Biopreservative Effects of Jack Fruit Seed and Bark on Fruit Juices

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Abstract: Fruit juices are drinks consumed worldwide as it contain vitamins, minerals, amino acids, dietary fibers, sugars and bioactive compounds which are important for the wellbeing and health of humans. Fresh fruits are minimally processed to obtain juices which render it perishable products and hence the need to preserve these juices. This study was aimed at investigating the biopreservative potential of jackfruit seed and bark on freshly prepared juices (watermelon, pineapple and pawpaw). The colony count was observed during the 96 hours storage period at 0 hour, 48 hours and 96 hours by standard spread plate count method. The samples were screened for total Heterotrophic Bacterial Count (THBC), Total Coliform Count (TCC), Total Lactic Acid Bacteria Count (LAB) and Total Fungal Count (TFC) according to the standard methods for the enumeration of bacteria and fungi. The microbial counts of the juices preserved with jack fruit seeds ranged from 1.0^{f} x 104 to 1.18^{b} x 10^{7} cfu/ml while those preserved with jackfruit bark ranged from 1.3^{e} x 10^{4} to 8.8^{e} x 106 cfu/ml. Jack fruit seeds exerted more biopreservative effects on the pineapple juice, while jackfruit bark had more biopreservative effects on the watermelon juice. The isolated microorganisms included *Escherichia coli*, *Bacillus* species, *Salmonella* species, *Staphylococcus aureus*, *Citrobacter* species, *Micrococcus* species, *Lactobacillus* species, *Rhizopus* species, *Aspergillus* species and *Penicillium* species. *Bacillus* sp were the most occurring bacteria in all fruit juices, followed by *Staphylococcus aureus* and *Lactobacillus* sp. while the most occurring fungi was *Penicillium* sp. Some of the microorganisms detected in this study can cause spoilage, food borne illness and pose great risks to human health, so there is need for mitigating the potential risks to consumer health and improving the quality of fruit juices by the addition of natural antimicrobials of plant such as jackfruit to fruit juices which are with little or no negative health consequences. In conclusion, jack fruit seed and bark has been found quite effective as a biopreservative and therefore, could serve as a good candidate for use in extending the shelf life of fruit juices.
Key word: Fruit juices, biopreservative, jackfruit, microbial count, human health

INTRODUCTION

The growing awareness on the need for healthy living and the consumption of healthy, safe and nutritive fruits and vegetables enhanced with natural sources as biopreservative rather than with synthetic preservatives has raised a need for wholesome and nutritive fruits (Qadri *et al*., 2015).

Fruits serve as excellent sources of vitamins, minerals, amino acids, dietary fibers, sugars and bioactive compounds which are important for the wellbeing and health of humans (Allaqaband *et al.,* 2022; Munekata *et al.,* 2023). Also, these components of fruits provide an excellent environment that supports the growth of a range of microorganisms. Examples of fruits include apple, banana, watermelon, pineapple, strawberry, grape, orange, mango, guava and pawpaw. The minimal processing of fresh fruits to make juices render them perishable products that will deteriorate rapidly compared with intact fruits (Mao *et al*., 2006).

Watermelon (*Citrullus lanatus* (Thunb.)) fruit which has about 93% water content is consumed worldwide. It is a rich source of vitamin C, A and B, amino acid, antioxidants and carotenoid lycopene which serve as important nutrients required for healthy living (Rani *et al*., 2019; Naz *et al*., 2014; Asante *et al*., 2020). Pineapple (*Ananas comosus* L.) fruit is a tropical fruit that is consumed worldwide. It contains calcium, potassium, vitamin A, C and carotene, organic acids, bromelain, phosphorus, carbohydrates, sugars, crude fiber, water and other essential compounds (Debnath, 2012; Lagnika *et al*., 2017) which are of immense benefits to human health. The fruits also possess antioxidant, therapeutic and medicinal properties. Pineapple fruits are either consumed fresh or as fresh pineapple juice. The fruits are also used in the production of different food items such as jam, syrup, jelly and pickles (Hossain *et al.,* 2015). Pawpaw (*Carica papaya* L.) fruit is commonly found in the tropical regions of the world. It is a good source of components such as minerals, iron, calcium, vitamins A, B and C which are beneficial to human health. Pawpaw fruits also have medicinal properties which aid in the treatment of various ailments (Dutta *et al*., 2010; Olusegun *et al*., 2016)

Jackfruit (*Artocarpus heterophyllus* Lam) belong to Moraceae family and grow mostly in countries such as India, Bangladesh, Sri Lanka, Burma, Malaysia and Brazil and some parts of Nigeria (Jagadeesh *et al*., 2007). The jackfruit is unusually large in size with each fruit weighing up to 30-35 kg. The ripe fruit consist of three parts which include: the skin (fibrous portion), the pulp (bulbs) and the seeds whose seed and pulp are edible (Albi and Jayamuthunagai, 2014). The pulp when ripe appears soft and yellow to brownish colour with distinct flavour which is sweetish and liken to banana-like flavour and taste (Elevitch and Manner, 2006). Jackfruit is a rich source of several high-value compounds that has potential beneficial physiological activities (Jagtap *et al*., 2010). It contains high level of proteins, starch, calcium, dietary fibre, vitamins and minerals (Ramli *et al*., 2021). Jackfruit has antibacterial, antifungal, antidiabetic, anti-cancer, anti-osteoporotic, anti-inflammatory, and antioxidant activities (Shanmugapriya *et al*., 2011; Khan *et al*., 2021; Palamthodi *et al*., 2021). Different parts of jackfruit tree are been used in ethno-medicine for the treatment of ailments such as diabetes, diarrhoea, dermatitis, malarial fever, asthma, tapeworm infection and anaemia (Araújo and Lima, 2010).

The presence of bioactive compounds in fruits enhances their beneficial effect (Galaverna *et al*., 2008; Vinuda *et al*., 2010). The functional significance of jackfruit (pulp) is associated with the phytochemicals it contains which include: phenolic compounds, carotenoids, flavonoids, volatile acids, sterols, and tannins (Ranasinghe *et al*. 2019; Chandrika *et al*. 2004; Amadi *et al*. 2018). In order to preserve and boost or improve the nutritional quality of fresh products, the use of "green" technologies is preferable (Davachi *et al*., 2021). Preserving food help to sustain it's freshness, quality and colour. Biopreservation involve the extension of shelf life and enhancement of food's safety by the use of natural or controlled microbiota and/or antimicrobial compounds. Hence, this study determines the preservative potential of jack fruits seeds and bark on some fruit juices.

MATERIALS AND METHODS

Sample collection: Fresh matured pineapple, watermelon and pawpaw fruits for juice extraction were purchased from a local market (isi-gate Market) in Umuahia while the jackfruit and its stem bark were obtained from a local farm located at Olokoro in Umuahia, both in Abia state, Nigeria. The Samples were authenticated in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Abia state and further transported in ice bags to the Laboratory for analysis.

Sample preparation: The Jackfruit was sorted, washed and sliced open and the seeds separated from the pulp manually using a sterile knife. The de-hulled seeds were washed with sterile water and dried while the Jackfruit stem bark was separated from the plant using sterile knife, washed with sterile water, and dried. The seeds and the bark were oven dried at 60^o C for 8 hours, according to the methods of Amadi *et al*. (2018). The dried seed and stem bark samples were ground into powdered form with the aid of an industrial blender (Philips HL1646 model) (Amadi *et al*., 2018).

Preparation of plant part extract: Fifty grams (50 g) each of the powdered samples was soaked in 500 ml distilled water in a conical flask, kept on a shaker for 24 hours. The extracts were filtered through Whatman filter paper (No.1) and concentrated to dryness with the aid of a rotary evaporator (Laborota 4000-efficient, Hedolph city, Germany). The extracts from each sample were kept in the refrigerator at 4^oC for analysis or prior to use (Silva *et al*., 2014 and Adeogun *et al*., 2016).

Production of fruit juices: Healthy watermelon, pineapple and pawpaw fruits with unbroken skin were thoroughly washed under a running tap water to remove dirt and then, rinsed with distilled water. The fruits were aseptically cut open with a sterile knife after surface disinfecting them with 70%v/v ethanol solution. The seeds were removed and the flesh (pulp) cut into chunks and scooped into a sterile blender, and blended with distilled water to make 1:1 (w/v) mixture. The juice was obtained by sieving the homogenized blend through a two-fold muslin cloth to get a clear juice (Boone *et al*., 2005; Gwana *et al*., 2014).

Experimental/ treatment set-up

Set-up A: 100% watermelon juice (Control), 95% of watermelon juice + 5% of jack fruit bark/seed, 90% of watermelon juice + 10% jack fruit bark/seed, 85% of watermelon juice + 15% of jack fruit bark/seed, 80% of watermelon juice + 20% of jack fruit bark/seed.

Set-up B: 100% Pineapple juice (Control), 95% of Pineapple juice + 5% of jack fruit bark/seed, 90% of Pineapple juice + 10% jack fruit bark/seed, 85% of pineapple + 15% of jack fruit bark/seed, 80% of pineapple + 20% of jack fruit bark /seed.

Set-up C: 100% Pawpaw juice (Control), 95% of Pawpaw juice + 5% of jack fruit bark/seed, 90% of Pawpaw juice + 10% jack fruit bark/seed, 85% of pawpaw + 15% of jack fruit bark/seed, 80% of pawpaw + 20% of jack fruit bark/seed.

Microbial study/analysis during storage: The colony counts were observed during the 96 hours storage period at 0 hour, 48 hours and 96 hours by standard spread plate counts method. The total Heterotrophic Bacterial Counts (THBCs), Total Coliform Counts (TCCs), Total Lactic Acid Bacteria Counts (LABs) and Total Fungal Counts (TFCs) were done according to the standard methods for the enumeration of bacteria and fungi (Michael and Joseph, 2004). The isolates were identified using standard microbiological (morphologically and biochemically) methods (Cheesbrough, 2006).

Statistical analysis: All data obtained were analyzed using one-way analysis of variance (ANOVA). Descriptive statistics in form of means and standard deviation were also used to assess the data. The significance differences between the variables at $p < 0.05$ were determined using Duncan's range test.

RESULTS

Enumeration of total microbial load (cfu/ml) in fruit juices during storage or preserved with jack fruit seed and bark

The bacteria and fungi population enumerated during the storage period is presented in Table 1 and Table 2. The total Heterotrophic Bacterial Counts (THBCs), Total Coliform Counts (TCCs), Total Lactic Acid Bacteria Counts (LABs) and Total Fungal Counts (TFCs) of fruit juices prepared from watermelon, pineapple and pawpaw and treated/preserved with different ratio of jack fruit seed and bark blend shows a decrease in the number of bacteria, coliforms and fungi present during the 96 hours storage period. The control (fruit juices without the jack fruit blend) which was run along with these to detect the efficacy of the bio-preservative effect of the jack fruit seeds showed an increase in the number of organisms present in the juices as the storage period advanced (Table 1and 2).

Characterization and identification of isolates

The morphological and biochemical studies on the bacteria and fungi isolates present during the storage period revealed isolates to be *Escherichia coli, Bacillus* species, *Salmonella* species, *Staphylococcus aureus, Citrobacter* species, *Micrococcus* species, and *Lactobacillus* species while the fungi isolates characterized were *Rhizopus* species, *Aspergillus* species and *Penicillium* species.

Microbial distribution during preservation or storage of fruit juices

Table 3 and 4 depicts the distribution of the isolates during the preservation period of 96 hours. *Bacillus* sp were the most occurring bacteria in all fruit juices analyzed, followed by *Staphylococcus aureus* and *Lactobacillus* sp. while the most occurring fungi was

Penicillium sp. During the preservation of the fruit juices with the seed blend, *Bacillus* sp occurred most with a distribution rate of 27.3% in both watermelon and pawpaw fruit juices preserved with 5% jack fruit seed while for the preservation with the bark, *Bacillus* sp also had the highest distribution of 25% in watermelon juice preserved with 5% jack fruit bark.

Table 1: Total microbial load (cfu/ml) of fruit juice preserved with jack fruit seed

Key: JFS- Jack Fruit Seed; WMJ- Watermelon juice; PPJ-Pineapple juice; PWJ-Pawpaw juice; NG- No growth, THBC-The total Heterotrophic Bacterial Count, TCC- Total Coliform Count, TFC- Total Fungal Count, LAB-Total Lactic Acid Bacteria Count, Values with different superscript down a column are significantly different from each other

Sample code	24hrs				48hrs				96hrs			
	THBC	TCC	TFC	LAB Count	THBC	TCC	TFC	LAB Count	THBC	TCC	TFC	LAB Count
A Control (100% WMJ)	$1.33^{a} \times 10^{7}$	$4.8^{b} \times 10^{4}$	$4.2^{a} \times 10^{4}$	NG	$1.58^{a} \times 10^{7}$	$7.8^{a} \times 10^{4}$	$5.1^{a} \times 10^{4}$	$2.8^{f} \times 10^{4}$	$2.13^{a} \times 10^{7}$	$8.4^{a} \times 10^{4}$	$5.8^{a} \times 10^{4}$	$3.4^{c} \times 10^{4}$
B (95%WMJ: 5% JFS)	$1.18^{b} \times 10^{7}$	$4.2^{c} \times 10^{7}$	$3.6^{b} \times 10^{4}$	NG	$1.02^{d} \times 10^{7}$	$3.9^{d} \times 10^{4}$	$3.1^{c} \times 10^{4}$	$1.7^{g} \times 10^{4}$	$8.4^{e} \times 10^{6}$	$3.6^{d} \times 10^{4}$	$2.3^{d} \times 10^{4}$	$1.0^{f} \times 10^{4}$
C (90%WMJ: 10% JFS)	$1.03^{d} \times 10^{7}$	$3.3^{d} \times 10^{4}$	$3.1^{c} \times 10^{4}$	NG	$8.8^{e} \times 10^{6}$	$2.8^{ef} \times 10^{4}$	$2.7^{d} \times 10^{4}$	NG	$7.1^{f} \times 10^{6}$	$2.5^{f} \times 10^{4}$	$1.8^{e} \times 10^{4}$	NG
D (85%WMJ: 15% JFS)	$8.3^{e} \times 10^{6}$	$2.6^{e} \times 10^{4}$	$2.2^{e} \times 10^{4}$	NG	$7.4^{f} \times 10^{6}$	$2.4^{g} \times 10^{4}$	$1.6^{f} \times 10^{7}$	NG	$6.9^{f} \times 10^{6}$	$1.8^{g} \times 10^{4}$	$1.1^{f} \times 10^{4}$	NG
E (80% WMJ: 20% JFS)	$7.7^{f} \times 10^{6}$	NG	NG	NG	$7.1^{f} \times 10^{6}$	NG	NG	NG	$6.2^{g} \times 10^{6}$	NG	NG	NG
A Control (100% PPJ)	$8.1^{e} \times 10^{6}$	$3.6^{d} \times 10^{4}$	NG	NG	$1.14^{c} \times 10^{7}$	$5.6^{c} \times 10^{4}$	$2.1^{e} \times 10^{4}$	$3.5^{d} \times 10^{4}$	$1.28^{c} \times 10^{7}$	$7.1^{c} \times 10^{4}$	$2.9^{c} \times 10^{4}$	$3.9^{b} \times 10^{4}$
B (95% PPJ: 5% JFS)	$7.3^{g} \times 10^{6}$	$2.4^{e} \times 10^{4}$	NG	NG	$6.9^{g} \times 10^{6}$	$2.3^{g} \times 10^{4}$	$1.3^{f} \times 10^{4}$	$2.4^{f} \times 10^{4}$	$6.4^{g} \times 10^{6}$	$2.2^{f} \times 10^{4}$	$1.1^{f} \times 10^{4}$	$2.1^{e} \times 10^{4}$
C (90% PPJ: 10% JFS)	$7.1^{g} \times 10^{6}$	NG	NG	NG	$6.7^{g} \times 10^{6}$	NG	NG	NG	$5.8^{h} \times 10^{6}$	NG	NG	NG
D (85% PPJ: 15% JFS)	$5.4^{h} \times 10^{6}$	NG	NG	NG	$5.1^{i} \times 10^{6}$	NG	NG	NG	$5.1^{i} \times 10^{6}$	NG	NG	NG
E (80% PPJ: 20% JFS)	$3.6^{i} \times 10^{6}$	NG	NG	NG	$3.2^{j} \times 10^{6}$	NG	NG	NG	$2.9^{j} \times 10^{6}$	NG	NG	NG
A Control (100% PWJ)	$1.16^{b} \times 10^{7}$	$5.2^{a} \times 10^{4}$	$2.6^{d} \times 10^{4}$	$6.8^{a} \times 10^{4}$	$1.42^{b} \times 10^{7}$	$6.4^{b} \times 10^{4}$	$3.8^{b} \times 10^{4}$	$7.3^{a} \times 10^{4}$	$1.68^{b} \times 10^{7}$	$7.8^{b} \times 10^{4}$	$4.3^{b} \times 10^{4}$	$8.4^{a} \times 10^{4}$
B (95% PWJ: 5% JFS)	$1.08^{c} \times 10^{7}$	$5.0^{a} \times 10^{4}$	$2.4^{d} \times 10^{4}$	$6.1^{b} \times 10^{4}$	$1.01^{d} \times 10^{5}$	$3.8^{d} \times 10^{4}$	$2.2^{e} \times 10^{4}$	$4.8^{b} \times 10^{4}$	$1.02^{d} \times 10^{7}$	$3.2^{e} \times 10^{4}$	$1.9^{e} \times 10^{4}$	$3.7^{b} \times 10^{4}$
C (90% PWJ:10% JFS)	$3.6^{f} \times 10^{6}$	$4.9^{ab} \times 10^{4}$	$2.4^{d} \times 10^{4}$	$4.2^{c} \times 10^{4}$	$7.4^{g} \times 10^{4}$	$3.3^{e} \times 10^{4}$	$2.0^{e} \times 10^{4}$	$3.9^{c} \times 10^{4}$	$2.7^{i} \times 10^{6}$	$3.0^{e} \times 10^{4}$	$1.8^{e} \times 10^{4}$	$3.4^{c} \times 10^{4}$
D (85% PWJ: 15% JFS)	$7.9^{g} \times 10^{5}$	$4.3^{c} \times 10^{4}$	NG	NG	$6.9^{h} \times 10^{4}$	$3.1^{e} \times 10^{4}$	NG	$3.1^{e} \times 10^{4}$	$6.2^{g} \times 10^{4}$	$3.0^{e} \times 10^{4}$	NG	$2.9^{d} \times 10^{4}$
E (80% PWJ: 20% JFS)	$7.4^{i} \times 10^{5}$	$4.0^{c} \times 10^{4}$	NG	NG	$6.1^{j} \times 10^{4}$	$3.0^{e} \times 10^{4}$	NG	$3.0^{e} \times 10^{4}$	$5.3^{i} \times 10^{4}$	$2.2^{f} \times 10^{4}$	NG	NG

Key: JFS- Jack Fruit Seed; WMJ- Watermelon juice; PPJ-Pineapple juice; PWJ-Pawpaw juice; NG- No growth, THBC-The total Heterotrophic Bacterial Count, TCC- Total Coliform Count, TFC- Total Fungal Count, LAB-Total Lactic Acid Bacteria Count, Values with different superscript down a column are significantly different from each other.

Table 2: Total microbial load (cfu/ml) of fruit juice preserved with jack fruit bark

Sample code	24hrs THBC	TCC	TFC	LAB Count	48hrs THBC	TCC	TFC	LAB Count	96hrs THBC	TCC	TFC	LAB Count
A Control (100% WMJ)	2.60^{a} x 10^{7}	8.1^{a} x 10^{4}	1.6^{c} x 104	NG	2.80^{a} x 10^{7}	8.4^{a} x 10^{4}	2.6^{b} x 10^{4}	2.2^{d} x 10^{4}	3.04^{a} x 10^{7}	8.8^{a} x 10^{4}	4.2^{b} x 10^{4}	3.7^{c} x 10^{4}
B (95%WMJ: 5% JFB)	1.51^{b} x 10^{7}	7.8^{b} x 10^{4}	NG	NG	1.26^{c} x 10^{7}	6.9^{b} x 10^{4}	1.9^{d} x 10^{4}	1.7^{f} x 10^{4}	1.13^{d} x 10^{7}	5.02^{b} x 10^{4}	2.1^{d} x 10^{4}	2.8^{d} x 10^{4}
C (90%WMJ: 10% JFB)	1.36^{d} x 10^{7}	7.0^{c} x 10^{4}	NG	NG	1.18^{e} x 10^{7}	6.4^{d} x 10^{4}	NG	NG	1.03^{e} x 10^{7}	3.9^{h} x 10^{4}	NG	NG
D (85%WMJ: 15% JFB)	1.08^{g} x 10^{7}	6.8^{c} x 10^{4}	NG	NG	8.3^{i} x 10^{6}	5.1^{e} x 10^{4}	NG	NG	8.0^{g} x 10^{6}	4.8^{f} x 10^{4}	NG	NG
E (80% WMJ: 20% JFB)	9.2^{h} x 10^{6}	5.3^{e} x 10^{4}	NG	NG	8.1^{i} x 10^{6}	4.7^{f} x 10^{4}	NG	NG	7.3^{h} x 10^{6}	4.2^{g} x 10^{4}	NG	NG
A Control (100% PPJ)	1.53^{b} x 10^{7}	6.8^{c} x 10^{4}	1.8^{c} x 10^{4}	NG	1.64^{b} x 10^{7}	7.2^{c} x 10^{4}	3.1^{a} x 10^{4}	3.3^{b} x 10^{4}	1.78^{c} x 10^{7}	7.7^{c} x 10^{4}	3.8^{c} x 10^{4}	4.2^{b} x 10^{4}
B (95% PPJ: 5% JFB)	1.43^{c} x 10^{7}	6.4^{d} x 10^{4}	1.8^{c} x 10^{4}	NG	1.16^{e} x 10^{7}	6.0^{d} x 10^{4}	1.7^{d} x 10^{4}	1.9^{e} x 10^{4}	1.03^{e} x 10^{7}	5.1^{e} x 10^{4}	1.3^{f} x 10^{4}	2.2^{e} x 10^{4}
C (90% PPJ: 10% JFB)	1.28^{e} x 10^{7}	5.3^{e} x 10^{4}	7.0^{e} x 10^{3}	NG	9.6^{g} x 10^{6}	4.7^{f} x 10^{4}	1.8^{d} x 10^{4}	NG	8.8^{f} x 10^{6}	4.3^{g} x 10^{4}	2.1^{d} x 10^{4}	NG
D (85% PPJ: 15% JFB)	8.6^{i} x 10^{6}	4.9^{f} x 10^{4}	NG	NG	8.1^{i} x 10^{6}	3.9^{g} x 10^{4}	NG	NG	7.2^{h} x 10^{6}	3.3^{i} x 10^{4}	NG	NG
E (80% PPJ: 20% JFB)	8.2^{j} x 10^{6}	4.3^{g} x 10^{4}	NG	NG	7.8^{j} x 10^{6}	3.1^{h} x 10^{4}	NG	NG	6.8^{i} x 10^{6}	2.6^{j} x 10^{4}	NG	NG
A Control (100% PWJ)	1.49^{c} x 10^{7}	4.7^{f} x 10^{4}	2.6^{a} x 10^{4}	3.1^{a} x 10^{4}	1.61^{b} x 10^{7}	5.2^{e} x 10^{4}	3.4^{a} x 10^{4}	4.2^{a} x 10^{4}	2.12^{b} x 10^{7}	6.3^{d} x 10^{4}	4.8^{a} x 10^{4}	5.7^{a} x 10^{4}
B (95% PWJ: 5% JFB)	1.26^{e} x 10^{7}	4.0^{g} x 10^{4}	2.2^{b} x 10^{4}	2.8^{b} x 10^{4}	1.22^{d} x 10^{7}	3.8^{g} x 10^{4}	2.1^{bc} x 10^{4}	2.4^{d} x 10^{4}	1.17^{d} x 10^{7}	3.1^{i} x 10^{4}	1.8^{de} x 10^{4}	2.4^{e} x 10^{4}
C (90% PWJ:10% JFB)	1.18^{f} x 10^{7}	3.7^{h} x 10^{4}	1.8^{c} x 10^{4}	2.8^{b} x 10^{4}	1.06^{f} x 10^{7}	3.3^{h} x 10^{4}	1.7^{d} x 10^{4}	2.8^{c} x 10^{4}	1.02^{e} x 10^{6}	2.4^{j} x 10^{4}	1.6^{e} x 10^{4}	2.5^{de} x 10^{4}
D (85% PWJ: 15% JFB)	9.6^{h} x 10^{6}	3.1^{i} x 10^{4}	1.8^{c} x 10^{4}	1.6^{c} x 10^{4}	8.8^{h} x 10^{6}	2.9^{i} x 10^{4}	2.3^{b} x 10^{4}	2.2^{d} x 10^{4}	8.0^{g} x 10^{6}	2.0^{k} x 10^{4}	2.0^{d} x 10^{4}	1.8^{f} x 10^{4}
E (80% PWJ: 20% JFB)	9.4^{h} x 10^{6}	2.6^{j} x 10^{4}	1.3^{d} x 10^{4}	1.3^{c} x 10^{4}	7.9^{j} x 10^{6}	1.8^{j} x 10^{4}	1.3^{e} x 10^{4}	1.9^{e} x 10^{4}	7.0^{i} x 10^{6}	1.8^{k} x 10^{4}	1.0^{f} x 10^{4}	1.5^{f} x 10^{4}

Key: JFB- Jack Fruit Bark; WMJ- Watermelon juice; PPJ-Pineapple juice; PWJ-Pawpaw juice; NG- No growth
THBC-The total Heterotrophic Bacterial Count, TCC- Total Coliform Count, TFC- Total Fungal Count, LAB-Total Lactic Acid Bacteria Count, Values with different superscript down a column are significantly different from each other.

Table 3: Distribution of microorganisms in 96 hours jackfruit seeds preserved juices

Samples	*E.coli*	*Bacillius* sp	*Salmonella* sp	*Staphylococcus aureus*	*Citrobacter* sp	*Micrococcus* sp	*Lactobacillus* sp	*Rhizopus* sp	*Aspergillus* sp	*Penicillium* sp	Total
WMJ 100:0 (Control)	1(4.5)	5 (21)	1(4.5)	3 (13)	3 (13)	2 (9)	3 (13)	0 (00)	3 (13)	2 (9)	23 (100)
WMJ 95%: JFS 5%	0 (00)	3(27.3)	1(9.1)	2(18.2)	1(9.1)	1 (9.1)	2(18.2)	0 (00)	1(9.1)	0 (00)	11 (100)
WMJ 90%: JFS 10%	0 (00)	2 (50)	0 (00)	1 (25)	0 (00)	0 (00)	1 (25)	0 (00)	0 (00)	0 (00)	4 (100)
WMJ 85%: JFS 15%	0 (00)	1(100)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	1 (100)
WMJ 80%: JFS 20%	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)
PPJ 100:0 (Control)	1(4.8)	4 (19)	3(14.3)	4 (19)	3(14.3)	2 (9.5)	0(00)	1 (4.8)	0(00)	3(14.3)	21(100)
PPJ 95%: JFS 5%	0 (00)	2 (25)	1(12.5)	2 (25)	1(12.5)	1(12.5)	0(00)	0(00)	0(00)	1(12.5)	8(100)
PPJ 90%: JFS 10%	0 (00)	2(66.7)	0(00)	1(33.3)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	3(100)
PPJ 85%: JFS 15%	0 (00)	1(100)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	1(100)
PPJ 80%: JFS 20%	0 (00)	0 (00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
PWJ 100:0 (Control)	1 (4)	6 (24)	2 (8)	3 (12)	2 (8)	1 (4)	3 (12)	2 (8)	2 (8)	3 (12)	25 (100)
PWJ 95%: JFS 5%	1(9.1)	3(27.3)	1 (9.1)	1 (9.1)	0(00)	0(00)	2(18.2)	1(9.1)	0(00)	2(18.2)	11(100)
PWJ 90%: JFS 10%	0 (00)	2 (50)	0(00)	0(00)	0(00)	0(00)	1 (25)	0(00)	0(00)	1 (25)	4(100)
PWJ 85%: JFS 15%	0 (00)	1(100)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	1(100)
PWJ 80%: JFS 20%	0 (00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)

Key: WMJ = watermelon Juice; PPJ = Pineapple Juice; PWJ = Pawpaw juice; JFS = Jack Fruit Seed

Table 4: Distribution of microorganisms in 96 hours jackfruit bark preserved juices

Samples	*E.coli*	*Bacillus* sp	*Salmonella* sp	*Staphylococcus aureus*	*Citrobacter* sp	*Micrococcus* sp	*Lactobacillus* sp	*Rhizopus* sp	*Aspergillus* sp	*Penicillium* sp	Total
WMJ 100:0 (Control)	1(4)	4 (16)	2(8)	4 (16)	4 (16)	3 (12)	2 (8)	1 (4)	1 (4)	3 (12)	25 (100)
WMJ 95%: JFB 5%	0 (00)	3(25)	1(8.3)	3(25)	2(16.7)	1 (8.3)	1(8.3)	0 (00)	0 (00)	1 (8.3)	12 (100)
WMJ 90%:JFB 10%	0 (00)	1(33.3)	0(00)	1(33.3)	0 (00)	0 (00)	1(33.3)	0 (00)	0 (00)	0 (00)	3 (100)
WMJ 85%:JFB 15%	0 (00)	1(100)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	1 (100)
WMJ 80%:JFB 20%	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)
PPJ 100:0 (Control)	1(5.5)	3(16.7)	3(16.7)	2(11.1)	2(11.1)	3(16.7)	1(5.5)	0 (00)	0(00)	3(16.7)	18(100)
PPJ 95%: JFB 5%	0 (00)	2(25)	1(12.5)	1(12.5)	1(12.5)	1(12.5)	0(00)	0(00)	0(00)	2(25)	8(100)
PPJ 90%: JFB 10%	0 (00)	1(33.3)	0(00)	1(33.3)	0(00)	0(00)	0(00)	0(00)	0(00)	1(33.3)	3(100)
PPJ 85%: JFB 15%	0 (00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
PPJ 80%: JFB 20%	0 (00)	0 (00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
PWJ 100:0 (Control)	1 (4.3)	4(17.4)	1 (4.3)	2 (8.7)	1 (4.3)	2 (8.7)	3(13.1)	2 (8.7)	3(13.1)	4(17.4)	23 (100)
PWJ 95%: JFB 5%	0 (00)	2(22.2)	0 (0.0)	1(11.1)	0(00)	1(11.1)	1(11.1)	1(11.1	1(11.1)	2(22.2)	9(100)
PWJ 90%: JFB 10%	0 (00)	1 (50)	0(00)	0(00)	0(00)	0(00)	0 (00)	1(00)	0(00)	1 (50)	2(100)
PWJ 85%: JFB 15%	0 (00)	1(100)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	1(100)
PWJ 80%: JFB 20%	0 (00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)

Key: WMJ = watermelon Juice; PPJ = Pineapple Juice; PWJ = Pawpaw juice; JFS = Jack Fruit Bark

DISCUSSION

Fruits such as pineapple and watermelon contain high concentrations of vitamins, minerals, amino acids, and sugars, thereby providing an excellent environmental condition for the development of a wide range of microorganisms that can lead to the spoilage of these fruits (Rahman *et al*., 2011; Khanam *et al*., 2018). Hence, to minimize or prevent the spoilage of these fruits, the use of natural preservatives is imperative.

In this study, the bacterial and fungal population enumerated showed a decrease in the microbial counts of fruit juices treated with jackfruit seeds and bark during the 96 hours storage period.

As the days of storage progressed, the rate of deterioration of the fruit juices declined**.** Microbial populations were lower in the preserved juices compared to unpreserved/ untreated fruit juices (controlled samples). This may be as a result of the contents in jackfruit which can inhibit and cause decline in the growth of the microorganisms in the juices (Burci *et al*. 2018). This is in accordance with the findings of Fauzi, *et al*. (2013), which states that jackfruit wood has antimicrobial substances which can affect microorganism activities and act as bacteriostatic in lower concentration and as bactericidal in high concentration. It may also be due to the synergistic effects of the bioactive phytochemical constituents in the extracts. This agrees with the findings of Adeogun *et al*. (2016) who attributed the activity of natural antimicrobials present in *Thaumatococcus daniellii* to be due to the synergy among the avalanche of phytoconstituents present in the plant extracts which was also observed in this study.

The various concentrations of the treatment ratios is directly proportional to the microbial loads obtained as the higher the treatment concentration, the lower the microbial load. This suggests that the higher the concentration of treatment addition, the more effective the preservation of the stored juice would be. The decrease in the bacterial load could also be as a result of the decrease in the pH of the juice as the storage period progresses as most bacteria such as those present in this study do not grow at low pH, while the reduction in fungi load could be due to deposited antimicrobials (Ike *et al*., 2020). From this study, *Escherichia coli*, *Bacillus* species, *Salmonella* species, *Staphylococcus aureus*, *Citrobacter* species, *Micrococcus* species, *Lactobacillus* species, *Rhizopus* species, *Aspergillus* species and *Penicillin* species were isolated. These contaminating microorganisms comprises of both spoilage and potential pathogenic organisms. The presence of microorganisms in the fruit juices may be as a result of the quality of the raw materials used, the equipment used for processing, handling practices employed, unsanitary storage conditions used and the environment (Afroz *et al*., 2013). In this study, the presence of *E. coli* and *Salmonella* was identified only in very few numbers and in a smaller part of the samples. The presence of *E. coli* and *Salmonella* in fruit juices are of great concern as these pathogens are of feacal origin and have been associated with a lot of outbreaks related to fruit juices (Raybaudi-Massilia *et al*., 2009). *Bacillus* species were detected across all the fruit juice samples investigated and were the most occurring microorganism in all fruit juices preserved and analyzed. This could be due to the presence of their endospores which are able to tolerate environmental stresses (Willey *et al*., 2008; Ndip and Njom, 2019). *Staphylococcus* sp are common contaminants that are often introduced into foods from handlers of the foods and the environment. The presence of *Staphylococcus* spp in the juice could be attributed to post process contamination. Lactic acid bacteria (LAB) regularly occur in juices that are unpasteurized (Oliveira *et al*., 2006). *Lactobacillus* and *Leuconostoc* have been reported as important spoilage microorganisms in products that are acidic (Keller and Miller, 2006). Lactic acid bacteria (LAB) produces lactic acids in fruit juices along with small amounts of acetic and gluconic acids, ethanol and carbon dioxide, thereby resulting in the alteration of the flavour of the juice (Jay and Anderson, 2001) and reduction in the pH of the juices. Moulds which are also spore formers and common food contaminants from the environment are able to cause spoilage of fruit juices by causing loss of juice cloud (Lawlor *et al*., 2009). Amidst these, some moulds such as *Aspergillus* and *Penicillium* species have the potential of producing mycotoxins which pose great risks to the health of humans who consume the juices. *Rhizopus* species are also known to cause the spoilage of fresh fruits, juices and vegetables (Moss, 2008). Hence, in order to prevent spoilage and food borne illness, there is need to control the existence/ presence of these organisms in fruit juices even in low numbers (Mudgil *et al.,* 2004; Oranusi *et al.,* 2007).

In general, the distribution of microbial population during storage was quite low and some totally disappeared at the end of the 96 hours of storage. Less numbers of microorganisms were isolated compared to unpreserved fruit juices (controlled samples). The low microbial counts could be associated to the antimicrobial effect of jackfruit seed and bark. The added ratios of the jackfruit seeds and bark might have prevented or halted the growth of microbes. Sousa *et al*. (2021) have reported the bactericidal (antimicrobial) effect of jackfruit dry leaf extracts against *E. coli,* methicillin resistant *S. aureus* and *S. enterica.* Also, Ruiz-Monta´nez *et al.* (2015) and Burci *et al*. (2018) have reported the effective antimicrobial activity of jackfruit against microorganisms such as *Streptococcus mutans, Streptococcus pyogenes, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Penicillium italicum* and *Candida albicans* which are similar to the microorganisms isolated from this study.

CONCLUSION

The demand for fresh wholesome fruit juices has been on the increase because of their health benefits. The major challenge with fresh fruit juices is the presence of microorganisms which are potentially hazardous to public health and their limited shelf life resulting in continuing usage of synthetic chemicals as preservatives. Hence, by addressing these concerns, the potential risks to consumer health can be mitigated and the safe consumption of fruit juices ensured by the addition of natural antimicrobials of plant such as jackfruit to fruit juices which are with little or no negative health consequences. Jack fruit seed and bark extracts has been found quite effective as a bio-preservative, therefore could serve as a good candidate for use in extending the shelf life of fruit juices. This is based on the ability of the extracts to effectively prevent or inhibit the growth of spoilage and pathogenic microorganisms isolated in this study and making the consumption of wholesome juices realistic. This will make maintaining the quality of fruit juices and avoiding food borne disease outbreaks an easy task.

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