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Microbiological quality of some locally-produced fruit juices in Ogun State, South western Nigeria

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Contaminated foods and drinks are the source of various food borne conditions due to gastroenteritis in human. For microbiological assessment of fruit juices, 120 fruit juice samples (24 each of avocado, papaya, pineapple, grape and orange) were collected. The spread plate method was used for the isolation of bacteria on appropriate selective media. Fungi were isolated on SDA with 0.1g chloramphenicol. All isolates were characterized using standard methods. The total viable count for bacteria, yeast, moulds, pH and titratable acidity of fruit juices were also determined. Questionnaire was used to obtain preliminary information on hygienic and safety practices of fruit juice makers and handlers. Isolated bacteria were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella sp*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Enterobacter sp*, *Salmonella sp*, *Streptococcus sp*, *Proteus sp* and *Serratia sp*. Fungi isolated were *Rhizopus spp*., *Fusarium spp*., *Penicillium spp*., *Mucor spp*., *Aspergillus spp*. and *Saccharomyces cerevisiae*. The highest titratable acidity was recorded in grape juice (0.245) and the lowest in avocado (0.081). The mean total viable count was highest in papaya juice ($6.5x10^4$ cfu/ml) and lowest in grape juice ($4.0x10^4$cfu/ml). Yeast count was highest in orange juice ($3.5x10^4$cfu/ml) and lowest in grape juice ($2.0x10^4$cfu/ml). Papaya and grape juices recorded the lowest mold count ($2.7x10^4$cfu/ml). In conclusion, the fruit juices had higher microbial loads than the specifications set for fruit juices in some parts of the world. Presence of pathogenic species in some of these fruit juices should is of paramount concern.

Keywords: Fruit; juices; quality; heath; risk; bacteria; fungi; hygiene.

INTRODUCTION

Juices are the aqueous liquids expressed or otherwise extracted usually from one or more fruits or vegetables, purees of the edible portion of one or more fruits or vegetables, or any concentrates of such liquids or purees (Fraternale, 2011). The consumption of fruit juices could have both positive and negative effect on the part of consumers. Fruit juices are well recognized for their nutritive value, mineral, and vitamin contents. Fruit juices processed under hygienic condition could play important role in enhancing consumers' health through inhibition of breast cancer, congestive heart failure (CHF), and urinary tract infection (Saenz and Sepulveda, 2001; Hyson, 2011). In many tropical Countries, they are common man's beverages and are sold at all public places and roadside shops. There are reports of food borne illness associated with the consumption of fruit juices at several places (Chumber *et al*., 2007; Muinde and Kuria, 2005; Lewis *et al*., 2006, Ghosh *et al*., 2007; Mosupye and Holy, 2000). Food borne diseases are harmful illness mainly affecting the gastrointestinal tract and are transmitted through consumption of contaminated food or drink. Such juices have shown to be potential sources of bacterial pathogens notably *E. coli* 0157:H7, species of *Salmonella*, *Shigella*, and *S. aureus* (Buchmann *et al*., 1999; Sandeep *et al*., 2001; Barro *et al*., 2006). In Sagamu, South western Nigeria, there is always a great demand for fresh vegetable and fruit juices. While most restaurants and café serve juices in apparently hygienic conditions, their microbiological qualities still remain questionable. This is also extended to those in roadside shops and recreational areas (parks) and busy market places (shopping malls, bus stations etc.). In developing countries like Nigeria, drinks, meals, and snacks sold by street food vendors are widely consumed by millions of

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people. Hence, the present study aimed to establish the hygienic status of street vended juices and their impact on street foods contamination with a view to assess their safety for human consumption and as possible sources of bacterial and fungal pathogens.

MATERIAL AND METHODS

Sample Collection and Survey

A total of 120 fruit juice samples (24 each of avocado, papaya, pineapple, grape and orange) were collected from six randomly selected cafés from among over 30 cafés/restaurants in Sagamu between April, 2013 and June, 2013. As some of the fruit juices vending cafés were serving either one, two or three types of the fruit juices, only those serving the maximum number of fruit juices were considered and six of them were selected for sampling. The fruit juices investigated in this study area were juices made from avocado, papaya, pineapple, grape and orange. Samples (25 ml each) of these fruit juices were separately collected in sterile flask and transported to the laboratory. Samples were processed within four hours of collection. Questionnaire was used to obtain preliminary information on the demographic characteristics of the fruit juice makers, servers, and cares being taken during processing of the fruit juices. All the personnel's involved in the processing and/or serving of the fruit juices in the selected cafés/restaurants were included.

Sample Processing and Microbial Analysis

10 ml of the sample was diluted in 90 ml of sterile buffered peptone water and mixed well. The samples were homogenized and appropriate dilutions of up to 10-8 were plated in duplicates on surfaces of respective media for microbial count using the spread plate technique. Aerobic mesophilic bacteria (AMB) were counted on plate count agar (PCA) after incubation at 32^0C for 48 hours; violet red bile agar (VRBA) was employed for the enumeration of coliforms after incubation for 48 hours at 32oC. Purplish red colonies surrounded by reddish zone of precipitated bile were counted as *coliforms*. *Enterobacteriaceae* were counted on MacConkey agar after incubated at 32^oC for 48 hours. Pink to red purple colonies with or without haloes of precipitation were counted as member of *Enterobacteriaceae*. *Streptococcus sp* was isolated on blood agar plates with characteristic colonies showing β-haemolysis confirmed as *streptococci*. *Bacillus cereus* was isolated on mannitol/eggs yolk/polymyxin agar (MYP). MYP was prepared using peptone (Oxoid, U.K.), meat extract (Oxoid, U.K.), D-mannitol, sodium chloride, phenol red, agar-agar, egg yolk (Oxoid, U.K.) and polymyxin B sulphate (Pfizer). The plates were incubated at 32°C for 24 h (Subbannayya *et al*., 2007). All catalase positive, motile organisms with ellipsoidal spores and positive V-P reaction were confirmed as B. cereus. Staphylococci were counted on mannitol alt agar (MSA) after incubation at 32^0C for 48 hours (Titarmare et al. 2009). Similarly, yeasts and molds were isolated on Sabouraud's dextrose agar (SDA) plus 0.1g chloramphenicol incubated at 25-28^0C for 3-5 days. Smooth (non-hairy) colonies without extension at periphery (margin) were counted as yeasts. Hairy colonies with extension at periphery were counted as molds. After enumeration, bacterial colonies were randomly picked from countable plates of PCA and MacConkey agar plates, and further purified by repeated plating on PCA (Holt *et al*., 1994). Cell morphology, Gram's reaction, colony characterization and biochemical characterizations of isolates were performed according to standard procedures (MacFaddin, 1980; Cowan and Steel, 1985; Holt *et al*., 1994).

Determination of pH

pH was measured using digital pH meter (Nig 333, Naina Solaris LTD, India) after homogenizing 10 ml of the fruit juices in 90 ml of distilled water (Erkmen and Bozkurt, 2004; Ferrati, 2005).

Titratable Acidity

Standard method was used to measure titratable acidity (Antony and Chanrda, 1997; Ferrati, 2005). The fruit juice sample (5ml) was homogenized in distilled water (20ml) and filtered through whatman No.1 filter paper. Two to three drops of phenolphthalein were added to 20ml of the filtrate as indicator and titrated against 0.05M NaOH to determine the end point of phenolphthalein. Titratable acidity was expressed as g lactic acid/100g of juice and calculated using the formula:

$$TA = \frac{MNaOH \times ml\ NaOH \times 0.09 \times 100}{ml\ juice\ sample}$$

Where, TA = Titratable acidity; MNaOH = Molarity of NaOH used; ml NaOH = amount (in ml) of NaOH used; 0.09 = equivalent weight of lactic acid.

RESULTS AND DISCUSSIONS

In Nigeria, street foods provide an affordable source of nutrients to many sectors of population. Street-vended fruit juices are well appreciated by consumers, because of their taste, low price, and availability at right time (FAO, 1988; Ohiokpehai, 2003). However, street foods are frequently associated with diarrhoeal diseases due to their improper handling and serving practices (WHO,

Table 3: pH and Titratable acidity (TA) of fruit juices in Sagamu, Ogun State.

Sources of fruit juices	Sample	pH	TA
Avocado	24	4.34±0.14	0.081
Grape	24	3.95±0.01	0.245
Orange	24	3.30±0.02	0.127
Papaya	24	3.90±0.01	0.110
Pineapple	24	3.50±0.001	0.223

Where, TA= Titratable acidity (g lactic acid/100g fruit juice

2002; Barro *et al*., 2006; Bello *et al.,* 2013). The source of fruits used for the processing of juices was majorly from the open market as this constituted 79.19% of this case study, while only 20.83% got their fruits directly from producers who were their routine suppliers. Fruit juices' producers made use of both ripened and over-ripened fruits, but with preference to ripened fruits as this constituted 75% of the case. The temporary storage sites of fruits were shelves (33.33%), baskets (41.67%) and refrigerators (10%). However, 15% of the juice producers had no special storage site for their fruits. Reasons for proliferation of microorganisms in fruit juices could also be attributed to the fact that the most juice producers lacked special training in food hygiene and safety. , as ilt was established in this study that 54.17% of the juice makers did not have such training; 75.83% were not examined medically as to their fitness and eligibility to produce juices and very few (17.5%) had the knowledge of symptoms as a result of eating contaminated foods. However, a lot of them (85.83%) had the knowledge that microorganisms can contaminate fruits and fruit juices, and 87.5% had education higher than primary education, 9.17% actually acquired primary education while only 3.33% had no formal education. Greater percentage (65%) of fruit juice makers interviewed were males and 75 (62.5%) of them were younger than 35 years (Table1).

Ten bacterial genera were isolated from the fruit juices and these were characterized as *Staphylococcus aureus*, *E. coli*, *Klebsiella sp*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Enterobacter sp*, *Salmonella sp*, *Streptococcus sp*, *Proteus sp* and *Serratia sp*. (Table 2). The mean pH of fruit juices investigated in this study ranged from 3.30 + 0.02, as in the case of orange juices, to 4.34 ± 0.14 in avocado. The highest titratable acidity was recorded in grape juice and the lowest in avocado juice (Table 3). The pH of fruit juices is usually too low with good potentials of inhibiting the growth of pathogenic bacteria (Uzeh *et al*., 2007), although some molds and yeasts could tolerate the acidity. Thus, the high magnitude of members of *coliforms* and other *Enterobacteriaceae* in these juices could be due to the high water activity of ready-to-serve juices (Antony and Chanrda, 1997; Ferrati, 2005). Products with high water activity possess good amount of unbound water molecule that supports growth and survival of microorganisms. However, the low acidity (i.e. higher pH) and viscosity of avocado, besides its nutrient content, makes it good medium for growth of microorganisms. The mean total viable count was highest in papaya juice (6.5x10^4 cfu/ml) and lowest in grape juice (4.0x10^{4}cfu/ml). Mean total *coliform* count was highest in avocado juice (4.0x10^{4}cfu/ml) and lowest in orange juice (1.5x10^{4}cfu/ml). The mean *staphylococcal* counts ranged from 1.0x10^{4}cfu/ml, as in the case of grape juice, to 3.5x10^4 cfu/ml, in avocado juice. *Salmonelli* were present in papaya and pineapple juices (1.0x10^{4}cfu/ml in both cases) but absent in the remaining fruit juices. Yeast count was highest in orange juice (3.5x10^{4}cfu/ml) and lowest in grape juice (2.0x10^{4}cfu/ml). Papaya and grape juices recorded the lowest mold count (2.7x10^{4}cfu/ml) while avocado juice recorded the highest (4.0x10^{4}cfu/ml) (Figure 1). The range of microbial counts recorded in this study (1.0x10^4 – 6.5x10^4 CFU/ml) was relatively lower than the microbial loads (102 - 105 CFU/ml) reported in earlier work of Lateef *et al.,* (2004). To the authors' knowledge, there was no specification set for the permissible level of microbes in fruit juices being served in Nigeria. However, the recommended specifications for fruit juices served in the Gulf region suggests that the maximum count permitted for total colony count of coliforms, yeast and molds are 1x10^4, 100, and 1.0x103 CFU/ml, respectively (Gulf Standards, 2002).This is similar to result and conclusion of Mahale *et al.,* (2008) in their study on microbiological analysis of street vended fruit juices from Mumbai city in India. He reported poor hygiene conditions of street vended fresh juices and the potential danger it posed to the consumers. Grape juice had lowest microbial load in this study (Figure 1). This could be attributed mainly to the very low pH observed (3.94- 4.04). In addition, conditions under which the juice was processed, stored, and/or served might have contributed to the betterment of the product. In fact, its low pH did not inhibit the growth of acid tolerant yeasts and molds, and this allowed their proliferation to level as high as 3.5 x 10^4 and 4.0 x 10^4 log CFU/ml, respectively. Some members of the *Enterobacteriaceae* family were encountered in all the five fruit juices. Of the one hundred and twenty fruit juice samples examined, 84 (70.0 %) yielded enteric bacteria which included *Klebsiella spp*,, *Enterobacter spp*, *Serratia spp*., *Proteus spp*., *Salmonella spp*. and *E. coli*. Fungal isolates included *Rhizopus spp*., *Fusarium spp*., *Penicillium spp*., *Mucor* spp., *Aspergillus spp*. and *Saccharomyces cerevisiae* (Table 4). Ten genera of bacteria were isolated from the

Table 1: Demographic study of fruit juice processing conditions in Ogun State, Nigeria.

Features	Number/Percentage of Respondents
Source of fruits	
Open Market	95 (79.17%)
Directly from producers	25 (20.83%)
Nature of fruit	
Ripened	90 (75%)
Over-ripened	30 (25%)
Temporary storage site of fruit	
Shelf	40 (33.33%)
Basket	50 (41.67%)
Refrigerator	12 (10%)
No special storage	18 (15%)
Training in food hygiene and safety	
Yes	55 (45.83%)
No	65 (54.17%)
Medical examination before commencement of work	
Yes	29 (24.17%)
No	91 (75.83%)
Knowledge that microorganisms can contaminate fruits/fruit juices	
Yes	103(85.83%)
No	17 (14.17%)
Knowledge of symptoms as a result of eating contaminated foods	
Yes	21 (17.5%)
No	99 (82.5%)
Education status of juice makers	
Acquired or completed primary Education	11 (9.17%)
Higher than primary education	105 (87.5%)
No formal education	4 (3.33%)
Sex	
Male	66 (55%)
Female	54 (45%)
Age	
Younger than 35	75 (62.5%)
Older than 35	45 (37.5%)

fruit juices and percentage occurrences ranged from 3% to 14%, while six fungal genera were isolated with percentage frequency ranging from 3% to 11%. Among bacterial isolates, *Staphylococcus aureus* had the highest percentage frequency (14%) while *Streptococcus spp* had the lowest. For fungal isolates, *Aspergillus niger* had the highest percentage occurrence (11%), followed by *Saccharomyces cerevisiae* (8%) and *Penicillium spp*

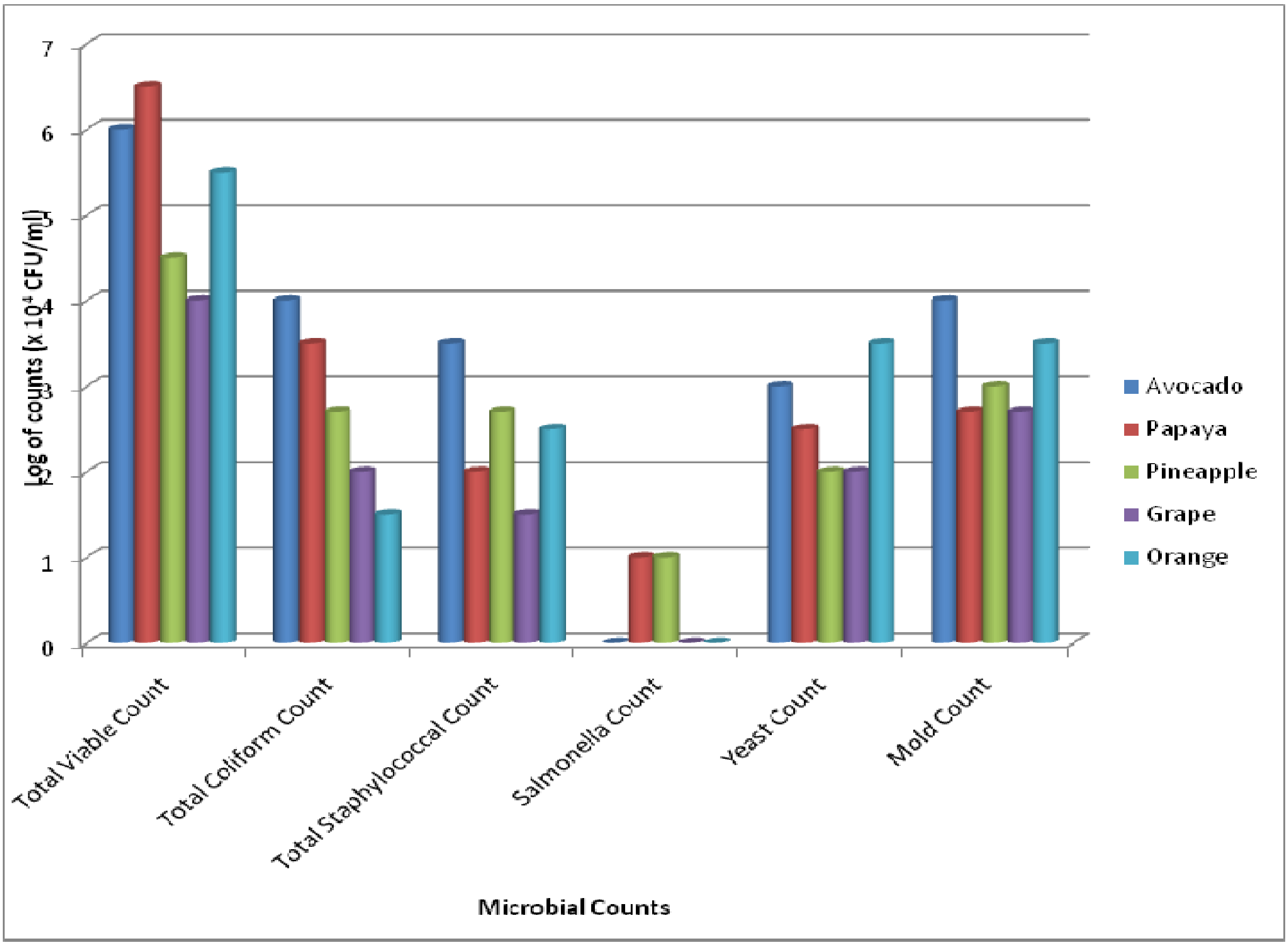

Figure 1: Microbial loads of fruit juices in Sagamu, Ogun State.

Table 4: Composite table of microorganisms isolated from fruit juices sold in Ogun State, Nigeria

Juice	Isolates
Avocado	*Klebsiella* spp., *Enterobacter* spp., *Bacillus* cereus, *Serratia* sp., *Staphylococcus aureus*, *Penicillium* spp. and *Aspergillus niger*
Papaya	*Pseudomonas aeruginosa*, *Klebsiella* spp., *S. aureus*, *Enterobacter* spp., *Proteus* spp., *Bacillus* cereus, *Streptococcus* spp and *Penicillium* spp.
Pineapple	*Salmonella* spp., *Bacillus cereus*, *S. aureus*, *E. coli*, *Saccharomyces cerevisiae, Fusarium* spp., *Penicillium* spp. *Aspergillus niger* and *Rhizopus* spp.
Grape	*S. aureus*, *Klebsiella* spp., *Salmonella* sp., *Aspergillus niger, Saccharomyces cerevisiae*
Orange	*E. coli*, *S. aureus*, *Serratia* sp., *Proteus* spp., *Saccharomyces cerevisiae, Mucor* spp. and *Aspergillus niger.*

Table 2: Morphological and biochemical characteristics of bacteria isolates from some fruit juices in Ogun State, Nigeria

Parameters	A	B	C	D	E	F	G	H	I	J
Gram's reaction	+	-	+	-	+	+	-	+	-	-
Catalase test	+	+	+	+	+	-	-	-	-	-
Citrate test	-	+	+	+	+	+	+	+	-	+
Oxidase test	-	-	+	+	-	-	-	-	-	-
Coagulase test	+	-	-	-	-	-	-	-	-	-
Indole test	-	+	-	-	-	-	-	-	+	-
Urease activity	+	-	+	-	-	-	-	-	+	-
Cellular morphology	Cocci	Straight rods	Rods	Rods	Rods	Cocci	Rods	Cocci	Rods	Rods
Growth on blood agar (colony)	Creamy white	circular	large white	greenish	N/A	Creamy	N/A	β-haemolysis	N/A	N/A
Growth on Mannitol salt agar	Bright yellow	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Growth on MacConkey agar	N/A	Red/ Pink	Mucoid	Pale	Pink	Pink	-	-	-	-
Motility	+	+	-	+	+	-	+	+	+	+
Glucose	A/G	A	A/G	A/G	A	A/G	A/G	A/G	A/G	D
Lactose	A	A/G	A/G	A	A	A/G	-	A/G	-	A/G
Sucrose	A/G	A	A/G	A/G	A	A/G	-	A/G	A	A/G
Mannitol	A/G	A/G	D	A/G	A	A	A/G	A/G	-	A
Maltose	A/G	A/G	N/A	A/G	A	A/G	A/ G	A/ G	A/G	A/G

Most probable organism: A - *Staphylococcus aureus,* B - *E. coli,* C – *Klebsiella* sp, D – *Pseudomonas aeruginosa,* E- *Bacillus cereus,* F – *Enterobacter* sp, G - *Salmonella* sp, H - *Streptococcus* sp, I - *Proteus sp* and J- *Serratia* sp.
- = No Growth, + = Growth, N/A = Not applicable, A = Acid Production, A/G = Acid and Gas Production.

(8%) while the lowest was recorded for *Fusarium spp* (3%), *Rhizopus* spp (3%) and *Mucor spp* (3%) (Figure 2).

Relatively high loads of different microbial groups, including *coli* forms and other *Enterobacteriaceae*, were recorded from the fruit juices examined in this study. Some members of the *Enterobacteriaceae* family were encountered in all the five fruit juices. The presence of *Staphylococcus spp* in almost all the juice samples can be attributed to contamination via handling. This may be due to poor personal and domestic hygiene indicating lack of knowledge of hygienic practices and safety of food products (Tambekar *et al.,* 2009; Bello *et al.,* 2013). Although the growth of *Staphylococcus spp* can be affected by the low pH of fruit juice samples, it is apparent that the vegetable juices having near neutral pH could be safe heaven for *staphylococci* to proliferate (Mudgil *et al*., 2004). The spoilage of acidic foods is most often due to contamination of the foods with aerobic acid

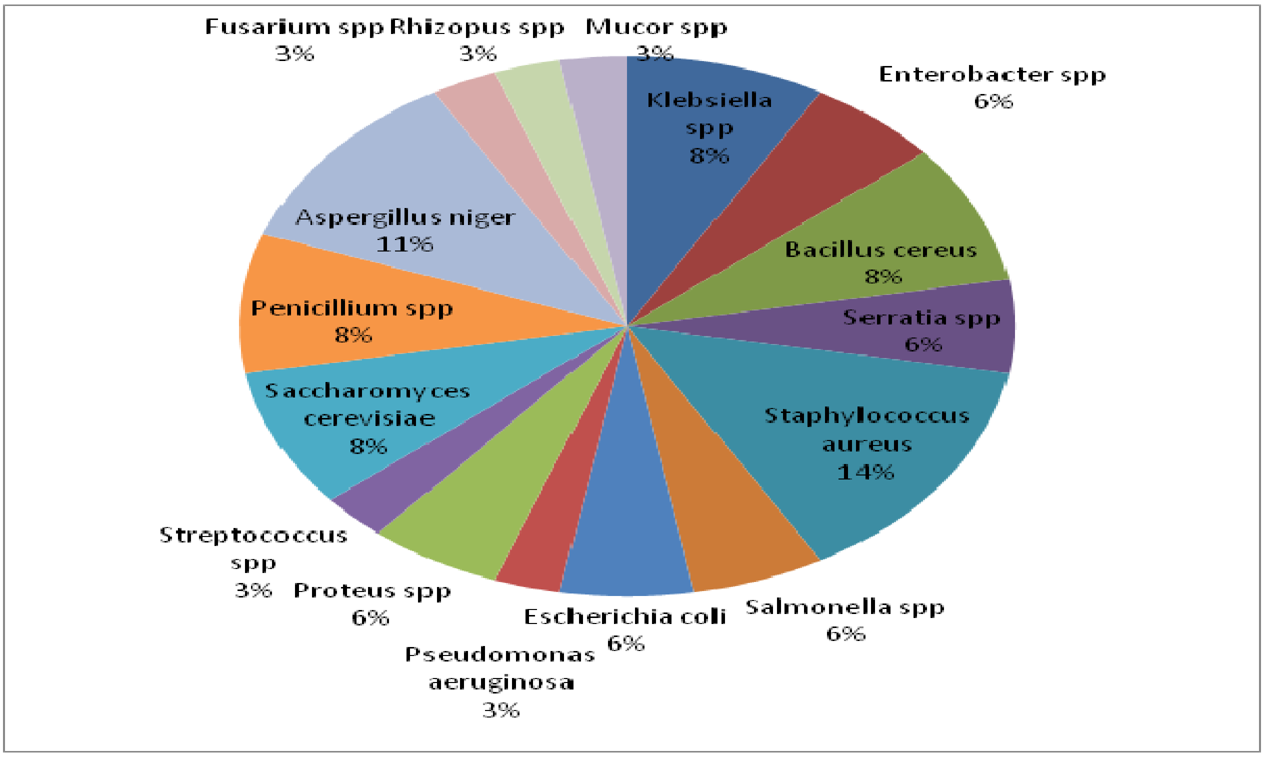

Figure 2: Percentage occurrence of microorganisms in some fruit juices in Ogun State, Nigeria

tolerant bacteria as well as yeasts and moulds (Hatcher *et al.* ,1992). Although reports on the microbiology of fruit juices were scanty, *Serratia* and *Enterobacter spp* had been reported to dominate the early phases of fermentation of Nigerian palm wine (Hatcher *et al.,* 1992). The occurrence of pathogenic bacteria in fresh fruit and vegetable products had been reported by Alzamora *et al.,* (2000), which include Listeria monocytogenes, *Aeromonas hydrophila*, and *Escherichia coli* O157: H7. These bacteria are found in both fresh and minimally processed fruit and vegetable products. Listeria monocytogenes is able to survive and grow at refrigeration temperatures on many raw and processed vegetables, such as ready-to-eat fresh salad vegetables, including cabbage, celery, raisins, fennel, watercress, leek salad, asparagus, broccoli, cauliflower, lettuce, lettuce juice, minimally-processed lettuce, butterhead lettuce salad, broad-leaved and curly-leaved endive, fresh peeled hamlin oranges, and vacuum-packaged potatoes. Adesetan *et al*., 2013 also reported *Staphylococcus aureus*, *Micrococcus sp*, *Bacillus* subtilis, *Lactobacillus spp*, *Streptococcus spp*, *E. coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Serratia plymuthica*, *Serratia ficaria*, *Proteus mirabilis* and *Enterococcus faecalis* in their study on street-vended pineapples, pawpaw, watermelons and coconut.

CONCLUSION

The fruit juices investigated in this study had higher microbial load than the specifications set for fruit juices in some parts of the world. On the basis of the gulf standards, it is clear that the colony counts of the microbial groups in our fruit juices exceeded the standard by considerable margin. These high counts, however, may pose hazard to the health of consumers especially if pathogenic species are present in the fruit juices to be consumed. Street vendors were mostly uninformed of good hygienic practices (GHP) and causes of diarrhea diseases which could increase the risk of street food contamination. They were also unaware of food regulations as well as lacking supportive services such as water supply of good and adequate quality, waste disposal systems which enhance their ability to provide safe food.

RECOMMENDATION

The practice of consuming fruit and vegetable juices cannot be stopped on unhygienic grounds, and juice makers cannot also be prohibited from selling such items, since it is a source of their livelihood. However, government health agencies must adopt measures to educate the vendors on food safety and hygienic practices. Regular monitoring of the quality of fruit and vegetable juices for human consumption must also be enforced. There is need to educate the juice makers and retailers on the hazards associated with the cultivation of non-chalant attitudes to hygienic processing, display and packaging of these juices. Control measures should be inculcated during sales of products, and these include displaying juices in glass cabinets, washing hands at regular intervals, disallowing customers from picking up and returning products with bare hands and/or without thorough hand-washing procedures. There should, also, be regular training/retraining and health education of handlers handlers in all aspects of food hygiene and safety.

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