Research Article

Microbiological and chemical quality assessment of some commercially packed fruit juices sold in Nigeria.

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Abstract

The microbiological and chemical qualities of some commercially packed fruit juices sold in Nigeria were assessed. A total of 30 fruit juice samples were collected including Orange, Lemon, Pineapple, Punch and Guava juice. Samples were screened for total aerobic mesophilic bacterial counts which ranged from $1.4x10^4$ to $2.6x10^5$cfu/ml. The fungal count ranges from $1.4x10^3$ to $1.7x10^5$cfu/ml, while the coliform counts ranges from $1.1x10^4$ to $6.0x10^4$cfu/ml. The isolated microbes include *S. aureus, B. subtilis, P. caseicolum and species of Saccharomyces, Enterobacter, Corynebacterium, Aspergillus, Rhizopus, Streptococcus, Acetobacter, Staphylococcus, Bacillus* and *Lactobacillus.* The total microbial counts are within acceptable standards for human consumption. *Bacillus, S. aureus Saccharomyces and Penicillium* was the most prevalent organisms isolated. All the samples were of acidic pH ranging from 3.20 to 4.50. Titrable acidity was 0.19 to 0.48; sugar (% sucrose) was 0.30 to 13.00. Heavy metals were not detected in any of the samples. The level of bacterial counts, fungal count and the absence of heavy metals conform to the standard specifications of National Agency for Food and Drug Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON). The presence of coliforms in some of the fruit juice calls for strict adherence to GMP and effective HACCP applications.

Key words: Coliform count, Fungal count, Good manufacturing practice (GMP), HACCP, Heavy metals, Total aerobic count.

Introduction

Fruit juice is unfermented but fermentable liquid or juice intended for direct consumption, obtained from the edible portion of sound, appropriately mature and fresh fruit by mechanical extraction process and preserve, exclusive by chemical and physical means (Woodroof and Luh, 2000; FAO/WHO, 2005). The juice may have been concentrated and later reconstituted with water suitable for the purpose of maintaining the essential composition and quality factor of the juice. The addition of sugar or acids can be permitted but must be endorsed in the individual standard (FAO/WHO, 2005). Juice is classified as pure or pulp and are prepared to contribute vitamins to diet, serve as pleasant tasting beverage drink or as a form of preservation technique (Salunkhe and Kadam, 1995).

Water is the predominant component of fruit juice, Carbohydrate including sucrose, fructose, glucose and sorbitol varies (Heldman and Lund, 1998). It contain small amount of protein, no fat, cholesterol and unless the pulp is included, contains no fiber (Parish, 1991; Pao *et al.,* 2000). Fruit juices are rich in Minerals and Vitamin A and C, and could be fortified with more minerals and vitamins. The anti-oxidant components of fruit juice have beneficial long term health effects, such as decreasing the risk of cancer and heart disease (Boyer and Liu, 2004; AICR, 2010). Increased iron absorption from food by Ascorbic acid is essential for children who consume diet with low iron bioavailability (Salunkhe and Kadam, 1995; Heldman and Lund, 1998; Ray, 2001).

The combination of raw fruit materials, food ingredients, juice preparation and processing methods, provides the manufacturer with practically infinite range of possible juice products varying in quality, price and value. The liquid nature of juice and the availability of many natural and manufactured juice constituents offer a blending opportunity, many mixing options, versatility of juices and enormous temptation to cut corners by economic adulteration. The ease with which juice can be altered with sugar, water or inferior juices has and continues to attract unethical suppliers. Such unethical practices can be dangerous, as there have been many tragic incidences of poisonous ingredients being added to juice (Nagy and Wade, 1995; Ashurst, 1995).

Chemical reactions involving oxygen, mental caution and other juice constituents can occur to modify sensitive pigment, taste or aroma substances. Chemical contamination can also occur from the environment via pesticide chemicals and the use of non-food grade equipment in the processing line, impact fruit juice (Kiritsy *et al.*, 1996).

Barring chemical and enzymatic changes, serious safety problem associated with juice consumption has risen, contamination of juice with pathogenic micro-organisms. Microbial deterioration is the major concern of fruit juices because the ubiquitous nature of microbes dictates that they contaminate the product via equipment and facilities, normal fruit micro flora, food handlers. Fruit juice has increasingly been the source of serious food poisoning outbreak and fatalities. Unpasteurized juice have been implicated in outbreaks due to *spp* of *Salmonella; E. coli, Clostridium, Cryptosporidium* and Hepatitis A (Southgate, 1995; Parish, 1997; De Rover, 1998).

In a processed fruit juice, bacteria are the most diversified micro-organisms causing its spoilage. Lactic acid bacteria *Acetobacter* and *Acetomonas* found on fruit surfaces comprise the most frequent spoilers of fruit juice because they exist on the surface of plant and fruits growing at the expense of secreted plant materials (Frazier and Westhoff, 1998). *Enterobacter spp* is commonly found on visually all types of unsound fruits in wash water. The presence of coliform mostly of the *E. aerogenes* type in both fresh and frozen fruit juices has been attributed to their being natural flora of fruits which may be introduced into the fruit juice if improperly processed (Frazier and Westhoff, 1998).

Most fruit juices are acidic enough and have sufficient sugar to favour the growth of yeast (Hooves, 1997). Mould are generally considered to be the least important group of micro-organisms causing spoilage in fruit juice because of their limitation, inability to grow in the absence of air (Parish, 1991), with the exception of few mould such as *Penicillium* and *Aspergillus* spore forming (Parish and Higgin, 1989).

Lapses in food safety do not only adversely impact the health of consumers; fragrant safety errors have and will ruin the reputation and financial health of the offending food company. To this end, this research work was undertaken to assess the microbiological and chemical qualities of fruit juices sold in Nigeria.

Materials and Methods

Sample Collection:

Sample selection was based on popularity (most demanded) and only samples within the expiry date as stipulated on the labels by manufacturers was analyzed. Three each of ten different brand of commercially available fruit juice packed either in pet bottle and tetra pack or cans were purchased from street vendors, and shopping malls between the months of May and August 2011. Information on the labels was recorded to include manufacturer's address, brand name, manufacture and expiry dates, batch number, NAFDAC number, type of preservative(s) and compositions. Samples were kept in the refrigerator at 4^0C before commencement of analysis.

Microbiological Analysis:

Prior to analysis, fruit juice samples were brought out of the refrigerator and allowed to assume the laboratory room temperature of 28 ± 2^0C. The surface of the containers was cleaned with cotton wool soaked in 70% Ethanol and then with tincture of iodine. The samples were shaken vigorously to mix up well. From each of the samples, aliquots of 1.0ml was collected and inoculated into 9ml of peptone water in screw caped test tubes for pre-enrichment (resuscitation) of organisms and into test tubes containing Lactose broth with inverted Durham tubes for coliform test by method of Speck (1976). Incubation of tubes was for 24 hours at 37^0C. Samples (0.2ml) was inoculated by spread plate methods onto Nutrient agar (Difco), MacConkey agar (Oxoid) and Sabauroud Dextrose agar (Fluka, Germany) for total aerobic mesophilic count, coliform count and fungal count respectively. Plates were incubated for 24 - 48hours at 37^0C for colony formation, except however, Sabouraud Dextrose agar (SDA) that was left for 24-72 hours at 28 ± 2^0C. At the expiration of the incubation time, the colonies were counted using colony counter (Stuart Scientific, UK).

After pre-enrichment, samples were plated out on Nutrient agar, MacConkey agar and Sabouraud Dextrose agar for 24 hours incubation at 37^0C and 28 ± 2^0C. Colonies were isolated and purified by repeated sub-culturing for further identification. Distinctive morphological properties of each pure culture such as colony form, elevation of colony and colony margin were observed. Further identification of bacterial isolates was based on the methods of Jolt *et al.* (1994). Fungal isolates were identified based on macroscopy and microscopy (Tsuneo, 2010).

Chemical Analysis:

The chemical analysis was carried out in Alpha Laboratories, Apapa, Lagos, Nigeria. Prior to the analysis, the samples were thoroughly shaken. The pH of the samples was determined using pH meter (pH 211 microprocessor, Hanna instruments). The ash content, titrable acidity, soluble solid, total solid, heavy metals were determined by the methods as described by AOC (1980). The sugar content (reducing sugar and total sugar) were determined by Lane and Eynon's method as described by Kirk and Sawyer (1997). Measurement of specific gravity was by method of UNIDO (2004) while the determination of Benzoic acid and Ascorbic acid was by the methods as described by Pearson (2001) and Kirk and Sawyer (1997) respectively.

Results

All the samples were adequately labeled with NAFDAC registration number, manufacturer's address, batch member, manufacture and expiry dates. The preservative for some of the products are not indicated. The total aerobic mesophilic count, coliform count and fungal counts is depicted in Table 1. Microbial contaminants were found in all the samples analyzed. Coliform was however, detected in only three brands. The table also revealed that microbial counts were below 10^6cfu/ml in all the samples and *S. aureus Saccharomyces, Bacillus and Penicillium spp* were the major contaminants. The table shows that there were no difference in the microbial load of the juices manufactured in Nigeria (samples with 'N' starting the code) and the imported fruit juices (samples with 'I' starting the code).

The chemical composition of the fruit juice is shown in Table 2. All the samples are of acidic pH ranging from 3.20 – 4.50 with percentage titrable acidity (as citric acid) of 0.19 – 0.48. Heavy metals (Arsenic, Copper, Lead, Iron, and Cadmium) were not detected in any of the samples. All the chemical compositions analyzed were within the standard acceptable range when compared with the 2008 standard specification of Standard Organization of Nigeria (SON, 2008).

Benzoic acid was detected in samples NDAN; NCHP; IRUB and IARI even though it was not indicated by the manufacturers. The level detected however, is within acceptable standard specification.

Discussion and Conclusion

Appropriate labeling of the analyzed samples is an indication that the samples were genuine, in conformity to good manufacturing practice (GMP) and to SON and NAFDAC standards.

Microbial contaminants of the fruit juice were below 10^6cfu/ml thus within acceptable limit for human consumption (ICMSF, 1974). The presence of Benzoic acid coupled with the low pH of the juice could be responsible for keeping the microbial load in check within acceptable level.

The presence of microbial contaminants in all the products could be a reflection of the quality of the raw materials, processing equipments, environment, packaging materials and the personnel's in the production process. The fungal count in all the products is of the order $10^3 – 10^5$. This finding corroborates the result of Rahman *et al.* (2011).

The low count could be associated to the suppressive effect of Benzoic acid and the limitations of growth in the absence of air (Parish, 1991). Fungi are common environmental contaminants and the moulds bear resistant spores that easily contaminate surfaces and can resist the juice condition (Parish, 1991). Fungi are the major causes of spoilage of fruits and vegetables (ICMSF, 1998), the presence of aflatoxin and other mycotoxin producing mould contamination on fruit surfaces have been reported to generally end in the juice (Riby *et al.,* 2001).

The absence of coliform in the products except, NGDI, NCHP and IRUB further confirms the products conformity to standards. *Enterobacter* and *Streptoccus spp* are commonly found in wash water, the presence of coliform mostly of the *Enterobacter* type in fruit juice has been attributed to their being natural flora of fruits which may be introduced into the fruit juice if improperly processed (Frazier and Westhoff, 1998). Safe Food Consumption Standard prohibit coliforms in fruit juice (Andres *et al.,* 2004), NGDI, NCHP and IRUB are therefore unfavourable for consumption.

That *Saccharomyces spp, S. aureus* and spore bearing bacillus and moulds are the major contaminants it could be explained by the fact that the low pH of the juice favour the yeast and mould (Parish and Higgin, 1989; Tournas *et al.*, 2006). *S. aureus* are common contaminants often from the food handlers and environment. *S. aureus* are facultative and tolerant; post process contamination could explain their presence in the juice. The bacillus and moulds are spore bearers and common food contaminants from the environment. The presence of these organisms though in low amount need to be controlled to prevent spoilage and food borne illness (Mudgil *et al.*, 2004; Oranusi *et al.,* 2004, 2007).

The fruit juice manufactured in Nigeria and those imported had microbial contaminants of equal magnitude, this shows that these products can be effectively produced in Nigeria to match standards obtained anywhere in the world. Importation of such products should therefore be discouraged to encourage local manufacturers and create job opportunities.

The pH of 3.20 – 4.50 recorded for the fruit juices conform to the standard description for acid foods (pH 3.0 – 4.60) (James, 1992; Kirk and Sawyer, 1997). The low pH may have inhibited the growth and proliferation of the contaminants. Sugar concentration in fruit juice is not high enough to inhibit bacterial growth, the inhibiting factor for bacteria appear to be acidity rather than sugar.

All the analyzed chemical parameters; total acidity, sugar, ethyl alcohol, total solid, Ascorbic acid, Benzoic acid and heavy metals conform to the standard specifications (NIS, 1987; SON, 2008). The titrable acidity reported in this work is in agreement with that reported by Rizzon and Miele (2012). The soluble solid as determined by the refract meter at 20^0C uncorrected for acidity and read as ^{0}Brix on the international sucrose scale (NIS 235 – 1987) conform to the reports of Rizzon and Miele (2012). The values reported for percentage total soluble solid could be associated to high fructose corn syrup (HFCS) often used as sweetening agent by some manufacturer. The level of Ascorbic acid reported is within standard specification and in agreement with the reports of Malo *et al.* (2006), Gazdik *et al.* (2008) and Okiei *et al.* (2009). The detection of Benzoic acid in samples NDAN, NCHP, IRUB and IARI even though within accepted standard specification could be a deliberate attempt of the manufacturers at deceiving the consumer with claims of natural product free of any preservative. This act negates the concept of GMP and calls for concern.

The microbial and chemical properties of some analyzed fruit juices sold in Nigeria complied with standard specifications, however, the presence of coliform in some products and the concealing of information on presence of Benzoic acid preservative in four of the products calls for concern and suggest intensified effort by the regulatory agencies to enforce GMP and effective HACCP application.

Table 1. Total microbial counts (cfu/ml) and microbial isolates of fruit juices

Sample code and type	TAMC	TFC	TCC	Microorganisms isolated
NMKI -Lemon juice	$1.4x10^4$	$1.2 x10^4$	-	*S. aureus, Saccharomyces spp, Bacillus spp*
NGDI -Lemon juice	$6.0x10^4$	$2.3 x10^4$	$2.8 x10^4$	*S. aureus, Enterobacter spp, Rhizopus*
NMKI -Orange juice	$1.9 x10^4$	$4.0 x10^3$	-	*Bacillus spp, Saccharomyces spp*
NLAV -Orange juice	$5.4 x10^4$	$1.4 x10^3$	-	*Staphylococcus, P. caseicolum, Saccharomyces spp*
NDAN -Pineapple juice	$9.6 x10^4$	$4.3 x10^4$	-	*Bacillus spp, Aspergillus spp*
NFAL -Citrus burst	$1.1 x10^5$	$1.0 x10^5$	-	*B. subtils, Penicillium spp, Corynebacterium spp*
NFRN -Orange juice	$1.5 x10^5$	$1.4 x10^5$	-	*Saccharomyces spp, Aspergillus & Penicillium spp*
NCHP - Pineapple juice	$1.8 x10^5$	$1.7 x10^5$	$6.0 x10^4$	*Enterobacter spp, Penicilliun spp, Rhizopus*
IRUB -Guava juice	$2.6 x10^5$	$1.6 x10^5$	$1.1 x10^4$	*Streptococcus spp, Bacillus spp, Acetobacter spp*
IARI -Punch juice	$2.1 x10^4$	$1.4 x10^4$	-	*S. aureus, Lactobacillus spp, Aspergillus spp*

TAMC= total aerobic mesophilic count; TFC= total fungal count; TCC= total coliform count; - = no growth; N-begins code of samples manufactured in Nigeria; I-begins code of imported samples

Table 2. Chemical properties of fruit juice

Properties	NMKI Lemon juice	NGDI Lemon juice	NMKI Orange juice	NLAV Orange juice	NDAN Pineapple juice	NFAL Citrus juice	NFRN Orange juice	NCHP Pineapple juice	IRUB Guava juice	IARI Punch juice
Ph	3.62	3.44	3.54	3.44	4.50	4.43	4.43	3.98	3.98	3.20
Titrable acid	0.30	0.34	0.30	0.34	0.19	0.20	0.19	0.19	0.48	0.45
Soluble solid (%)	1.01	1.00	2.80	1.00	11.00	11.40	12.68	13.40	8.60	8.50
Sugar (% sucrose)	11.86	0.30	1.50	0.30	10.80	11.00	9.00	13.00	7.60	7.55
Total solids mg/kg	20.20	2.70	15.30	2.70	22.00	30.00	27.20	26.00	42.35	42.00
Ascorbic acid mg/100ml	77.48	35.22	20.25	35.22	2.60	19.37	35.22	4.64	35.22	25.00
Benzoic acid	15.25	99.12	26.25	99.12	64.00	-	-	62.00	122.00	80.00
Ethyl alcohol	-	-	-	-	-	-	-	-	-	-
Arsenic µ/l	-	-	-	-	-	-	-	-	-	-
Copper µ/l	-	-	-	-	-	-	-	-	-	-
Lead µ/l	-	-	-	-	-	-	-	-	-	-
Fe µ/l	-	-	-	-	-	-	-	-	-	-

- = no growth; N-begins code of samples manufactured in Nigeria; I-begins code of imported samples

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REFERENCES

AICR (American Institute for Cancer Research). 2010. Cited 2011 Dec. Available from http://www.aicr.org/site/page server.

Andres SC, Giannuzzi L, Zaritzky NE. 2004. Temperature effect on microbial growth in film packed apple cubes with preservative added orange juice. *International Journal of Food Science and Technology,* 39:927-933.

AOAC. 1980. Official Method of Analysis. Association of Official Analytical Chemist 13th ed. Washington D.C pp 22-30.

Ashurst PR. 1995. Production and Packaging of non-carbonated fruit juices and fruit beverages. Blackie academic and professional London pp 399.

Boyer J, Liu R. 2004. Apple Phytochemicals and their health benefits. *Nutr. J.,* 3:5 doi:10. 1186/1475-2891-3-5.

De Rover C. 1998. Microbiology safety evaluations and Recommendation on fresh produce. *Food control*, 9(6): 321-347.

FAO/WHO. 2005. Codex general standard for fruit juices and nectars CODEXSTAN 247-2005.

Frazier WC, Westhoff DC. 1998. Food microbiology 4th ed. Mc Graw Hill, New Delhi pp 196-215.

Gazdik Z, Zikka O, Petrolova J, Homa A, Reznicek VI, Belelova M, Kizck R. 2008. Determination of vitamin C (Ascorbic acid) using high performance liquid chromatography coupled with electro chemical detection. *Sensors,* 8:7079-7112.

Heldman DR, Lund DB. 1998. Handbook of Food Engineering Mercel Dekker, New York pp 756.

Hoover DG. 1997. Manually Processed Fruits and Vegetable: Reducing microbial loads by non-thermal physical treatment. *Food Technology Journal,* 51(6):66-69.

ICMSF (International Commission on Microbiology Specification for Food). 1994. Sampling for microbiological Analysis. Principles and Specific Application, University of Toronto press, Toronto pp 1-18.
ICMSF. 1998. Microbial Ecology of Food Commodities. Microorganisms in Foods. Blackie Academic and Professional.
James MJ. 1992. Spoilage of Miscellaneous Food in Modern Food Microbiology 4th ed. Nostraned Reinhold, New York pp 245-246.
Jolt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST. 1994. Berger's Manual of Systemic Bacteriology, 9th ed. Williams and Wilkins Co. Baltimore, Maryland, p.786.
Kiriksy MC, Levgi SM, Warren JJ, Chowdhury NJR, Marshal T. 1996. Assessing Fluoride Concentrations of Juices and Juice Flavoured Drinks. *JAM Dentist Association,* 127:895-902.
Kirk RS, Sawyer B. 1997. Composition and Analysis of Food, Fruit and Vegetable Products 9th ed. Pp 237-239.
Melo EA, Lima VL, Macnel MIS, Lactano AC, Leal FL. 2006. Ascorbic acid and total carotenoid content in common fruits and vegetables. *Braz. Journal of Food Technal.,* 9:89-94.
Mudgil S, Argawal d, Ganguli A. 2004. Microbiological analysis of Street vended Fresh Squeezed carrot and Kinnow Mandarin Juice in Patiala City, India. *Internet Journal of Food Safety,* 3:1-3.
Nagy S, Wade RL. 1995. Method to detect adulteration of Fruit Juice Beverages. Ag Science Inc. Auburn dale Forida.
NIS (Nigeria Industrial Standard). 1987. Standard for Fruit Juices, NIS Standards, Ikoyi, Lagos.
Okiei W, Ogunlesi M, Azeeze L, Obakachi V, Osunsanmi M, Nkechor G. 2009. The voltametric and titrimetric determination of Ascorbic acid levels in Tropical Fruit Sample. *Int. Journal Electro. Chem Sci.,* 4:276-287.
Oranusi S, Onyeike E, Galadima M, Umoh VJ. 2004. Hazard analysis critical control points of foods prepared by families in Zaria, Nigeria. *Nig. J. Microbiol.*, 18(1-2):346-362.
Oranusi S, Galadima M, Umoh VJ, Nwanze PI. 2007. Food safety evaluation in boarding schools in Zaria, Nigeria, using the HACCP system. *Scientific Research and Essay,* 2(10):426-433.
Pao S, Fellers PL, Brown GE, Chambers M. 2001. Formulation of Fresh Squeezed Unpasteurized Citrus Juice Blend. *Fruit Processing Journal,* 7:268-271.
Parish ME, Higgins DP. 1989. Yeast and Mould Isolated from Spoiling Citrus Products. *Journal Food Production,* 52:261-263.
Parish ME. 1991. Microbial Concern in Citrus Juice Processing. *Journal of Food Technology,* 45(4) 128-132.
Parish ME. 1997. Public Health and Non Pasteurized Fruit Juices. *Critical Review in Microbiology,* 23(2) 109-119.
Pearson N. 2001. Composition and analysis of Food, Fruits and Vegetable Products, FAO of United Nation Rome Paper 4/7 pp 237-239.
Rahman T, Hasan S, Noor R. 2011. An Assessment of Microbiological Quality of Some Commercially Packed and Fresh Fruit Juices available in Dhaka City: A Comprehensive Study. *Stanford Journal of Microbiology,* 1(1):13-18.
Ray B. 2001. Fundamental Food Microbiology. CRC Press, New York pp 14-19.
Riby JE, Fujisaws T, Ferreria SHP. 2001. Physico-Chemical Suability of Nature or Pre-Sweetened Frozen Passion Fruit Juice. *Braz. Arch. Biol. And Tech.*, 42(4):393-396.
Rizzon LA, Miele A. 2012. Analytical characteristics and discrimination of Brazilian commercial grape juice, nectar and beverages. *Ciencia e Tecnologia de Alimentos*
Salunkhe DK, Kadam SS. 1995. Handbook of Fruit Science and Tech. Mercel Dekkar New York 614.
SON (Standard Organisation of Nigeria). 2008. *SON Standard Bulletin* Ikoyi, Lagos.
Southgate DA. 1995. Digestion and Metabolism of Sugar. *AM. Journal Clinical Nutrition,* 62:2035-2115.
Speck ML. 1976. Compendium of methods for microbiological examination of foods,
American Public Health Association. Washington DC pp 277 – 328.
Tournas VH. 2005. Moulds and Yeasts in Fresh and Minimally Processed Vegetables and Sprouts. *International Journal of Food Microbiology,* 99: 71-77.
Tsuneo W. 2010. Pictorial atlas of Soil and Seed Fungi: Morphologies of Cultural Fungi and Key to Species. Third edition CRC Press 2010.
UNIDO (United Nations Industrial Development Organization). 2004.UNIDO Technology Manual: small-scale fruit and vegetable processing and products production methods, equipment and quality assurance practices. UNIDO, Vienna, 2004.
Woodroof FG, Luh BS. 2000. Commercial Fruit Processing. AVI Publishing Company Mc Westport Comm. Pp234-229.