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Research Article

MICROBIOLOGICAL AND PHYTOCHEMICAL EVALUATION OF JACKFRUIT AND SOURSOP

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Abstract

This research was conducted to study the microbiological and phytochemical composition of jackfruit and sour-sop smoothies derived from jackfruit and sour-sop. Whole jackfruit and sour-sop with precut jackfruit were purchased from Eke-Awka Market, washed, peeled, cut, and blended with a sterile blender to achieve a jackfruit and sour-sop smoothie. Qualitative phytochemical screening was carried out on the smoothie samples for the detection of saponins, tannins, phenolics, alkaloids, steroids, triterpenes, phlobatannins, glycosides, and flavonoids. A microbiological evaluation of jackfruit and soursop smoothies was also carried out to determine the bacteria and fungi isolates present in the smoothie sample. The total viable bacteria count of 9.1 x $10^3$and the total fungi count of 5.9 x 10^3 were obtained from the study of the smoothie sample. *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis*, *Enterobacter* spp., *Shigella* spp., and *Pseudomonas aeruginosa* were the bacteria isolates obtained, while the fungal isolates obtained were *Saccharomyces cerevisiae, Penicillium* spp., and *Aspergillus* spp. The bacteria isolated were all gramme-negative, rod-shaped bacteria from the family Enterobacteriaceae. Biochemical characteristics of bacteria isolates, such as gramme staining, catalase tests, coagulase tests, sugar fermentation tests, motility tests, and citrate tests, were carried out. The bacteria isolates were all catalase positive and all coagulase negative, the former indicating the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide when broken down, and the latter indicating the absence of *Staphylococcus aerus*. Phytochemical analyses of jackfruit were carried out, indicating the presence of saponin, tanin, steroids, flavonoids, terpenoids, coumarins, glycosides, triterpenes, phenolics, and alkaloids, and the absence of anthocyanin, amino acids, and phlobatannin. This research helps to exploit the microbiological and phytochemical composition of jackfruit and soursop smoothies. The research is also helpful in determining the microbial contaminants in smoothies purchased to eradicate food poisoning.

Keywords: Jackfruit, Sour-sop, Phytochemicals, Nutrient profile.

INTRODUCTION

Fruit juices are important sources of nutrients and contain several important therapeutic properties that may reduce the risk of various diseases. They contain large amounts of antioxidants, vitamins C and E, and possess pleasant taste and aroma (Abbo *et al.,* 2006). Juices produced from tropical fruits have increasingly gained global importance due to their health effect. The juice may be produced from single fruit or combination of fruits and sold by the street vendors. Also, most fruits and berries have the potential to produce wine (Okeke *et al.,* 2015). Jackfruit (*Artocarpusheterophyllus*), a member of the family *Moraceae* is the largest tree-borne fruit. Mainly grown in tropical countries, it is native to India, also known as Jacquier (French), khnaor (Cambodia), langka (Philippine), or khanoon (Thailand), is one of the local non-seasonal tropical fruits that is widely cultivated throughout the Southeast Asia region. Jackfruit is extensively planted for local and export markets. It is rich in carbohydrate, fiber, potassium and carotene. It has a fibrous, thin, soft and musky flesh, and emits a strong aroma when it ripens (Saxena *et al.,* 2011).The fruit is oval-shaped and spiny, but its most distinguishing feature is its mass, typically 10–30 kg (Haque *et al.,* 2015), which is considered the world largest fruit (Peng *et al.,* 2013). The pulp is golden-yellow, and is arranged in fleshy bulbs (30–35% of the fruit's weight), each containing a single seed (Swami *et al.,* 2014).

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Jackfruit bulbs (JFBs) are edible, and can be described as slightly acidic, creamy, smooth, fibrous, sweet, and highly fragrant, similar to other tropical fruits like banana or pineapple (Prakash *et al.,* 2009). They are rich in sugars, mainly sucrose, fructose and glucose (Ong *et al.,* 2006), minerals, dietary fiber, carboxylic acids, and vitamins. Jackfruit crops are economically important for most countries that cultivate them. Jack fruit bulbs can be consumed fresh as ingredient in salads, or processed into fruit bars, cakes, jams, ice cream, chutney, jelly, juices, nectars, and fermented beverages among others (Fernandes *et al.,* 2011). Past findings have reported that jackfruit is rich in phenolic compounds which contributing to its multiple pharmacological properties for the treatment of anti-diabetic, inflammatory, wound healing, and fungal infection (Baliga *et al.,* 2011). Besides possessing a unique flavor, jackfruits also rich in phytochemical compounds serve as vital components for various health-promoting benefits (Jagtap *et al.,* 2010). Soursop (*Annonamuricata*), also called guanabana is a fruit with an acidic taste, closely related to custard apple. It is a small evergreen tree, member of the custard apple family; *Annonaceae*. *Annonamuricata* or soursop which originated from tropical Mexico, Central America, Caribbean, South America and sub-Saharan African countries now have spread and is grown in many countries, including some areas in Southeast Asia such as Indonesia, Malaysia, Philippines, and Vietnam (Morton, 1987). Soursop tree grows as high as 6 m (20 ft). It is cultivated for its edible fruits, which are large, juicy, and dark green, and have short, fleshy spines. Soursop

fruit seeds are small and have a glossy black colour (Morton, 1987). Soursop fruit contains various types of nutrients beneficial to human health such as vitamins C, B_1, and B_{12}. Soursop fruit is also rich in carbohydrates, particularly fructose (Taylor, 1998). The Nutrient value per 100 grams serving includes: Vitamin C (20.6 g), Calcium (14 g), Iron (0.6 g), Calories: (66 g), Dietary Fibre (3.3 g), Protein (1 g), Cholesterol (0 mg), Sodium (14 mg), Sugars (13.54 g), Total Carbohydrate (16.84 g), Total Fat (0.3 g), Saturated Fat (0.05 g), Monounsaturated Fat (0.09 g) and Polyunsaturated Fat (0.06 g) (Umme *et al.,* 1997). Soursop can be eaten fresh as fruit; made into cakes, ice cream, preserved, beverages and for flavouring. The young soursop, where the seeds are still soft, is used as a vegetable. The fermented fruit is also used to make an apple cider-like drink (Lutchmedial *et al.,* 2004). Owing to the discovery of soursop as an anticancer agent, (Aspreyand Thornton, 1995) the fruit can be preserved by converting it to a stable product like wine (Okigbo, 2009).

Soursop (*Annonamuricata L.*) is a juicy fruit, commonly found in the Southern part of Nigeria, and mostly eaten as fresh fruit (Abbo *et al.,* 2006). However, soursop can become a potential source of raw material for juice drink production. The juice can be extracted and packaged for human consumption. Freshly processed soursop juice drink can be consumed immediately or stored in the refrigerator or treated with preservatives. Freshly expressed juice is subject to rapid microbial growth, fermentation, enzymatic or chemical and physical deterioration (Anaukwu *et al.,* 2015; Okoli *et al.,* 2023). Therefore, any possible way of processing that will minimize these undesirable reactions and enhance the inherent quality of the starting fruit should be encouraged, and a number of processing techniques are in operation in Nigeria (Agu *et al.,* 2013; Mbachu *et al.,* 2014). Occasionally, fresh fruits and vegetables can become contaminated with harmful microorganisms (Peggy and Amanda, 2001; Agu *et al.,* 2014; Anaukwu *et al.,* 2015). The fresh fruits are exposed to microbial contamination through a variety of sources. Environmental contamination such as water quality, soil fertility management, equipment sanitation and many other factors contribute to the risk of microbial contamination. In addition, the microbial contamination with fresh produce could be associated with human or animal feaces (Pradnya and Sonali, 2008).

According to survey, the total production of the fruit in the world is around $27x10^6$ ton per year (Hathaitip *et al.,* 2013). A survey in United Kingdom shows that 15-20% of fresh-cut fruits are lost each year due to the microbial contamination (Bond *et al.,* 2013). Jackfruit is rich in vitamins A, C and B-complex with abundant of fiber, minerals and energy. During the cutting process of fresh jackfruit, the microorganisms on the surface of a skin of jackfruit may be transferred into the flesh of fruit. These bacteria utilize the host via extracellular lytic enzymes that hydrolyzed these polymers to release water and the other intracellular constituents for use as nutrients for their growth (Margaret B *et al.,* 2009). As a result, the quality of the fruit is affected. This project was conducted to assess the microbiological evaluation of pre-cut jackfruit and soursop sold in Awka, Anambra State, Nigeria, in order to highlight the health implications of consuming such fruits. The aim of this work is to ascertain the microbiological and phytochemical properties of jackfruit and soursop smoothies; and also to evaluate the nutritional composition of jackfruit and soursop*per se.*

MATERIALS AND METHODS

Study Area

Awka Town is at Awka South L.G.A., and is one of the 21 existing LGAs of the state comprising the towns of Amawbia, Ifite-Awka, Ezinato, Isiagu, Mbaukwu, Nibo, Nise, Okpuno, Umuawulu, under its administration.

Methods

Collection of Samples

Samples were collected from Eke Awka market and Nkwo market. These two vending sites were chosen because they are the major markets relied upon by many fruit vendors for their sales. The fruits studied were jackfruit and soursop. Whole jackfruit, soursop and cut jackfruit were purchased in the market and put in a sterile bag. They were washed and cut with sterile knife and blended in to smoothies using sterile blender and was taken to the Laboratory in the Department of Applied Microbiology and Brewing, NAU, Awka for processing and analysis.

Microbiological Quality of Various Smoothie Samples

Sample Analysis

One millitre (1g) of the smoothie samples were weighed out aseptically and introduced into 9ml of sterile water for bacteria, it was properly shaken to homogenize the sample. A 10-fold serial dilution of each of the sample was carried out using distilled water as the diluents. 0.1ml of appropriate dilutions (10^{-1}) of the sample were inoculated by plate method into sterile plates of Nutrient agar (NA),Eosin-Methylene Blue Agar (EMB), Cetrimide Agar base (CAB) and *Salmonella-Shigella* agar (SSA) plates for the culture of bacteria. The culture plates were incubated at 37°C aerobically for 24-48hours for bacteria; while Saboraud Dextrose Agar (SDA) was used for fungi. Developing colonies on Nutrient agar for bacteria and SDA for fungi were counted to obtain total viable. Discrete colonies for the bacteria were obtained by sub culturing into Nutrient agar plates and were subsequently identified using standard methods**.**

Total Bacterial Count (TBC) and Total Fungal Count (TFC) were calculated thus:

$$TBC/TFC = \frac{(N)}{VD} \times 10$$

Where TBC: Total Bacterial Count
TFC: Total Fungal Count
TCC: Total Coliform count
V: Volume plated
D: Dilution Factor

Sample Preparation and Serial Dilution

1ml of the samples was pipetted using a sterile pipette and diluted in a test tube containing 9mls of sterile water for the stock solution. The content of the test tube was mixed properly. Serial dilution is a stepwise dilutions used to reduce a culture of cells to a more useable concentration. The sample and test tubes are arranged properly in rack. Each test tube

contained 9ml of water and it's labeled from 10^1-10^6(10^1,10^2,10^3,10^3, 10^4, 10^5,10^6).

1ml was taken from the stock to the first test tube already containing 9ml of water and mixed properly. Same procedure was used for the remaining test tubes in the series 10, 10,10, 10, 10, 10.

Innoculation of Samples by Pour-plate Methods

Pour plate method was done, using an aliquot of 0.1ml from each diluted samples ($10^{-1}$and 10^{-2}) diluent of bacteria suspension, the aliquots were aseptically transferred to the center of the sterile, labeled Petri dishes. Then the cooled Nutrient Agar, Cetrimide Agar, Salmonella shigella Agar and Sabouraud Agar at about 45°C were poured on them accordingly. These were mixed by swiveling the petri dish for 10seconds. The plates were allowed to solidify and inverted. The plates were incubated at room temperature for 48 hours to allow for bacterial growth and 72hrs for fungi growth, (Blackwell, 2011).

Isolation and Enumeration of Bacteria and Fungi

Each fruit sample was homogenized by introducing in sterile distilled water and blending using a sterile blender. Ten-fold serial dilution of the resultant homogenate was prepared by transferring 1ml of the suspension into 9 ml of sterile diluents. The total aerobic plate counts for the samples were determined by plating 0.1 ml of 10^{-1} and 10^{-2} dilutions on sterile Nutrient agar, Salmonella shigella Agar and Cetrimide Agar plates using the pour plate method. For the fungal counts, 0.1 ml of 10^{-1} and 10^{-2} dilutions were plated on Sabroud Dextrose Agar. The Nutrient agar plates were incubated at 37°C for 48 hrs to obtain the total viable bacterial counts, SDA plates were incubated at 25°C for 72 hrs to obtain the fungal counts. Discrete colonies appearing at the end of the incubation period were counted using a digital illuminated colony counter. Colonies of bacteria that developed on the plates were then identified using both morphological and biochemical methods (Fawole and Oso, 1986). The fungi were identified using colonial morphology and microscopy. The counts were expressed as colony forming units per gram (cfu/g) of the sample.

Characterization and Identification of Bacteria

Discrete colonies that developed after incubation were subcultured to obtain pure cultures which were stored at 40°C and used subsequently for microscopic characterization and biochemical analyses. The distinct colonies that developed in the pure culture plates were observed for the morphological and cultural characteristics including the nature of margin, elevation, shape, colour and transparency. The isolates were further characterized and identified following biochemical procedures as described by Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994) these included catalase test, Sugar fermentation test, coagulase test, Citrate test, Motility test.

Gram-staining Test

Gram staining was carried out to differentiate between gram-positive and gram-negative bacteria isolates. A drop of normal saline was place on a clean grease free glass slide and a colony in isolates was picked with a sterilized wire loop and emulsified and then allowed to air dry. The glass slide was pass over the flame three times to heat fix. The smear was flooded with crystal violet for 60 seconds and rinsed with distilled water. Lugol's iodine was added and allowed to stand for 60seconds then you wash off with distilled water. After washing you decolorize with acetone and rinsed immediately with distilled water. The smear was counter stained with safranin for 60seconds and rinsed with distilled water. The smear was then allowed to air dry after which oil immersion was added and viewed under microscope using x100 objective lens. Purple colour indicated Gram-positive organisms while Red or Pink indicates Gram-negative organisms.

Catalase test

This test is used to detect the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide when broken down. The container containing hydrogen peroxide solution was shaken to expel the dissolved oxygen. Two drop of 3% hydrogen peroxide was dropped on a clean glass slide followed by the inoculation of a 24-hour old culture on the slide. The presence of gas bubbles indicates a positive test while the absence of gas bubbles indicates negative reaction. (State *et al.,* 2015)

Coagulase test

This test is to detect the presence of coagulase, its used to differentiate species of *Staphylococcus* an enzyme that has the ability to coagulate blood. 5ml of blood was collected and centrifuged. A drop of physiological saline was put on a clean glass slide, followed by making a smear of a 24-hour old isolate of the test organism, and then a drop of human plasma was added into it to make a suspension. Clumping indicates a positive result which implies the ability of the test organisms to produce coagulase, an enzyme that coagulates blood plasma while for a negative result, no clumping was observed (Cheesbrough, 2000).

Sugar Fermentation Test

Each of the isolate was tested for its ability to ferment a specific sugar. 1g of the sugar and 2g of peptone water were dissolved in 200ml of water. 5ml of the solution were transferred into clean test-tubes using sterile pipettes. The test-tubes containing peptone water and sugar were added Durham's tube which were placed inversely and bromothymol blue as an indicator. These were sterilized for 10minutes and allowed to cool before inoculating the inocula. The test-tubes were incubated for 3days. The production of acid and gas or acid only indicated utilization of sugars. Acid production was indicated by change in colour of the medium from green to yellow while gas production was observed by presence of gas in the Durham's tubes (Acharya, 2016).

Motility test (Hanging Drop Method)

A loopful of 18-24 hour broth culture of the test bacteria was placed at the centre of a clean grease-free cover-slip. Carefully, the cover slip was inverted and placed over the concave portion of a hanging drop slide. The cover-slip/slide arrangement was observed for motility at X100 magnification on a compound microscope. Care was taken to not interprete

"drift" or "Brownian motion" as motility. Results were recorded as motile or non-motile.

Citrate test

A 24h old culture was inoculated into test tubes containing sterile Simmons Citrate agar slant and then incubated for 24hours. A positive test was indicated by a change from green to blue colour on the surface of the Simmons Citrate agar slant. No colour change indicated a negative reaction.

Fungal Identification

Isolation and Characterizaton of the Fungi

This was done based on the gross morphological appearance of fungal colonies on the SDA culture medium and the slide culture as described by Agu and Chidozie (2021) and lactophenol cotton for microscopic evaluation under X10 and X40 magnification of the microscope; with reference to the Manual of Fungal Atlases (Frey *et al.,* 1979, Barnett and Hunter, 2000, Watanabe, 2002, Ellis *et al.,* 2007).

Qualitative Phytochemical Screening

Phytochemical analysis extract was carried out using the method described by (Odebiyi and Sofowora, 1978) for the detection of saponins, tannins, phenolics, alkaloids, steroids, triterpenes, phlobatannins, glycosides and flavonoids.

Alkaloids: 1cm³ of 1%HCl was added to 3cm³ of the extracts in a test tube. The mixture was heated for 20 minutes, cooled and filtered. The filtrate was used in the following tests: 2 drops of Wagner's reagent was added to 1cm³ of the extracts. A reddish brown precipitate indicates the presence of alkaloids

Tannins: 1cm³ of freshly prepared 10% KOH was added to 1cm³of the extracts. A dirty white precipitate indicates the presence of tannins.

Phenolics: 2 drops of 5% $FeCl_3$ was added to 1cm³ of the extracts in a test tube. A greenish precipitate indicates the presence of phenolics

Glycosides: 10cm³of 50% H_2SO_4 was added to 1cm³of the extracts, the mixture was heated in boiling water for 15 minutes. 10cm³ of Fehling's solution was added and the mixture boiled. A brick red precipitate indicates the presence of glycosides.

Saponins: Frothing test: 2cm³ of the extract in a test tube was vigorously shaken for 2 minutes. Frothing indicates the presence of saponins.

Flavonoids: 1cm³of 10% NaOH was added to 3cm³ of the extracts. A yellow colouration indicates the presence of flavonoids.

Steroids: Salakowsti test: 5 drops of concentrated H_2SO_4 was added to 1cm³of the extracts. Red colouration indicates the presence of steroids

Phlobatannins: 1cm³of the extracts was added to 1% HCl. Ared precipitate indicates the presence of phlobatannins.

Triterpenes: 5 drops of acetic anhydride was added 1cm³of the extracts. A drop of concentrated H_2SO_4 was then added and the mixture was steamed for 1 hour and neutralized with NaOH followed by the addition of chloroform. A blue green colour indicates the presence of triterpenes.

Phytosterols (Finar, 1986): liberman-burchard's test: 50mg is dissolved in 2ml acetic anhydride. To this, one or two drops of conc. $H_2SO_4$is added slowly along the sides of the test tube. An array of colour changes shows the presence of phytosterols.

Fixed oils and fats (kokate, 1999) A small quantity is processed between two filter papers, oil stain on the paper indicates the presence of fixed oil.

Terpenoids: 5ml of aqueous extract of the sample is mixed with 2ml of CHCl3in a test tube 3ml of con. $H_2SO4$is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Amino acid (Yasuma and Ichikawa, 1953): Two drops of ninhydrin solution (10mg of ninhydrin in 200ml of acetone) are added to two ml of aqueous filtrate. A characteristic purple colour indicates the presence of amino acids.

Determination of Coumarin

Add 0.5 ml of 5N NaOH to the solution for 1 ml of the extract (0.5 g in 1ml of ethanol), heat at 80 0C for 5 min, cool, add 0.75 ml of 5 N H_2SO_4, mix thoroughly, add 0.25 g of anhydrous

$NaHCO_3$, mix, and transfer to the extractor. Rinse the flask with distilled water and transfer to the extractor and make up to 50 ml. extract for 3 hours with petroleum ether, remove the inner tube, and transfer the petroleum ether in the extractor to the extraction flask. Add 20 ml of water to the pet ether extract and carefully evaporate the pet ether in a water bath at 50-55^{o}C. Transfer the aqueous solution to a volumetric flask, make up to 50 ml with continuous mixing. pipette 25 ml into a flask and add 1% Na_2CO_3 solution, heat in a water bath at 85^{0}C for 15 min and cool. Add 5 ml of the diazonium solution, and let stand for 2 hours.

Estimation of Total Phenolic Content

The total phenolic content of sample was estimated according to the method of (Makkar *et al.,* 1997).The aliquots of the extract was taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially to the test tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 min. and the absorbance was recorded at 725 nm against the reagent blank. Using Gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1-10 µg/ml. using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/g of extract. (Makkar *et al.,* 2009).

Total Flavonoid Assay

Total flavonoid content was measured by aluminium chloride colorimetric assay.1ml of extracts or standard solution of

Quarcetin (500µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5% NaNO2 was added. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added. At 6th min, 2 ml of 1M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm. Total flavonoid content of the flower was expressed as percentage of Quarcetin equivalent per 100 g of fresh mass. (Talari, *et al*., 2012). The spectrophotometeric method of (Brunner, 1984) was used for the analysis of saponins. Briefly, 1g of the finely ground dried sample was weighed into a 250ml beaker and 100ml of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman No. 1 filter paper into a 100ml beaker containing 20ml of 40% saturated solution of $MgCO_3$. The resulting mixture was again filtered to obtain a clear colourless solution. One millilitre of the colourlessfilterate was pipette into a 50ml volumetric flask and 2mlof 5% Fe Cl_3 solution was added and made up to the marked level with distilled water. This was then allowed to stand for 30 minutes for a blood red colour to develop. 0-10ppm saponins standard was prepared from saponins stock solution. The standard solutions were treated similarly with 2ml of 5% $FeCl_3$ solution as earlier described. The absorbance of the samples as well as standard saponin solutions was read after colour development using a Jenway V6300 spectrophotometer at wavelength of 380nm.

Percentage saponin was calculated using the formula:

% saponin =Absorbance of sample × Average gradient× Dilution factor Weight of sample × 10,000

Alkaloids:

The quantitative determination of alkaloids was done by distillation and titrimetric methods as described by (Henry, 1973). Briefly, 2g of finely ground sample was weighed into 100ml beaker and 20mls of 80% absolute alcohol added to give a smooth paste. The mixture was transferred to a 250ml flask and more alcohol added to make up to 1g of magnesium oxide was then added. The mixture was digested in a boiling water bath for an hour and half under a reflux air condenser with occasional shaking. The mixture was filtered while hot through a Buchner funnel. The residue was poured back into the flask and redigested for another thirty minutes with 50ml alcohol after which the alcohol was evaporated. Distilled water was added to replace the lost alcohol. When all alcohol has evaporated, 3 drops of 10% HCl was added. The whole solution was later transferred into 250ml volumetric flask; 5ml of Zinc acetate solution and 5ml of potassium ferricyanide solution were thoroughly mixed together to give a homogenous mixture. The flask was allowed to stand for a few minutes, filtered through a dry filter paper and 10ml of the filterate was transferred into a separating funnel and the alkaloids present were extracted vigorously by shaking with five successive portions of chloroform. The residue obtained was dissolved in 10ml of hot distilled water and transferred into a Kjeldahl tube with the addition of 0.2g of selenium for digestion to a clear colourless solution. The clear colourless solution was used to determined Nitrogen using Kjeldahl distillation apparatus the distillate was back titrated with 0.01N HCl and the titre value obtained was used to calculate the % Nitrogen using the formulae:

%N = Titre value ×Atomic mass of Nitrogen× Normality of HCl × 100/ Weight of sample (mg) % Alkaloid = % Nitrogen × 3.26

Where 3.26 is a constant

Tannins:

The method of (Swain, 1979) was used to determine the quantity of tannins. 0.20g of sample was measured into a 50ml beaker 20ml of 50% methanol was added and covered with paraflim and placed in a water bath at 77-80˚C for 1 hour. It was shaken thoroughly to ensure uniform mixing. The extract was filtered using a double layered Whatman No. 41filter paper into a 100ml volumetric flask.20ml water was added and 2.5ml Folin-Denis reagent and 10ml of 17% Na_2 CO_3 were added and mixed properly. The mixture was made up to mark with water mixed well and allowed to stand for 20 minutes. A bluish-green colour will develop at the end of range. 0-10ppm was treated similarly as 1ml sample above. The absorbances of the Tannic acid standard solutions as well as samples were read after colour development on a spectronic 21D spectrophotometer at a wavelength of 760nm. % Tannin was calculated using the formula:

$$\% \text{ Tannin} = \text{Absorbance of sample} \times \frac{\text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Glycosides:

10ml of extract was pipette into a 250ml conical flask. 50ml Chloroform was added and shaken on a Vortex Mixer for 1 hour. The mixture was filtered into a conical flask. 10ml pyridine and 2ml of 2% sodium nitroprusside were added and shaken thoroughly for 10 minutes. 3ml of 20% NaOH was later added to develop a brownish yellow colour. Glycoside standard of concentration ranging from 0-5mg/ml were prepared from 100mg/ml stock glycoside standard. The series of standards 0-5mg/ml were treated similarly like the sample above.

The absorbances of sample as well as standards were read on a spectronic 21D Digital spectrophotometer at a wavelength of 510nm. % Glycoside was calculated using the formula:

$$\% \text{ Glycoside} = = \text{Absorbance of sample} \times \frac{\text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Steroids:

0.05g of sample extract was weigh into a 100ml beaker. 20ml of chloroform- methanol (2:1) mixture was added to dissolve the extract upon shaking for 30 minutes on a shaker. The whole mixture until free of steroids. 1ml of the filterate was pipette into a 30ml test tube and 5ml of alcoholic KOH was added and shaken thoroughly to obtain a homogenous mixture. The mixture was later placed in a water bath set at 37˚C-40˚C for 90 minutes. It was cooled to room temperature and 10ml of petroleum ether added followed by the addition of 5ml distilled water. This was evaporated to dryness on the water bath. 6ml of Liebermann Buchard reagent was added to the residue in dry bottle and absorbance taken at a wavelength of 620nm on a spectronic 21D digital spectrophotometer. Standard steroids of concentration of 0-4mg/ml were prepared from 100mg/ml stock steroid solution and treated similarly like the sample as above.

% steroid was calculated using the formula:

% steroid = Absorbance of sample × Average gradient × Dilution factor Weight of sample × 10,000 Phlobatannins: 0.05g of sample extract was weighed into 50ml beaker. 20ml of50% methanol was added and covered with paraflim and placed in a water bath set at 77-80˚C for 1 hour.

The mixture was properly shaken to ensure uniform mixing and later filtered through a Whatman No 1 filter paper into a 50ml volumetric flask using aqueous methanol to rinse, and make up to mark with distilled water.

1ml of the sample extract was pipette into a volumetric flask. 20ml water, 2.5ml Folin-Dennis reagent and 10ml of 17% sodium carbonate were added to the solution in the 50ml flask. The mixture was homogenized thoroughly for 20 minutes. 0-5mg/ml of phlobatannins standard concentration were prepared from 100mg/ml phlobatannins stock solution and treated like the sample above.

% phlobatannins was calculated using the formula:

Absorbance of sample × Average gradient × Dilution factor Weight of sample × 10,000

Anthraquinones:

The method of (Lewis, 1974) was used.

0.5g of sample was weighed into 250ml beaker and 60ml benzene added and stirred with a glass rod to prevent lumping. This was filtered into 100ml volumetric flask and 0.2% Zinc dust was added followed by the addition of 50ml hot 5% NaOH solution. The mixture was heated just below boiling point for five minutes and then rapidly filtered and washed once in water. The filterate was again heated with another 50ml of 5% NaOH to develop a red colour. Standard anthraquinone solution of range 0-5mg/l were prepared from 100mg/l stock anthraquinone and treated in a similar way with 0.2% Zinc dust and NaOH like the sample. The absorbances of sample as well as that of standard concentrations were read on a Digital Spectrophotometer at a wavelength of 640nm.

The percentage anthraquinone is calculated using the formula:

= Absorbance of sample × Average gradient × Dilution factor Weight of sample × 10,000

Triterpenes:

0.50g of sample was weighed into a50ml conical flask and 20ml 0f 2:1 chloroform-methanol mixture was added, shaken thoroughly and allowed to stand for 15 minutes. The supernatant obtained was discarded, and the precipitate was re-washed with another 20ml chloroform-methanol mixture for re-centrifugation.

The resultant precipitate was dissolved in 40ml of 10% Sodium Dodecyl Sulphate (SDS) solution.1ml of 0.01M ferric chloride solution was added to the above at 30 seconds intervals; shaken well, and allowed to stand for 30 minutes. Standard triterpenes of concentration range 0-5mg/ml were prepared from 100mg/l stock triterpenes solution from sigma-Aldrich chemicals, U.S.A. The absorbances of sample as well as that of standard concentrations of triterpenes were read on a digital spectrophotometer at a wavelength of 510nm.

The percentage of triterpenes was calculated using the formula:

= Absorbance of sample × Average gradient × Dilution factor Weight of sample × 10,000

Determination of total anthocyanins Total anthocyanin compounds of the samples were estimated using a UV-spectrophotometer by the pH differential method reported by (Abu Bakar *et al*., 2009) with slight modifications. Two buffer systems, potassium chloride buffer, pH 1.0 (0.0025 M) and sodium acetate buffer, pH 4.5 (0.4 M) were used. Briefly, 400 µl of extract (3 mg of ground beans in 10 ml absolute methanol) was mixed in 3.6 ml of corresponding buffer solutions and read against a blank at 510 and 700 nm. Absorbance

(Ad) was calculated as: Ad = (A510 -A700) pH1.0 -(A510 - A700) pH4.5 Anthocyanin concentration in the extract was calculated and expressed as cyaniding-3 lycoside (mg g-1) equivalent:

Ad × MW × DF × 1000 / (Ma × 1)

Where, Ad is difference in absorbance,

MW is a molecular weight for cyaniding-3-glucoside (449.2), DF is the dilution factor of the samples and Ma is the molar absorptivity of cyaniding-3-glucoside (26.900).

Results were expressed as mg of cyaniding-3-glucoside equivalents in 100 g of dried sample.

Determination of proanthocyanidins content was determined by vanillin-H_2SO_4 assay as described by (Chang *et al.,* 2007) with minor modifications. A volume, 1.0 ml aliquots of bean extract (3 mg in 10 ml absolute methanol) were mixed with 2.5 ml of 1.0% (w/v) vanillin in absolute methanol and then with 2.5 ml of 25% (v/v) sulfuric acid in absolute methanol to undergo vanillin reaction with polyphenols in bean species. The blank solution was prepared in the same procedure without vanillin. The vanillin reaction was carried out in a 25°C water bath for 15 min. The absorbance at 500 nm was read and the results were expressed as (+) catechin equivalent by a calibration method.

Determination of Moisture Content

This method is based on moisture evaporation, as described by Frank and Kingsley (2014). Here the aluminum dishes were washed dried in oven and in desiccators for cooling. The weight of each dish was taken. 5.0 g of ground samples of were weighed into a sterile aluminium dish, weight of the dish and weight of un-dried sample (in duplicate) were taken. This was transferred into an oven set at 80°C for 2 h and at 105°C for 3 h respectively. This was removed and cooled in desiccators. Then the weight was measured using a measuring scale balance. It was transferred back into the oven for another one hour and then reweighed. The process continued until a constant weight was obtained. The difference in weight between the initial weight and the constant weight gained represents the moisture content.

Calculation: The loss in weight multiplied by 100 over the original weight is percentage moisture content.

Moisture content (g/100 g)
= loss in weight ((W2-W3)/ (W2-W1)) x 100

Where W1= initial weight of empty crucible, W2= weight of crucible + food before drying, W3 = final weight of crucible + food after drying.
% Total solid (Dry matter) (%) = 100- moisture (%)
(AOAC 2005)

Ash Content

The ash represents the inorganic component (minerals) of the sample after all moisture has been removed as well as the organic material. The method is a destructive approach based on the decomposition of all organic matter such that the mineral elements may be lost in the process. Twenty grams (20 g) of each of the samples were weighed into a clean dried and cooled platinum crucible. It was put into a furnace set at 550 °C and allowed to blast for 3 h. It was then brought out and allowed to cool in desiccators and weighed again.

Calculation: Percentage weight is calculated as weight of ash multiplied by 100 over original weight of the samples used.

Ash content = (weight of ash/ weight of original sample used) x100.
Loss in weight ((W3-W1)/(W2-W1)) x 100

Where W1 = weight of empty crucible, W2 = weight of crucible + food before drying and or ashing, W3 = weight of crucible + ash.
(AOAC, 2005)

Determination of Lipid Content

The method employed was the soxhlet extraction technique described by (AOAC, 2005). 15 g of the samples were weighed and carefully placed inside a fat free thimble. This was covered with cotton wool to avoid the loss of sample. Loaded thimble was put in the Soxhlet extractor, about 200ml of petroleum ether were poured into a weighed fat free soxhlet flask and the flask was attached to the extractor. The flask was placed on a heating mantle so the petroleum ether in the flask refluxed. Cooling was achieved by a running tap connected to the extractor for at least 6hrs after which the solvent was completely siphoned into the flask. Rotary vacuum evaporator was used to evaporate the solvent leaving behind the extracted lipids in the soxhlet. The flask was removed from the evaporator and dried to a constant weight in the oven at 60°C. The flask was then cooled in a desiccator and weighed. Each determination was done in triplicate. The amount of fat extracted was calculated by difference.

Ether extracts (100g) dry matter = (weight of extracted lipids/ weight of dry sample) x100 (AOAC, 2005)

Protein Determination

Total protein was determined by the kjeldahl method by (AOAC 2005). The analysis of a compound of its protein content by kjeldahl method is based upon the determination of the amount of reduced nitrogen present. About 20 g of the samples were weighed into a filter paper and put into a kjedahl flask, 10 tablets of Na2SO4 were added with 1 g of CuSO4 respectively. Twenty millilitre (20 mL) of conc. H2SO4 were added and then digested in a fume cupboard until the solution becomes colourless. It was cooled overnight and transferred into a 500 mL flat bottom flask with 200 mL of water. This was then cooled with the aid of packs of ice block. About 60 to 70 mL of 40% of NaOH were poured into the conical flask which was used as the receiver with 50 mL of 4% boric acid using 3 days of screened methyl red indicator. The ammonia gas was then distilled into the receiver until the whole gas evaporates. Titration was done in the receiver with 0.01M HCl until the solution becomes colourless.

Calculation: The percentage protein is calculated as follows:

Vs – Vb x 0. 01401x N acid (6.25) x 100 Original wt of sample used

Where Vs = Vol (ml) of acid required to titrate sample, Vb = Vol (ml) of acid required to titrate blank, N acid = normality of acid (AOAC, 2005).

Crude Fiber

The bulk of roughages in food is referred to as fiber and is estimated as crude fiber. Twenty grams (20 g) of the different samples were defatted with diethyl ether for 8 h and boiled under reflux for exactly 30 min with 200 mL of 1.25% H_2SO4. It was then filtered through cheese cloth on a flutter funnel. This was later washed with boiling water to completely remove the acid. The residue was then boiled in a round bottomed flask with 200 mL of 1.25% sodium hydroxide (NaOH) for another 30 min and filtered through previously weighed couch crucible. The crucible was then dried with samples in an oven at 100°C, left to cool in a desiccator and later weighed. This was later incinerated in a muffle furnace at 600°C for 2 to 3 h and later allowed to cool in a desiccator and weighed. (AOAC, 2005)

Calculation = Weight of fiber = (C2-C3) y
% fiber = C2-C3 x 100 /Wt. of original sample

Carbohydrate Determination

Available carbohydrate (%) = 100- (protein (%) + Moisture (%) + Ash (%) + Fibre (%) + Fat (%)).
Energy or Caloric Value (KJ/100g) = (Protein X 16.7) + (Lipids X 37.7) + (Carbohydrate X 16.7)

RESULTS

Table 1. The Total Viable Bacteria and Fungi Count of Smoothie Samples Gotten from Whole Jackfruit and Soursop and Cut Jackfruit

	NA			SDA		
Sample	x	y	Mean TBC	x	y	Mean TFC
A	TNTC	TNTC	TNTC	195	158	$1.77 X10^4$
B	96	85	$9.1 X10^3$	57	61	$5.9 X 10^3$

Key: NA – Nutrient Agar; SDA- Sabouraud Dextrose Agar; TBC- Total Bacterial Count; TFC- Total Fungal Count; TNTC- Too Numerous To Count

Table 1 shows the total viable bacteria and fungi count of smoothie samples gotten from whole jackfruit and soursop and cut jackfruit. The mean total bacteria count of sample A is too numerous to count, while sample B is 9.1×10^3 while the mean total fungal of sample A is 1.77×10^4 while sample B is 5.9×10^3.

Table 2. Cultural and Morphological Characteristics of Bacteria Isolated from Smoothie Sample

Isolate	Form	Surface	Colour	Elevation	Opacity	Gram stain	Probable isolate
A	Circular	Glistening	Cream	Raised	Transparent	-ve Rod	*Proteus* spp
B	Circular	Smooth	Greyish/Colourless	Convex	Translucent	-ve Rod	*Shigella*spp
C	Irregular	Glistening	Cream	Raised	Opaque	-ve Rod	*Klebsiella*spp
D	Circular	Smooth	Whitish	Convex	Translucent	-ve Rod	*Escherichia coli*
E	Circular	Shiny	Whitish	Convex	Moist	-ve Rod	*Enterobacter*spp
F	Circular	Smooth	Greyish	Convex	Opaque	-ve Rod	*Pseudomonas* spp

Table 2 shows the cultural and morphological (both macroscopic and microscopic) characteristics of bacteria isolated from smoothie samples. Macroscopy by looking at the media plate for colour, form, elevation and margin of the colony formed on the plate. Microscopy by gram staining and identifying the microorganism under the microscope.

Table 3. Colonial and Microscopic Identification of the Fungi Isolate

Isolate	Colony Morphology	Microscopy	Probate Organism
A	White to cream-coloured smooth glabrous yeast-like colonies on SDA	Spherical to ellipsoidal budding blastoconidia, 2-6 x 3-7um in size. Ascospores may be produced on 5% malt extract or cornmeal agar after 5- 30 days at 25°C	*Saccharomyces cerevisiae*
B	Black centre, white fluffy regular margin and pale yellowish on SDA	Smooth coloured conidiophores and conidia. conidiophores are protrusions from The a septate and hyaline hyphae. The phialides produces conidia that have rough texture and dark brown coloured.	*Aspergillusflavus*
C	Yellow centre, white fluffy regular margin and pale yellowish on reverse SDA	Smooth coloured conidiophores are protrusions from a septate and hyaline hyphae. The phialides produces conidia that have rough texture and dark brown coloured.	*Aspergillusterreus*
D	The colonies are rapidly growing, flat, filamentous and velvety, wool or cottony in texture	Chains of single-celled conidia are produced in basipetal succession of phalides	*Penicillium*spp

Table 3 shows the colonial and microscopic identification of the fungi isolates when viewed under the microscope.

Table 4. Biochemical Characteristics of Identified Bacterial Isolate

Parameter	A	B	C	D	E	F
Catalase Test	+	+	+	+	+	+
Motility test	+	-	-	+	+	-
Citrate test	+	-	+	-	+	+
Coagulase test	-	-	-	-	-	-
Sugar fermentation test						
Glucose	+	+	+	+	+	-
Lactose	+	-	+	+	+	-
Sucrose	+	-	+	Var	+	-
Fructose	-	+	-	-	+	-
Maltose	+	var	+	-	+	+

Key:
+ = Positive
-= Negative
Var = Variable

A,B,C,D,E,F represent the most probate isolate which include *Proteus mirabilis, Shigella* spp, *Klebsiella pneumonia, Escherichia coli, Enterobacter* spp and *Pseudomonas aeroginosa* respectively.

Table 5. Phytochemical Analysis Result of Jackfruit

Test	Jackfruit
Saponin	+
Tanin	+
Steroids	+
Flavonoids	+
Terpenoids	+
Coumarins	+
Glycosides	+
Triterpenes	+
Anthocyanin	-
Phenolics	+
Aminoacid	-
Phlobatannin	-
Alkaloids	+

Key:
+ = positive
-= negative

Table 6. Phytochemical Composition of Jackfruit

Test	Jackfruit
Saponin	0.061616
Tanin	3.546296
Phenolics	6.246418
Phlobatanin	Nil
Steroids	64.24585
Flavonoids	34.5641
Coumarins	2.88655
Anthocyanin	Nil
Terpenoids	8.108523
Glycosides	5.17716
Triterpenes	26.6833
Alkaloids	12.02222

Table 7. Nutritional Analysis of Jackfruit

Sample	Crude fat %	Crude	fibre %	Moisture %	Ash %	Protein %	CHO %	CalorificcontentValue kj/100g
Jackfruit	1.0012	2.0853		65.0274	1.5474	7.3646	22.9741	506.6563
Soursop	1.0937	2.02223		64.8234	1.3843	7.4452	23.23117	512.2954

DISCUSSION

The results obtained from this study show that the samples were not wholesome despite their aesthetics. The pre-cut fruits were contaminated with *Escherichia coli,* This contamination may have been introduced at the point of processing and distribution. The presence of thermo-tolerant (44°C) *Escherichia coli* and other coliform bacteria is generally an indication of faecal contamination of the water often used by vendors for washing their utensils and hands before cutting the fruits. Table 1 shows the total viable bacteria and fungi count of smoothie samples gotten from whole jackfruit and soursop and cut jackfruit. The mean total bacteria count of sample A is too numerous to count, while sample B is 9.1 $\times 10^3$ while the mean total fungal of sample A is 1.77×10^4 while sample B is 5.9×10^3. Precut fruits could become contaminated with *Escherichia coli* by factors such as processing utensils in inadequate hygienic conditions as they are left uncovered, trays left open in unsuitable places for buyers and irregular hand-washing by the vendors. Cross-contamination of food during preparation has been identified as an important factor associated with food-borne illness (Wanyenya, *et al.,* 2004). In this survey, the utensils used for cutting the fruits were not investigated. However, pre-cut fruit vendors used plain water to wash their hands and knives used for cutting the sampled fruits. **Table 2** shows the cultural and morphological (both macroscopic and microscopic) characteristics of bacteria isolated from smoothie samples. Macroscopy by looking at the media plate for colour, form, elevation and margin of the colony formed on the plate. Microscopy by gram staining and identifying the microorganism under the microscope.

Escherichia coli and other bacteria isolated in this study could be associated with the general poor sanitary environmental conditions under which the fruits were handled (Little and Mitchell, 2004). The microbial quality of the fruits in their raw state, contaminated water or inadequate hand-washing by fruit vendors and the absence of individual sanitary practices are similar to the reports of (Kuminos and Copeland, 1972) who reported *Pseudomonas aeruginosa* infection in a hospital through vegetables. The probable bacteria isolate identified in the smoothie sample includes, *Proteus mirabilis*, *Shigella* spp*, Klebsiellapneumoniae, Escherichia coli, Enterobacter* spp and *Pseudomonas* spp respectively while probable fungi isolated include *Saccharomyces cerevisiae, Aspergillus* spp*,* and *Penicillium* spp.

Phytochemical analysis of jackfruit shows the presence of Saponin, Tannin, Steroids, Flavonoids, Coumarins, Glycosides, Triterpenes, Phenolics, and Alkaloids with the absence of Anthocyanin, Amino acid and Phlobatannin.

Conclusion

The results show that the smoothies of pre-cut fruits investigated were contaminated with potential bacterial pathogens. Parasite or cysts, ova and larvae were not detected from any of the pre-cut fruits sampled. The general distribution of the bacterial contamination of the pre-cut fruits sampled includes *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Enterobacter* spp*, Shigelladysentriae* and *Pseudomonas aeruginosa.* The fungal contamination of the precut fruits were *Saccharomyces cerevisiae, Penicillium* spp and *Aspergillus* spp. Reducing the risk of pathogenic microorganism can be through strict adherence to recommended good hygienic practice and hygiene levels for smoothie preparation.

Recommendation

Microbial contamination of precut fruits calls for improved surveillance system on fruits products and public health education and enlightenment of retailers and consumers. As a result of the occurrence of potential foodborne pathogens contamination of these pre-cut fruits studied before their purchase for consumption and the possible outbreaks of food poisoning, retailers and consumers are advised to wash fresh fruits properly before peeling, slicing or cutting. Also to handle and cut fruits with clean and sanitized utensils, surfaces and to store cut fruits at 4°C or below until sold or consumed.

REFERENCES

Abbo, E.S., Olurin, T., Odeyemi, G. (2006). Studies on the Storage Stability of Soursop (*Annonamuricata L.*) Juice. *African Journal of Biotechnology*, 5:108-112.

Adewole, S.O., and Ojewole, J.A. (2008). Protective Effects of *Annonamuricata Linn. (Annonaceae*) Leaf Aqueous Extract on Serum Lipid Profiles and Oxidative Stress in Hepatocytes of Streptozotocin-Treated Diabetic Rats. *African Journal of Traditional, Complement and Alternative Medicines,*6:30–41.

Agu, K.C. and Chidozie, C.P. (2021). An Improved Slide

Culture Technique for the Microscopic Identification of Fungal Species. *International Journal of Trend in Scientific Research and Development,* 6 (1): 243-254, URL: www. ijtsrd.com/papers/ijtsrd45058.pdf

Agu, K.C., Orji, M.U., Onuorah, S.C., Egurefa, S.O., Anaukwu, C.G., Okafor, U.C., Awah, N.S.,

Okafor, O.I., Mbachu, A.E. and Anyaegbunam, B.C. (2014). Comparative Bacteriological Analysis of Ready-to-Eat Vegetables Salad Sold by Various Food Vendors in Awka. *American Journal of Life Science Researches*, 2 (4): 458-465.

Agu, K.C.,Ukponmwan, I.O., Orji, M.U., Awah, N.S., Anaso, C.I. and Udemezue, O.I. (2013). Prevalence of Pathogenic Bacteria in Smoked Fish sold in Major Retail Markets in Benin, Nigeria. *International Journal of Applied Science and Engineering,*1 (1): 1- 4.

Ahmed, K. M., Malek, K. J., and Salamatullah, K. (1986). Nutritive Value of Food Stuff. *Institute of Nutrition and Food Science, University of Dhaka, Bangladesh,* 3:16-27.

Anaukwu, C.G., Nwangwu, F.C., Okafor, I.O., Ezemba, C.C. Orji, C.C., Agu, K.C. and

Archibong, J.E. (2015). Microbiological Analysis of Burukutu beverage produced in southern part of Nigeria. *European Journal of Experimental Biology*, 5(8):18-22

Anaukwu, C.G., Ugwuoke, G.O., Ekwealor, I.A., Okafor, O.C. and Agu, K.C. (2015). Preliminary Study of Bacterial Isolates from Indigenous Ready – To – Eat Salad Vegetables. *American Journal of Life Science Researches*, (3) 4: 282-286

Babitha, S., Sandhya, C., and Pandey, A. (2004). Natural Food Colorants. *Applied Botany Abstracts,* 23: 258-66

Baliga, M.S., Shivashankara, A.R., Haniadka, R., Dsouza, J., and Bhat, H.P. (2011). Phytochemistry, Nutritional and Pharmacological Properties of *Artocarpusheterophyllus* Lam (jackfruit): A review. *Food Research International*, 44:1800– 1811.

Barua, A.G. and Boruah, B.R. (2004). Minerals and Functional Groups Present in the Jackfruit Seed: A Spectroscopic Investigation. *International Journal of Food Sciences and Nutrition*, 55:479-483.

Berry, S.K., and Kalra, C.L. (1988). Chemistry and Technology of Jackfruit (*Artocarpusheterophyllus). A review. Indian Food Packer,*42(3): 62-76

Bond, M., Meacham, T., Bhunnoo, R. and Benton, T. G. (2013). Food Waste Within Global Food Systems. *A Global Food Security Report* ,81(1):61-75.

Brunner, J.H. (1984). Direct Spectrophotometer Determination of Saponin. *Analytical Chemistry*, 34: 1314-1326.

Burkill, H.M. (1997). The Useful Plants of West Tropical Africa. *Royal Botanic Gardens, Kew,*4:160-161.

Chang, M., Gao, H., Xu,L., Bao-ying, L., Zang, H., and Xian-hua, L.(2007). Cardioprotective Effects of Grape Seed Proanthocyanidins Extracts in Streptozocin Induced Diabetic Rats. *Journal of Cardiovascular Pharmacology,* 50(5):503-509.

Chowdhury, A.R., Bhattacharyya, A.K. andChattopadhyay, P. (2012). Study On Functional Properties Of Raw And Blended Jackfruit Seed Flour (A Non-Conventional Source) For Food Application. *Indian Journal of Natural Products and Resources*, 3(3):347-353.

Devasagayam, T.P.A., Tilak, J.C., Boloor, K.K., Ketaki, S., Saroj, S., Ghaskadbi, L. andLele, R.D. (2004). Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. *Journal of the Association of Physicians of India,* 52:291-297

Ejiofor, E. J., Beleya, E.A. and Onyenorah, N.I. (2014). The Effect Of Processing Methods On The Functional and Compositional Properties of Jackfruit Seed Flour. *International Journal of Nutrition and Food Sciences*, 3(3): 166-173.

FAO (2010). Tropical Fruits Medium-Term Prospects for Agricultural Commodities. *Trade And Markets Division Food and Agriculture Organization of the United Nations FAO*.

Fernandes, F. A. N., Rodrigues, S., Law, C. L., and Mujumdar, A. S. (2011). Drying of Exotic Tropical Fruits: *a comprehensive review. Food Bioprocess Technology*, 4:163–185.

Fernando, M.R., Wickramasinghe, N., and Thabrew, M.I. (1991). Effect of *Artocarpusheterophyllus* and *Asteracanthuslongifolia* on Glucose Tolerance in Normal Human Subjects and in Maturity-Onset Diabetic Patients. *Journal of Ethnopharmacology*, 31(3):277-282.

Finar, I,L. (1986).Organic Chemistry: The Fundamental Principles. *Longman Scientific and Technical, Harlow Essex,* 13:582-588.

Fleet, G.H. (2003). Yeast Interactions and Wine Flavour. *International Journal of Food Microbiology,* 86:11-22

Frank, C.O. and Kingsley, C.A. (2014). Proximate Composition, Physiological Changes during Storage, and Shelf Life of Some Nigerian Varieties of Yams (*Dioscorea* species). *Journal of Scientific Research and Reports*, 3 (4): 553-562.

Frank, C.O. and Kingsley, C.A. (2014). Role of Fungal Rots in Post-harvest Storage Losses in Some Nigerian Varieties of *Dioscorea* Species. *British Microbiology Research Journal*, 4 (3): 343-350

Goswani, C., Hossain, M.A., Mortuza, M.G. and Islam, R. (2010). Physicochemical Parameters Of Jackfruit Seeds in Different Growing Areas. *International Journal of Bioresearch*, 2(10):1-5.

Gunasena, H.P.M., Ariyadasa, K.P., Wikramasinghe, A., Herath, H.M.W., Wikramasinghe, P., and Rajakaruna, S.B. (1996). Manual of Jack Cultivation in Sri Lanka. *Forest Information Service, Forest Department*, 3:26-27

Haq, N. (2006). Fruits for the Future-Jackfruit (*Artocarpusheterophyllus*). Southampton Centre for Underutilised Crops. *Experimental Agriculture*,43(3):407-408.

Haq, N., and Hughes, A. (2002). Fruits for the Future in Asia. *International Centre for Underutilized Crops. University of Southampton. pp 234.*

Haque, M. A., Begum, R., Shibly, A. Z., Sultana, M. M., and Khatun, A. (2015). Influence of Jackfruit Pulp On the Quality and Shelf Life of Jackfruit Cake. *Journal Environmental Science and Natural Resources,* 8:59–64.

Hathaitip, R., Aphirak, P. and Tri, I. W. (2013). Physical Properties of Impregnated Cantaloupe and Apple Affected by Different Pressure Levels. *Asian Journal of Agriculture and Food Science*, 1 (4):192-199.

Henry, T.(1973). Introductory Microbiology. *New York: Willey*, *International journal of Microbiology,*46: 1372-1383.

Jacobo-Herrera, N.J., Jacobo-Herrera, F.E.,Zentella-Dehesa, A.,Andrade-Cetto, A., Heinrich, M., and Pérez-Plasencia, C. (2016). Medicinal Plants Used in Mexican Traditional Medicine for the Treatment of Colorectal Cancer. *Journal of Ethnopharmacology*, 179: 391–402.

Jagtap, U. B., Panaskar, S. N., and Bapat, V. A. (2010). Evaluation of Antioxidant Capacity and Phenol

Content in Jackfruit (*Artocarpusheterophyllus* Lam.) Fruit Pulp. *Plant Foods for Human Nutrition*, 65(2): 99– 104.

Ko, F.N., Cheng, Z.J., Lin, C.N., and Teng, C.M. (1998). Scavenger and Antioxidant Properties of Prenyl-avones Isolated from *Artocarpusheterophyllus*. *Free Radical Biology and Medicine*, 25(2): 160-168

Kokate, (1986). Preliminary Screening of Methanolic Extract from Different Parts of *Jatrophacuras(L).* An International Referred, Peer Reviewed and Indexed Quarterly Journal in Science, Agriculture and Engineering, 6:138-142.

Koroth, S. (2015). Jackfruit processing. Agriculture Information Connecting Growers and Buyers, *International Journal of Food microbiology*, 21:185-195.

Kuminos, S.D, and Copeland, C.E (1972). Introduction of *Pseudomonas aeruginosa* into Hospital through Vegetables. *Applied Microbiology and biotechnology*, 24(4): 567-570.

Lansky, E.P., and Newman, R.A. (2007). *Punicagranatum* (pomegranate) and Its Potential for Prevention and Treatment of Inflammation and Cancer. *Journal of Ethnopharmacology,* 1(9):177-206.

León-Fernández, A.E., Obledo-Vázquez, E.N., Vivar-Vera, M.D.A., Sáyago-Ayerdi, S.G., and Montalvo-González, E. (2015). Evaluation of Emerging Methods On the Polyphenol Content, Antioxidant Capacity and Qualitative Presence of Acetogenins in Soursop Pulp *(Annonamuricata L.*). *Journal of Revista Brasileira de Fruticultura,*39:358-362.

Lewis, C.E (1974). Cyclization of 1-Acetylanthraquinone. *The Journal of Organic Chemistry*, 40(3): 366-368.

Little, C.L, and Mitchell, R.T (2004). Microbiological Quality of Pre-Cut Fruits, Sprouted Seeds and Unpasteurized Fruit from Retail and Production Premises in UK. *Public Health*, 7(3): 184-190.

Lutchmedial, M., Ramlal, R., Badrie, N., and Chang-Yen, I., (2004). Nutritional and Sensory Quality of Stirred Soursop (*Annonamuricata L.*) Yoghurt. *International Journal of Food Science and Nutrition,* 55:407-414.

Makkar, H.P.S., Norsambuu, T., Lkhavatsere, S., and Becker, K. (2009). Plant Secondary Metabolites In Some Medical Plants of Mongolia Used for Enhancing Animal Health and Production. *Tropicultura*, 27 (3): 159-167.

Makkar, H.P.S., becker, k. and Barr, A.C (1997). Nutrients and Antiquality Factors in Different Morphological Parts of the *Moringaoleifera* Tree. *Journal of Agricultural Science*, 128(3): 311-322.

ManuSmith, K.R., Woodward, D., Campbell-Lendrum, D.D., Chadee, Y., Honda, Q., Liu, J.M., Olwoch, B., Revich, B., and R. Sauerborn (2014). Human health: Impacts, Adaptation and Vulnerability. Exit Part A: Global and Sectoral Aspects,), *Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, 11: 709-754.

Many Functional Components as Related to Human Health: *A Review. Comprehensive Reviews in Food Science and Food Safety,* 11(6):565-576.

Margaret, B., Thomas, R. H., Hong, Z. and Frederick, B. (2009). Microbiological Spoilage of Fruits and Vegetables. and*Food Microbiology Food Safety*, 12:135-183

Matin, A. (2015). A poor man's fruit: Now a miracle food (https://en.wikipedia.org/ wiki/

Mbachu A.E., Etok , C.A., Agu, K.C., Okafor, O.I., Awah, N.S., Chidi-Onuorah, L.C., Ekwueme, V.C., Okpala, J., Ogbue, M.O. and Ikele, M.O. (2014). Microbial Quality of Kunu Drink sold in Calabar, Cross River State, Nigeria. *Journal of Global Biosciences*, 3 (2): 511-515.

Measey, M. (2010). Indonesia: A Vulnerable Country in the Face of Climate Change. *Global Majority E-Journal*, 1:31-45.

Morton, J. F. (1987). Fruits of Warm Climates. Miami. *Coral Gables Publishers*, 239-240

Mukprasirt, A., and Sajjaanantakul, K. (2004). Physicochemical Properties of Flour and Starch from Jackfruit Seed. *International Journal of Food Science and Technology,*39:271-276.

Mushumbusi, D.G (2015). Production and Characterization of Jackfruit Jam. A Thesis Submitted Food Science of Sokoine University of Agriculture Morogoro, Tanzania. *Fiji Agricultural Journal*, 57:252-258.

Nualla-Ong, S., Chetpattananondh, P. and Yamsaengsung, R. (2009). Extraction of prebiotics from jackfruit seeds. *Journal of Engineering*, 36:213–20.

Odebiyi, O.O and Sofowora, E.A. (1978). Phtochemical Screening of Nigerian. Medicinal plants part II:Iloydia, 41:1-25.

Odoemelam, S.A. (2005). Functional Properties of Raw and Heat Processed Jackfruit Seeds Flour. *Pakistan Journal of Nutrition*, 4(6):366-370.

Okeke, B.C., Agu, K.C.,Uba, P.O., Awah, N.S., Anaukwu, C.G., Archibong, E.J., Uwanta, L.I., Ezeneche, J.N Ezenwa, C.U. and Orji, M.U. (2015). Wine Production from Mixed Fruits (Pineapple and Watermelon) Using High Alcohol Tolerant Yeast Isolated from Palm Wine. *Universal Journal of Microbiology Research*, 3 (4): 41-45.

Okoli, F.A., Okonkwo, N.N., Agu, K.C., Nwobu, W.C., Uwanta, L.I., Ifediegwu, M.C.,

Umeoduagu, N.D. (2023). Identification of Potential Microbial Contaminants from Stored Pap. *International Journal of Progressive Research in Engineering Management and Science,*Vol. 03, Issue 09, September 2023, pp : 409-415

Ong, B. T., Nazimah, S. A. H., Osman, A., Quek, S. Y., Voon, Y. Y., Hashim, D. M., Chew, P. M., and Kong, Y. W. (2006). Chemical and Flavour Changes in Jackfruit (*Artocarpusheterophyllus* Lam.) Cultivar J3 during ripening. *Postharvest Biology and Technology*, 40:279–286.

Padre, S. 2014. Farmers' role in popularizing, marketing jackfruit lauded: A Critical Review of Jackfruit (*Artocarpusheterophyllus*), A Versatile But Underutilized Food Source Of Fiji And Other Pacific Islands. Fiji Agriculture Journal 2018, 57(1):5-18

Peggy, V. L. and Amanda, S. (2001). Safe Handling of Fresh Fruits and Vegetables. *Critical Reviews in Food Science and nutrition,* 52(7):595-610.

Peng, S., Lin, L., Ouyang, L., Zhu, B., Yuan, Y., Jing, W., and Li, J. (2013). Comparative Analysis of Volatile Compounds Between Jackfruit (*Artocarpusheterophyllus L.*) Peel and Its Pulp. *Advanced Materials Research,*781:1413–1418.

Pieme, C.A., Kumar, S.G., Dongmo, M.S., Moukette, B.M., Boyoum, F.F., Ngogang, J.Y., and Saxena, A.K.(2014). Antiproliferative Activity and Induction of Apoptosis by *Annonamuricata (Annonaceae)* Extract On Human Cancer Cells. *BioMed Central Journal of Complement and Alternative Medicine,*14 (1): 1-10.

Pradnya, A. J. and Sonali, P. P. (2008). Microbiological Analysis of Fresh Vegetables and Fruits And Effect Of Anti-Microbial Agents On Microbial Load. *Italian Journal of Food Science*, 27(3):336-344.

Prakash, O., Kumar, R., Mishra, A., and Gupta, R. (2009). *Artocarpusheterophyllus* (Jackfruit): *an overview. Pharmacognosy Reviews,* 3(6):353–358.

Saxena, A., Bawa, A.S. and Raju, P.S. (2011). Jackfruit (*Artocarpusheterophyllus Lam*.) In Yahia, E.M. (Ed.). Postharvest Biology and Technology of Tropical and Subtropical Fruits. *Woodhead Publishing,*2: 275-299.

Siddappa, G.S. (1957). Development of Productsfrom Jackfruit - Canned Jackfruit, Frozen Canned Jackfruit and Jackfruit Jam. *Journal of Scientific and Industrial Research*, 9(11): 166-199.

Singh, A., Kumar, S., and Singh, I.S. (1991). Functional Properties of Jackfruit Seed Flour. *Lebensmittel-Wissenschaft Technology*, 24: 373-374.

Stukin, S. (2016). Is jackfruit the next big meat substitute? http://www.nationalgeograph-ic.com/people-and-culture/food/the-plate/jackfruit-sustainable-vegetarian-meat-sb-stitute/

Swain,T.(1979). Tannins and Lignins in Rosenthal, G.A.,Jasen, D.H(ed). Herbivores, their Interaction with Secondary Plant Metabolites. *Academic Press Newyork*, 34: 657-682.

Swami, B.S., Thakor, N. J., Haldankar, P. M., and Kalse, S.B. (2012). Jackfruit and Its Many Functional Components as Related to Human Health: *A Review. Comprehensive Reviews in Food Science and Food Safety*, 11(6):565-576.

Swami, S. B., Thakor, N. J., Orpe, S., and Kalse, S. B. (2014). Development OfOsmo-Tray Dried Ripe Jackfruit Bulb. *Journal of Food Research and Technology*, 1(2):77–86.

Talari S., Rudroju S., Penchala S. and Nanna Rama S. (2012) Quantification Of Total Phenolics and Total Flavonoid Contents in the Extracts of *OroxylumindicumL.Kurz.Asian Journal of Pharmaceutical and Clinical Research,*5:177-179.

Theivasanthi, T. and Alagar, M. (2011). An Insight Analysis Of Nano Sized Powder Of Jackfruit Seed. *Nano Biomedicine and Engineering,* 3(3):163-168.

Tulyathan, V., Tananuwong, K., Songjinda, P, and Jaiboon, N. (2002). Some Physicochemical Properties of Jackfruit (*Artocarpusheterophyllus Lam*) Seed Flour and Starch. *Journal of Science Asia,* 28: 37–41.

Umesh, J.B., Panaskar, S.N., andBapat, V.A. (2010). Evaluation of Antioxidant Capacity and Phenol Content in Jackfruit (*Artocarpusheterophyllus Lam*.) Fruit Pulp. *Plant Foods for Human Nutrition,*65:99-104

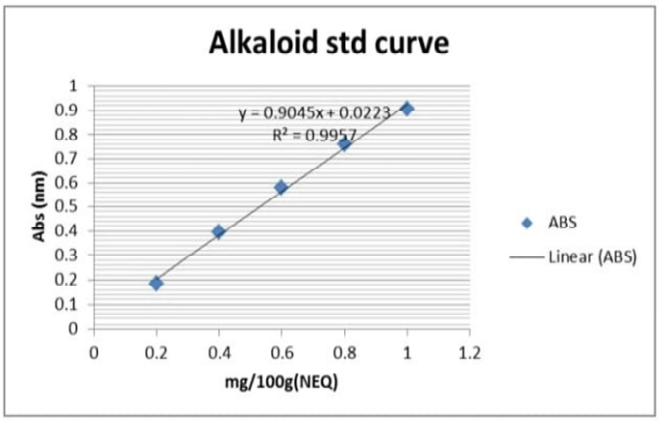
Umme, A., Bambang, S.S., Salmah, Y., Jamilah, B. (2001). Effect of Pasteurization on Sensory Quality of Natural Soursop Puree Under Different Storage Conditions. *Food Chemistry*, 75(3):293-301.

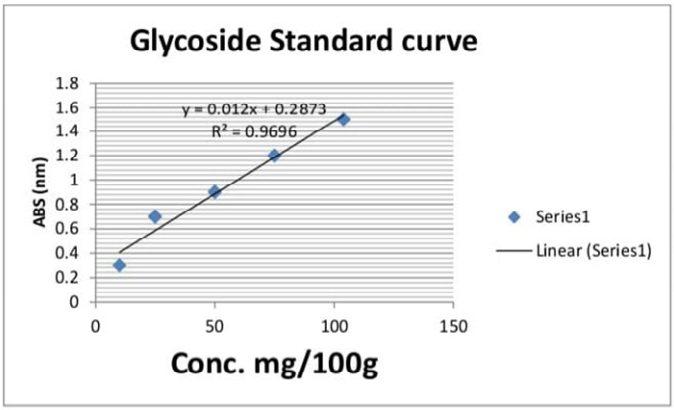
Wanyenya, I., Muyanja, C., and Nasinyama, G.W (2004). Kitchen Practices Used in Handling Broiler Chickens and Survival of Campylobacter Species On Cutting Surfaces in Kampala, Uganda. *Journal of Food Protection*, 67:1957-1960.

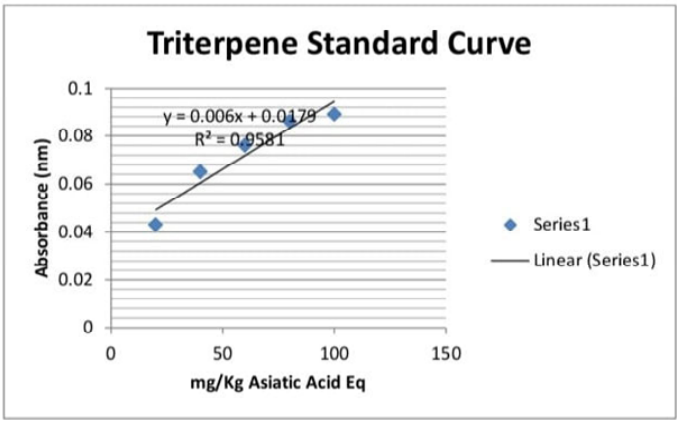
Williams, J.T., and Haq, N. (2002). Global Research on Underutilized Crops. An Assessment Of Current Activities and Proposals for Enhanced Cooperation. Southampton, UK.

Yasuma, A., and Ichikawa, T. (1953). Ninhydrin-Schiff and Alloxan-Schiff Staining: A New Histochemical Staining Method for Protein. *The journal of Laboratory and Clinical Medicine*,10:1-100.

APPENDIX I







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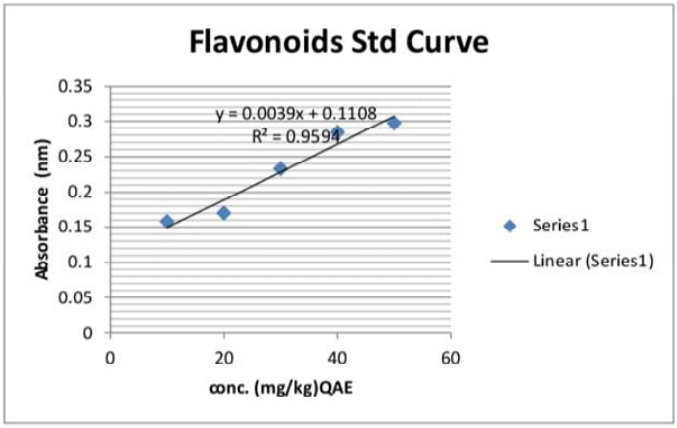

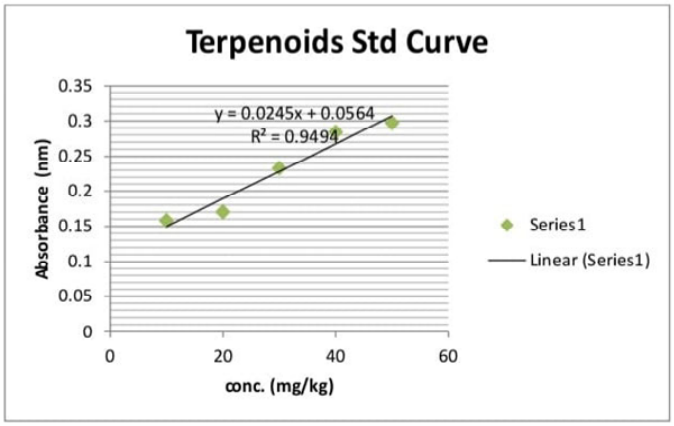

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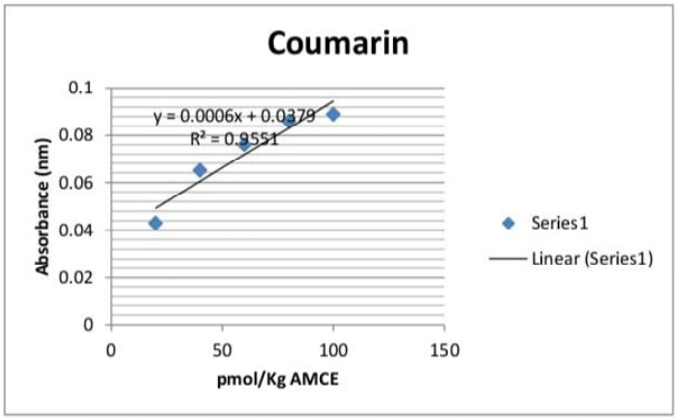

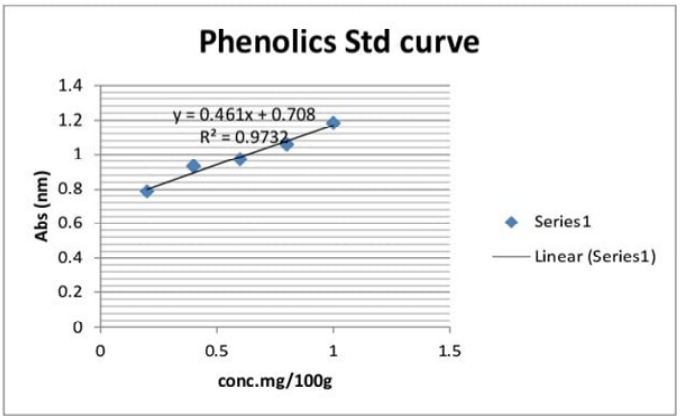

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