European Journal of Nutrition & Food Safety

13(8): 10-22, 2021; Article no.EJNFS.74572
ISSN: 2347-5641

Bacteriological and Nutritional Quality of *Irvingia gabonensis* Fruit Juice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2021/v13i830439
Editor(s):
(1) Dr. Kristina Mastanjevic, Josip Juraj Strossmayer University of Osijek, Croatia.
Reviewers:
(1) Ifedolapo Olufunke Olanrewaju, Covenant University, Nigeria.
(2) Kouassi Kouadio Benal, Université Nangui Abrogoua, Côte d'Ivoire.
Complete Peer review History, details of the editor(s), Reviewers and additional Reviewers are available here: https://www.sdiarticle5.com/review-history/74572

Original Research Article

Received 06 September 2021
Accepted 12 October 2021
Published 30 November 2021

ABSTRACT

Aims: This study was undertaken to examine the bacteriological and nutritional quality of *Irvingia gabonensis* fruit juice locally produced and stored for 28 days at 4^0C.
Study Design: Four groups of the *Irvingia* fruit samples were prepared and stored at 4^oC. The samples were analysed at different intervals, beginning from day 0, to day 28. The juice was also observed for onset of spoilage and turbidity during sampling days.
Place and Duration of Study: This study was conducted in Biological Sciences, Faculty Of Science, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State between July 2019 to September 2019.
Methodology: The microbiological analysis was done using culture dependent methods.
Results: The results revealed the weighted mean of the total heterotrophic bacterial count increased as the storage days' increases (3.76 ± 0.04 at day 0 to 4.03 ± 0.02 at day 28). Similar results were obtained for the population of coliform bacteria (from 3.59 ± 0.03 to 3.89 ± 0.02), *Staphylococcal* counts (3.73 ± 0.01 to 3.98 ± 0.00) and *pseudomonads'* counts (3.64 ± 0.02 to 3.88 ± 0.07). A total of 240 bacterial isolates were isolated throughout the storage days. However,

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Escherichia coli recorded the highest percentage of occurrence while *Bacillus sp.,* had the least. The proximate analysis of the juice samples indicated a decline in the fibre content (0.84- 0.72), protein (7.46- 6.53), carbohydrate (64.3 – 55.6), ash (2.46 – 2.18), and fat 23.41 – 21.1). It also showed that freshly prepared *Irvingia gabonensis* fruit juice is of high quality.

Conclusion: The degradation of the nutrients is suggested to result from the bacterial activities in the stored juices. There is also an increase in the bacterial population as the storage days' increase. The presence of contaminating bacteria was found to deplete the nutritional content of the fruit juice from their metabolic activities.

Keywords: Bacteriological; nutritional; quality; Irvingia gabonensis; fruit juice; bacteria.

1. INTRODUCTION

Globally, it has been posited that forest trees produce over 50 percent of the fruits by humans, and a vast number of it come from cultivated sources [1]. Relatively little number of these tropical food trees are being cultivated around the world as commodity crops. According to the report of Dawnson *et al* [2], these commodity crops include; e.g. Cocoa (*Theobroma cacao*), Coffee (*Coffea spp*.), oil palm (*Elaeis guineensis*), and Bush mango (*Irvingia gabonensis*) etc.One high point of fruit trees is that they are a rich source of proteins, fat, vitamins, and other nutrients that are essential to humans [3]. Stadlmayr et al., [4] underscored that high nutritional value is a major feature of a vast number of African indigenous fruit trees.

Irvingia gabonensis is one of the forest trees that have generated a lot of interest in recent times, basically because of the nutritional, economic and medicinal values it provides. Thus, in Nigeria, the *Irvingia gabonensis* seeds make up a significant part of the rural diet. The tree is highly valued for its root, seeds, fruit, hardwood and high value for the leaves and fruit. Interestingly, the root and fruit have numerous applications in medicine, food and industries [5].

Also known as the African mango or bush mango tree, *Irvingia gabonensis* is found throughout the tropical forests of Africa and its cultivation is common on farms in central and western Africa. *Irvingia gabonensis* prefers habitats such as moist lowland tropical forest that is below 1000m altitude, mean annual temperatures of 25-32^{o}C and with annual rainfall of 1500-3000mm. Similarly, it has been suggested that *Irvingia gabonensis*is better adapted to acid Ultisols in high-rainfall areas than to less acidic Alfisols. It also prefers well-drained sites. Generally, about 2-3 trees grow together. However, in some regions gregarious growth has been reported [5].

Irvingia gabonensis have a typical pattern of growth. The tree grows to a height of about10 to 40 m and it has a flared base 3m in height. The leaves are elliptical, and the foliage is dense and dark green. From February to March, the yellow to white flowers occur in bundles or clusters. The fruit emerge during the rainy season, usually from July to September. Maturity of the tree is attained after 10 to 15 years, and it is characterised by flowering. However, it is important to note that the flowering and fruiting of *Irvingia gabonensis* vary according to the region or geographic location. The flowering is from March to June in Nigeria, and there are two fruiting seasons; from April to July and September to October [6].

Irvingia gabonensis is a non-timber forest product, and it's made up of leaves, tree trunk (stem), fruits and roots. The fruit comprises of a fleshy part and the nut, which is made up of a hard shell and the kernel/seed makes up the fruit. The seeds have an outer brown testa (hull) and two white cotyledons [5]. Two varieties have been identified in Nigeria. These are;*Var gabonensis* and *Var excels* [7] The *Irvingia gabonensis*is an edible African indigenous fruit tree. It was stated by Atangana et al., [8] in their study that it produces fruits and seeds that are edible. Some of the common names of *Irvingia gabonensis* include bush mango, African mango, wild mango or Dikanut plant etc.

The propagation of *Irvingia gabonensis* is mainly by the seeds. In selecting the seeds to be used for propagation, the fruit size, taste of the seed, quantity of yield, early maturation, good sliminess of kernels and the ease of kernel extraction are some criteria that are given careful consideration. It takes more than 14 days for seeds of *Irvingia gabonensis* to germinate, and they are first extracted from the fruit and dried for at least 2 days. In this way, 80 percent rate of germination can be achieved.

The phytochemical screening of the plant materials revealed the presence of tannins, saponins, alkaloids and anthraquinones and the absence of cardiac glycosides. In a study by Onimawo *et al*., [9], analysis of the pulp recorded 80 % moisture, 1 % crude protein, 1 % crude fat, 1 % mineral ash, 0.5 % crude fiber, and 11 % carbohydrate. The high moisture content of the edible pulp provides proof for its use in the production of juice, while the low ash content indicates a low mineral content. The pulp is also an excellent source of calcium (262 mg per 100 g) and vitamin C (66.7 mg per 100 ml) [9].

The seeds are known to be a good source of nutrients. It contains vitamins and minerals such as calcium, magnesium, potassium, sodium, phosphorus, and iron [10]. The presence of phytochemicals has also been documented. They are also rich in the phytochemical, tannins, mainly made up of ellagic acid [10]. The ripe fruit of *Irvingia gabonensis* is green and the edible mesocarp is soft and juicy with bright orange colour. The consumption of the tasty mesocarp as dessert snack or fruit is common in Western and Central Africa owing to its health promoting potentials [11]. Apart from the popular application of the kernels as soup thickener, the kernel oil has been reported to be potential materials for drug binding, confectionary edible fat and cosmetics [12]. Tradition and modern medicine have witnessed the applications of the plant and edible fruit of *Irvingia gabonensis* for the treatment of different illnesses [13].

Etebu [14] asserted that the sweet and juicy pulp is an inherent characteristic of the fruit of *Irvingia gabonensis*, and they can be consumed fresh. Juice, jelly, wine, jam, etc. are some products that can be prepared with the fruit pulp. The *Irvingia gabonensis* have been extensively used for the production of juice. The rate of extraction of the juice from the pulp was suggested to be about 75%, with the sugar concentration of the juice being comparable with that of pineapple and orange juice, but with higher ascorbic acid content [15]. It has been established that the extraction rate and nutrients is far superior to other fruits used for making juices. For example, Jain [16] reported that the concentration of ascorbic acid in the juice is nearly three times that of *Dacryodes edulis*, and *Chrysophyllum albidum*. This makes *Irvingia gabonensis* a good local source of vitamin C (Onimawo, 2002) [9]. The fruit pulp of *Irvingia gabonensis* is a good candidate for juice preparation because of the presence of flavonoids and dietary fiber [9].

There has been an increase in the production, trade and applications of the trade in African mango [17]. As a result, there is an urgent need to avoid wastage of the fruits after harvest. A good option to prevent wastage is the processing of the fruit into juice. This will preserve the fruit, reduce post-harvest losses and equalise availability in between seasons in addition to providing essential vitamins, minerals, and phytochemicals to diets. While this is a great innovation, the microbiological quality of the fruit juice and its shelf life becomes a matter of great concern. For natural fruit products such as the *Irvingia gabonensis* fruit drink to meet consumers need, it is required to have a good shelf-life, acceptable microbiological quality and good nutrient content.

Foodborne illness is commonly caused by certain chemicals and bacteria or their toxins, which are poisonous toxins produced by pathogenic bacteria. The most common foodborne pathogenic bacteria are *Bacillus cereus, Escherichia coli, Shigella spp, Salmonella app, Staphylococcus aureus, Campylobacter jejuni, Streptococcus pyogenes* etc [18]. Numerous illness and even deaths have resulted from the contamination of juices by pathogenic bacteria. The presence of spoilage bacteria also reduces the shelf life of juice products [19]. This study is designed to examine the Bacteriological quality of locally prepared *Irvingia gabonensis* fruit juice which seeks to determine and ascertain the load of bacteria in the fruit juice at different storage periods, Cultivate, isolate and identify spoilage bacteria associated with *Irvingia gabonensis* fruit juice, determine the nutritional value of the fresh fruit drinks at different storage periods and to ascertain the shelf-life of refrigerated *Irvingia gabonensis* fruit juice.

2. MATERIALS AND METHODS

2.1 Sample Collection

This study was conducted in Department of Biological Sciences, Faculty of Science, Niger Delta University Wilberforce Island Bayelsa State in Amassoma community. The *Irvingia* fruits were purchased from farmers in the riverine area of the community GPS Coordinates: $4^0$58'6.132''N $6^0$5'58.92''E. The fruits were put into clean sac bags and were transported to the laboratory immediately for preparation of the *Irvingia gabonensis* fruit juice and microbiological and proximate analysis.

2.2 Sterilisation of Materials

The materials used for this study were sterilised to discriminate contamination microorganisms during the bacteriological analysis of the samples. Materials suitable for sterilization by means of moist heat sterilisation were autoclaved. These materials include the glass wares and nutrient media and cotton wool. They were autoclaved at 121^0C for 15 minutes to ensure sterility of the materials to be used. Materials not suitable for autoclaving such as droppers and glass rods were disinfected with 70% ethanol. Also, the bench was disinfected with ethanol before and after work, to maintain a clean and safe working environment.

2.3 Preparation of *Irvingia gabonensis* Fruit Juice

The *Irvingia gabonensis* fruits were washed in a basin of clean water. The fruits were peeled manually with a sharp stainless knife, and blended to produce juice. The prepared juice was stored in refrigerator at 4°C. No preservatives or additives were added to the pulp.

2.4 Preparation of Nutrient Media

The nutrient media used for this study were sterilised by autoclaving. Nutrient agar, Macconkey agar, and Mannitol salt agar were used for the cultivation and enumeration of the bacterial population of the samples. Other nutrient media were used for biochemical test of the isolates. Kliger iron agar was used for the detection of lactose and glucose fermentation, gas and hydrogen sulfide production. Simmon citrate agar was used for the detection of citrate utilisation as a sole carbon source. Tryptone water was used for the detection of Indole production.

The powder media were weighed and dissolved in distilled water according to the manufacturer's instructions. The dissolved media were autoclaved at 121^0C for 15 minutes, following standard operation procedures.

NUTRIENT AGAR- For the cultivation less fastidious microorganisms

Composition: agar- 15.00 g
Yeast extract: 1.50 g
Beef extract: 1.50 g
Sodium chloride: 5.00 g
Peptic digest of animal tissue: 5.00 g

MACCONKEY AGAR- For the selective isolation and differentiation of lactose fermenting and lactose non-fermenting enteric bacteria

Composition: Peptic digest of animal tissue: 20.00 g

Lactose: 10.00 g
Bile salts: 5.00 g
Sodium chloride: 0.05 g
Neutral red: 0.05 g
Agar: 13.50 g

MANNITOL SALT AGAR- For selective isolation of pathogenic *Staphylococci*

Composition: Agar- 15.00
Proteose peptone- 10.00 g
Sodium chloride- 75.00 g
D-mannitol- 10.00 g
Phenol red- 0.025 g
Beef extract- 1.00 g

SIMMON CITRATE AGAR- For the differentiation of gram negative bacteria based on the basis of citrate utilization

Composition: Agar- 15.00 g
Sodium chloride- 5.00 g
Sodium citrate- 2.00 g
Dipotassium phosphate- 1.00 g
Ammonium dihydrogen phosphate- 1.00 g
Magnesium phosphate- 0.20 g
Bromothymol- 0.08 g

KLIGER IRON AGAR- For differential identification of gram negative enteric bacilli based on fermentation of dextrose lactose and hydrogen sulfide (H_2S) production.

Peptone- 15.000 g
Agar- 15.000 g
Lactose- 10.000 g
Proteose peptone- 5.000 g
Sodium chloride- 5.000 g
Beef extract- 3.000 g
Yeast extract- 3.000 g
Dextrose- 1.000 g
Sodium thiosulphate- 0.300 g
Ferrous Sulphate- 0.200 g
Phenol red- 0.024 g

TRYPTONE WATER- For the detection of microbial indole formation

Composition: Peptone from Casein- 10.0 g
Sodium chloride- 5.0 g

2.5 Experimental Design

Four groups of the *Irvingia gabonensis* fruit samples were prepared and stored at 4^{o}C. The samples were analysed at different intervals, beginning from day 0, to day 28. The juice was also observed for onset of spoilage and turbidity during sampling days.

2.6 Assessment of Bacteriological Quality of *Irvingia gabonensis* Fruit Juice

This part of the study was conducted over four weeks (day 0, day 7, day 14, day 21 and day 28). The same procedures and standards were followed in all the days of analysis to obtain uniform and accurate data. The *Irvingia gabonensis* fruit juice samples were analysed to assess the bacteriological quality. A bacterial stock solution was prepared by transferring 1 ml of the sample to a test tube containing 10ml of normal saline. Thereafter, a ten-fold serial dilution was done to obtain acceptable colony counts ranging from 30-300 [20]. From the stock bacterial solution, 1ml of the sample was collected and transferred into the first dilution tube. From the first dilution tube, 1ml was transferred to the second tube. The samples were diluted just twice.

Plating of the *Irvingia gabonensis* fruit juice samples was done with the third dilution factor in triplicates using pour plate method. 1ml of the inoculum was aseptically collected with a syringe and poured into a sterile petri dish. Thereafter, 20ml of the molten nutrient media was poured into the dishes aseptically. The plates were swirled to distribute the inoculum evenly in the medium to achieve an even colony distribution after incubation. After solidification of the medium, the plates were inverted and incubated at 37^{0} C for 24 hours in aerobic condition. After the incubation time, the plates were observed for the number of colonies and colony morphology. The number of colonies was counted and expressed in cfu/ml [21].

2.7 Isolation of Bacteria

To identify some of the bacteria associated with the *Irvingia gabonensis* fruit juice, subculturing of the bacterial colonies was performed. The colonies were randomly selected and were picked off with sterile wire loop. The colonies were sub-cultured on fresh nutrient agar plates by streaking colonies on the agar surface using the three loop method. Four parallel lines were dragged from the inoculum pool. This process was repeated until the three-loop pattern was completed. The sub cultured plates were inverted and incubated at 37^{o}C under aerobic condition to obtain pure isolates [21].

2.8 Characterisation and Identification of Bacterial Isolates

2.8.1 Gram Staining Technique

- Colonies from different pure culture plates were emulsified into a drop of distilled water on a slide and a thin preparation was made.
- The smear was allowed to air dry
- The smear was covered with crystal violet stain for 60sec and was rapidly washed off with clean water.
- The Lugol's iodine was added for 60sec and was washed off.
- The smear was decolorised with alcohol and washed off rapidly
- The smear was counter stained with seferanine for 60sec and washed off.
- The smear was examined microscopically under the x100 objective lens. [22].

2.8.2 Catalase Test

This test was performed in test tubes, 3ml of hydrogen peroxide was discarded into sterile test tubes using a sterile glass rod, and colony of the pure culture was picked and dipped into the test tube and observed for production of gas bubbles [22].

2.8.3 Citrate Utilisation Test

10 ml of Simmon citrate slants were prepared in test tubes as slants. Using a wire loop, the test isolate was picked off and streaked on the slope of the medium. The test tubes were inoculated at 37^{o}C for 24 hours [22].

2.8.4 Kliger Iron Agar Slant Test

10 ml of Kliger Iron Agar was prepared in test tubes as slants. Using a stab, the butt of the test tubes was first inoculated. Thereafter, the slope was streaked with the test organism with a wire loop. Tubes were closed with cotton wool and incubated at 37^{o}C for 24 hours. At the end of the incubation period, the color changes, blackening and cracking of the medium were observed

in the tubes and results were interpreted appropriately.

2.8.5 Indole Test

10 ml of thee tryptophan broth was prepared in tubes. Using a wire loop, the medium was inoculated with the test organism and incubated for 48 hours. Thereafter, five drops of Kovac reagent was added to the medium [22].

2.8.6 Oxidase test

A piece of filter paper was placed in a sterile petri dish and 3 drops of freshly prepared oxidase reagent was added. Using a plastic wire loop, a colony of the test organism was smeared on the filter paper [22].

2.9 Proximate Analysis

The standard analytical procedures for food analysis were adopted for the determination of moisture content, crude protein, crude fibre, lipids (fat), carbohydrate, ash and pH.

2.10 Statistical Analysis

The statistical package used was (IBM SPSS Statistics version 23). All data were subjected to statistical analysis. Values were reported as mean ± standard error of mean (SEM). The statistical package used was SPSS. The ANOVA was done with the log (cfu), using adjusted sum of squares (SS) for tests. The population was examined in 5 different storage days (DAY 0-28).

3. RESULTS

The analysis of variance (ANOVA) of the Bacterial population of the *Irvingia gabonensis* fruit juice at the different storage periods is represented in Table 1. The assessment of the total heterotrophic Bacterial Population was done using culture dependent methods. All the storage days had significant bacteria growth. However, the table shows differences in the weighted mean. Day 14 is shown to record the highest mean, while the least mean was recorded at day 0.

Table 1. Population of heterotrophic bacteria isolated for *Irvingia gabonensis* fruit juice stored for 28 days

Storage period	Mean ± SEM (log cfu)	Weighted mean
0	3.76 ± 0.04	5755.50
7	3.85 ± 0.06	7080.60
14	4.03 ± 0.01	10716.22
21	4.04 ± 0.02	10716.24
28	4.03 ± 0.02	10716.23

Table 2. Population of coliform bacteria isolated for *Irvingia gabonensis* fruit juice stored for 28 days

Storage period	Mean ± SEM (log cfu) (log cfu)	Weighted mean
0	3.59 ± 0.03	3891.52
7	3.77 ± 0.01	5889.46
14	3.87 ± 0.02	7414.15
21	3.91 ± 0.02	8129.35
28	3.89 ± 0.02	7763.52

Table 3. Population of *pseudomonas* bacteria isolated for *Irvingia gabonensis* fruit juice stored for 28 days

Storage period	Mean ± SEM (log cfu) (log cfu)	Weighted mean
0	3.64 ± 0.02	4368.21
7	3.78 ± 0.07	6026.77
14	3.78 ± 0.01	6062.68
21	3.92 ± 0.02	8318.69
28	3.88 ± 0.07	7586.95

Table 4. Population of *Staphylococcal* bacteria isolated for *Irvingia gabonensis* fruit juice stored for 28 days

Storage period	Mean ± SEM (log cfu) (log cfu)	Weighted mean
0	3.73 ± 0.01	5371.37
7	3.82 ± 0.01	6607.95
14	3.88 ± 0.01	7586.80
21	3.93 ± 0.01	8512.40
28	3.98 ± 0.00	9550.93

Table 5. Proximate analysis of *Irvingia gabonensis* fruit juice stored for 28 days

DAH	Fibre (mg/100ml)	Protein (mg/100ml)	Carbohydrate (mg/100ml)	Ash (mg/100ml)	Ph	Fat (mg/100ml)
DAY 0	0.84	7.46	64.3	2.46	7.23	23.41
DAY 14	0.78	7.12	62.8	2.38	6.81	23.12
DAY 21	0.73	6.86	57.2	2.26	6.33	21.4
DAY 28	0.72	6.53	5.5.6	2.18	5.08	21.1

The population of coliform bacteria isolated from the fruit samples is represented in table 2 above. The examination of the population of the coliform group as shown in table 2 shows an increase in the population, from 3.59 ± 0.03 at day 0 to 3.89 ± 0.02 at day 28. However, the highest weighted mean was recorded in day 21. This implies the growth of the coliform group was peak at that storage period.

The population of the *Pseudomonads* Bacteria at the different storage periods was ascertained using Centramide agar. The population of the *Irvingia gabonensis* fruit juice was assessed using ANOVA of the log (cfu) obtained from the counts. Table 3 shows the weighted mean of the log (cfu) of the Bacterial population in the different storage days. The least mean as shown in Table 3 above, was recorded at day 0 (3.64 + 0.02). The highest weighted mean was recorded at storage day 21 (3.92 + 0.02). This indicates variations in the population of the Pseudomonads bacteria in the *Irvingia gabonensis* fruit juice. The results presented in the table indicate there is an increase in the weighted mean of the Pseudomonads population.

The variations of the population of the *Staphylococcal* Bacteria population in the *Irvingia gabonensis* fruit juice samples were assessed by performing an analysis of variance (ANOVA). The results of the analysis are presented in Table 4. The table show marked differences in the weighted mean of the *Staphylococcal* Bacteria population at the different storage periods. The results indicate that day 8 had the highest weighted mean, while day 0 recorded the least mean. This indicates that there was an increase in the *Staphylococcal* Bacteria population from day 0 to day 28.

The figure shows the percentage of occurrence of the 240 bacterial isolates isolated from this study. The figure shows the highest occurrence was recorded by *Escherichia coli,* while the least was recorded by *Bacillus sp.* however, it is indicated that only seven different bacteria isolates were obtained and successfully identified from the bacteriological analysis.

Table 5 represents the nutritional analysis of the fruit samples stored for 28 days. The ash, fibre, protein, carbohydrate, and fat content are shown to decline as the storage period increases. The pH of the *Irvingia gabonensis* fruit juice also declined from 7.23 to 5.08. This implies a more acidic medium at the 28^{th} day of storage. Remarkably, all the parameters analysed had similar pattern of decline.

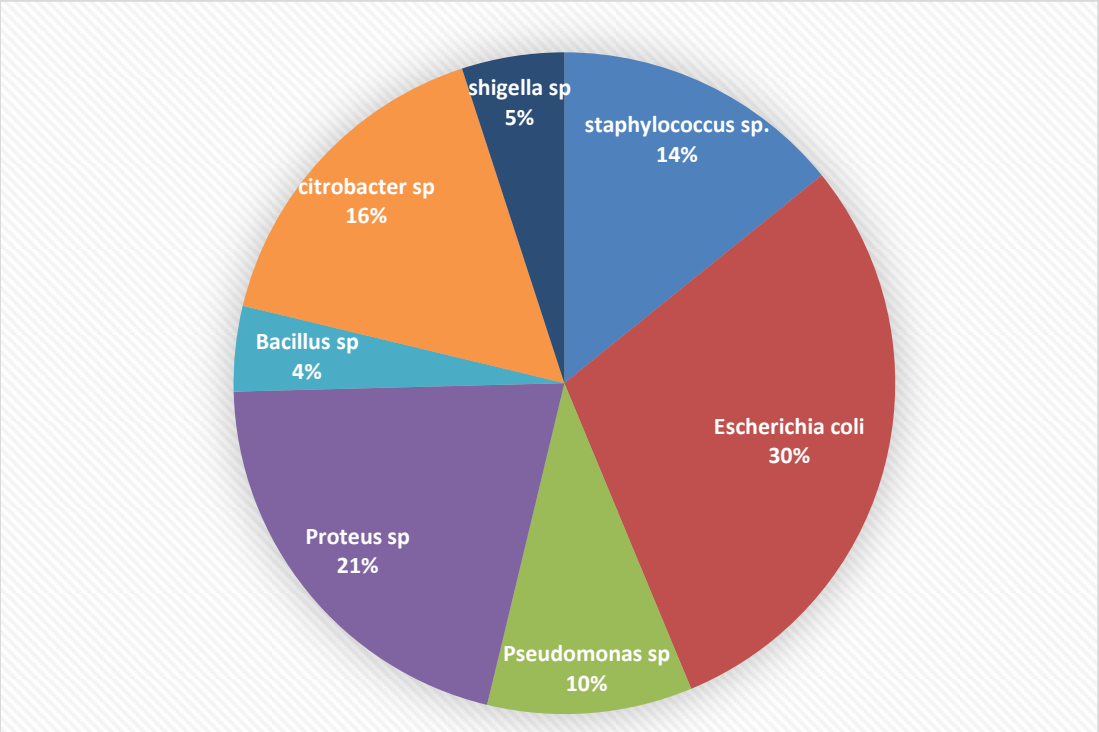

Fig. 1. Percentage of Occurrence 240 Bacterial Isolates isolated from *Irvingia gabonensis* fruit juice in storage

4. DISCUSSION

The results obtained from this study indicate the bacteriological quality of *Irvingia gabonensis* fruit juice and the effects of storage, on its nutritional quality. *Irvingia gabonensis* fruit juice provides a source of rich nutrients to the body. The nutritional properties of *Irvingia gabonensis* has been well established in several studies. However, the presence of microorganisms in the finished fruit juice results to deterioration and spoilage. The spoilage of the *Irvingia gabonensis* fruit juice is even more critical because of the nutrient rich content. This study was undertaken to ascertain the bacteriological quality of *Irvingia gabonensis* fruit juice, which is one of the most recent investigations into the bacteriological quality of locally produced *Irvingia gabonensis* fruit juice. There have been few investigations into the bacteriological quality of *Irvingia gabonensis* fruit juice.

Bacteria are well known for their ubiquitous nature. The implication of this is the presence of bacterial cells in fruit drinks. The nutritional versatility of bacteria has enabled them to use and degrade a wide range of nutrients. This suggests that the presence of bacteria in the *Irvingia gabonensis* fruit juice can impact on its nutritional composition. This study was undertaken to examine the bacteriological quality of freshly prepared *Irvingia gabonensis* fruit juice, and to assess the effects of storage on the bacterial population and its nutritional composition.

The cultivation and enumeration of the bacteria associated with the juice samples was done using culture dependent methods. Different groups of bacteria having medical and spoilage importance were targeted, including coliforms, pseudomonads, staphylococcal species, and the general heterotrophic bacterial population. The assessment of the bacterial population and nutritional composition was done at four different intervals (Day 0, 7, 14, 21 and 28 respectively).

Regarding the total heterotrophic bacteria, nutrient agar was used for their cultivation and enumeration. The results for the analysis of variance total heterotrophic count for the samples are presented in Table 1. The table shows that the weighted mean of the bacterial counts ranged from 3.76 ± 0.04 to 4.04 ± 0.02. The highest mean value was recorded at day 21, while the lowest count was recorded at day 0. This implies that there was a change in the population of bacteria in the fruit juice samples.

Several studies have revealed that the fruits of *Irvingia gabonensis* harbor a high load of bacteria [23]. The presence of such loads of bacteria after harvest may however affect the bacteriological quality of the fruit juices to be produced, and in turn affect its shelf life. Similarly, the investigation by Etebu et al., [14] suggests that the diversity of bacterial species is dependent on the storage period. Although their work was done with the wastes of *Irvingia gabonensis*, the pattern of bacteria growth is similar with the findings of our study. We observed that there was an increase in the bacterial population as the storage days' increases. Thus, we ascertained that the bacterial population and diversity is dependent on the storage days. A separate work by Etebu and Tungbulu et al., [24] further emphasised that, the combination of culture dependent and molecular assays used showed that bacteria were not isolated from fermenting *Irvingia gabonensis* fruits wastes after 6 days of harvest. The absence or very limited presence of bacteria cells in *Irvingia gabonensis* products after prolonged storage may be as a result of the changes in the medium or fruits such as the pH. In our study, the examination of the pH of the samples indicates a decline or decrease in pH, which indicates a transition to a more acidic medium. However, bacteria are very sensitive to changes in pH. The changes in the pH of the juice samples are presented in table 5. The table indicates that the values decreased from 7.23 at day 0 to 5.08 at day 28. As expected, we recorded a change in the population of bacteria. Table 1 shows a decline in the mean bacterial population count. As such, we suggest that as the storage days' increases, the metabolic activities of the bacteria, which cause a degradation of the nutrients available in the fruit juice samples, will affect the acidity of the medium, which in turn will impact on the population of bacteria. This supports the work of Etebu and Tungbulu [24], which concluded that the number of bacterial phyla obtained from *Irvingia gabonensis* fruits were dependent on the postharvest period or days after harvest/storage days.

Apart from the pH of the fruit juice, several factors can influence the growth of bacteria in fruit juices. Notably, Omemu and Aderoju [25] suggested that the storage temperature is an important factor that could contribute to the increase in contamination of stored fruit juices. This implies that storing fruit juices may not necessarily keep off the bacteria population from growing if the right temperatures are not used. As the storage days' increases, the population of the bacteria will thus increase until the medium becomes too harsh or unsuitable for their continuous growth and survival. In relation to the effects of the storage temperature on the growth of micro organisms and nutritional profile of the fruit juices. Helen and Henrietta [26] made an investigation using monkry Kola. Their study was aimed at evaluating the effect of storage time and temperature on some physicochemical properties of juice and jam developed from two varieties of monkey kola (*Colaparchycarpa, Cola lepidota*). It was observed that the pH, total solid, soluble solid, titratable acidity, and total plate counts varied with storage time and temperature; the parameters studied were more stable in samples stored at refrigeration temperature (12^{o}C) than those stored at ambient temperature ($29\text{-}32^{o}$C). However, there was no significant change in specific gravity throughout storage period. Under ambient storage at week 4 *C. parchycarpa*jam had the least total plate count (42.5 Cfu/g). Under refrigeration (12^{o}C) *Colalepidota* jam had the least (22.5 Cfu/g) plate. *C.parchycarpa* juice was more stable than *Cola lepidota* juice at both ambient and refrigeration temperatures. From the result of this study, it can be concluded that the shelf-life of the products (jam and juice) can be extended by storing them in the refrigerator at suitable temperatures.

In our study, we were interested in ascertaining whether there will be significant differences in the population of bacteria in the samples using statistical tools. Table 1 shows there were significant bacterial growth in all the days (0-28). However, the correlation between the successive days of storage was ascertained by using statistical tools (IBM SPSS Statistics 20). The results for the correlation analysis indicate significant and positive relationships between the different days of storage. Table 2 presents the results for the correlation analysis. The results indicate that there was a significant difference between the bacterial population at Day 0 and day 7, with p value of 0.0000. There was similar positive correlation between day 0 and 14, 0 and 21, 0 and 28, with p values < 0.05. Other comparisons between 7 and 14, 7 and 21, 7 and 28 also indicated significant differences in the bacterial population. However, comparison of the other days showed no significant correlation. The positive correlation in most of the storage days supports the notion that the storage period and temperature exerts great influence on the population of bacteria. The availability of

nutrients such as proteins and carbohydrates creates a perfect nutrient rich environment for the proliferation of the bacteria. This indicates that time is an essential factor to consider when stocking *Irvingia gabonensis* fruit juice. As this may have an impact on the shelf life.

Food safety is dependent on the presence or absence of toxic chemicals and pathogenic microorganisms. According to WHO [21] in order to ascertain the microbiological safety from food borne pathogens, widespread use of groups or species which are easily enumerated and whose presence in food indicates exposure conditions that might introduce hazardous organisms and/or allow their growth are used. These groups are referred to as indicator organisms. In relation to ascertaining the safety of the fruit juice, the presence of three groups of bacteria was evaluated- coliform, pseudomonads and staphylococcal bacteria.

The enumeration of the coliform bacteria was done by standard plate count method and most probable number (MPN) method. Coliforms are a group of gram negative, non sporing rod bacteria, usually found in the intestine of warm blooded animals. The laboratory analysis of the Irvingia fruit juice shows the presence of coliform bacteria. Table 2 represents the results for the ANOVA of the total plate count of coliform bacteria in the samples at different time intervals. Although, to the best of our knowledge, there are no records of investigations into the presence of coliform in locally produced Irvingia fruit drink, however, it has been suggested that fruit juice may be contaminated by bacteria from various sources, including the water used for preparation, the utensils, the processor and from harvest conditions [27,28]. Similarly, Rashed and Adeniji [29] asserted that a number of factors can act as sources of bacterial contamination of fruit juices. These factors may "include the use of unhygienic water for dilution, dressing with ice, prolonged preservation without refrigeration, and unhygienic environments". A study conducted by Mattioli et al., [30] reported that both water and hands are important means of bacteria-pathogen transmission.

However, the presence of coliform, mostly of the Enterobacter type in some of the fruit juices have been reported to be natural flora of fruits which may be introduced into the fruit juice if improperly processed [31].

The detection of coliform bacteria is of public health concern, as these bacteria may pose health risks to the consumers. The results for the total coliform bacteria count show variances in the population of the coliforms at the different storage days. The mean count of the coliform bacteria ranged from 3.59 for day 0 to 3. 91 for day 21 respectively. This suggests that there was an increase in the number of coliform bacteria, as storage days' increases. However, at day 0, there was a reduction in the number of coliform bacteria. This may be as a result of the deaths of the bacteria. The correlation analysis of the variations of the coliform bacteria suggests that the duration of storage is a major factor that influences the population of coliform bacteria. The correlation of the population between day 0 and 7, 14, 21, and 28 respectively, indicates a positive and significant relationship. We recorded similar results in the correlation analysis of day 7 and days 14, 21, and 28. However, we recorded contrasting correlation results while comparing the other storage days. This may imply that, factors beyond the storage days or duration may impact on the population of the coliform bacteria in the fruit juice samples.

As a part of the bacteriological and safety evaluation of the Irvingia fruit drink, our investigation examined the presence of Pseudomonads at the different day intervals. The results for the mean count of the Pseudomonads are presented in table 3. The results indicate that the highest count of Pseudomonads was recorded in day 21, while the lowest mean count was recorded in day 0. This implies that as the storage days' increases, the population of the pseudomonads also increases. We examined the correlation between the population of Pseudomonads at the different storage days and the analysis indicates a positive and significant relationship between day 0 and 7, 14, 21 and 28. Population of Pseudomonads at the different storage days and the analysis indicates a positive and significant relationship between day 0 and 7, 14, 21, and 28. Similarly, the comparison of between day 7 and 14, 21, and 28 also produced positive and significant differences. However, it was a different case for the correlation analysis of the other storage days.

The results for the total coliform count are shown in table 4. The total coliform count was significant in all DAH. This finding agrees with the findings of Saranraj et al., [32] that among the groups of bacteria commonly found on plant vegetation are

mainly coliforms or precisely faecal coliforms, such as *Klebsiella* and Enterobacter.

The study further investigated the nutritional composition of the Irvingia fruit juice samples. Examinations into the crude fibre content, crude protein, carbohydrate, ash, pH, and fat were done at the different days of analysis. The nutritional composition of the fruit juice samples was observed to be declining as the storage days' increases. The proximate analysis of the Irvingia fruit juice is represented in Table 5. The table shows that the crude fibre of the stored juice declined from 0.84 to 0.71, crude protein declined from 7.46 to 6.53, carbohydrates from 64.3 to 55.6, ash declined from 2.46 to 2.18 and the pH from 7.23 to 5.08. The results obtained are expected because the presence of bacteria in the juice samples set a pace for the biodegradation of the nutrients available. The degradation of the nutrients is time, and microbial population dependent. Thus, the decline in the nutritional composition. In other related works, the nutritional composition of the Irvingia fruit has yielded similar results. A study conducted by Onojah et al., [33] investigated the Proximate Composition, Mineral Composition, Anti-nutrient factors and Vitamin Composition of *irvingia gabonensis* and *irvingia wombolu* seeds using standard analytical techniques. Moistures, ash, crude fat, crude fibre, protein and carbohydrates content of irvingia *wombolu* and *gabonensis* were (%): 4.85 ± 0.57 and 6.28 ± 1.5, 1.51 ± 0.12 and 1.71 ± 0.05, 53.8 ± 0.98 and 58.7 ± 0.29 and 9.30 ± 2.94 respectively.

5. CONCLUSION

This study examined the bacteriological quality and nutritional composition of *Irvingia* fruit juice. The use of *Irvingia gabonensis* in diet and medicine is widely known and accepted. This is as a result of the presence of phytochemicals and nutrients it contains. However, the value for its nutrients and phytochemicals, the successful use of *Irvingia gabonensis* in the production of fruit drink will depend on the microbial safety and shelf life.

The study shows that bacteria could contaminate the fruit juice from various sources ranging from the water used for processing, the handler; the utensils used and could even come from post-harvest sources. The presence of bacteria in the fruit juice impacts on its nutritional value and safety. The results obtained from our investigation reveals that the populations of bacteria present in the fruit juices will increase as the storage days' increases. As such, the degradation of the nutrients in the juice samples will also become accelerated. This will lead to a decline/decrease in the nutritional properties and pH. The degradation of the fruit juice samples from day 7 indicates that the fruit juice prepared without the use of preservatives will have a short shelf life. This however, will have various market implications, including a reduction in the value.

The results of proximate analysis are an indication of the nutritional benefits of consuming *Irvingia gabonensis* fruit juice, as it contains essential minerals and nutrients such as protein, carbohydrate, and lipids.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/74572