillus brevis* and *Paenibacillus cellulositrophicus* were isolated from Chi-exotic and Happy-hour juices, while *Paenibacillus septentrionalis*, was similarly isolated from Capri-sun and Ribena. *Bacillus smithii*, *Bacillus mycoides*, *Bacillus coagulans* and *Bacillus aryabhattai* were seen only in Capri-Sun fruit juice. *Bacillus thuringiensis* was seen only in Chi-exotic. *Lysinbacillus fusiformis* was only seen in Happy-hour fruit juice, while *Bacillus firmis*, *Bacillus acidifier*, *Bacillus simplex*, *Paenibacillus assamensis* and *Lysinibacillus alkalisoli* were only isolated from in Ribena. The heterotrophic bacterial counts were within normal limits for anticipated and permitted microbial load for fruit juice and there were no detectable counts of coliforms, *E. coli*, *Salmonella Shigella* and *Staphylococccus aureus* in all juices sampled in this study.

The findings of acceptable levels of heterotrophic bacterial counts and isolation of Bacillus species during this study has been similarly recorded in another study in which the microbiological quality of commercially packed fruit juices sold in South-East Nigeria were assessed (Ogodo *et al.*, 2016). A similar study revealed a total aerobic mesophilic bacterial count which was within acceptable standards for human consumption and the isolated microbes included *Bacillus* species as the most prevalent organism (Oranusi *et al.*, 2012). Contrary to findings of acceptable level of bacterial count obtained in this study, a bacteriological analysis of some commercially packed and fresh fruit juices available in Jessore city, found viable bacteria within the range of 10^{3} to 10^{8}CFU/ml.

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Nineteen samples exhibited the presence of staphylococcai. Total coliforms were detected in seventeen samples within the range of $x10^3$ to $x10^6$ CFU/ml which were further identified to be *Escherichia coli, Klebsiella* spp. and *Enterobacter* species (Kader *et al.*, 2014). In another study, the microbiological status of industrially processed fruit juices sold in Onitsha main market showed high microbial loads consisting of bacteria such as *Bacillus* sp. (Braide *et al*., 2012).

This study revealed the presence of *Bacillus* species which portends health risk to consumers as some species produce potent toxins associated with food borne illnesses. Most cases of food-borne outbreaks caused by the *B. cereus* group have been associated with concentrations above 10^5CFU/g. In this study, the highest mean population of heterotrophic bacteria obtained in the black currant fruit juice (Ribena juice) was low. However, cases of both emetic and diarrhoeal illnesses have been reported involving lower levels of *B. cereus,* hence antibiotic sensitivity was set-up during this study (EFSA BIOHAZ Panel, 2016).

Members of the *B. cereus* group displayed resistance to antibiotics. The most common resistance is against beta-lactam antibiotics (e.g., penicillin, ampicillin, and oxacillin) due to the presence of beta-lactamase genes (Chen *et al*., 2003), as also observed in this study. Most *B. cereus* group members are also resistant to cotrimoxazole (Ombui *et al*., 1996; Rather *et al*., 2012), as observed in this study. Resistance to other antimicrobials is variable and strain-dependent (Chaves *et al*., 2011; Chon *et al*., 2012; Ikeda *et al*., 2015; Luna *et al*., 2007; Turnbull *et al*., 2004;). The four most regularly found resistances were to clindamycin (lincosamide) (up to 60% of the strains), tetracycline (10–33%) and levofloxacin (fluoroquinolone) (ca. 10%), as similarly observed in this study (EFSA BIOHAZ Panel, 2016). Although, multidrug resistance was observed in this study, the multidrug resistance index (MAR index) obtained for all isolates in this study is greater than 0.2., meaning that in high-risk source of contamination is where antibiotics should be used.

The findings from the proximate compositions of the fruit juices revealed Happy-hour and Ribena had similar lowest ash content, while Chi-exotic juice had the highest ash content.

Capri-sun had the highest protein while Happy-hour and Ribena had the least protein content amongst the juices. Chi-exotic, had the highest fat content, Capri-sun and Happy-hour had same values of fat, while Ribena had the least fat content. Chi-exotic had the highest carbohydrate content while capri-sun had the least carbohydrate content. Results also showed that despite the differences in the values of carbohydrate and fat content of the juice samples, there was no statistically significant difference However, there was a significant statistical difference observed in the crude protein and Ash contents amongst the fruit juices studied.

The findings for ash content from this study, is similar to that obtained from the study done by Chuku and Akani (2015), in which sweet orange had the highest ash content. Also, the ash content of Capri-sun, Happy-hour and Ribena are similarly low as the ash content of apple juice observed in a study done by previous researchers (Ekanem and Ekanem, 2019). Similarly low values of crude protein were observed in studies by past researchers (Ekanem and Ekanem, 2019). Although, the low value of protein obtained from Capri-sun orange, is in contrast with previous study in which single strength orange juice had higher protein the value is seemingly about the same as that obtained for Ribena (Eziaghighala *et al.*, 2010). The fat content of all fruit juices sampled generally varied from a previous study which showed low concentrations of lipid (Ekanem and Ekanem, 2019). The values of fat content obtained from this study were lower than that obtained from the study conducted by past workers, in which sour orange had the highest values for lipid (Chuku and Akani, 2015). The values obtained for carbohydrate content of the fruit juice samples in this study were lower than those obtained from past research on the proximate composition of lime (*Citrus limonia),* which showed that lime recorded the highest value for carbohydrate (Chuku and Akani, 2015).

In conclusion, the counts of total heterotrophic bacteria in the fruit juices studied were within standard acceptable limits. However, spore bearing *Bacillus* isolated from this study, which were multidrug resistant, have pathogenic potential. These *Bacillus* species may result in spoilage before expiry date resulting to economic loss, food borne illness as well as could pose antimicrobial resistance challenges in hospital setting if not routinely checked and controlled.

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All the packaged fruit juice studied contained sufficient amount of carbohydrate, protein and lipids, and as such, were safe for human consumption. Government authorised institutions should diligently continue to monitor and control the quality of packaged fruit juices to ensure their healthiness.

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