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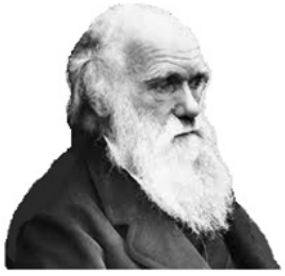

World News of Natural Sciences

WNOFNS 14 (2017) 97-105 EISSN 2543-5426

Physicochemical and microbiological analysis of canned and bottled fruit juices sold in Owerri Metropolis

Ihenetu Stanley Chukwuemeka[1,*] and **Ihenetu Francis Chukwuebuka**[2]

[1]Imo State University, Owerri, Nigeria

[2]Federal University of Technology, Owerri, Nigeria

*E-mail Addresses: Ihenetustanley@yahoo.com , Astancos41@gmail.com

ABSTRACT

This study was designed to investigate the physiochemical and microbiological analysis of canned and bottled fruit juices sold in Owerri metropolis. The samples were subjected to standard microbiological analysis and physiochemical parameters. The titratable acidity of the fruit juice samples ranges from 0.15% to 0.31%, the ash content of all the samples ranges from 0.32% to 0.63%. The total solid of all the samples ranges from 4.10% to 12.25%. The moisture content of the samples ranges from 2.32% to 4.81% and the pH ranges from 3.0 to 4.01. The total bacteria count (CFU/ml) of all the fruit juice samples ranges from 1.1×10^2 to 4.1×10^2. The total fungi count (CFU/ml) ranges from 0.0×10^2 to 1.2×10^2. Bacteria isolates from the samples includes Bacillus sp. (34.7%), followed by *Enterococcus sp*. (17.3%), *Aspergillus sp.* (21.7%) and *Lactobacillus sp.* (26.0%). Fungi isolates were identified as *Penicillum sp*. and *Saccharomyces sp*. The Standard Organization of Nigeria and NAFDAC should define more specifically the quality control standards of locally manufactured commercial juices, stating clearly the minimum and tolerable numbers and types of microorganisms.

***Keywords*:** canned fruit juices, bottled fruit juices, physiochemical analysis, microbiological analysis

1. INTRODUCTION

Juice is a liquid naturally contained in fruit or vegetable tissue. Juice is prepared by mechanically squeezing or macerating fresh fruits or vegetables without the application of

heat or solvent [1]. For example, orange juice is the liquid extract of the fruit of the orange tree. Juice may be prepared in the home from fresh fruits and vegetables using variety of hand or electric juicers [2,4]. Juice may be 0market in concentrate form, sometime frozen, requiring the user to add water to constitute the liquid back to its original state. However, concentrates generally have a noticeable different taste than their comparable "fresh squeezed" versions [5]. Other juices are reconstituted before packaging for retail sale. Fruit juice consists of 100% pure juices and generally has no added ingredients [6,7].

Fruit Juice

According to Turkish Food Codex, fruit juice is described as an unfermented but fermentable product obtained from fresh, ripe and healthy fruits [8]. It can be produced using a single type of fruit or mixed fruits. The juice has the characteristics of the fruit which it is made [9]. The major component of the fruit juice is water. The other most common constituent is carbohydrates which comprise sucrose, fructose, glucose and sorbitol. Also, limited amount of protein and minerals 5 Especially citrus fruits and juices are good sources of ascorbic acid, folic acid, vitamin B1, thiamine and potassium [10-12].

2. MATERIALS AND METHODS

MICROBIAL ANALYSIS

2. 1. Study Area

The study was carried out in Owerri, the capital of Imo State. Owerri is the capital of Imo state Nigeria, set in the heart of Igbo land. Owerri consist of three local government area including Owerri municipal, Owerri north and Owerri west, it has an estimated population of about 750,000 as of 2006 census and is approximately 100 square kilometers in area [13].

2. 2. Sample Collection

Six (6) samples of canned and bottled orange, apple, and pineapple fruit juices used in this study were purchased from different locations in Owerri. Some of them were bought from street hawkers, Ekeonunwa market and Imo State University campus. The samples consisting of two (2) orange canned and bottled juices labeled A1 and A2, two (2) apple canned and bottled juices labeled B1 and B2 and two (2) pineapple canned and bottled juices labeled C1 and C2, were analyzed within four hours of purchases from the different sources.

2. 3. Sample Analysis (Serial Dilution / Culture)

Serial Dilution method (spread plate/pour plate technology)

Procedure:

• Test tubes containing 9ml peptone water each were labeled A-F

• Using the sterile peptone water and separate sterile pipettes or string, serial dilution of the samples each were prepared as follows

A. 1g of the mashed sample were introduced into 9ml sterile Peptone Water and homogenized = 10^1 dilution.

B. 1ml of A into 9ml sterile Peptone Water of B and homogenized = 10^2 dilution

C. 1ml of B into 9ml sterile Peptone Water of C and homogenized = 10^3 dilution

D. 1ml of C into 9ml sterile Peptone Water of D and homogenized = 10^5 dilution

E. 1ml of D into 9ml sterile Peptone Water of E and homogenized = 10^6 dilution

F. 1ml of E into 9ml sterile Peptone Water of F and homogenized = 10^6 dilution [14].

After the serial dilution, 0.5ml of the serially diluted samples each were inoculated into freshly prepared Nutrient agar (for Total Heterotrophic Plate Count), MacConkey agar (for Coliform count) and *salmonella shegella* agar for salmonella spp. respectively using a sterile syringe or pipette and spread plate method were adopted. Nutrient Agar and MacConkey agar culture plates were incubated at 37 °C for 24hours while SDA were incubated at 37 °C for 3-4 days. The Nutrient Agar and MacConkey Agar plate were checked for growth after 24hours of incubation. They were counted and the discreet colonies were sub-cultured into a fresh prepared Nutrient Agar plate to get a pure culture. The Sub-cultured plate was incubated for 24 hours and was checked for pure culture. The pure culture growth were used for Gram staining and for Biochemical characterization of the organism which include Urease test, Citrate utilization test, Indole test, Motility test, Methyl-red test, Coagulase test, Sugar fermentation test and Catalase test. A stock culture was prepared using a vigeou bottle: this stock culture was used in storing the organism for further biochemical characterization if need be. The SDA plate were checked for growth after 3-4 days; counted and were identified by its colonial and morphological characteristics.

2. 4. Bacterial identification

The MacConkey, Blood agar, Nutrient Agar, Salmonella-Shigella agar (SSA) plates were examined for bacterial growth. Growth characteristics and other colonial morphology such as lactose fermentation, formation of mucoid colonies of the bacteria were carefully recorded. Less than five identical colonies for a particular organism growing on a plate were ignored [15].

2. 5. Fungi Identification

The pure isolated fungi were identified using cultural and morphological features according to the most documented keys in fungal identification [16].

PHYSICO-CHEMICAL ANALYSIS

Physiochemical analysis was carried out using the methods of Association of Official Analytical Chemists (A.O.A.C.) and [17].

2. 6. pH

The pH meter was first standardized in buffer solution of 4, 14, and 7. Aliquot of 5 ml each of the juice sample was used to determine the pH using pH meter at 20 °C.

2. 7. Titratable acidity

Aliquot of 5 ml of each sample was titrated with 0.1M of sodium hydroxide (NaOH) using phenolphthalein as the indicator. The percentage acidity as citric acid was determined as described in the Association of Official Analytical Chemists. The percentage titratable acidity was calculated using the relation below:

$$\text{Total titratable (\% citric acid)} = \frac{\text{Titre x 0.1m NaOH}}{\text{Volume of juice used}} \times 100$$

2. 8. Ash content

The ash content is the inorganic matter or constituents contained in fruit juices. It is the soft grey powder that remains after the dried solids from orange juice has been ignited at 550 °C in a muffle furnace for several hours. Ash content serves as a measure of the inorganic salts that were present the original material.

2. 9. Total solids

Ten milliliters of each of sample was weighed into a crucible and dried at 70 °C for 2 hours in a vacuum oven. The calculation of the total solids was done as follows:

$$\frac{W_3 - W_1}{W_2 - W_1} \times 100$$

where
W1 = weight of crucible
W2 = weight of sample + crucible before drying
W3 = weight of sample + crucible after drying

2. 10. Moisture content

Aliquot of 10 ml of each sample was added into a silica dish. This was immersed in a water bath until the water was completely evaporated. The dried solids in the silica dish is then removed from the water bath and dried at 70 °C in a vacuum oven for 3 hours and was then placed in the desiccator for 10 minutes for cooling. The content of the dish was weighed and the moisture content calculated as follows.

$$\text{\% moisture content} = \frac{\text{weight of sample after drying}}{\text{weight of sample before drying}} \times 100$$

3. RESULT

3. 1. Physiochemical characteristics

The results of physiochemical measurements were shown in Table 1.

Table 1. Results of Physical Measurements

Fruit samples	pH	T.A (%)	A.C (%)	T.S (%)	M.C (%)
A1	3.22	0.30	0.32	8.12	2.32
A2	3.00	0.32	0.39	5.45	3.62
B1	3.04	0.29	0.45	5.22	4.52
B2	3.25	0.31	0.63	4.10	4.12
C1	4.01	0.18	0.42	12.25	4.15
C2	3.85	0.15	0.37	8.68	4.81

KEY:
pH = Hydrogen ion concentration, T.A = Titratable acidity, A.C = Ash content, T.S = Total solid, M.C = Moisture content, A1 = canned orange juice, A2 = bottled orange juice, B1 = canned apple juice, B2 = bottled orange juice, C1= canned pineapple juice, C2 = bottled pineapple juice.

3. 2. Total Heterotrophic Bacterial Count and Total fungi count (CFU/ml)

Total bacteria count and total heterotrophic bacteria count of some fruit juice samples are shown in Table 2.

Table 2. Total bacteria count (CFU/ml) of some fruit juice samples

Fruit juice samples	Total bacteria count (CFU/ml)	Total fungi count (CFU/ml)
A1	1.1×10^2	0.3×10^2
A2	2.1×10^2	0.9×10^2
B1	3.2×10^2	1.0×10^2
B2	2.4×10^2	1.1×10^2
C1	4.1×10^2	0.0×10^2
C2	2.0×10^2	1.2×10^2

Key:
A1 = canned orange juice, A2 = bottled orange juice, B1 = canned apple juice, B2 = bottled orange juice, C1= canned pineapple juice, C2 = bottled pineapple juice.

3. 3. Frequency of occurrence of bacterial isolates

The frequency of occurrences of bacteria isolates obtained from some of canned and bottled fruit juices are shown in Fig. 1. *Bacillus* sp. (34.7%) was most predominant. This was followed by *Enterococcus* sp. (17.3%). *Aspergillus* sp. (21.7%) and *Lactobacillus* sp. (26.0%) were least predominant.

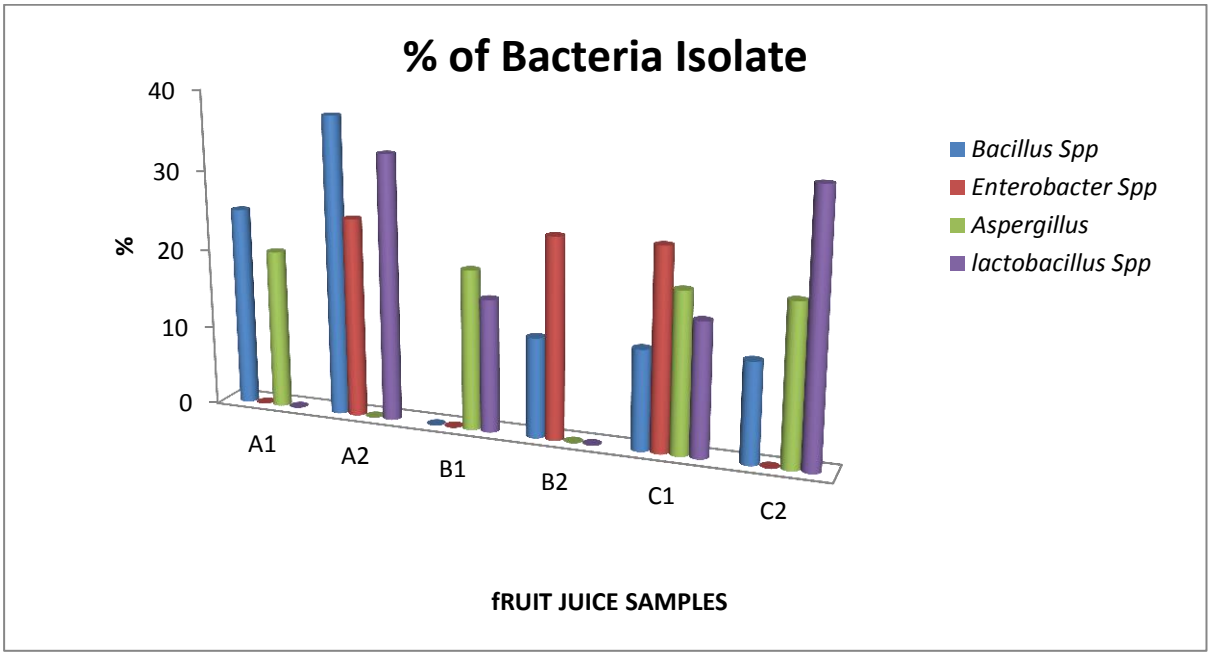

Fig. 1. Bar chart showing Frequency of occurrence of bacterial isolates

3. 4. Frequency of occurrence of fungi isolates

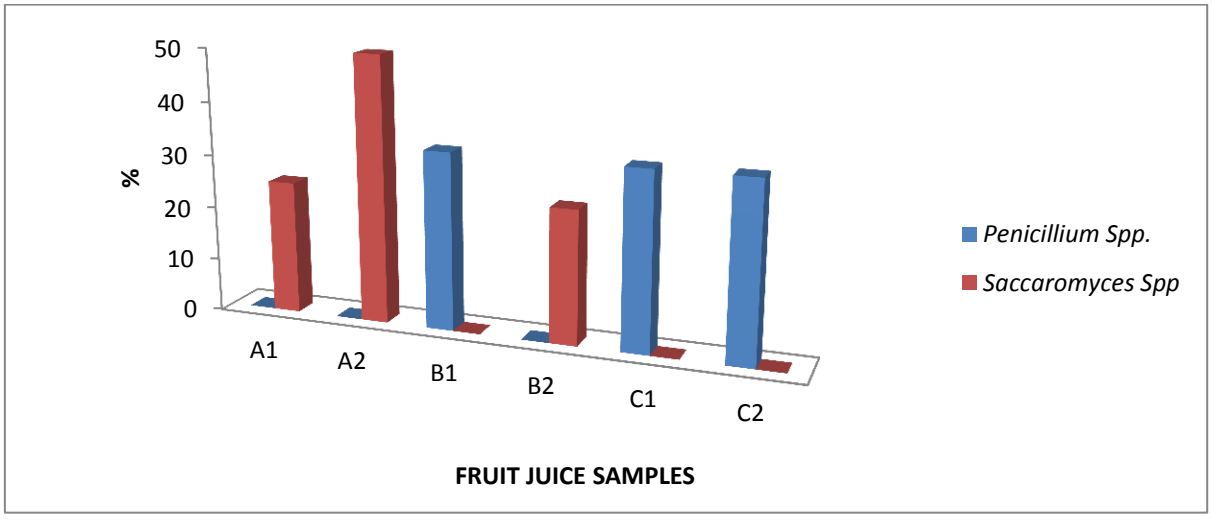

Fig. 2. Percentage frequency of occurrence of fungi isolates

Based on the cultural and morphological features of most documented keys in fungal identification, fungi isolates were identified as *Penicillum* sp. and *Saccharomyces* sp. Fig. 2 shows the frequency of occurrences of fungi isolates obtained from the canned and bottled fruit juice.

4. DISCUSSION

The results of microbiological and physico-chemical evaluation of the canned and bottled fruit juice are presented in tables 1and 2 also presented in fig 1and 2. Many microorganisms are found in fruit juice and soft drinks as environmental or raw material contaminations either during their growing in fields, orchards, vineyards or greenhouse or during harvesting, post-harvest handling and distribution. But relatively few can grow within the acidic and low oxygen environment, yeast are the most significant group of microorganisms associated with spoilage of fruit juice and soft drinks. According to WHO (2003), a food is deemed to be adultered if its content is composed in whole or in part of any poisonous or deleterious substance, which renders its contents injuries to health. The result obtained from table 1 show that the pH of 3.00 to 4.01, it was recorded that the pH of pineapple canned juice had the highest pH measurement which might be as a result of the fruit. The titratable acidity of the fruit juice samples ranges from 0.15% to 0.31%, the ash content of all the samples ranges from 0.32% to 0.63%. The total solid of all the samples ranges from 4.10% to 12.25%. The moisture content of the samples ranges from 2.32% to 4.81%.

Table 2 shows the total bacteria count and total heterotrophic bacteria count of all the fruit juice samples analyzed. The total bacteria count (CFU/ml) of all the fruit juice samples ranges from 1.1×10^2 to 4.1×10^2. The total fungi count (CFU/ml) ranges from 0.0×10^2 to 1.2×10^2.

From the results obtained in the present study, it was shown that the mean bacterial counts of the canned and bottled fruit juice do not exceed the maximum recommended standards by the International Commission on Microbiological Specification of Foods (ICMSF, 1978). According to this agency, the acceptable limit of bacteria in food products should not exceed a maximum of 10^3 cfu/ml. On the other hand, all the results of the fungal counts from all the canned and bottled fruit juice analyzed were within the acceptable limit.

Frequency of occurrences of bacteria isolates obtained from some canned and bottled fruit juice shows the percentage of the following bacteria, *Bacillus* sp. (34.7%) which is more predominant in all the fruit juices.

This was followed by *Enterococcus* sp. (17.3%). *Aspergillus* sp. (21.7%) and *Lactobacillus* sp. (26.0%). Based on the cultural and morphological features of most documented keys in fungal identification, fungi isolates were identified as *Penicillum* sp. and *Saccharomyces* sp. The presence of these bacteria may be due to the unhygienic environmental conditions and poor handling. *Bacillus* species are spore formers whose spores could survive high temperatures of processing [17]. The thermoduric nature of the spores of these microbes ensures survival at pasteurization temperatures [18] and hence their presence in the packaged fruit juice samples that were not subjected to heat treatment during processing.

5. CONCLUSIONS

The average counts for bacteria of the canned and bottled fruit juice samples examined are generally below the maximum allowable limit in foods to be marketed for consumption (10^3 cfu/g). With the number of isolated bacteria and fungi from the different packaged fruit juice sold in Owerri, it can be concluded that different bacterial and fungal species occur within fruits and materials used for the production of the juice as well as poor sanitation, extraction, raw material contaminations (often from insect damage), lack of both proper heat sterilization and adequate quality control during processing of fruit juice. Some of the fungal isolates especially *Penicillium* sp. have the potential to induce rot on fresh fruits which might have a remarkable effect on the value of the fruit especially in the food industry as well as on human health. The study has also shown that these packaged fruit juices are not sterile and thus can favour the growth of microorganisms when conditions become favourable, which could pose a public health risk to their consumers.

The Standard Organization of Nigeria and NAFDAC should define more specifically the quality control standards of locally manufactured commercial juices, stating clearly the minimum and tolerable numbers and types of microorganisms. Good agricultural manufacturing practices and application of hot water and chemicals sanitizers such as chlorine dioxide, ozone and per acetic acid should be used on surfaces of orange fruits prior to juice extraction in order to avoid and reduce microbial contaminants of fruit products.

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(Received 25 September 2017; accepted 10 October 2017)