Microbiological Analysis of some Packaged Fruit Juices sold in Port Hacourt Metropolis, Nigeria

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Abstract: Fifteen (15) samples of packaged fruit juices which include pineapple, orange, and apple juice were analyzed for there microbial content using standard microbiological techniques. The fruit juices were purchased from street hawkers in Port Harcourt Metropolis, Nigeria. Total heterotrophic bacteria count of some of the packaged fruit juice samples ranged from $3.5x10^2$ to $7.1x10^3$ CFU/ml (for orange juice), $4.2x10^2$ to $6.6x10^4$ CFU/ml (for apple juice), and $3.0x10^2$ to $9.0x10^4$ CFU/ml (for pineapple juice). Total fungi count of some of the packaged fruit juice samples ranged from $1.5x10^2$ to $2.5x10^2$ CFU/ml (for orange juice), $2.0x10^2$ to $4.2x10^2$ CFU/ml (for apple juice) and $0.0x10^2$ to $2.2x10^2$ CFU/ml (for pineapple juice). Bacteria isolates obtained from the packaged fruit juices include; *Micrococcus* sp. (26.7%), *Flavobacterium* sp. (13.3%), *Bacillus* sp. (57.1%), *Lactobacillus* sp. (13.3%). The results also showed that of the fungi isolates obtained from packaged fruit juice, *Penicillum* sp. (57.1%) was predominant over *Saccharomyces* sp (42.9%). No coliform bacteria were observed in all packaged fruit juice samples. None of the fruit juice samples showed any growth of *Salmonella*, *Shigella* and *Vibrio* species. With the number of isolated bacteria and fungi from the different packaged fruit juice sold in Port Harcourt, it can be concluded that different bacterial and fungal species occur within fruits and materials used for the production of the juice as well as poor sanitation, extraction, raw material contaminations (often from insect damage), lack of both proper heat sterilization and adequate quality control during processing of fruit juice. Some of the fungal isolates especially *Penicillium* sp. have the potential to induce rot on fresh fruits which might have a remarkable effect on the value of the fruit especially in the food industry as well as on human health. [Odu NN and Adeniji AO. Microbiological Analysis of some Packaged Fruit Juices sold in Port Hacourt Metropolis, Nigeria. *Nat Sci* 2013;11(4):30-40]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 7

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1. INTRODUCTION

Juice is a liquid naturally contained in fruit or vegetable tissue. Juice is prepared by mechanically squeezing or macerating fresh fruits or vegetables without the application of heat or solvent. For example, orange juice is the liquid extract of the fruit of the orange tree (Doyle, 1991). Juice may be prepared in the home from fresh fruits and vegetables using variety of hand or electric juicers (FAO, 2002). Juice may be market in concentrate form, sometime frozen, requiring the user to add water to constitute the liquid back to its "original state" (Doyle, 1991). However, concentrates generally have a noticeable different taste than their comparable "fresh squeezed" versions (Brothier, 1999). Other juices are reconstituted before packaging for retail sale (Brandon and Ferreiro, 1998).

Fruit juice consists of 100% pure juices and generally has no added ingredients (USDA, 2000). Although some minor exceptions exist like in cases where salt is added to Tomato juice to ensure the final product is of an acceptable taste (Tetra Pak, 2000). Also the juice may have been concentrated and later reconstituted with water suitable for the purpose of maintaining the essential composition and quality of the juice. The addition of sugar or acid can be permitted but must be endorsed in the individual standard (FAO, 2002).

Fruit juices accounts for more than 90% of the total fruit production in Nigeria. The western part of Nigeria is the principal fruit juice producing region in the country (FAO, 2002). In fact, the concept of maintaining a fruit desired for juicing in its whole, intact form until the juicing is needed continues to be a sound principle. Even today, maintaining the fruit intact is one of the easiest ways of preserving juices quality (Brandom and Ferreiro, 1998).

It has been known that fruits constitute commercially and nutritionally important indispensable food commodity (Al-Hindi et al., 2011). Fruits play a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet and that can help to keep a good and normal health (Al-Hindi et al., 2011). Fruits are widely distributed in nature (Al-Hindi et al., 2011). One of the limiting

factors that influence the fruits economic value is the relatively short shelf-life period caused by pathogens attacked (Al-Hindi et al., 2011). It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Droby, 2006; Zhu, 2006; Al-Hindi et al., 2011). In developing countries, postharvest losses are often more severe due to inadequate storage and transportation facilities (Al-Hindi et al., 2011).

Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts and splits that occur during growing or harvesting (Durgesh et al., 2008). Contamination from raw materials and equipments, 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 (Victorian Government Department of Human Services 2005; Oliveira *et al.*, 2006; Nicolas *et al.*, 2007; Durgesh et al., 2008).

Fungal fruits infection may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer (Al-Hindi et al., 2011). Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal decayed (Singh and Sharma, 2007; Al-Hindi et al., 2011). Yeasts and moulds are more favored as spoilage agents of fruit juices compared to bacteria because of the physical and chemical properties of the fruit juices (Obire et al., 2008; Okigbo and Obire, 2009).

Some of these properties include the low pH of fruit juices, the positive oxidation reduction potential of the fruit juices and the rich nutrient composition of the juice (Obire et al., 2008; Okigbo and Obire, 2009). Although the processing of the fruit juice has been maintained at a considerable hygienic standard, a variety of yeasts, moulds and some bacteria are still able to find their way into these industrially produced juices (Tetra pak, 2000). It may therefore seem apparent that these potential spoilage organisms originate from the raw fruits used for processing or from the processing equipment. In developing nations like Nigeria, it has not been possible to have control over the processing of hawked foods, because most of the vendors lack the adequate knowledge of food processing and handling practices (Essien et al., 2011). As such, there is likely to be a high risk of chemical and microbial contamination (Essien et al., 2011). A large number of lactic acid bacteria, coliforms, molds and yeast have been reportedly implicated in food spoilage as they use the carbohydrate content of the foods for undesirable fermentation processes (Amusa *et al*., 2005; Essien et al., 2011).

Freshly squeezed fruit and vegetable juices have little or no process steps that reduce pathogen levels, if contaminated (Victorian Government Department of Human Services 2005; Durgesh et al., 2008). There are few published works on the health risk, especially in the Third World, that could arise from the consumption of soft drinks directly from the orifice of the opened bottles (Kigigha and Jonathan, 2012). Hoffmann *et al*. (1997 cited in Kigigha and Jonathan, 2012) carried out the microbiological survey of non-alcoholic carbonated beverages while Griffiths *et al*. (1997 cited in Kigigha and Jonathan, 2012) carried out an analysis of the quality of the ingredients used in the Soft-drink Industries. In 2005, Amusa *et al.* carried out studies on the microbiological and nutritional quality of a hawked locally brewed soft drink called *zobo* in Nigeria while Oranusi *et al.* (1994 cited in Kigigha and Jonathan, 2012) studied the microbial contaminants of commercially bottled non-alcoholic drinks produced in Nigeria. Kigigha and Jonathan (2012) carried out microbiological assessment of opened soft drink bottles for pathogenic bacteria associated with drinking directly from the orifice in Nigeria.

The aim of this study was to determine the microbiological quality of some packaged fruit juice sold in Port-Harcourt Metropolis, Nigeria. It also evaluated the sanitary quality of marketed packaged fruit juice. This would provide a background microbiological data for development methods that would effectively reduce the microbial load of fruit juice including those considered to constitution spoilage threat and potentials health hazards to subsequent consumer.

2. MATERIALS AND METHOD

2.1. Sources of Packaged Fruit Juice Samples

Fifteen (15) samples of packaged orange, apple, and pineapple fruit juices used in this study were purchased from different locations in Port Harcourt. Some of them were bought from street hawkers, Rumuokoro market, Abuja and Choba campuses. The samples consisting of five (5) oranges, five (5) apples, and five (5) pineapples packaged fruit juices were analyzed within four hours of purchases from the different sources.

2.2. Sample Analysis

Five milliliters (5 ml) of each fruit juice were mixed with 45 ml of buffered peptone water and homogenized by manual shaking. The liquid phase then forms the stock sample from which dilutions were made to obtain 10^{-1}, 10^{-2}, 10-3 up to 10^{-10} dilutions. After mixing each tube with the dilution, 0.1 ml of it was transferred onto a sterile plate count agar (PCA) (Oxoid Ltd, Basingstoke Hants, England)

then spread on the agar surface and immediately placed in an incubator. The plates were incubated at 37^{O}C over-night. The remaining stock samples were incubated at 37^{O}C for 4 hours after which they were subcultured onto Blood Agar, MacConkey Agar (MCA), Nutrient Agar, Sabouraud Dextrose Agar, Plate Count Agar (PCA), Salmonella-Shigella agar (SSA), Thiosulphate citrate bio salt agar (TCBS), Mannitol salt agar (MAS) (Oxoid Ltd, Basingstoke Hants, England) plates and incubated at 37^{O}C overnight.

2.3. Total Heterotrophic Bacterial Count

After overnight incubation, growth on the PCA showing 30-300 colonies was counted. Bacterial counts were ex-pressed as colony-forming-units per ml of fruit juice sample analyzed.

2.4. Total and faecal coliforms

The technique used was the most probable number or multiple tube fermentation technique to detect the presence of both total and faecal coliform. The test was performed using three columns of test tubes; each column having three test tubes. The test is usually carried out in three stages test procedures presumptive, confirmatory and complete test.

2.5. Total Salmonella-Shigella count

Sterile Salmonella- Shigella agar plates were used, by aseptically transferring 0.1ml aliquots from 10^{-1}, 10^{-2}, 10-3 up to 10^{-10} dilutions. These were incubated at 37^{0}C for 24 hours. Black colonies indicate Salmonella while pale pink colony indicates Shigella.

2.6. Total Vibrio count

Sterile thiosulphate citrate bile salt (TCBS) agar plates were used, by aseptically transferring 0.1ml aliquots from from 10^{-1}, 10^{-2}, 10-3 up to 10^{-10} dilutions, these were incubated at 37^{0}C for 24 hours. Green and yellow colonies indicate *Vibrio* species

2.7. Total fungi count

Sabouraud medium was used to which 0.1ml of lactic acid was added to 100ml of cooled agar base at about 45^{0}C. A sterile 1ml pipette was used to transfer 0.1ml of the juice sample into the Sabouraud agar plate's samples (in duplicate). The inoculum was spread using a sterile glass rod and the plates were allowed to stay inverted and incubated for 48 hours for yeast and 96 hours for moulds at room temperature plate yielding counts of 30 -300 colonies were chosen (Cheesbrough, 2000).

2.8. Bacterial identification

The MacConkey, Blood agar, Nutrient Agar, Salmonella-Shigella agar (SSA), Thiosulphate citrate bile salt agar (TCBS), Mannitol salt agar (MAS) plates were examined for bacterial growth. Growth characteristics and other colonial morphology such as lactose fermentation, formation of mucoid colonies of the bacteria were carefully recorded. Less than five identical colonies for a particular organism growing on a plate were ignored. When more than five similar colonies were counted on a plate, then five isolated identical colonies on either the blood agar, MacConkey agar, Nutrient agar, Salmonella-Shigella agar, Thiosulphate citrate bile salt agar, or Mannitol salt agar plates were picked carefully, one by one and inoculated into buffered peptone water in sterile microtitre wells. Culture from each microtitre well was re-inoculated onto a Nutrient agar (Oxoid Ltd, Basingstoke, Hamspshire, England) to obtain pure growth. Organisms which were identified to be the same from the microtitre wells were grouped as one isolate from the food sample analyzed. Bacterial identification was done using the pure culture on the nutrient agar plates. The bacterial isolates were identified by comparing their characteristics with those of known taxa, as described by Jolt et al. (1994) and Oyeleke and Manga (2008).

2.9. Fungi Identification

The pure isolated fungi were identified using cultural and morphological features according to the most documented keys in fungal identification (Samson and Varga, 2007).

3. RESULT ANALYSIS

3.1. Total Heterotrophic Bacterial Count

Total heterotrophic bacteria count of some fruit juice samples are shown in Table 1. It shows that the total heterotrophic bacteria count of the orange fruit juice samples ranged from $3.5x10^{2}$ CFU/ml to $7.1x10^{3}$ CFU/ml. Total heterotrophic bacteria count of apple fruit juice samples ranged from $4.2x10^{2}$ CFU/ml to $6.6x10^{4}$ CFU/ml. Total heterotrophic bacteria count of pineapple fruit juice samples ranged from $3.0x10^{2}$ CFU/ml to $9.0x10^{4}$ CFU/ml.

Table 1: Total heterotrophic bacteria count (CFU/ml) of some fruit juice samples

Locations	Fruit juice		
	Orange	Apple	Pineapples
1	$5.6x10^{2}$	$6.6x10^{4}$	$9.0x10^{4}$
2	$7.1x10^{3}$	$5.3x10^{3}$	$6.9x10^{3}$
3	$1.9x10^{3}$	$1.2x10^{4}$	$4.2x10^{3}$
4	$5.2x10^{2}$	$4.2x10^{2}$	$3.0x10^{2}$
5	$3.5x10^{2}$	$4.4x10^{2}$	$1.1x10^{4}$

3.2. Total and faecal coliforms, Salmonella-Shigella and Vibrio counts

No coliform bacteria were observed in all packaged fruit juice samples. None of the SSA plates showed any black colonies of Salmonella or pale pink colonies of Shigella. None of TCBS agar plate showed any green and yellow colonies of *Vibrio* species

3.3. Total fungi count

As shown in Table 2 indicates that the total fungi count of orange fruit juice samples ranged from $1.5x10^2$ CFU/ml to $2.5x10^2$ CFU/ml. Total fungi count of Apple fruit juice samples indicate that the count ranged from $2.0x10^2$ CFU/ml to $4.2x10^2$ CFU/ml. Total fungi count of pineapple fruit juice samples indicate that the count ranged from $0.0x10^2$ CFU/ml to $2.2x10^2$ CFU/ml.

Table 2:Total fungi count (CFU/ml) of some fruit juice samples

Locations	Fruit juice		
	Orange	**Apple**	**Pineapples**
1	$2.5x10^2$	$2.0x10^2$	$2.2x10^2$
2	$2.0x10^2$	$2.0x10^2$	$0.0x10^2$
3	$2.5x10^2$	$4.2x10^2$	$2.0x10^2$
4	$1.5x10^2$	$1.5x10^2$	$1.5x10^2$
5	$2.0x10^2$	$1.4x10^2$	$0.0x10^2$

3.4. Bacterial identification

Based on the results of the gram reaction and biochemical test performed, fifteen bacterial isolates belonging to four genera were identified as *Bacillus* sp, *Micrococcus* sp, *Flavobacterium* sp and *Lactobacillus* sp.

3.5. Frequency of occurrence of bacterial isolates

Table 3: Frequency of occurrences of bacteria isolates obtained from some of the packaged fruit juice. *Bacillus* sp. (57.1%) was most predominant. This was followed by *Micrococcus* sp. (26.7%). *Flavobacterium* sp. (13.3%) and *Lactobacillus* sp. (13.3%) were least predominant.

Table 3: Frequency of occurrence of bacterial isolates

Bacterial isolates	No. (%)
Flavobacterium sp.	2(13.3)
Lactobacillus sp.	2(13.3)
Bacillus sp.	7(46.7)
Micrococcus sp.	4(26.7)
Total	**15(100.0)**

3.5. Fungi Identification

Based on the cultural and morphological features of most documented keys in fungal identification, fungi isolates were identified as *Penicillum* sp. and *Saccharomyces* sp.

3.6. Frequency of occurrence of fungi isolates

Table 4: Frequency of occurrences of fungi isolates obtained from some of the packaged fruit juice. *Penicillum* sp. (57.1%) was predominant over *Saccharomyces* sp (42.9%).

Table 4: Frequency of occurrence of fungi isolates

Fungi isolates	No. (%)
Penicillum sp.	4(57.1)
Saccharomyces sp.	3(42.9)
Total	**7(100.0)**

4. DISCUSSION

Many microorganisms are found in fruit juice and soft drinks as environmental or raw material contaminations either during their growing in fields, orchards, vineyards or greenhouse or during harvesting, post-harvest handling and distribution. But relatively few can grow within the acidic and low oxygen environment, yeast are the most significant group of microorganisms associated with spoilage of fruit juice and soft drinks. According to WHO (2003), a food is deemed to be adultered if its content is composed in whole or in part of any poisonous or deleterious substance, which renders its contents injuries to health.

Most fruit contains bacterial counts of 1 X 10^5 cfu/cm^2 on their surface (Splittstosser 1979; Harrigan 1998; Al-Jedah *et al.*, 2002; Durgesh et al., 2008). Improper washing of fruits adds these bacteria to juices leading to contamination (Durgesh et al., 2008). In addition lack of appreciation of basic safety issues by vendors contribute to augmentation of the microbial loads (Durgesh et al., 2008). These include use of crude stands and carts, unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust (Lewis *et al.*, 2006; Durgesh et al., 2008).

The result obtained from this study showed that the total heterotrophic bacteria count of the packaged fruit juice samples ranged from $3.5x10^2$ to $7.1x10^3$ CFU/ml (for orange juice), $4.2x10^2$ to $6.6x10^4$ CFU/ml (for apple juice), and $3.0x10^2$ to $9.0x10^4$ CFU/ml (for pineapple juice). Total fungi count of the fruit juice samples ranged from $1.5x10^2$ to $2.5x10^2$ CFU/ml (for orange juice), $2.0x10^2$ to $4.2x10^2$ CFU/ml (for apple juice) and $0.0x10^2$ to $2.2x10^2$ CFU/ml (for pineapple juice). None of the fruit juice samples showed any growth of *Salmonella*, *Shigella* and *Vibrio* species. The bacterial count was low for some of the packaged and comparatively higher for some others especially in pineapple juice. The microbial safety of commercial ice used in drinks was evaluated by Lateef *et al.* (2006) in Nigeria and it was found that microbial loads of these ice samples ranged from 1.88-3.20 x 10^4 cfu/ml which was largely above the recommended loads of more than 500 and 1000 cfu/ml for ice obtained from manufacturing plants and retail outlets respectively (Durgesh et al., 2008).

The result of the study also showed that the total fungal count of the packaged fruit juice ranged from $1.5x10^2$ to $2.5x10^2$ CFU/ml (for orange juice), $2.0x10^2$ to $4.2x10^2$ CFU/ml (for apple juice) and $0.0x10^2$ to $2.2x10^2$ CFU/ml (for pineapple juice). Some of the fruit juices showed no fungal growth.

From the results obtained in the present study, it was shown that the mean bacterial counts of the packaged apple and pineapple fruit juice samples from location 1, apple fruit juice samples from location 3 and pineapple fruit juice samples from location 5 obviously exceeded the maximum recommended standards by the International Commission on Microbiological Specification of Foods (ICMSF, 1978). According to this agency, the acceptable limit of mesophilic aerobic bacteria in dried food products should not exceed a maximum of 10^3 cfu/ml. On the other hand, all the results of the fungal counts from all the packaged fruit juice from the locations analysed were within the acceptable limit. However, the counts are considerably high since no microorganism should be recovered in any food meant for human consumption (FAO, 1979; 1993; WHO, 2003; Kawo and Abdulmumin, 2009).

The high fungal counts suggest the presence of fermentative organisms (Zocklein, 1990 cited in Kawo and Abdulmumin, 2009). This is confirmed by the presence of yeast, *Saccharomyces* sp. which was also isolated in these packaged fruit juice samples. Thus, the presence of *Penicillium* species in packaged fruit juice samples examined in the present study could result in the production of toxic substances (mycotoxins), which could lead to health hazards for the consumer (Frazier and Westhoff, 1978 and Weinzirl, 1992 cited in Kawo and Abdulmumin, 2009). *Penicillium* sp. produces mycotoxins that are harmful to man and may result in 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).

No coliform bacteria were observed in all packaged fruit juice samples used in this study. Coliforms are rare in fruit juices. A very high occurrence of false positives result due to species of *Erwinia* and other coliform types associated with plants, these are not human or animal "fecal coliforms." Nevertheless, coliforms have been reported to retain viability in frozen concentrates but die off rapidly in fresh or reconstituted juices. Thus, coliforms are of little or no public health significance in fresh or frozen citrus products. And, even though spores of *Clostridium botulinum* cannot germinate or grow, this does not rule out the importance of maintaining high sanitary standards in processing plants. Further, the rapidity at which lactic acid bacteria can grow during processing requires good sanitary practice to prevent spoilage. Though coliforms were not reported in packaged fruit juice samples examined in this study, it has been reportedly associated with tap water popularly consumed in some towns in Nigeria (Adegoke *et al*., 1993 cited in Essien et al., 2011). A number of studies from different countries have shown presence of *E. coli*, coliforms and a variety of microorganisms like *Streptococcus pyogenes*, *Streptococcus equi*, *Pseudomonas aeruginosa*, *Staphylococcus* spp, *Micrococcus* spp etc (Moyer *et al*., 1993; Vieira *et al*., 1997; Nichols *et al*., 2000; Lateef *et al*., 2006; Durgesh et al., 2008). Amusa and Ashaye (2009) also reported that the presence of coliforms in hawked kunun drink samples in south-western Nigeria were as a result of the use of contaminated water, containers, as well as dirty environment where the kunun samples were being processed and even hawked. Essien et al. (2011) also reported coliforms in hawked kunun drink samples in Port Harcourt however, no coliform was found associated with the laboratory prepared kunun drink samples where sterile water was used (Essien et al., 2011).

Coliforms are indication of unsanitary conditions, unhygienic practices during or after production and poor quality of source of water used (Durgesh et al., 2008). If the source water used is of poor quality, harmful microorganisms may persist in ice since the process of freezing cannot destroy them (Durgesh et al., 2008). When ice is thawed the surviving microorganisms though may be injured, tend to recover their viability so that when the ice melts into the juices, they may be able to survive these too (FEHD 2005; Durgesh et al., 2008).

Other previous studies have also reported that most ready-to-eat foods were contaminated with enteric bacteria and other potential food poisoning organisms with bacterial counts higher than the acceptable levels. In a study in Kumasi, Ghana, Feglo and Sakyi (2012) reported that the mean bacterial count of cocoa drinks analyzed was 6.16 $\log_{10}$ cfu/ml much higher than the national acceptable reference of <5.0 $\log_{10}$ cfu/ml. This level of contamination could be due to the unhygienic production practices as the preparation involves manual mixing of the cocoa powder with sugar and probable non-potable water collected from streams nearby when municipal water

supply is interrupted (Feglo and Sakyi, 2012). The viable bacterial counts of bottled drinks and juice were 3.7 CFU/ml and 4.1 CFU/ml, respectively in a study by Abdalla et al. (2009).

The generally observed high microbial counts in this study could be attributed to the influence of environmental factors on the microbial populations, which have been shown to play a significant role in affecting the quality of food products (Abdullahi et al., 2005; Shamsuddeen and Ameh, 2008; Shamsuddeen et al., 2008; Oyeyi and Lum-nwi, 2008; Wada-kura et al., 2009; Kawo and Abdulmumin, 2009). The ways these products are handled in an open air environment are no exception (Kawo and Abdulmumin, 2009).

Identification of isolates showed the presence of *Bacillus* sp, *Micrococcus* sp, *Flavobacterium* sp., *Lactobacillus* sp., *Penicillum* sp. and *Saccharomyces* sp., particularly important is the *Bacillus* sp. *Bacillus* sp. is known causative agent of food poisoning and intoxication (FAO, 1979; Adams and Moss, 1995 cited in Kawo and Abdulmumin, 2009). The presence of some of these organisms are not surprising as most of them are known to thrive in medium rich in fermentable substrates such as sugars which often led to the production of acids after fermentation (Essien et al., 2011).

The presence of these bacteria may be due to the unhygienic environmental conditions and poor handling. *Bacillus* species are spore formers whose spores could survive high temperatures of processing (Essien et al., 2011). The thermoduric nature of the spores of these microbes ensures survival at pasteurization temperatures (Essien et al., 2011) and hence their presence in the packaged fruit juice samples that were not subjected to heat treatment during processing. The ropiness associated with the fermented drink has been associated with the presence of both *Pseudomonas spp*. and *Bacillus subtilis* (Adegoke *et al*., 1993 cited in Essien et al., 2011).

The presence of *Bacillus* sp. (46.7%) in almost all the fruit juice may be attributed to its ability to form spores which are heat resistance. In addition, its immediate source is usually the plant equipment, but it may also have originated from one of the major ingredients of fruit juice e.g. sugar (Banwart, 1989). The presence of *Bacillu*s sp in the juices samples which are food borne pathogens implied associated health hazards. This is an agreement with the work of some others (Efiuvwevwere and Akoma, 1991; Hayes, 1995). *Bacillus* sp. was also reported in bottled drinks and juice in a study by Abdalla et al. (2009).

The presence of *Bacillus* species in this study might also be attributed to poor handling. Several food samples have been reported to contain some of these organisms (Blackey and Priest, 1980 cited in Kawo and Abdulmumin, 2009; Frazier and Westhoff, 1978 cited in Kawo and Abdulmumin, 2009; Aboloma, 2008). Blackey and Priest (1980 cited in Kawo and Abdulmumin, 2009) reported that *Bacillus* species is common in soils and vegetation and has been isolated in several countries from wide variety of routine samples of food. The occurrence of this bacterium in the present study is therefore not surprising because of the way the packaged fruit juice products are handled in an open market in a dusty and muddy environment (Kawo and Abdulmumin, 2009). Its presence therefore could be due to the contamination from many sources, which may include soil, air and water (Kawo and Abdulmumin, 2009). The organism might have come in during processing; an observation that goes to support Pederson (1979 cited in Kawo and Abdulmumin, 2009) according to whom spores of molds and Bacillus abound in air and water.

Most of the bacteria isolated has implicated in fermentation of different carbohydrate food in Nigeria. They include *Bacillus sp., Flavobacterium, Micrococcus,* and *Lactobacillus* species (Odunfa, 1985 and Aderiye and Ogunjobi, 1998 cited in Obire et al., 2008). These microbes; *Lactobacillus plantarum*, *Bacillus cereus*, *Micrococcus acidiophilis* and *Bacillus subtilis* had the highest rate of occurrence was also reported in a study by Essien et al. (2011) in Port Harcourt, Nigeria with *Bacillus* species being most predominant. Kigigha and Jonathan (2012) also reported *Bacillus* and *Micrococcus* species in opened soft drink bottles. The most common bacteria that are transmitted from food handlers include the Genera *Micrococcus* and *Staphylococcus* (Jay, 1987 cited in Kigigha and Jonathan, 2012).

Lactobacillus sp were also isolated from the fruit juices, due to their acid tolerant nature. They are also carbon dioxide tolerant and achieve high growth rates in the presence of high sugar content (Efiuvwevwere et. al, 1991). *Lactobacillus* species identified in the packaged fruit juice samples are advertised and listed as probiotic microorganisms (BgVV, 1999 cited in Obire et al., 2008). Probiotics are certain living microorganisms; sufficient amounts of which reach the intestines in an active form to exert health (Ezendam and Loveren, 2006; Obire et al., 2008). BgVV (1999 cited in Obire et al., 2008) stated that depending on the amount ingested and taking into account the best-before date, a regular-in most cases daily-intake of 10^8 and 10^9 probiotic microorganisms is necessary to achieve probiotic action in the human organisms. Odunfa and Adeyeye (1985 cited in Essien et al., 2011) reported that *L.*

plantarum was the predominant organism in the fermentation responsible for lactic acid production.

Micrococcus sp were among the organisms isolated from the fruit juice. It may have contaminated the fruit juice through the soil, water, air, the skin of workers during processing and even inadequately cleaned and sanitized equipment (Banwart, 1989). Odunfa and Adeyeye (1985 cited in Essien et al., 2011) reported that *S. lactis and Micrococcus acidiophilis* are known to be involved in fermentation of agricultural produce. The presence and the activities of these fermenters might be responsible for the souring of taste usually observed if the drinks are not consumed within six hours of processing.

Flavobacterium sp was also isolated from some of the juice samples. This collaborates with the findings of Obire et al. (2008) who also isolated *Micrococcus* and *Flavobacterium* species from fruits. Schmidt-Lorenz (1978 cited in Kigigha and Jonathan, 2012) observed that microorganisms, which have simple nutritional requirements such as *Flavobacterium* species and *Pseudomonads*, could multiply in stored spring and mineral water at room temperature. Comparatively, *Saccharomyces cerevisiae* and *Penicillium* species were common to the packaged fruit juice with *Penicillium* species having the highest frequency of occurrence/isolation in the fruit juice samples. This is contrary to the findings of Obire et al. (2008) who reported *Saccharomyces cerevisiae* predominance over *Penicillium* species. *Penicillium* and *Saccharomyces* species have been implicated in fermentation of fruits and other food items (Pelczar *et al.*, 1993 and Battock and Azam-Ali, 1998 cited in Obire et al., 2008). Kolawole *et al.* (2007) reported that *Saccharomyces cerevisiae* were isolated from *burukuto* and *pito* (fermented indigenous alcoholic beverages). Olorunfemi and Adetuyi (2005) actually isolated two different yeasts suspected to be *Saccharomyces* from naturally fermented pineapple. According to Gow-chin and Hsin-Tan (1996 cited in Obire et al., 2008), yeast growth was favoured by the presence of sugar and acid pH. Fruit juices are readily fermented by yeast while acid pH discourages most bacterial growth (Obire et al., 2008).

The presence of fungi in some of the packaged fruit juice samples indicates that the handling of fruits and the extraction of juices leaves a lot to be desired with respect to sanitary practices. *Penicillum* sp. and *Saccharomyces* sp. were also isolated from the samples. This may be due to contamination of the surface of fruit by these organisms which end up in the fruit during processing (FAO, 2002). The fungi isolated in this study are mostly contaminants. The surrounding air, packaging materials and the personnel concerned with the packaging processes could all serve as sources of these contaminants (Kawo and Abdulmumin, 2009). This agrees with Kawo and Abdulmumin (2009), Aboloma (2008) as well as Akinyosoye and Nwosisi (1994) who isolated these organisms and reported that they could be contaminants from air or materials used in processing. The isolation of these organisms gives serious cause for concern because Aspergillus species is specifically known to produce mycotoxins (Adams and Moss, 1995 cited in Kawo and Abdulmumin, 2009), which cause food intoxication in man and other animals (Kawo and Abdulmumin, 2009).

The study equally shows that some of the isolated fungi were the causative agents of rot in fresh healthy fruits (Amusa et al., 2003; Okigbo and Obire, 2009). The involvement of *Penicillium* sp., in the deterioration of tropical fruits has long been identified (Okigbo and Obire, 2009; Akintobi et al., 2011). It is generally known fact that wine is the alcoholic product of the fermentation of any fermentable fruit or vegetable by action of yeasts and various tropical fruits have been used for wine production (Okigbo, 2003; Omole, 2005; Okunowo and Osuntoki, 2007; Okigbo and Obire, 2009). Culture of *Saccharomyces cerevisiae* has been utilized in production of wine, in which it utilizes the sugar present in the juice and converted it to ethyl alcohol and carbon dioxide (Okigbo and Obire, 2009).

In addition, since the fruit juice was not pasteurized, it is likely that some other microorganisms were acting on the fruit juice thereby disrupting the yeast activity (Okigbo and Obire, 2009). When sugar was added as the only supplement in another fermenting juice, it gave a higher alcoholic content, indicating that the levels of nutrients for the yeasts to utilize have been increased (Okigbo and Obire, 2009). Many factors however, contributed to the presence of pathogenic microorganisms in these packaged juices (Okigbo and Obire, 2009). Lack of pasteurization is one of these factors. The pasteurization which is a process by which pathogenic organisms in the fluid is killed by heat (Okigbo and Obire, 2009). This indicates that lack of pasteurization is one of the major risks factors involved in juice production (FAO, 1999; Okigbo and Obire, 2009). So it means that the pasteurization has killed the harmful microorganisms thereby drastically avoiding their toxic components. The importance of pasteurization cannot be overemphasized (Okigbo and Obire, 2009).

Food spoilage problems occur with minimally processed concentrated frozen citrus products. These are prepared with little or no heat treatment, and major spoilage can be caused by *Lactobacillus* sp,

Bacillus sp and *Micrococcus* sp which produce dactyl butter flavors. Source of fruit and fruit juice are commonly contaminated with yeast and moulds often from the insect damage. Fallen fruit should thus be avoided where possible. Additional contamination may come from equipment coming in contact with food from packaging materials and from personnel (Abdullahi et al., 2005; Mohammed et al., 2005; Rogo and Kawo, 2005; Aminu et al., 2006; Shamsuddeen and Ameh, 2008; Shamsuddeen et al., 2008; Kawo and Abdulmumin, 2009). Generally, poor sanitary conditions and the environment being highly charged with spoilage and pathogenic flora could be the source of contamination to food items exposed to it (Kawo and Abdulmumin, 2009). Thus, retailers of food products, which include sweets, have been implicated in the spread of food-borne diseases (Abdullahi et al., 2005; Shamsuddeen and Ameh, 2008; Shamsuddeen et al., 2008; Oyeyi and Lumnwi, 2008; Wada-kura et al., 2009; Kawo and Abdulmumin, 2009). Various products have been implicated in food poisoning due to their quality, composition and general handling (Kawo and Abdulmumin, 2009).

Furthermore, the issue of safety and wholesomeness (safety), food plays a special role in the prioritization of control during manufacture and handling of food (Juhaniaková et al. 2013). According to Association of Food and Drug Officials (AFDO, 1990), simple packaging or repackaging operations can bring about an opportunity for the contamination or recontamination with pathogens if strict aseptic conditions are not adhered to (Juhaniaková et al. 2013). Testing for these organisms at specific control points provides the best means of quality control (Juhaniaková et al. 2013). Constant surveillance and good manufacturing practice are the best methods for prevention of contamination (Kačániová and Juhaniaková, 2011; Juhaniaková et al. 2013).

5. CONCLUSION

The study showed the presence of *Bacillus* sp, *Micrococcus* sp, *Flavobacterium* sp., *Lactobacillus* sp., *Penicillum* sp. and *Saccharomyces* species. *Saccharomyces* species isolated from these fruit juice samples can be screened for leavening ability. *Penicillium* species could be useful for the appropriate food and drug industries. Probiotic microorganisms may also be isolated from the packaged fruit juice. The average counts for bacteria of the packaged fruit juice samples examined are generally below the maximum allowable limit in foods to be marketed for consumption (10^3 cfu/g) except for the those from few locations while the fungal counts are all within acceptable limit. However, the average ranges obtained for the bacteria indicated a public health concern as they showed counts far above this limit. These high counts are suggestive of heavy bacterial contamination of the packaged fruit juice during handling since they are liquid, which could have contributed to the development as well as multiplication of these contaminants.

With the number of isolated bacteria and fungi from the different packaged fruit juice sold in Port Harcourt, it can be concluded that different bacterial and fungal species occur within fruits and materials used for the production of the juice as well as poor sanitation, extraction, raw material contaminations (often from insect damage), lack of both proper heat sterilization and adequate quality control during processing of fruit juice.

Some of the fungal isolates especially *Penicillium* sp. have the potential to induce rot on fresh fruits which might have a remarkable effect on the value of the fruit especially in the food industry as well as on human health. The study has also shown that these packaged fruit juices are not sterile and thus can favour the growth of microorganisms when conditions become favourable, which could pose a public health risk to their consumers.

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