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Full Length Research Paper

Microbiological status of processed fruit juice sold in the commercial city of Onitsha

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The microbiological status of industrially processed fruit juices sold in Onitsha main market was determined using standard methods. Fourteen (14) brands of the samples consisting of seven single fruits and seven mixed fruit juices were repeatedly subjected to bacteriological and mycological screening for six months. Isolates were characterized colonially, microscopically and biochemically, and their identity confirmed with reference to standard manuals. The processed fruit juices investigated showed high microbial loads consisting of bacteria such as *Bacillus* sp, *Staphylococcus* sp, *Enterococcus* sp *Pseudomonas* sp, *Micrococcus* sp and *Corynebacterium* sp. The Yeasts and moulds isolated are *Saccharomyces cerevisiae*, *Saccharomyces* var *ellipsoideus*, *Penicilluim caseicolum*, *Penicilium notatum*, *Rhizopus stolonifer* and an unidentified *Saccharomyces* species. Some of the isolates are normal commensals and or contaminants from the fruits and the environment. The presence of *Staphylococcus aureus, Bacillus* and *Penicillium* species portends health risk to consumers as some species produce potent toxins associated with food borne illnesses and mycotoxicoses. The Total Viable Count reveals a high microbial population across all the samples. These values are quite higher than the microbiological limits for fruit juices and nectars. Poor sanitary conditions and failure to adhere to good manufacturing practices during processing could influence the high microbial load. Recommendations were made to reduced the microbiological contamination and promote quality assurance of the products.

Key Words: Fruit Juice, Microorganisms, health implication

INTRODUCTION

Juice is the liquid that is naturally contained in fruit or vegetable tissue. It is prepared by mechanically squeezing or macerating fruit or vegetable flesh without the application of heat or solvents (Hollis *et al*., 2009). For example, orange juice is the liquid extract of the fruit of the orange tree (Wikipedia, 2011). Nigeria is the largest market in sub-Saharan Africa, with a population nearing 150 million people and growing at three percent annually. Despite a huge number of consumers, Nigeria's agricultural sector is under-developed and the country remains a major importer of food and agricultural products, including fruit juice concentrates and premix (GAIN Report, 2009).

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Most fruit juices contain sufficient nutrients that could support microbial growth. Several factors encourage, prevent, or limit the growth of microorganisms in juices. The most important are water activity (*aw*), low pH, hygienic practice and storage temperature and concentration of preservative (Troller, 1983; Jay, 1987).

The low pH of fruit juices greatly limits the number and types of bacteria that can survive or grow. Storage of products at refrigerator temperature or below is not always best for the maintenance of desirable quality of some fruits (Matchis and Liston, 1968). Water used for juice preparation can be a major source of microbial contaminants such as coliforms, faecal coliforms, faecal streptococci, etc (Gill *et al*., 1996).

The consumption of fruit juices could have both positive and negative effect on the part of consumers (Tsige *et al*., 2008). Fruit juices processed under hygienic condition could play important role in enhancing consumers' health

Table 1: Total Viable Count on Nutrient agar

S/No.	Sample Code	Total Colony Count (CFU/ml)	Colony Count Range (CFU/ml)
1	SBa	2.7×10^9	$1.6 \times 10^9 – 3.8 \times 10^9$
2	M6	2.4×10^9	$1.5 \times 10^9 – 3.3 \times 10^9$
3	SBl	2.4×10^9	$1.3 \times 10^9 – 3.3 \times 10^9$
4	SPe	1.5×10^9	$1.2 \times 10^9 – 1.8 \times 10^9$
5	SGu	1.7×10^9	$1.1 \times 10^9 – 2.4 \times 10^9$
6	M4	4.16×10^{10}	$3.76 \times 10^{10} – 4.56 \times 10^{10}$
7	Sap	1.4×10^9	$1.2 \times 10^9 – 1.6 \times 10^9$
8	M5	2.98×10^9	$2.96 \times 10^9 – 3.0 \times 10^9$
9	SOr	$>1.2 \times 10^9$	1.2 X 109 – TNTC
10	SPi	3.45×10^8	$7.0 \times 10^7 – 602 \times 10^8$
11	M3	TNTC	TNTC
12	M1	2.0×10^8	$2.0 \times 10^7 – 3.8 \times 10^8$
13	M2	3.5×10^8	$9.0 \times 10^7 – 6.1 \times 10^8$
14	M7	TNTC	TNTC

Key: TNTC= Too numerous to count

through inhibition of breast cancer, congestive heart failure (CHF), and urinary tract infection (Dennison, 1996; Saenz and Sepulveda, 2001). In absence of good manufacturing practice, however, the nutritional richness of fruit juices makes the product good medium for microbial growth, vehicle of foodborne pathogens and associated complications (Al-Jedah and Robinson, 2002). Fruit juices contaminated at any point of processing could be the source of infections pathogens (Tsiga *et al.*, 2008). This study reports on the microbiological status of some brand of fruit juice processed and sold in Onitsha, Nigeria.

MATERIALS AND METHODS

Collection of Samples

A total of fourteen (14) processed fruit juice samples, consisting of seven mixed and seven single fruit juices were bought from Onitsha Main Market. The samples were kept in the refrigerator before analysis.

Sample processing

Ten milliliters (10 ml) of the samples were diluted with 90 ml of sterile distilled water and mixed well to obtain 10^{-1} dilution. Serial dilutions were prepared and spread plate technique was used on appropriate selective media.

Microbiological analysis of samples

Microbiological analysis included enumeration and identification of microbiological contaminants in the samples (FDA, 2001). The fruit juice samples were cultured by employing spread plate method described by Uriah (2004) as the best for bacterial enumeration of food samples. The plates were incubated at 37°C for 24-48 hours before observation for Total Viable Count (TVC) on Nutrient Agar and total conforms on MacConkey Agar. The Potato Dextrose Agar plates for Yeast and moulds were incubated for 5 days. All inoculated plates were maintained at the requisite time and temperature. Total bacteria counts were done with Gallenkamp digital colony counter (Harrigan and McCance, 1990; Pelczar and Chan, 1977). The mean number of colonies counted was expressed as Colony Forming Units (CFU)/ml. Subculture was carried out to obtain pure isolates and discrete colonies. Identification of organisms was done on the basis of morphological, biochemical and cultural characteristics. Bacterial isolates were analysed for Gram character and their motility and various biochemical tests were performed by inoculating small portion of well-isolated colony into a series of media such as Sugar Fermentation, Indole Test, Methyl Red Test, Voges Proskauer Test, Urease Test, Citrate Utilization Test and Catalase Test (Cheesbrough, 2000; Uriah, 2004). Fungi isolates were characterized on the basis of pigmentation, sporulation, mycelia arrangement and microscopically (Abbey, 2007; Harrigan and McCance, 1990; Efiuvwevwere, 2000). The identities of the isolates were confirmed with reference to standard bacteriological and mycological manuals (Buchanan and Gibbon, 1974; Barnett and Hunter, 1987).

RESULTS

The examination of the microbiological quality of processed fruit juice sold in Onitsha main market revealed generally high bacterial and fungal counts. Table 1 show the Total Viable Count obtained from the Nutrient Agar. The Total Coliform Count obtained from MacConkey Agar is shown on table 2, while the Fungi Counts from the Potato Dextrose Agar is shown on table 3. The result of the Microscopic and Biochemical characteristics of bacterial isolates is shown in Table 4. Cultural and morphological characteristics of the fungal

Table 2: Total Coliform Count on MacConkey agar

S/No.	Sample Code	Total Coliform Count (CFU/ml)	Coliform Count Range (CFU/ml)
1	SBa	7.0×10^6	7.0×10^6
2	M6	8.0×10^6	8.0×10^6
3	SBl	1.5×10^7	1.5×10^7
4	SPe	3.0×10^6	3.0×10^6
5	SGu	2.0×10^6	2.0×10^6
6	M4	4.0×10^6	4.0×10^6
7	Sap	N.G.	N.G.
8	M3	7.5×10^6	$5.0 \times 10^6 – 1.0 \times 10^7$
9	M5	1.57×10^8	$1.05 \times 10^8 – 2.08 \times 10^8$
10	M7	4.5×10^6	$2.0 \times 10^6 – 7.0 \times 10^6$
11	SPi	2.0×10^6	2.0×10^6
12	SOr	5.0×10^6	$1.0 \times 10^6 – 4.0 \times 10^6$
13	M1	3.5×10^6	$3.0 \times 10^6 – 4.0 \times 10^6$
14	M2	3.0×10^6	$3.0 \times 10^6 – 3.0 \times 10^6$

Key: N.G. = No Growth.

Table 3: Total Fungal Count on Potato Dextrose agar

S/No.	Sample Code	Total Colony Count (CFU/ml)	Colony Count Range (CFU/ml)
1	SPe	1.95×10^7	$1.5 \times 10^7 – 2.4 \times 10^7$
2	SGu	2.2×10^7	$1.1 \times 10^7 – 3.3 \times 10^7$
3	Sap	7.5×10^6	$5.0 \times 10^6 – 1.0 \times 10^7$
4	M6	$>3.84 \times 10^8$	3.84×10^8 – TNTC
5	M4	3.4×10^7	$3.0 \times 10^7 – 3.8 \times 10^7$
6	SBa	1.35×10^7	$1.2 \times 10^7 – 1.5 \times 10^7$
7	SBl	1.4×10^7	1.4×10^7
8	M3	1.22×10^7	$7.6 \times 10^6 – 1.68 \times 10^7$
9	M5	$>3.04 \times 10^7$	3.04×10^7 – TNTN
10	M7	3.0×10^6	$1.1 \times 10^6 – 4.9 \times 10^6$
11	SOr	1.4×10^7	$2.5 \times 10^6 – 2.9 \times 10^6$
12	SPi	9.5×10^5	$7.0 \times 10^5 – 1.2 \times 10^6$
13	M1	1.29×10^7	$7.0 \times 10^6 – 1.88 \times 10^7$
14	M2	4.8×10^6	$4.5 \times 10^6 – 5.1 \times 10^6$

Key: TNTC= Too Numerous To Count

Table 4: Microscopic and Biochemical and Characteristics of Bacterial isolates

Colony Code	Grams Reaction/ Morphology	Lac	Glu	Suc	Cat	Oxi	Coag	Mot	In	VP	MR	Cit	Urease	Most Probable Identity
FJS1	+ S in chains	+	+	+	+	-	-	-	-	-	+	+	ND	*Enterococcus* sp
FJS2	- S in clusters	+	+	+	+	-	+	-	-	+	-	+	+	*Staphylococcus* sp
FJS3	+ R in short bead-like chains	-	+	-	+	-	ND	+	-	+	-	+	-	*Bacillus* sp
FJS4	+ S in tetrads	-	-	-	+	-	-	ND	-	-	+	+	+	*Micrococcus* sp
FJS5	- Rod shapes	+	+	+		+		+					+	*Psudomonas* sp
FJS6	+ R in club Shapes	-	+	-	+	-	+	-	+	+	-	+	+	*Corynebacterium* sp

KEY: Lac, Lactose; Glu, Glucose; Suc, Sucrose; Cat, Catalase; Oxi, Oxidase; Coag, Coagulase; Mot, Motility; In, Indole; VP, Voges Proskaur; MR, Methyl Red; ND, Not Done; S, Spherical, R, Rod, +, Positive, -, Negative.

isolates are shown in table 5. The microbiological quality of the fruit juice samples are distinctively presented in table 6.

DISCUSSION

Despite the potential benefits offered by fruit juices,

Table 5: Characterization of Fungi Isolates

Colony Code	Cultural characteristics	Morphological characteristics	Isolates
FJS7	Greenish white, flat and irregular in shape, dull and dry.	Septate hyphae, conidia arranged like mob-head	*Penicillium caseicolum*
FJS8	Circular powdery green colony	Hyphae septate. Conidia attached to a vesicle via a sterigma	*Penicillium notatum*
FJS9	Creamy flat colonies that are dull and dry	Gram positive spherical/oval budding cells	*Saccharomyces cerevisiae*
FJS10	Creamy flat round moist and shiny colonies	Gram positive large ellipsoidal budding cells	*Saccharomyces ellipsoideus*
FJS11	Orange round colonies that are dull and dry	Gram positive large spherical budding cells	*Saccharomyces* sp
FJS12	White filamentous colonies	Possess non-septate hyphae. Spores enclosed in a sporangium	*Rhizopus stolonifer*

Table 6: Bacteria and fungi isolates from the samples

Sample Code	Bacteria Isolates	Fungi Isolates
SOr	*Staphylococcus* sp*, Bacillus* sp, *Micrococcus* sp and *Pseudomonas* sp	*Saccharomyces ellipsoideus, Saccharomyces cerevisiae, Saccharomyces* sp and *Penicillium notatum*
SBa	*Enterococcus* sp, *Staphylococcus* sp and *Bacillus* sp	*Saccharomyces ellipsoideus*
M1	*Staphylococcus* sp and *Bacillus* sp	*Saccharomyces ellipsoideus*
M4	*Bacillus sp, Micrococcus* sp and *Staphylococcus* sp	*Saccharomyces ellipsoideus* and *Saccharomyces* sp
SBl	*Bacillus* sp*, Enterococcus* sp and *Staphylococcus* sp	*Rhizopus stolonifer, Saccharomyces cerevisiae* and *Saccharomyces* sp
M3	*Corynebacterium* sp and *Bacillus* sp	*Saccharomyces cerevisiae* and *Saccharomyces* sp
SGu	*Bacillus* sp*, Staphylococcus* sp and *Enterococcus* sp	*Penicillium caseicolum*, *Penicillium notatum* and *Saccharomyces cerevisiae*
M2	*Staphylococcus* sp*, Corynebacterium* sp, *Bacillus* sp*, Pseudomonas* sp and *Micrococcus* sp	*Saccharomyces ellipsoideus, Saccharomyces cerevisiae,* and *Saccharomyces* sp
M6	*Bacillus* sp, *Corynebacterium* sp and *Staphylococcus* sp	*Saccharomyces ellipsoideus* and *Saccharomyces cerevisiae*
SPe	*Staphylococcus* sp*, Bacillus* sp and *Enterococcus* sp	*Saccharomyces cerevisiae, Saccharomyces* sp and *Penicillium caseicolum*
M5	*Bacillus* sp and *Staphylococcus sp*	*Penicillium caseicolum,* and *Saccharomyces ellipsoideus*
M7	*Bacillus* sp*, Enterococcus* sp and *Staphylococcus* sp	*Saccharomyces ellipsoideus, Saccharomyces cerevisiae* and *Saccharomyces* sp
Sap	*Bacillus* sp	*Rhizopus stolonifer*, *Saccharomyces ellipsoideus*
SPi	*Bacillus* sp, *Staphylococcus* sp, *Enterococcus* sp and *Micrococcus* sp	*Penicillium caseicolum* and *Saccharomyces cerevisiae*

people are developing concerns on their safety and quality especially due to the high number of brands in the Nigerian market. In this research project, the processed fruit juices that were investigated showed high microbial loads (Tables 1,2,3) consisting of bacteria such as *Bacillus* sp, *Staphylococcus* sp, *Enterococcus* sp *Pseudomonas* sp, *Micrococcus* sp and *Corynebacterium* sp (Table 4). The Yeasts and moulds isolated are *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* var *ellipsoideus*, *Penicillium caseicolum*, *Penicillium notatum*, *Rhizopus stolonifer* and unidentified *Saccharomyces* sp (Table 5). Two (2) fruit juice samples namely SOr and M2 have the highest microbial population with 8 isolates. While the samples with the least microbial population are M1 and SAp with 3 isolates each (Table 6). *Bacillus* sp and *Staphylococcus* sp were the dominant species. *Pseudomonas* and *Corynebacterium* were among the least isolated from the samples analysed. Six (6) fungi were identified in the samples with the yeasts, *Saccharomyces* species as the dominant species. *P. notatum* and *R. stolonifer* were isolated from two samples only. The Microbiological limits in fruit juices and nectars according to UNBS (2009) is maximum of 10^3CFU/g Total Plate Count and 30 CFU/g maximum for yeasts and moulds. According to the UNBS (2009) standard, *Escherichia coli* should not be detected

in the fruit juices and nectars. In another development, the Good Manufacturing Practices (GMP) standard limit for yeasts in fruit juices is $<10^3$ CFU/ml for unpasteurized fruit juices and <10 CFU/ml for pasteurized fruit juices (Development and use of Microbiological criteria for foods, 1997), though the maximum acceptable level is 10^6 CFU/ml. Based on this standard limit, it can be deduced that the microbial load of the fruit juice samples analysed are quite high.

Al-Jedah and Robinson (2002) had reported on a number of factors responsible for contamination of fruit juices. Prior to processing, most fruit contains bacterial counts of 1 X 10^5 CFU/ml on their surface. These microorganisms may reflect in the final fruit juice products. Improper washing of fruits adds these bacteria to juices leading to contamination. In addition, lack of appreciation of basic safety issues by fruit processors contribute to augmentation of the microbial loads. These include use of unsterilized extractors, homogenizers and other equipments 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 (Lewis *et al.*, 2006). The presence of species of *Bacillus* across all the fruit juice samples investigated maybe due to the presence of their endospores. These structures are extraordinarily resistant to environment stresses such as heat, ultraviolet radiation, chemical disinfectants and desiccation (Willey *et al*., 2008). It is worthy of note that these processed fruit juices may be diluted and/or reconstituted from the juice concentrates. The concentrates can be a vehicle for endospores of *Bacillus* sp and other commensals and pathogens.

All the fruit juice samples investigated was devoid of foodborne pathogens such as *Salmonella, Shigella* and *E coli,* although some lactose fermenting bacteria were isolated on MacConkey agar medium. Indicator organisms of faecal pollution (such as faecal coliforms, faecal Streptococci) were also not present in the samples, however, the isolation of *Bacillus* sp, *Staphylococcus* sp and species of *Saccharomyces*, *Penicillium*, *Rhizopus*, *Corynebacterium* and *Pseudomonas* shows general poor quality processing of the products. The health implications of some the bacteria isolated from the samples had been reported (Prescott *et al.*, 2002: Pelczar *et al*., 1986; Perry and Staley, 1997; Nester *et al*., 1998; Eisenberg *et al*., 1975). *Pseudomonas* species cause food deterioration and spoilage even at refrigeration temperature (Prescott *et al*., 2002; Frazier and Westhoff, 1978; Jay, 1987; Pelczar *et al*., 1993). Some species of *Rhizopus* and *Penicillium* have been reported to produce potent mycotoxins responsible for various mycotoxicosis in humans (Abbey, 2006; Efiuvuwevwere, 2000). Fruit Juice M7 and M2 have the highest TVC with colonies too numerous to count. These high TVC could be a reflection of poor GMP in the processing of the products. SOr and M2 have the highest microbial diversity and both samples are products from the same manufacturer. The application of HACCP in this organization is advised and the Critical Control Points of the processing of the fruit juices have to be assessed.

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