MICROBIAL QUALITY AND PHYSICOCHEMICAL PROPERTIES OF SOME CARBONATED COMMERCIAL FRUIT JUICES IN IBADAN METROPOLITAN MARKET OYO STATE, NIGERIA. OLAITAN

[1]Abiodun Josiah, [2]MAKINDE, Sunday Clement, [3]OGUNMADE, Taiwo Oludare, [4]BANKOLE Isaac Sewanu, [5]AKINYEMI Abosede Atinuke, [6]OGUNLUSI, Rapheal Olusola, [7]OGUNBANWO, Cecilia Mojisola, [8]OLAJUWON Mistura Ojuolape

[1,4]Department of Science and Technology Education, Lagos State University Ojo Lagos, Nigeria.
[2]Department of Botany, Faculty of Science, Lagos State University Ojo Lagos, Nigeria.
[3,5,6,7]Department of Chemistry, Lagos State University of Education Oto/Ijanikin Lagos, Nigeria.
[8]Department of Chemistry, Louisiana State University, United State of America.

Abstract- **Three commercially produced fruit juices namely Oranges, Pineapple and Blackcurrant were purchased from open market in Ibadan Metropolis in Oyo State, Nigeria to examine their quality parameters. Physico-chemical and Microbiological examinations were carried out on these three fruit juices. The results obtained from physico-chemical analysis showed that orange juice had the highest pH value of 3.15 followed by pineapple with moderate pH value of 2.87 while the blackcurrant had the lowest pH value of 2.28. The total titratable acidity of all the samples was also determined with the blackcurrant having the highest total titratable acidity content of 0.63g/100g and followed by pineapple with 0.55g/100g while orange juice had the lowest value of 0.19g/100g. The Microbiological examination however, revealed elevated level of coliform counts. All the three samples had coliform counts ranging between 11 and 14 MPN/100ml with orange juice having the highest coliform count. Other bacteria species found in the samples include *Bacillus spp, Escherichia coli, Staphylococcus aureaus, Streptococcus spp* and *Salmonella spp, Enterobacter spp, Shigella spp, Lactobacillus planetarium, L. brevis* and *L. perulens.* Only four fungal species were isolated and identified. These were *Aspergillus niger, Rhizopus stolonifer, Saccharomyces cereviceae, Ochrocosis gallopova, A. flavin* and *A. glaucus*. The presence of coliform in all the three fruit juices showed the possibility of faecal contamination which is of serious concern from the public health point of view. It is therefore suggested that the possible source(s) of contamination need(s) need to be examined in order to protect the poor consumer from the danger of food poisoning and food borne-infection.**

Keynotes: **Physico-chemical, Microbiological examinations, juice, coliform counts, food poisoning.**

Introduction

Non-alcoholic beverages, particularly fruit drinks play a major role on the diets of people in both developed and developing countries. These beverages are regarded as after meal drinks or refreshing drinks during the dry season in rural and urban centers. Tropical fruits such as pineapples and oranges have been founding this regard to be useful as the main raw materials in the beverages (Osuntogun and Aboaba, 2004). Based on fruits antioxidant potential, they are used as indicators for healthy nourishment as well as protection factors of the human body against oxidative destruction (Costescu *et al*., 2006). Fruits have been shown to contain high amount of minerals, low ash, moisture and crude fibre (Wall, 2006) and are sources of vitamin A, B and C groups, low protein, sugar and lipid (Ogbonna *et al.*, 2013). Fruit juices are liquid, non-alcoholic products with certain degree of viscosity and clarity obtained through mashing or breaking up of fruits with or without sugar or carbon dioxide addition (Costescu *et al.*, 2006).

Orange (*Citrus cinensis*) belongs to the genus citrus of the family *Rutaceae*. It is a distinguished, widely consumed fresh fruits and particularly appreciated for its sharp-flavoured taste. Its pulp is a very good source of vitamin C providing 64% of the daily requirement of an individual (USD, 2014). Apart from vitamin C content of orange juice, it's also rich in folic acid, potassium and an excellent source of bioactive antioxidants and they are important trade commodities in most countries (Vasavada, 2003).

Pineapple (*Ananas comosus*) is an economically important plant in the *Bromelanceae* family which encompasses about 50 genera and 2000 species mostly epiphytic (De-Carvalho *et al.*, 2008). The worldwide total pineapple

production is between 16 –19 million tons (Fernandes *et al.*, 2008). Pineapple and its juice is nonalcoholic drink and the demand continues to increase mainly due to increasing awareness of its health benefits (Nwachukwu and Ezejiaku, 2014). Orange juice have an proximate composition of 81.2 – 86.2% moisture, 13 – 19% total solid of which glucose, sucrose and fructose are the main compositions, 0.4% fibre and a rich source of vitamin C. Pineapple also contains polyphenolic compounds and exhibit antioxidant activity (Hossain and Rahman , 2011). Its pulp is juicy and fleshy with the stem serving as a supporting fibrous core. It is an excellent source of antioxidant, vitamin C which is needed for the collagen synthesis in the body. Pineapple juice is widely consumed around the world, mostly as canning industry by-products and in the blend composition to obtain new flavours in beverage and other products (De-Carvalho *et al.*, 2007).

Blackcurrants are largely grown in a temperate climate in Europe, North America, Asia, Australia, and New Zealand. According to the International Blackcurrant Association, Poland is the world leader in the production of blackcurrant: more than half of the volume of the berries is collected in this country. Due to the high content of bioactive compounds, blackcurrants are a valuable raw material in the production of juices, jams, nectars, dried foods, liqueurs, and wines. Blackcurrant fruits are rich in health-promoting biologically active substances such as fibre, vitamin C, and polyphenols e.g, anthocyanins, phenolic acids, flavanols, flavonols, and proanthocyanidins Cortez *et al.*, 2019. However, the qualitative and quantitative composition of blackcurrant bioactive compounds has been revealed to vary among cultivars and growing years (Mattila *et al.*, 2016). Furthermore, the processing operations significantly affect the final composition profile of blackcurrant products, e.g juices (Laaksonen *et al.*, 2014).

Microorganisms such as *L. monocytogenes* have been reported to be capable of surviving in raw fruit and vegetable juices (Barbosa *et al*., 2013), and *A. acidoterrestris* is increasingly being reported as a cause of spoilage of fruit juices (Oteiza *et al*., 2014). *B. cereus* and *S. aureus* have also been isolated from fruit products (Carvalho *et al*., 2007). Species of *Alicyclobacillus* are acid-tolerant and heat resistant bacteria (thermo-acidophilic bacteria, TAB) that cause spoilage of fruit juices stored at room temperature. Spores of *Alicyclobacillus species* are known to survive heat pasteurization processes applied to fruit, vegetable, and fruit/vegetable-based beverages, fruit concentrates, sugar, sugar syrups, tea, isotonic drinks (sports drinks), and other low pH products (Murray *et al.*, 2007). Some strains of the bacterium can grow at pH 2.0 and at temperatures as high as 70°C. Germination of spores, followed by vegetative cell growth can occur within a few days or be delayed well into the expected shelf life of products. Medicinal or disinfectant off odours attributable to guaiacol and halogenated phenols produced by *Alicyclobacillus* (Jensen and Whitfield, 2003) can be detected at concentrations as low as 2 ng/ml of beverage (Gocmen *et al.*, 2005).

Materials and Method

Collection of Samples

Three commercially available fruits were used for this study. Orange fruit was obtained from Sango Motor Park in Ibadan while pineapple and Blackcurrant fruits were purchased at Agbeni market in Ibadan, Oyo State, Nigeria. The fruits were then transported in a sterile polythene bag and taken directly to the Biology Department Laboratory of the Polytechnic Ibadan for processing including extraction of their juices and further for their physico-chemical analysis.

Physico-Chemical Analysis

Determination of pH and Titratable Acidity (TTA)

The pH of the fermented fruits was determined at the time of fermentation and 24-hourly up to 72hours using pH meter (Jenway 33510). Similarly total titratable acidity by titration with 0.1N sodium hydroxide solution (results expressed in grams citric acid per 100ml of sample) was determined as described by AOAC (1995).

Bacteriological Analysis of Fruit Juice Samples

The fruit samples were thoroughly washed and rinsed with clean water, each fruit was processed separately. 10g of the solid sample (orange fruit, pineapple and blackcurrant) were chopped gradually with sterile cutter and then pulverized to paste using a sterile mortar and pestle. The paste were appropriately diluted in a normal saline. Orange fruit, pineapple and blackcurrant were squeezed using a squeezer till considerable amount of liquid juice was obtained. 1ml of diluted sample was transferred into 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10} micro pipette. The 10^{-10} dilution is then inoculated into a sterile petri-dish using an auto pipette and 15ml of nutrient agar is poured and carefully homogenized. The tenth dilutions of the samples were also cultured on differential and selective media for bacteria cultivation in order to isolate bacteria entero-pathogen. The cultured plate was incubated overnight with temperature of 37^0c as carried out in the study conducted by Dubey (2005).

Identification of Mould Isolates

The mould isolates were identified by initial sub-culturing as pure cultures. Smear was made with a sterile inoculating needle with lactophenol cotton blue reagent on a clean grease free slide. A cover slip was applied and further examined under the oil immersion objectives of the light microscope at magnification X_{40}. The main characters employed in the identification of moulds are then summarized (Odetunde *et al.*, 2014).

Statistical Analysis

The results of the study were compiled for obtaining mean and standard deviation. Analysis of variance was used for determining significant differences between samples.

Result and Discussion

The study revealed that both bacteria and mould are associated with the analyzed fruit drinks (Table 1). This result corroborates the findings of Olaitan *et al*., (2021) who isolated similar bacteria and mould from fruit juices. There were 6 bacteria isolates from orange drink, 6 pineapples and 5 blackcurrants while there are 2 fungi isolated from orange drink, 6 fungi from pineapple and 2 fungi from blackcurrant drink.

From the result obtained, it was evident that pH has effect on the number and types of organisms isolated. Blackcurrant drink which had the lowest pH contained two bacterial isolates each. However, blackcurrant contained two species of *Aspergillus ssp* (Table 1). This result is in agreement with the earlier submission that moulds in preference to bacterial tend to grow in an acidic medium (Prescott *et al*., 1999). The result of analysis shows that *Lactobacillus planetarium, L. brevis, L. perulens, Streptococcus spp and Escherichia coli* are only associated with blackcurrant drinks of lower pH (Table 1) which was in agreement with the submission of (Back *et al*., 1999). The relatively low level of coliform recorded in blackcurrant and pineapple drink may be attributed to the low pH values because many members of coliform groups do not survive at low pH values (Pilo *et al.*, 2009). Table 2 showed that the pH of blackcurrant and pineapple juice was <3.0 which is acidic (Titarmare *et al.*, 2009). In another study by Pilo *et al*. (2009), orange flavored juices were reported to have pH levels ranging from 2.72 to 3.14.

These indicate that blackcurrant drink had more shelf life than other two drinks. The pH obtained for the pineapple drink is lower than the ones earlier reported. The result of analysis in the present study depicts that blackcurrant drink has the highest total titratable acidity while orange drink has the least (Table 2). This result shows that there is an inverse relationship between pH and total titratable acidity of the fruit drinks when analyzed. Table 3 shows that orange juice has the highest coliform count which ranges from 12 to 75 MPN/100ml amongst others, while pineapple juice 9.0 to 43 MPN/100ml and blackcurrant has the lowest coliform count which ranges from 6.0 to 43 MPN/100ml.

Table 1: **Microbial Analysis of some Commercial Fruit Drinks**

Fruit Drinks	Identified Organisms Bacteria Organisms	Fungi Organisms
Orange Drink	*Bacillus spp, Escherichia coli, Staphylococcus aureaus, Streptococcus spp, Salmonella spp* and *Shigella spp*	*Aspergillus niger, A. flavus*
Pineapple Drink	*Bacillus spp, Staphylococcus aureaus, Streptococcus spp, Enterobacter spp, Shigella spp* and *Lactobacillus spp*	*Campilobacter jejuni, A. niger, Rhizopus stolonifer, Saccharomyces cereviceae, Ochrocosis gallopova* and *A. flavin*
Blackcurrant Drink	*Lactobacillus planetarium, L. brevis, L. perulens, Streptococcus spp and Escherichia coli*	*A. niger* and *A. glaucus*

Table 2: pH and Titratable Acidity of some Commercial Fruit Drinks

Fruit Drinks	pH (% Citric Acid)	Total Titratable Acidity	Coliform counts (MPN/100ml)
Orange Drink	3.15 (0.05)[a]	0.19 (0.09)[a]	14 (3.00)[a]
Pineapple Drink	2.87 (0.05)[b]	0.55 (0.03)[b]	11 (2.6)[b]
Blackcurrant Drink	2.28 (0.07)[b]	0.63 (0.02)[b]	11 (2.0)[b]

Values are means, Standard deviation (n=3) in parenthesis. Means values within a column with different letters are significantly different at P<0.05.

Table 3: Coliform count of Orange Fruit Drinks

Isolate code	Positive Volume 10.0	1.0	0.1 (ml)	Coliform counts (MPN/100ml)
A	3/3	1/3	0/3	43

B	1/3	2/3	1/3	20
C	0/3	0/3	3/3	9

Isolate code	Positive Volume 10.0	1.0	0.1 (ml)	Coliform counts (MPN/100ml)
A	3/3	1/3	1/3	75
B	2/3	3/3	1/3	36
C	0/3	1/3	3/3	12

Table 4: Coliform count of Pineapple Fruit Drinks

Table 5: Coliform count of Blackcurrant Fruit Drinks

Isolate code	Positive Volume 10.0	1.0	0.1 (ml)	Coliform counts (MPN/100ml)
A	3/3	1/3	0/3	43
B	2/3	2/3	0/3	21
C	0/3	0/3	2/3	6.0

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