

Full Length Research Paper

# Microbiological quality assessment of some National Agency for Food and Drug Administration and Control (NAFDAC) approved fruit juices sold in Ilorin metropolis

Olaniyi, O. O.

Department of Microbiology, Federal University of Technology, Akure, Nigeria.

Accepted 4 June, 2013

This study was carried out to investigate on the microbiological qualities of some NAFDAC approved fruit juices sold in Ilorin metropolis. The brand of fruit juices assessed was coded as LL, LC and LB. The total viable counts, associated microorganisms and physicochemical properties of 10 randomly selected samples from each of the brand were determined. The highest mean bacterial load of 7.8 cfu/ml was found in fruit juice coded LL, while the lowest mean bacterial load of 3.5 cfu/ml was recorded for LB. The highest mean fungal load of 6.1 sfu/ml was observed with LL and the lowest mean load of 2.2 sfu/ml was found in LB. LL had no coliform growth, while LC and LB had the same mean load of 0.7 cfu/ml. The bacterial isolates identified from the samples were *Bacillus* sp., *Micrococcus* sp., *Proteus* sp., *Escherichia coli* and *Lactobacillus* sp, while the fungal isolates were *Aspergillus nidulans*, *Aspergillus niger*, *Cladosporium* sp. and *Schzosaccharomyces pombe*. The pH values for LL, LC and LB ranged from 3.78 to 3.83, 2.40 to 2.43 and 2.39 to 2.41, respectively. LC had the highest mean total titratable acidity which ranged from 0.14 to 0.66, followed by LB ranging from 0.10 to 0.16, while LL had the lowest values ranged from 0.07 to 0.13. *Bacillus* sp. is the most frequently encountered in all the samples with a percentage occurrence of 46.67%, while *Lactobacillus* sp., *Micrococcus* sp. and *Escherichia coli* had the lowest percentage occurrence of 36.67% each. In case of fungi, *Cladosporium* sp had the high percentage occurrence of 43.33%, while *A. nidulans* had the least percentage occurrence of 23.33% of all the samples examined.

**Key words:** Fruit juice, microbial quality, microbial load, microbial contamination.

## INTRODUCTION

Traditionally, fruit juices are considered susceptible to spoilage only by yeast, mycelial fungi and lactic acid bacteria (Chang and Kang, 2004). Foods with a pH lower than 4 are considered as high in acid and are generally regarded as not being susceptible to spoilage by a variety of microorganisms (Jay, 1998). The low pH is considered sufficient to prevent the growth of almost all bacteria spore formers. Spores of *Clostridium botulinum* cannot germinate or produce the lethal *botulinum* toxin in an environment with a pH below 4.6 (Chang and Kang, 2004). The microorganisms present in fruit juice often originate from the natural flora of the raw materials used

for the preparation and those introduced during the course of the processing (Splittstoesser et al., 1994). The number and types of organisms are determined by the properties of the food product and activity of the organisms in the product. In some cases, the microorganisms have no discernable deleterious effects and the food is consumed without harm (Yeh et al., 2004). In other cases, however, the presence of microorganisms have manifested in form of spoilage, food-borne illness and fermentation (Yeh et al., 2004). During the heat treatment of foods, pathogens and most non spore forming microorganisms are killed, but a heat process

**Table 1.** Physical characteristics of samples.

Sample	Condition of the pack	Visual observation of the sample
LL	Intact plastic bottle	Normal colour of pale orange and no foreign particles
LC	Intact plastic bottle	Normal colour of caramel and no foreign particles
LB	Intact paper pack	Normal colour of pale orange and no foreign particles

LL, A brand of fruit juice; LC, a brand of fruit juice; LB, a brand of fruit juice.

**Table 2a.** Total bacterial and coliform counts (cfu/ml) and fungal counts (sfu/ml) of the fruit juice LL.

Brand/sample	TBC	TFC	TCC
LL1	17	5	ND
LL2	ND	8	ND
LL3	ND	9	ND
LL4	13	6	ND
LL5	16	1	ND
LL6	18	7	ND
LL7	6	6	ND
LL8	8	4	ND
LL9	ND	6	ND
LL10	ND	9	ND
Mean	7.8	6.1	0

TBC, Total bacterial count; TFC, total fungal count; LL, a brand of fruit juice; ND, not detectable.

sufficient to destroy all the microbial spores will have a detrimental effect on the organoleptic quality of the product (Walls and Chuyata, 2000). The purpose of this study is to evaluate the microbiological quality of some brands of fruit juice sold in Ilorin metropolis, Nigeria.

The presence of 'registration number' from 'regulatory agencies' may not necessarily give assurance of quality, as these can be faked.

## MATERIALS AND METHODS

### Brands of fruit juice used

The fruit Juices employed in this study were manufactured by companies in Nigeria and were purchased randomly at different locations in Ilorin metropolis. A total of 3 brands (LL, LC and LB) were used in the study and each brand contains 10 samples of different batches.

### Physical observation of the samples

The packed juices were examined for leakage or bulged ends and changes in the colorations of the liquid content.

### Determination of pH of the samples

The pH of the various samples was immediately determined using

sterile probes of the pH meter (Corning 35).

### Determination of total titratable acidity (TTA)

This was determined according to the method of Akharayi and Omoya (2005). Twenty-five millilitres of the samples with three drops of 1% phenolphthalein as an indicator was titrated against 0.1 M NaOH. The end point was recorded when pink colour was noted. The TTA was then calculated as lactic acid thus: volume of 0.1 M NaOH × 100 over volume of weight of sample used in titration.

### Microbiological analysis

The experiment was carried out without further diluting of the fruit juice. This was done to determine the presence of viable microorganisms in the fruit juices at the concentration at which they were consumed. Microbiological analysis included identification and enumeration of potential pathogens which was carried out according to standard procedures (Buchanan and Gibbons, 1974). The total colony count was done by pour plate method using nutrient agar for bacteria and potato dextrose agar for fungi (Lateef, 2004; Lateef et al., 2005). The presence of faecal coliforms was determined by using eosine methylene blue agar (Titarmare et al., 2009). All inoculated at the requisite time and temperature. The bacterial isolates were then identified following standard microbiological procedures as described by Buchanan and Gibbons (1974) and Cheesbrough (2002), while fungal isolates were identified based on the taxonomic schemes and descriptions by Ainsworth et al. (1973) and Mislivec et al. (1992).

## RESULTS AND DISCUSSION

Physical characteristics of samples from the 3 brands were examined for leakage or bulged ends and changes in the coloration of the content (Table 1). The samples from the brand of fruit juice coded as LB were intact in paper pack, while LL and LC were intact in plastic bottles. The visual observation of samples from all the brands (LL, LC and LB) retained their normal colour without any suspended foreign particles. The physical characteristics of all the samples are in line with good manufacturing practices as stated in fruit juice and nectar regulations Act 2005. Fruit juices are well appreciated by consumers because of their taste, nutritional value and availability at the right time. In spite of these potential benefits offered, concerns over their safety and quality have been raised. Result of this study (Table 2a, b and c) indicated the presence of bacteria, fungi and coliform in some of the samples selected randomly from the brands. At the concentration at which they were consumed, samples from

**Table 2b.** Total bacterial and coliform counts (cfu/ml) and fungal counts (sfu/ml) of the fruit juice LC.

Brand/sample	TBC	TFC	TCC
LC1	3	8	1
LC2	5	2	ND
LC3	ND	5	2
LC4	13	5	1
LC5	ND	2	ND
LC6	2	ND	ND
LC7	16	3	2
LC8	ND	7	ND
LC9	4	6	1
LC10	ND	ND	ND
Mean	4.3	3.8	0.7

TBL, Total bacterial count; TFC, total fungal count; LC, A brand of fruit juice; ND, not detectable.

**Table 3a.** pH and total titratable acidity determination of the fruit juice LL.

Brand/sample	pH	TTA (ml)
LL1	3.80	0.07
LL2	3.81	0.13
LL3	3.79	0.08
LL4	3.80	0.07
LL5	3.83	0.07
LL6	3.80	0.10
LL7	3.78	0.08
LL8	3.78	0.11
LL9	3.79	0.13
LL10	3.80	0.13
Mean	3.80	0.10

LL, A brand of fruit juice; pH, hydrogen ion concentration; TTA, total titratable acidity

**Table 2c.** Total bacterial and coliform counts (cfu/ml) and fungal counts (sfu/ml) of the fruit juice LB.

Brands/sample	TBC	TFC	TCC
LB1	20	3	1
LB2	ND	5	ND
LB3	ND	1	2
LB4	ND	4	1
LB5	1	4	ND
LB6	ND	1	ND
LB7	ND	3	2
LB8	ND	ND	ND
LB9	12	ND	1
LB10	2	1	ND
Mean	3.5	2.2	0.7

TBL, Total bacterial counts; TFC, total fungal counts; LB, a brand of fruit juice; ND, not detectable.

**Table 3b.** pH and total titratable acidity determination of the fruit juice LC.

Brand/sample	pH	TTA (ml)
LC1	2.40	0.16
LC2	2.42	0.14
LC3	2.41	0.16
LC4	2.42	0.21
LC5	2.43	0.16
LC6	2.42	0.17
LC7	2.40	0.17
LC8	2.43	0.16
LC9	2.41	0.16
LC10	2.42	0.17
Mean	2.42	0.17

LC, A brand of fruit juice; pH, hydrogen ion concentration; TTA, total titratable acidity.

from LL recorded the highest mean total bacterial count of 7.8 cfu/ml followed by LC (4.3 cfu/ml), while the least mean value of 3.5 cfu/ml was recorded for LB. The mean total fungal counts for the samples from LL, LC and LB were 6.1, 3.8 and 2.2 sfu/ml, respectively. The samples from LC and LC indicated the presence of coliform, while LL had no growth after 24 h of incubation. The values obtained in this study for total bacterial and fungal counts fall below the range of  $10^2$  to  $10^5$  cfu/ml reported for microbial populations in fruit juices (Hatcher et al., 1992). However, the presence of bacteria and fungi could be an indication of spoilage. Also, low or high bacterial and fungal counts may be indicative of improper hygiene and may perhaps be a result of poor quality fruit being used

(Lateef et al., 2004).

According to the microbiological guidelines as stated by Hatcher et al. (1992), still and carbonated beverages must be free of coliforms. The presence of coliform is not only an indication of poor hygienic quality of this juices but also places consumers at high risk of contacting food borne infections. The mean pH and titratable acidity (TTA) are shown in Table 3a, b and c. The results of pH determination showed that the samples from all the brands were acidic in nature. Samples collected from brand LB had the lowest mean pH of 2.40, while the highest mean pH of 3.80 was observed with samples from LL. The acidic nature of juices probably favours the growth of yeasts. In this study, a strain of

**Table 3c.** pH and total titratable acidity determination of the fruit juice LB.

Brand/samples	pH	TTA (ml)
LB1	2.40	0.10
LB2	2.39	0.16
LB3	2.39	0.13
LB4	2.39	0.10
LB5	2.41	0.12
LB6	2.40	0.16
LB7	2.41	0.10
LB8	2.40	0.13
LB9	2.40	0.13
LB10	2.41	0.10
Mean	2.40	0.12

LB = A brand of fruit juice, pH = Hydrogen ion concentration, TTA = Total titratable acidity.

**Table 4.** Percentage occurrence of bacterial and fungal isolates from the samples.

Bacterial isolates	% occurrence	Fungal isolates	% occurrence
<i>Bacillus</i> sp.	46.67	<i>Aspergillus niger</i>	40.00
<i>Proteus</i> sp.	40.00	<i>A. nidulans</i>	23.33
<i>Lactobacillus</i> sp.	36.67	<i>Schizosaccharomyces pombe</i>	33.33
<i>Micrococcus</i> sp.	36.67	<i>Cladosporium</i> sp.	43.33
<i>Escherichia coli</i>	36.67		

*Schizosaccharomyces* was isolated from the fruit juices. The isolation of these yeasts from fruit juice has been previously reported (Deak and Beuchat, 1993; Lateef et al., 2004). The pH of fruit juices is usually too low for the growth of pathogenic bacteria (Lateef et al., 2004; Jackson et al., 2010), but the incidence of such bacteria in the juices used in this study might not be unconnected with the nature of the juices. All the juices are ready-to-serve fruit juices and the water activity (*aw*) values are sufficiently high to allow microbial growth (Lateef et al., 2004). There was a slight variation in the TTA of the samples examined and this could be attributed to the differences in chemical constituents.

Microbial isolates and their percentage occurrence from the samples are shown in Table 4. The microbial isolates consisted of five bacteria and four fungi. The bacterial isolates were *Bacillus* sp, *Proteus* sp, *Lactobacillus* sp, *Micrococcus* sp and *Escherichia coli*, while the fungal isolates included *A. niger*, *A. nidulans*, *Schizosaccharomyces pombe* and *Cladosporium* sp. Frazier (1984) observed that the natural microflora plus contaminating microorganisms from the soil constitute the organisms found on the surface of healthy fruits. The presence of notable bacterial pathogens such as *Bacillus* sp, *Proteus* sp, *Micrococcus* sp and *E. coli* in fruit juices is considered a safety concern. *B. cereus*, an aerobic

spore former is associated with food borne illnesses (Lateef et al., 2004), while *B. subtilis* has been associated with food borne diseases. Some strains of *E. coli* synthesize heat stable enterotoxins and are responsible for diarrhoeal disease in humans and domestic animals. There is no justification for processed ready to eat food being contaminated with these organisms, and their presence even in small numbers results in such foods being of unacceptable quality or potentially hazardous (PHLS, 2000). The processing units of the juices are likely primary causes of bacterial and fungal load. The maintenance of proper hygienic conditions and use of good quality fruit and water will certainly improve the microbiological quality of these juices, and make them acceptable to quality conscious markets both locally and abroad. In this line, the establishment of quality control unit/laboratory becomes imperative to detect contamination of either the raw materials or the products early enough.

In addition, operators in these sectors should utilize the technical assistance of NAFDAC towards attaining acceptable quality standard.

## Conclusion

The study indicates that all brands of fruit juices exa-

mined are contaminated which might be due to poor unhygienic conditions related to washing of utensils, use of contaminated water and ice, poor personal and domestic hygiene. The practice of consuming fruit and vegetable juices cannot be stopped on unhygienic grounds or prohibited from selling such items, since it is a source of their livelihood. However, government health agencies must adopt measures to educate the producers on food safety and hygienic practices. Regular monitoring of the quality of fruit juices for human consumption must also be enforced.

## REFERENCES

- Ainsworth GC, Sparrow FK, Sussman AS (1973). *The Fungi* Vol.1 VA:A Taxonomic Review with keys; Ascomycetes and Fungi imperfecti, London: Academic Press. pp. 13-67.
- Akharayi FC, Omoya FO (2005). Physicochemical determination and sensory evaluation of wine produced from selected tropical fruits. *Biosci. Biotechnol. Res.* 3:21-28.
- Buchanan RE, Gibbons NE (1974). *Bergey's Manual of Determinative Bacteriology*, Baltimore. Williams and Wilkins Co. 8<sup>th</sup> edn. pp. 34-89.
- Chang S, Kang D (2004). *Alicyclobacillus* spp. in the fruit juice Industry: History, Characteristics and Current Isolation/Detection Procedures. *Crit. Rev. Microbiol.* 30: 55-74.
- Cheesbrough M (2002). *Biochemical Tests to Identify Bacteria*. In: *Laboratory Practice in Tropical Countries*, Cheesbrough M (eds). Cambridge edn. pp. 63-70.
- Deak T, Beuchat LR (1993). Yeasts associated with fruit juice concentrates. *J. Food Prot.* 56:777-782.
- Frazier WC (1984). Wine and wine yeasts. In *Food Microbiology* 2<sup>nd</sup> Edition, Mc Grawhill New York. pp. 418.
- Gilbert RJ, de Louvois J, Donovan T, Little C, Nye K, Ribeiro CD, Richards J, Roberts D, Bolton FJ (2000). Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. *PHLS Advisory Committee for Food and Dairy Products. Commun. Dis. Public Health* 3(3):163-167.
- Hatcher WS, Weihe JL, Splittstoesser DF, Hill EC, Parish ME (1992). Fruit Beverages. In: *Compendium of methods for the microbiological examination of foods*. Vanderzant C, Splittstoesser D.F (eds). American Public Health Association, Washington, DC.
- Jackson C, Ibezim E, Akeem A, Udofia M, Odo H (2010). Determination of bioload of commercially available brands of fruit juices in Uyo, Nigeria. *Res. Pharm. Biotechnol.* 2(4): 51-53.
- Jay JM (1998). *Intrinsic and Extrinsic Parameters of Foods that affect Microbial Growth*. In: *Modern Food Microbiology*, 5th ed. Chapman and Hah., New York. pp. 354-355.
- Lateef A (2004). The Microbiology of a Pharmaceutical effluent and its Public health implications. *World J. Microbiol. Biotechnol.* 20: 167-171.
- Lateef A, Oloke JK, Gueguim Kana EB (2004). Antimicrobial resistance of bacterial strains isolated from orange juice products. *Afr. J. Biotechnol.* 3(6): 334-338.
- Lateef A, Oloke JK, Gueguim Kana EB (2005). The Prevalence of bacterial resistance in clinical, food, water and some environmental samples in Southwest Nigeria. *Environ. Monit. Assess.* 100: 59-69.
- Mislivec PB, Beuchat LR, Cousin MA (1992). Yeasts and molds In: *Compendium of methods for the microbiological examination of foods*. Vanderzant C, Splittstoesser DF (eds). American Public Health Association, Washington, DC.
- PHLS Advisory Committee for Food and Dairy products (2000). Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. *Comm. Disease and Public Health.* 3:163-167.
- Splittstoesser DF, Churey JJ, Lee CY (1994). Growth characteristics of aciduric spore-forming bacilli isolated from fruit juices. *J. Food Prot.* 57: 1080-1083.
- Titarmare A, Dabholka P, Godbole S (2009). Bacteriological Analysis of Street Vended Fresh Fruit and Vegetable Juices in Nagpur City, India. *Internet J. Food Safe.* (11):1-3.
- Walls I, Chuyate R (2000). Isolation of *Alicyclobacillus acidoterrestris* from fruits juices. *J. AOAC Int.* 83: 1115-1120.
- Yeh JY, Ellis H, Chen J (2004). Influence of calcium lactate on the fate of spoilage and pathogenic microorganisms in orange juice. *J. Food Prot.* 67:1429-1433.