International Journal of Biological Research, 4 (1) (2016) 21-24

International Journal of Biological Research

Website: www.sciencepubco.com/index.php/IJBR
doi: 10.14419/ijbr.v4i1.5798
Research paper

Microbiological and physicochemical properties of commercial seal tampered refrigerated fruit juices

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Abstract

Microbial and physicochemical properties of seven branded, seal tampered refrigerated fruit juices were carried out in this study using standard methods. Coliform counts ranged from 2.079 to3.093 $\log_{10}$cfu/ml over the storage period with pineapple juice and citrus juice having the highest and least coliform count respectively. Total bacteria count in the juice ranged from 7.009 to 8.243 $\log_{10}$cfu/ml. Citrus fruit juice however had the highest staphylococcal count while pineapple juice had the least (2.344 to 3.881$\log_{10}$cfu/ml). Also, osmophilic yeast count ranged from 2.017 to 3.903$\log_{10}$cfu/ml, having the highest load in orange fruit juice and lowest load in citrus fruit juice. The pH of the juice samples ranged from 2.9 to 4.2 during the period of refrigeration. Conductivity was highest in apple fruit juice and lowest in orange nectar pulp fruit juice. The total dissolved solids ranged from 0.29 to 1.95 over storage and was recorded highest in apple juice and lowest in orange nectar pulp fruit juice. Turbidity ranged from 5.8-200. These results indicate a reduction in the quality of fruit juices after 5 days of opening and thus reveals that both spoilage and pathogenic organisms could proliferate in juices despite refrigeration.

Keywords: *Fruit Juices; Coliforms; Yeasts; Bacteria; Staphylococci.*

1. Introduction

Over the years, humans and many animals have become dependent on fruits as a source of food (Lewis, 2002). Fruits can be classified as either juicy fruits such as lemon, orange, lime, tangerine and pomelo or pulpy fruits such as mango, pineapple, avocado, pear, guava, pawpaw, sour soup and banana (Abalaka, *et al*., 2013). Fruits are generally high in fiber, water, vitamin C and sugars, which varies widely from traces in lime to 61% of the fresh weight of the date fruit (Hulme, 1971). However, due to the high perishability of fruits, it is essential to process it to a form that can be stored for longer period hence the idea of making fruit juices (Ndife *et al*., 2013).

Fruit juice is a non-fermented and non-sparkling fruit or vegetable beverage, obtained by the dilution in potable water of the juice, pulp or vegetable extract of the fruit of origin, with or without sugar (Piló *et al*., 2009). Juices can be obtained either by mechanical extraction processes or by reconstitution of concentrated fruit juice with clean water. Fruit juices are becoming a vital part of the modern diet in many communities Ghenghesh *et al*. (2005), accounting for more than 90% of the total fruit production in Nigeria (Odu and Adeniji, 2013). While homemade juices are usually consumed immediately, large scale commercially made juices are intended to stay for a period of time and are usually concentrated or preserved so as to stay consumable for a period of time. The low pH and high sugar content often is inhibitory to most organisms and more importantly pathogenic, toxigenic organisms including spore-formers like *Clostridium botulinum* (Olaniyi, 2013).

The microorganisms present in fruit juice often originate from the natural flora of the raw fruits used for the preparation and those introduced during the course of the processing (Splittstoesser *et al*., 1994; Yeh *et al*., 2004; Olaniyi, 2013). There have been several reports of incidences of food borne illnesses associated with the consumption of fruit juices (Sandeep *et al*., 2001; Ahmed *et al*., 2009; Sharma, 2013; Olorunjuwon *et al*., 2014) with the commonly implicated etiological agents being *E. coli* O157: H7 (Frank *et al*., 2004), and *Salmonella* (Cook *et al*. 1998; CDC, 1999).

Most of these outbreaks were however as a result of consumption of unpasteurized juices but an outbreak of botulism was recorded in pasteurized carrot juice consumption in USA in 2006 (CDC, 2006). During the heat treatment, pathogens and most non–spore–forming microorganisms are usually killed, but a heat process sufficient to destroy all the microbial spores will have a detrimental effect on the organoleptic quality of the product (Walls and Chuyata, 2000). In most cases fruit juices are prepared at home and are kept in the refrigerators or the industrial packed one are open, served and the remaining kept in the refrigerators. This study therefore aimed at determining the effect of refrigeration on microbial load and physicochemical properties of seal tampered commercially fruit juices.

2. Materials and methods

2.1. Sample collection

Seven brands of fruit juices were purchased from different supermarkets in Ado-Ekiti, Nigeria and the details on the juice pack were taken as shown in Table 1

Table 1: Brands of Juice Used for the Analysis with Their Manufacturing and Expiry Date

Name of fruit juice	Designation	Shelf Life From Date Bought
CHV apple juice	A	8 months
CHV orange and mango juice	B	8 months
DNS guava juice	C	11 months
CHE orange nectar with pulp	D	7 months
CHV pineapple	E	11months
CHV orange juice	F	7 months
FVA citrus juice	G	5 months

2.2. Microbiological analysis

The microbial quality of the juice samples were monitored for five days of storage in the refrigerator using the method described by Fawole and Oso (2001). Inoculum were plated on Nutrient agar (Oxoid), Eosin methylene blue (Oxoid), Malt-extract agar (Oxoid), and Mannitol salt agar (Oxoid) to determine the total heterotrophic bacteria, total coliform count, Osmophilic yeast count and staphylococcal load count respectively. The agar plates were incubated at 37^{o}C for 24h for bacteria and at 26^{o}C for 72hrs for fungi.

2.3. Physicochemical analysis

The pH, conductivity (EC), total dissolved solids (TDS), and the turbidity of the samples were evaluated every 24hours for a 5 day period (AOAC. 2005). The pH and TDS were measured using HANNA pH/EC/TDS meter H19813-0 while the turbidity was measured by turbidometer TBN 80120-1(Shanghai China Instrument and meter Limited).

3. Results

Table 2 shows the coliform load of stored commercial fruit juice samples under refrigeration conditions for 5 days. The coliform load value reduced in sample A from day 1 till day 3 but increased from day 4. Samples B and G coliform values increased from day 1 till day 2 but in sample B, it declined on day 3 till day 4 before increasing on day 5 while sample G values were erratic, increasing and declining again while in samples B and G, it peaked on day 2. Samples C and F's coliform loads was highest on days 3 and 4 respectively. The coliform count (log_{10} CFU/mL) ranged from 1.023 to 3.049 all across the samples (Table 2).

Table 2: Coliform Load (Log_{10} CFU/ml) of Opened and Refrigerated Commercial Fruit Juice Samples

Samples	Days of refrigeration after opening				
	1	2	3	4	5
A	3.000	2.944	2.778	2.857	2.881
B	2.079	3.049	2.903	2.778	2.944
C	2.833	2.806	2.903	2.881	2.857
D	3.033	2.857	2.944	2.924	2.903
E	3.093	2.778	2.903	2.778	2.903
F	2.857	2.505	2.000	2.903	2.857
G	2.602	3.017	2.778	2.944	2.748

The osmophilic yeast count revealed an increase in count in sample A from day 1 till day 5 except on day 4 where there was a decline. Samples B and E increased in count on day 2 but decreased on day 3 only to increase on day 4 with subsequent decrease on day 5. Also, sample C's count was erratic decreasing on days 2 and 4 but increased on days 3 and 5 respectively. The count in samples D, F and G were similar in pattern, reducing in value from day 1 till day 3 while sample D's count increased from day 4 but samples F and G increased on day 5 of sampling (Table 3).

The staphylococcal count of the commercially sold fruit juice samples revealed that the count of sample A increased from day1 to day 4 before declining on day 5. Sample D had high counts on days 1 and 2 but declined afterwards. This was also noticed in sample E. sample B and F had the highest counts on day 2 and the least counts on day 3 while sample C and G had their lowest counts on day 2 and highest counts on days 1 and 4 respectively (Table 4).

Table 3: Osmophilic Yeast Count (Log_{10} CFU/ml) of Opened and Refrigerated Commercial Fruit Juice Samples

Sample	Days of refrigeration after opening				
	1	2	3	4	5
A	2.017	3.009	3.107	3.017	3.903
B	2.944	3.000	2.93	2.944	2.881
C	2.982	2.064	2.857	2.832	2.925
D	3.049	3.017	2.748	2.806	2.881
E	3.000	3.924	2.602	2.903	2.778
F	3.064	3.017	2.924	2.778	2.857
G	3.000	2.945	2.881	2.806	2.881

Table 4: Staphylococcal Load (Log_{10} CFU/ml) of Opened and Refrigerated Commercial Fruit Juice Samples

Sample	Days of refrigeration after opening				
	1	2	3	4	5
A	2.602	3.079	3.681	3.881	3.778
B	3.079	3.176	2.602	2.778	2.681
C	2.681	2.301	2.681	2.944	2.681
D	3.881	3.903	2.748	2.832	2.778
E	3.505	3.380	2.857	2.806	2.505
F	3.301	3.602	2.344	2.602	2.602
G	3.602	2.447	2.903	2.681	2.806

The total bacterial counts of the samples showed a relatively high bacterial load. There was marked reduction in the population observed in the initial load of samples A and C on day 2 while other samples still maintained a high population with all but sample B increasing on day 2. There was marked reduction on day 4 in sample F but it increased on day 5 while the lowest counts was observed on day 5 for sample B, day 4 for sample D, day 3 for samples E and G. Day 3 recorded the highest bacterial population in samples D and G with others showing a relatively high population in comparison with other days (Table 5).

Table 5: Total Bacterial Count (Log_{10} CFU/ml) of Opened and Refrigerated Commercial Fruit Juice Samples

Sample	Days of refrigeration after opening				
	1	2	3	4	5
A	8.017	7.199	7.217	8.103	8.000
B	8.033	8.009	8.049	8.029	7.029
C	8.049	7.025	8.207	7.017	8.103
D	8.009	8.209	7.201	7.037	7.964
E	8.093	8.093	7.207	7.944	8.182
F	8.000	8.049	8.199	6.964	8.093
G	8.079	8.243	7.009	8.049	8.017

The physicochemical properties of the fruit juices were also monitored for the five days of refrigeration. The pH across the samples ranged from 3.1 to 4.1. The pH generally dropped in the samples on day 2 except in sample C where there was an increase steadily till day 3 before it declined. Samples E and F also had a drop in pH till day 3 before increasing on day 4. The pH of other samples A, B, D and G dropped on day 2 with intermittent increase and decrease in values till day 5. There was also intermittent fluctuations in the values of the other parameters (conductivity, Total dissolved solids and turbidity) with the patterns similar to what was observed in the pH readings over 5 days. The turbidity markedly reduced in all samples.

Table 6: Physicochemical Analysis of Opened and Refrigerated Commercial Fruit Juice Samples

Day	Parameters	Commercial fruit juice samples A	B	C	D	E	F	G
	pH	3.6	3.5	3.5	3.2	3.7	4.1	3.8
	Conductivity	2.5	0.67	2.12	0.91	1.55	1.80	1.19
1	Total dissolved solids (g/l)	1.86	0.43	1.56	0.65	1.22	1.32	0.59
	Turbidity NTU (1:10)	176.9	45.0	102.0	55.0	19.6	104.2	194.1
	pH	3.3	3.0	3.8	2.9	3.5	3.7	3.1
	Conductivity	2.03	0.64	2.21	0.91	1.41	1.85	1.04
2	Total dissolved solids(g/l)	1.48	0.42	1.63	0.65	1.02	1.35	0.74
	Turbidity NTU (1:10)	90.6	13.0	57.6	30.0	12.4	98.3	44.1
	pHp pH	3.4	3.2	`4.2	2.8	3.3	3.2	3.5
3	Conductivity	2.24	0.77	0.40	1.09	1.49	2.01	1.20
	Total dissolved solids(g/l)	1.66	0.55	0.29	0.78	1.09	1.45	0.86
	Turbidity NTU (1:10)	120.1	5.8	200	40	22.2	85.2	128.7
	pH	4.1	3.8	3.0	3.7	4.1	3.5	3.8
	Conductivity	2.04	1.45	0.61	1.35	1.84	1.43	2.20
4	Total dissolved solids(g/l)	1.74	1.05	0.43	0.97	1.35	1.03	1.62
	Turbidity NTU (1:10)	140.0	12.3	107.5	49.3	93.4	20.5	63.6
	pH	3.7	3.6	3.1	3.5	3.7	3.6	3.5
	Conductivity	2.60	1.48	0.62	1.30	1.30	1.47	2.09
5	Total dissolved solids(g/l)	1.95	1.069	0.438	0.938	1.28	1.06	1.52
	Turbidity NTU (1:10)	105	13.2	129.0	73.7	145.2	19.9	157.7

Keys: A-CHV Apple juice, B- CHV Pineapple juice, C-CHE Orange nectar pulp juice, D-DNS Guava juice, E-CHV Orange juice, F-FVA Citrus juice, G-CHV Mango and orange juice.

4. Discussion

In spite of the potential benefits offered by fruit juices, concerns over their safety and quality have been raised (Jackson *et al*., 2010). The processing units serve as potential source of bacterial and fungal contamination of fruit juices (Lateef and Yusuf, 2002). In this study we found out that coliform count of the samples analysed were relatively high ranging from 124 CFU/ml in Pineapple juice to 1240 CFU/ml in orange juice as shown in Table 2. Safe Food Consumption Standard prohibit coliforms in fruit juice (Andres *et al*., 2004), hence the juices were unsafe for consumption. Also, it is well above the regulatory specification of <3 CFU/ml for total coliform counts in fruit juices (SON, 2008). The presence of coliform 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, Oranusi *et al*., 2012).

Table 3 showed that the osmophilic yeast count ranged from 60 CFU/ml in citrus juice to 1280 CFU/ml in apple fruit juice. 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 (Braide *et al*., 2012), though the maximum acceptable level is 10^6 CFU/ml. It can then be deduced that the values recorded in this research exceeds that of the GMO standard as the juices were meant to have been pasteurized. Water and environment may play a major role in the fungi contamination of pineapple especially during washing of fruits (Abalaka *et al*., 2013). The presence of yeasts is expected due to its preference for sugar and low pH, which highly favor yeast proliferation (Adams and Moss, 1995). High values observed in apple juices can reduce the shelf life the juice Sutherland *et al*. (1995) and may result in off flavors in the juice when consumed.

The results shown in Table 4 revealed the staphylococcal load of the fruit juices analysed. The load ranged from 120 to 8800 cfu/ml. Coagulase positive staphylococci may cause human diseases through the production of toxins. Effective levels of toxin formation however require a large number of microorganisms (approximately 10^5 to 10^6 CFU/mL of food) (IDF, 1994) hence, the risk of intoxication from consumption of these juices is highly unlikely. The presence of *Staphylococcus* spp. in all the juice samples can is an indication to contamination during handling which reflects poor personal and domestic hygiene as well as lack of knowledge of hygienic practices and safety of food products (Tambekar *et al*., 2009; Bello *et al*., 2013).

The total bacterial count in all juice samples were also considerably high, ranging from 9.2×10^6cfu/ml to 17.5×10^7 CFU/ml and this exceeded the maximum limit in the SON (2008) specification for commercial fruit juices (<2.0 x 10^2 CFU/100 ml) (Table 5). It was also discovered that the bacterial count peaked on the third day of storage in all the samples except sample D and G which peaked on the 2nd day. Most fruit contains bacterial counts of 1×10^5 CFU/cm on their surface (Al-Jedah *et al*., 2002; Durgesh *et al*., 2008, Odu and Adeniji, 2013) hence; improper washing of fruits adds these bacteria to juices leading to contamination (Durgesh *et al*., 2008). The high count recorded is indicative of poor production and processing conditions Ezeama, (2007) and poses risk to consumers' health which should not be taken lightly (Dietary Guideline for Americans, 2005).

Table 6 shows the results of the physicochemical analysis of the fruit juice samples. The pH range from 2.9 to 4.2 indicating an acidic condition that promotes yeast growth and some bacteria species (Deak and Beuchat, 1993). It agrees with the pH range observed by Ndife *et al*. (2013) who recorded a pH range of 3.23 to 4.08. A report by Kareem and Adebowale, (2007) showed that the main acid in orange juice is citric acid. Food acids determine the prominent microflora in foods and to a large extent, the shelf stability of the juice (Ezeama, 2007). Acidic fruit juice is more yeasts and molds than mould (Jay, 2000). The turbidity ranged from 5.8 to 200 NTU during storage which is indicative of potential microbial activity. Statistical analysis of the results using univariate analysis of variance at 0.05% confidence limit showed significant difference between the samples.

5. Conclusion

The occurrence of higher than accepted limit of microbial contamination of fruit juice is alarming and reflects poor good manufacturing practices. Awareness program regarding the maintenance of hygiene and sanitation during processing should be effected. Quality control should be ensured also to forestall disease outbreak as a result of fruit juice consumption.

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