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The Antimicrobial Efficacy and Quality Changes in Pineapple Juice Treated with Natural Preservatives

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Authors' contributions

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

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ABSTRACT

The study investigated effects of natural preservatives on the quality of fresh pineapple fruit juice. The natural preservatives used are ginger, garlic, moringa seed and cloves and were processed into powder. The treatment of pineapple with the natural preservatives was at a concentration of 20% with 3 minutes dipping time. The enumeration and identification of microorganisms on the fresh pineapple fruit, shows that the total bacteria count was $1.8{\times}10^5$ colony forming unit per gram (CFU/g) and $4.6{\times}10^3$ CFU/g respectively. The identified bacteria are *staphylococcus aureus* and *streptococcus spp* and identified fungi were *Aspergillus Niger, penicillin spp and Aspergillus perfrigens* respectively. Moringa seed extract exhibited moderate antibacterial activity, with zones of

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inhibitions (ZOIs) of 16 mm against *Staphylococcus aureus* and 15 mm against *Streptococcus* spp. Garlic (GL) showed the least antibacterial activity among the natural preservative samples, with 9mm zones of inhibition against *Staphylococcus aureus* and 4 mm against *Streptococcus* spp. Clove extract displayed significant antibacterial activity, with 14 mm zones of inhibition against *Staphylococcus aureus* and 19 mm against *Streptococcus* spp. Ginger extract exhibited slight antibacterial activity, with zones of inhibition of 4 mm against both bacterial strains. Chemical composition of the treated pineapple juice result ranges from 0.77-0.865% TTA, 64.12-63.10 Vit C, 26.50-25.06 Brix Content,3.20-3.39 pH and 13.23-13.17 TSS respectively. The control (100% pineapple Juice) displayed balanced properties serving as an effective baseline3. Storage studies, ambient stored samples were analyzed at 0 day and 21 days intervals. All samples were subjected to standard analytical methods. Results showed increase in number of bacteria and fungi with increase in the number of days. The use of cloves, garlic, ginger, and moringa seeds as natural antimicrobial agents in pineapple juice is effective in controlling microbial growth, thus enhancing the safety and shelf life of the juice. These treatments also influence the chemical and sensory properties of the juice, contributing to consumer acceptability.

Keywords: Pineapple; antimicrobial; storage stability; zones of inhibition.

1. INTRODUCTION

Pineapple (*Ananas comosus)* is a tropical fruit known for its distinct sweet and tangy flavor. Pineapple offers several nutrients and potential health benefits. They are a good source of various vitamins and minerals. Pineapple contain vitamin C, manganese, vitamin B6, thiamin, and folate, among others [1]. Pineapples are a rich source of bromelain, a mixture of enzymes with potential digestive and anti-inflammatory properties. According to Daba et al. [2] Bromelain has potential benefits in improving digestion and reducing inflammation. The high vitamin C content in pineapple, plays crucial role in boosting the immune system and promotion skin health [3]. It is also a good source of vitamin B6, manganese and dietary fiber [1]. Additionally, it contains bromelain enzyme with potential anti-inflammatory properties [1]. David et al. [4] consumption of pineapple juice regularly may have several health benefits. Its antioxidant rich profile may help combat oxidative stress and reduce the risk of chronic disease. Bromalin found in pineapple juice has been investigated for its potential anti-inflammatory and digestive benefits [5].

Ginger (Zingiber officinale), a well-known spice, is celebrated for its unique flavor profile and medicinal properties. Studies have highlighted ginger's potential as a natural preservative due to its antimicrobial and antioxidant effects. Ginger extracts have been found to inhibit the growth of spoilage bacteria and molds, making them a promising ingredient for extending the shelf life of food products [6,7].

Cloves (Syzygium aromaticum), the aromatic flower buds of the clove tree, have been employed for centuries not only for their culinary uses but also for their potent antimicrobial and antioxidant properties. Research suggests that clove extracts and essential oils exhibit strong antibacterial, antifungal, and antioxidant activities, making them effective natural preservatives in various food products [8,9].

Garlic (Allium sativum), with its distinctive pungent aroma and flavor, has been utilized not only for its culinary value but also for its remarkable antimicrobial properties. Allicin, a key compound in garlic, has been shown to exhibit strong antibacterial and antifungal effects. Garlic extracts and garlic-derived compounds have the potential to serve as natural preservatives in a range of food products [10,11].

Moringa Seed (*Moringa oleifera)*, often referred to as the "drumstick tree," has on gained attention for its various health benefits. Moringa seeds, in particular, contain bioactive compounds with antioxidant and antimicrobial properties. These properties suggest that moringa seed extracts can function as natural preservatives, helping to extend the shelf life of food products [2,12].

2. MATERIALS AND METHODS

2.1 Materials

The fresh root of ginger (*Zingiber officinale*) cloves, garlic, moringa seed and pineapple fruit was purchased from

Wurukum Market, *staphylococcus aureus spp,* and *streptococcus spp* were collected from Federal Medical Centre Wadata, Makurdi, Benue State and taken to the Food Science and Technology Laboratory, Joseph Sarwaun University for processing and analysis.

2.2 Preparation of Ginger Powder

The gingers were washed several times with tap water (portable water). They were peeled, rewashed with tap water, sliced into fillets of 2-3 diameter thick. They were oven dried at 50^{O}C for 12 h. The dried gingers were milled into powder using kitchen blender (model: Binatone BLG-452), and sieved through a sieve of pore size 0.5 mm [13]. The sieved ginger powder was stored in well-sealed plastic container at room temperature in a closed cupboard to avoid UV light.

2.3 Preparation of Moringa Seed Powder

Matured moringa seeds were manually removed from the seed kernels and dried using sun drying (for 7days). The dried seeds were milled in a clean blender. The moringa seed powder were sieved using a sieve of 0.5 mm mesh size, to obtain a fine powder [1]. The flow chart for the production of moringa seed powder is shown in Fig. 2.

2.4 Preparation of Garlic Powder

The method of Douglas et al. [14] was used in preparation of the garlic powder, as shown in Fig. 3. The outer cover of the garlic rhizomes were peeled, after which they were washed with clean water, sliced with sharp knife and oven dried at 55ºC for 24 h. The garlic rhizomes were grinded into powder using electric grinder. The powder was sieved with a 0.5 mm mesh.

Fig. 1. Flow chart for preparation of Ginger
Source: [13] with modification

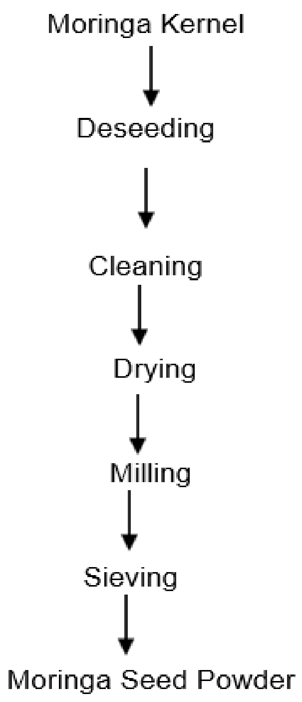

Fig. 2. Flowchart for the preparation of Moringa Seed Powder
Source: [1] modified

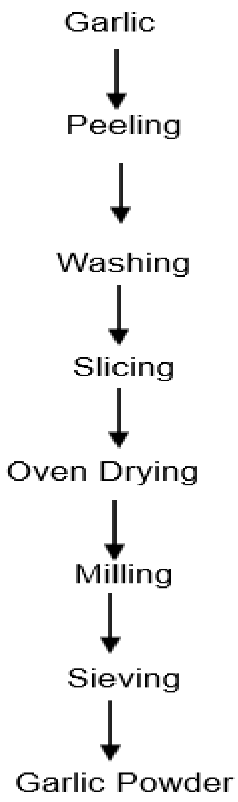

Fig. 3. Flow chart for the preparation of Garlic powder
Source: [14] with slight modification

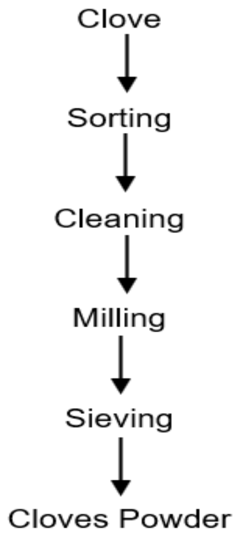

Fig. 4. Flow chart for preparation of cloves powder
Source: [15] with slight modification

2.5 Preparation of Cloves Powder

Cloves powder was prepared according to the method of Kumar and Tanwar [15] as shown in Fig. 4. Cloves were sorted, cleaned and milled into powder. They were further stored in air-tight container until further usage.

2.6 Formulation of Pineapple Fruit Treatment with the Natural Preservatives

Sliced fresh pineapple fruit was dipped into 20% of the natural preservatives solution for 3 minutes and removed and put into juice extractor to obtain pineapple fruit juice. The juice was packed, label and refrigerated.

2.7 Preparation of Fresh Pineapple Juice

The pineapple fruits were washed and rinsed thoroughly with potable water. The fruits were peeled with sterile stainless knife. The pineapple fruits were cut into small pieces with a sterile knife and homogenized in a clean electric Juicer [16].

2.8 Enumeration, Insolation and Identification of Microorganisms on Pineapple Fruit

A ten-fold serial dilution of each of the samples were carried out. Spread plate technique was employed by inoculating 0.1ml of the appropriate dilutions on plate count agar plate [17] for enumeration of bacteria and on potato dextrose agar for fungal count. The agar plates were incubated at 30 oC for 24-48 h for bacterial count and at 26 oC for 48-72 h for fungal count. Each sample was inoculated in duplicate agar plates and the mean values of bacterial and fungal counts were recorded as colony forming unit per ml (Cfu/ml). the bacterial and fungi isolates were characterized based on their cultural and morphological features as described by Cheebrough [18]. Bacterial isolates were identified following Gram reaction, spore staining, motility test and biochemical tests (indole, methyl red, Voges Proskauer, citrate, catalase, coagulase, oxidase, urease, sugar-fermentation).

2.9 Determination of Antimicrobial Properties of the Natural Preservatives

From the identification of microorganisms on pineapple fruits, the known microorganisms (*Staphylococcus aureus spp*, and (*streptococcus spp)* were collected from Federal Medical Centre Wadata, Makurdi Benue State. Using Agar well diffusion method, 0.1ml of each organism were taken into 9.9ml of sterile distilled water to obtain 1:100 of the dilution of the organism. 0.2 ml of each of the diluted organism was put into the prepared sterile nutrient agar. It was poured aseptically into the sterile plate and allowed to solidify for 45 mins. Using a sterile cork borer of 6 mm diameter, the wells were made including the controls. The duplicates were made to ascertain the results obtained. The plates were allowed on the bench for about 2 h to allow the extract to diffuse properly into the nutrient agar. The plates were incubated uprightly for at 37 ^{o}C for 24 h and the zone of inhibition was measured in mm using a ruler [18].

2.10 Physiochemical Properties of the Treated Pineapple Juice

2.10.1 Determination of titratable acidity of the pineapple juice

Association of Official Analytical Chemists [19] A 10 g sample of pineapple juice was weighed. Then, the sample was transferred to a 500 ml Erlenmeyer flask. The sample was diluted to 250 ml with deionized water. Using a standard solution of 0.1 N sodium hydroxide (Hanns, analytical grade), the sample was titrated to the end point. The end point was determined using pH meter and phenolphthalein indicator. 1 ml of phenolphthalein indicator was added to the sample and titrated to faint pink end point. The volume of 0.1 N sodium hydroxide used was recorded. The total acidity was calculated using equation (1) and expressed as concentration of citric acid (g/l). The measurement was repeated three times.

% Acid (as anhydrous citric acid) = Volume of 0.1 N NaOH (ml) × 0.64 / 10 eqn (1)

2.10.2 Determination of pH of the treated pineapple juice

pH was determined using [19] procedure. Each juice (25ml) was pipette into a beaker and inserted pH probe of the pH meter into it after the pH has been standardized in buffer 4 and 7 solution at 25^{o}C, it was titrated against 0.1N NaOH solution until the pH meter displayed 8.10 at the end point.

2.10.3 Determination of vitamin C of the treated pineapple juice

Association of Official Analytical Chemists [19] 1ml of juice sample was mixed with 9 ml of metaphosphoric acid-acetic acid solution. The mixture was centrifuge to remove any solids. 1 ml of bromine water was added to the sample mixture and mix well. It was allow to stand for 1-2 mins to allow oxidation to occur. 1 ml of chromogen solution was added and mix well. It stand for 30-60 mins to allow color development. The absorbance of the colored complex was measured at 520 nm using a spectrophotometer.

Vitamin C Content (mg/100mL) = (Absorbance of Sample / Absorbance of Standard) x (Concentration of Standard / Dilution Factor) x 100 (2)

2.10.4 Determination of total soluble solid of the pineapple juice

Method described by Association of Official Analytical Chemists [19] was adopted for determination of total soluble solid. Total soluble solid of the pineapple juice was determined by using a hand refract meter (Erma, Japan) with solid scale in the range of 0 to 32 ^{o}Bix.

2.10.5 Determination of ^{O}Brix content of the pineapple juice

Association of Official Analytical Chemists [19] Clarifying the juice sample by centrifugation, 1:10 of the sample was diluted with water to obtain a suitable concentration. 1 mL of the diluted sample was mixed with 1 mL of cupric sulfate solution and 1 mL of alkaline tartrate solution in a test tube. 1 mL of reducing sugar standard solution was added. The mixture was incubated at room temperature (20-25°C) for 10-15 minutes. The mixture turned yellow or orange due to the formation of a colored complex. using a spectrophotometer, the absorbance of the reaction mixture at 420 nm (or 450 nm) was measured.

Calculation:

Reducing Sugar Content (%) = (Absorbance of Sample / Absorbance of Standard) x (Concentration of Standard / Dilution Factor) x 100 (3)

2.11 Sensory Analysis of the Treated Fresh Pineapple Juice

The sensory evaluation was carried out by 10 panelists composed of 5 males and 5 females. The evaluation was based on quality parameters such as sweetness, tartness, color, odor and overall acceptability (using questionnaire) by the panel of 10 testers. The test panelists were asked to rate the different pineapple juice presented to them on a 9-point hedonic scale with the ratings of: 9= like extremely; 8 = like very much; 7= like moderately; 6 = like slightly; 5 = neither like nor dislike; 4= dislike slightly; 3 = dislike moderately; 2 = dislike very much and 1= dislike extremely [20].

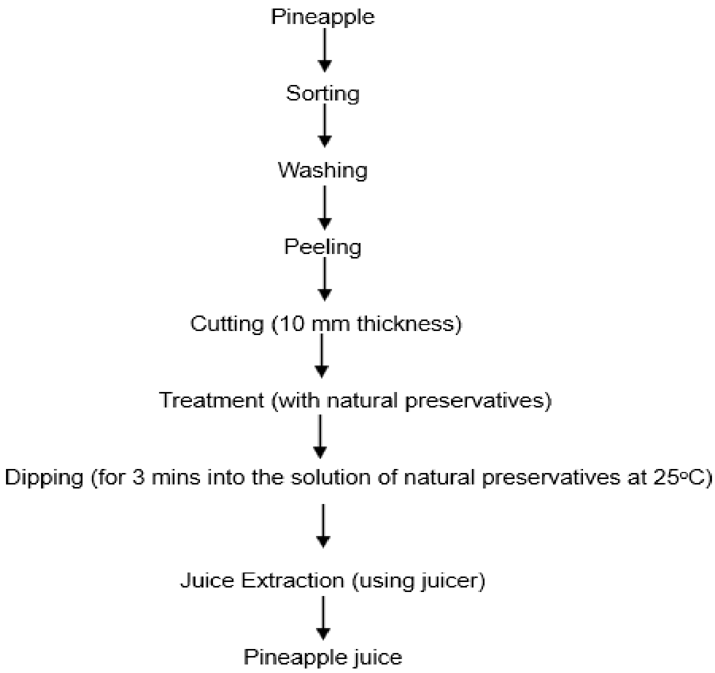

Fig. 5. Flow chart for production of pineapple juice
Source: [16] with slight modification

2.12 Storage Stability of the Treated Fresh Pineapple Juice

The fresh pineapple juice sample containing different concentrations of the natural preservatives, was stored at 4^oC temperature for 21 days. The control sample was 200ml of the pineapple juice without any treatment.

2.13 Statistical Analysis of the Treated Fresh Pineapple Juice

The effect of treatments on the different parameters was analyzed using analysis of variance (ANOVA) as described by Iwe [21]. Least significant difference (LSD) was used to ascertain differences between samples at p 0.05.

3. RESULTS AND DISCUSSION

3.1 Enumeration of Microorganisms on Pineapple Fruit

The microbiological population observed in this study is summarized in Table 1. The process of counting the number of microorganisms in a sample is known as microbial enumeration. Microbial enumeration is very essential to sectors such as pharmaceuticals, food production, and environmental monitoring [22]. The total bacteria counts ranges from 1.8×10^5 colony forming units (cfu/ml) while the total fungal count ranges from 4.6×10^3 respectively. The pineapple fruit has a significant amount of bacteria, as seen by the Total Bacterial Count (TBC) of 1.8×10^5 CFU/ml. This result is in line with research [18] which similarly found that handling procedures and environmental exposure led to high bacterial burdens on fresh food. According to Cheebrough [18], there were 10^4–10^6 CFU/ml of bacteria on a variety of fruits and vegetables, indicating how common bacterial contamination is in fresh produce. Despite being lower than the TBC, the Total Fungal Count (TFC) of 4.6×10^3 CFU/g indicates a considerable presence of fungal growth on the pineapple fruit. De Oliveira et al. [23] also discovered fungal counts on tropical fruits ranging from 102 to 10^4 CFU/ml, with higher counts frequently connected to poor storage. Similar levels of fungal contamination have been recorded.

3.2 Antimicrobial Effects of the Natural Preservatives

3.2.1 Antibacterial activity test of the natural preservatives

The antibacterial activity test findings (Table 2) compare the effectiveness of natural preservatives (Moringa seed, garlic, cloves, and ginger) against the pathogens found in pineapple fruit, *Staphylococcus aureus* and *Streptococcus spp.* The diameter of the clear zone surrounding the disc where bacterial growth is stopped is indicated by the zones of inhibition (ZOI), which are measured in millimetres. Chemicals with antibacterial action are those that kill or inhibit the growth of bacteria locally [24].

3.2.2 Antifungi activity testing of the natural preservatives

*Aspergillus Niger, Penicillium spp.*and *Aspergillus perfrigen*s were the fungal species that the natural extracts of moringa seed, garlic, cloves, and ginger were tested against and they were the identified fungi in pineapple fruit.The findings of the testing indicate that these extracts lacked any antifungal action. This implies that these extracts did not successfully stop these fungal species from growing as shown in Table 2. On the other hand, a known antifungal medication called fluconazole had shown antifungal activity against each of the three fungal species, demonstrating its effectiveness in preventing fungal development. There could be a number of reasons for the natural extracts' lack of antifungal action, including the extract's composition, concentration, and solvent type. Furthermore, the result shows that the fungus species found in pineapple fruit are resistant to the bioactive substances found in the extracts. The results of this investigation are consistent with Singh et al. [25] who revealed that garlic extract showed no antifungal action against Aspergillus perfrigens, while [26] found that extract from moringa seeds had no effect against *Aspergillus Niger*. Furthermore, [27] also found that cloves extract had no antifungal activity against *penicillium spp.*

Table 1. Result of Enumeration of microorganisms on pineapple fruit

Sample	TBC(CFU/g)	TFC(CFU/g)
Pineapple fruit	1.8×10^{5}	4.6×10^{3}

Key: TBC= Total Bacterial Count; TFC= Total Fungi Count

3.3 Physiochemical Properties of the Treated Pineapple Juice

The results shown in Table 4 demonstrate that the addition of natural preservative blends to pineapple juice significantly affects its physicochemical properties. The findings shows that the type and concentration of spice and herb blends used can either enhance or diminish the quality and nutritional value of pineapple juice. Serving as the standard for comparison, the control sample consisted of 100% pineapple juice and showed moderate levels of acidity, vitamin C, and sugar content, all of which are characteristic of natural pineapple juice [28] assessment of unprocessed pineapple juice revealed similar results of pineapple juice with moderate acidity, vitamin C and Sugar content.

Sample B revealed a little greater acidity (TTA) and a pH that was comparable to the control, indicating a slightly more acidic nature that might enhance preservation but might also have an impact on flavour. The vitamin C content was marginally lower than the control, consistent with the finding by Jones and Zhang [29] that exposure to specific spices may cause vitamin C to degrade. Additionally, the °Brix value was marginally lower, suggesting a minor decrease in sweetness. Sample C showed a notable increase in vitamin C content and °Brix, pointing to an improvement in taste and nutritional value. This increase is due to the high vitamin C content of moringa seeds as stated by White and Green [30] As the TTA and pH readings were comparable to the control, there was no discernible change in acidity. Sample D produced the highest TTA while preserving high vitamin C and °Brix levels. According to Smith and Patel [28] the preservation and nutritional enhancing qualities of these substances, the combination of cloves and moringa seed appears to improve the juice's acidity and nutritional profile. Sample E had the most acidic profile, as indicated by the highest TTA. The lowest amount of vitamin C was found, as a result of vitamin C's instability when components from garlic and ginger are present as a stated by Jones and Zhang [29] A less sweet profile was indicated by the lower TSS and °Brix values.

3.4 Sensory Attributes of the Treated Pineapple Juice Sample

The Table 4 shows that while adding natural preservative mixes to pineapple juice alters its sensory qualities, these changes are not always bad. All sensory qualities showed that the control sample, which was 100% pineapple juice without any modification, scored the highest. This outcome is anticipated since it captures the true, unadulterated flavour profile of pineapple juice, which is typically well-liked by customers. Smith and Patel [28] reported similar results,

Table 2. Results of antibacterial testing activity of the natural preservatives

Sample	Conc. Mg/ml	(ZOI mm) *staph aureus*	(ZOI mm) *strep spp*
CP	200	19	29
MS	200	16	15
GL	200	9	4
CL	200	14	19
GG	200	4	4

Key: CL= Ciproflocaxin (control); MS= Moringa seed; GL= Garlic; CL= Cloves; GG= Ginger; ZOI= Zones of inhibition

Table 3. Results of antifungi testing activity of the natural preservatives

Samples	Conc. Mg/ml	*Aspergilius niger*	*Penicillin spp*	*Aspergilius perfrigens*
FL	200	19	17	20
MS	200	—	—	—
GL	200	—	—	—
CL	200	—	—	—
GG	200	—	—	—

Key: CL= Ciproflocaxin (control); MS= Moringa seed; GL= Garlic; CL= Cloves; GG= Ginger;
— = No Zones of inhibition

Table 4. Sensory attributes of the treated pineapple juice

Samples	Color	Tartness	Sweetness	Aroma	General acceptability
A	$9.00^{a}\pm0.00$	$8.20^{a}\pm0.42$	$8.70^{a}\pm0.43$	$9.00^{a}\pm0.00$	$9.00^{a}\pm0.00$
B	$7.60^{b}\pm0.52$	$7.30^{b}\pm0.48$	$7.40^{b}\pm0.52$	$7.90^{b}\pm0.32$	$7.60^{bc}\pm0.52$
C	$6.80^{c}\pm0.42$	$6.70^{c}\pm0.48$	$6.60^{c}\pm0.52$	$6.60^{c}\pm0.52$	$7.40^{bc}\pm0.52$
D	$6.80^{c}\pm0.42$	$7.30^{b}\pm0.68$	$7.60^{b}\pm0.52$	$7.30^{c}\pm0.48$	$7.90^{b}\pm0.52$
E	$6.90^{c}\pm0.74$	$6.50^{c}\pm0.53$	$6.60^{c}\pm0.52$	$7.00^{c}\pm0.82$	$7.10^{c}\pm0.74$

Values are means ± standard deviations of duplicate determinations. Means in same column with same superscripts do not differ significantly (p>0.05)
Key: A= 100% Pineapple juice without treatment (control); B =20% Cloves and Ginger; C = 20% Garlic and Moringa Seed; D =20% Cloves and Moringa Seed; E = 20% Ginger and Garlic

Table 5. Physiochemical properties of the treated pineapple juice

samples		Parameters			
	TTA	VIT C	^{O} BRIX	PH	TSS
A	$0.77^{b}\pm0.04$	$64.12^{c}\pm1.39$	$26.50^{ab}\pm2.12$	$3.20^{a}\pm0.10$	$13.23^{ab}\pm0.04$a
B	$0.83^{ab}\pm0.02$	$63.73^{c}\pm0.81$	$25.35^{b}\pm0.35$	$3.13^{a}\pm0.01$	$13.09^{b}\pm0.12$
C	$0.78^{ab}\pm0.04$	$70.56^{b}\pm0.14$	$28.22^{a}\pm0.21$	$3.23^{a}\pm0.14$	$13.26^{a}\pm0.03$
D	$0.83^{ab}\pm0.01$	$67.12^{a}\pm0.16$	$28.08^{a}\pm0.09$	$3.22^{a}\pm0.03$	$13.14^{ab}\pm0.04$
E	$0.87^{a}\pm0.01$	$63.10^{c}\pm0.01$	$25.06^{b}\pm0.07$	$3.40^{a}\pm0.42$	$13.17^{ab}\pm0.04$

Values are means ± standard deviations of duplicate determinations. Means in same column with same superscripts do not differ significantly (p>0.05)
Key: A= 100% Pineapple juice without treatment (control); B =20% Cloves and Ginger; C = 20% Garlic and Moringa Seed; D =20% Cloves and Moringa Seed; E = 20% Ginger and Garlic; TTA= Titratable acidity; TSS= Total soluble solid

emphasizing the natural attractiveness of unprocessed fruit liquids. In comparison to the control, the addition of 20% cloves and ginger marginally lowered the sensory scores. Scores for colour tartness and aroma suggest a discernible but not overpowering shift. Strong, distinctive flavours like those of ginger and cloves are known to change the sensory profile of food products [29]. The juice continued to be generally well-received despite the modifications. The combination of moringa seed and garlic received the lowest ratings for both colour and aroma. The lower aroma score was caused by the strong, pungent flavour of garlic [30]. Despite the well-known health advantages of moringa seed, the general acceptance was likewise lower, indicating that sample C might not be ideal for pineapple juice. Sample D received an intermediate score. Compared to the garlic pairings, the aroma was less altered, suggesting that cloves could somewhat disguise the moringa flavour. Strong spice flavours have been shown to affect sensory perception, according to Smith and Patel [28], however general acceptability remained lower than the control. Given the potent and occasionally overpowering character of garlic, the combination of ginger and garlic produced reasonably high scores for acidity and sweetness but a lower score for aroma [31]. In spite of this, the overall acceptance was fairly high, indicating that most of the panel found the sensory experience to be generally positive.

The microbial load of the control sample, which was made up of pure pineapple juice devoid of any preservatives, increased steadily over the course of the 21-day storage period.The CFU count increased to 3.5 x 10^4 CFU/mL on day 21, showing a notable increase in microbial

Table 6. Total bacteria plate count of the treated juice during storage (CFU/ml) × 10

Storage duration in days		Samples			
	A	B	C	D	E
O	2.4	2.0	1.5	1.2	1.7
7	2.7	2.4	1.8	1.4	2.1
14	3.1	2.7	2.4	1.8	2.6
21	3.5	3.0	2.7	2.2	2.8

Table 7. Total fungi plate count of the treated juice during storage (CFU/ml) × $10^{2.}$

Storage Duration (Days)		Samples			
	A	B	C	D	E
O	1.6	1.4	1.2	1.0	0.9
7	1.9	1.7	1.5	1.2	1.0
14	2.3	2.0	1.8	1.4	1.2
21	2.4	2.2	2.1	1.7	1.5

Table 8. Titratable acidity content of the treated pineapple juice during storage

Storage Days		TTA			
	A	B	C	D	E
O	0.77^a±0.04	0.83^a±0.02	0.78^a±0.04	0.83^a±0.01	0.87^a±0.01
7	0.77^a±0.04	0.83^a±0.02	0.78^a±0.04	0.83^a±0.01	0.87^a±0.01
14	0.73^a±0.05	0.71^b±0.01	0.72^a±0.00	0.70^b±0.01	0.75^b±0.01
21	0.29^b±0.00	0.31^c±0.03	0.29^b±0.02	0.31^c±0.01	0.29^c±0.01

Values are means ± standard deviations of duplicate determinations. Means in same column with same superscripts do not differ significantly (p>0.05)
Key: A= 100% Pineapple juice without treatment (control); B =20% Cloves and Ginger; C = 20% Garlic and Moringa Seed; D =20% Cloves and Moringa Seed; E = 20% Ginger and Garlic; TTA= Titratable acidity

Table 9. Vitamin C content of the treated pineapple juice during storage

Storage Days		VIT C			
	A	B	C	D	E
O	64.12^a±1.39	63.73^a±0.81	70.56^a±0.14	67.12^a±0.16	63.10^a±0.01
7	64.12^a±1.39	63.73^a±0.81	70.56^a±0.14	67.12^a±0.16	63.10^a±0.01
14	61.14^a±1.46	60.17^b±0.39	67.89^b±0.345	63.96^b±0.20	59.39^b±0.01
21	57.46^b±0.09	55.57^c±0.96	65.84^b±1.00	56.04^c±0.76	51.87^c±0.65

Values are means ± standard deviations of duplicate determinations. Means in same column with same superscripts do not differ significantly (p>0.05)
Key: A= 100% Pineapple juice without treatment (control); B =20% Cloves and Ginger; C = 20% Garlic and Moringa Seed; D =20% Cloves and Moringa Seed; E = 20% Ginger and Garlic

development. The results of Smith and Patel [28] who noticed comparable patterns in fruit juices that had not been treated, are in line with this tendency. Throughout the storage period, there was less microbiological development in sample B addition than in the control. The CFU count was 3.0 × 10^4 CFU/mL by day 21. Although their efficacy waned with time, cloves and ginger's antibacterial qualities assisted in slowing down microbial development. This result is consistent with the research conducted by Jones and Zhang [29], which similarly observed that several natural preservatives gradually lose their antibacterial activity over time. Microbial growth in sample C was found to be reasonably stable, with the CFU count progressively rising to 2.7 × 10^4 CFU/mL by day 21, reason been that sample C have potent antibacterial qualities, the combination of the two seeds seems to be useful in preserving lower microbial levels over the course of storage [30]. During the course of the 21-day storage period. sample D maintained comparatively low microbial growth and produced the lowest initial CFU count. The CFU count was 2.2 × 10^4 CFU/ml by day 21, indicating that the combination of moringa seed and cloves had potent antibacterial properties. This confirms research by Smith and Patel [28] regarding the ability of moringa and cloves to preserve food. By day 21, the amount of microorganisms growing in sample E had increased to 2.8 x 10^4 CFU/mL, indicating a moderate rise in microbial growth. This shows better microbiological stability than the control, even if it is higher than samples C and D. Garlic has well-established antibacterial qualities, yet the addition of ginger may have somewhat reduced its potency [29].

Fungal counts in the control sample increased steadily over the course of 21 days, peaking at 2.4 CFU/ml. This demonstrates how pineapple juice naturally ages in the absence of preservatives. Wuyts et al. [32] found that untreated fruit juices with high sugar content and high nutrient availability exhibited rapid microbial growth. On day 21, Sample B had 2.2 CFU/ml, somewhat lower fungal levels than the control. Ginger and cloves are well recognized for their antibacterial qualities. It has been demonstrated that eugenol in cloves and gingerol in ginger prevent fungal development [33,34] With 2.1 CFU/ml, Sample C fungal levels were even lower. Allicin, which is found in garlic, has been shown to have antifungal qualities [35]. Glucosinolates and isothiocyanates, which are found in moringa seeds, are also known to have antifungal properties [36]. On day 21, sample D had the lowest fungal levels, measuring only 1.7 CFU/ml, making it the most successful. The improved preservation was probably made

Table 10. pH value of the treated pineapple juice during storage

Storage Days	pH				
	A	B	C	D	E
O	3.20^b±0.20	3.13^c±0.01	3.23^c±0.01	3.22^c±0.03	3.22^c±0.03
7	3.20^b±0.20	3.13^c±0.01	3.23^c±0.01	3.22^c±0.03	3.22^c±0.03
14	3.20^b±0.03	3.83^b±0.00	3.80^b±0.02	3.83^b±0.02	3.85^{ab}±0.00
21	4.72^a±0.01	4.63^b±0.01	4.64^a±0.01	4.61^a±0.01	4.56^a±0.01

Values are means ± standard deviations of duplicate determinations. Means in same column with same superscripts do not differ significantly (p>0.05)
Key: A= 100% Pineapple juice without treatment (control); B =20% Cloves and Ginger; C = 20% Garlic and Moringa Seed; D =20% Cloves and Moringa Seed; E = 20% Ginger and Garlic

Table 11. Total soluble solid of the treated pineapple juice during storage

Storage Days	TSS				
	A	B	C	D	E
O	13.23^a±0.04	13.85^a±0.12	13.26^a±0.02	13.21^a±0.04	13.17^a±0.04
7	13.23^a±0.04	13.85^a±0.12	13.26^a±0.02	13.21^a±0.04	13.17^a±0.04
14	13.17^a±0.00	12.15^b±0.09	11.49^b±0.08	10.94^b±0.00	10.47^b±0.15
21	12.48^b±0.34	10.68^c±0.06	10.41^c±0.00	10.34^b±0.64	9.76^c±0.33

Values are means ± standard deviations of duplicate determinations. Means in same column with same superscripts do not differ significantly (p>0.05)
Key: A= 100% Pineapple juice without treatment (control); B =20% Cloves and Ginger; C = 20% Garlic and Moringa Seed; D =20% Cloves and Moringa Seed; E = 20% Ginger and Garlic; TSS= Total soluble solid

possible by the antibacterial properties of moringa seeds and the synergistic action of eugenol from cloves [37,38]. Additionally, Sample E fungal counts effectively decreased, reaching 1.5 CFU/ml by day 21. Broad-spectrum antibacterial action is provided by the combination of allicin and gingerol, which is in line with research by Singh and Ranganathan [39] that highlighted the beneficial effects of mixing several natural antimicrobial agents.

Day 0 (the start of the storage period) revealed no discernible variations in TTA values across all treatments ($p > 0.05$). Similar TTA values were shown by the treated samples (B, C, D, and E) and the control (A), suggesting that the initial addition of preservatives did not instantly change the juice's acidity. The ability of pineapple juice to act as a buffer and the early equilibrium between organic acids and other ingredients are responsible for its stability [40]. Over all treatments, by Day 7, the TTA values had not changed much from Day 0. This implies that there was no interaction between the natural preservatives and the juice ingredients that would have changed the acidity during the first week. The capacity of these preservatives to preserve the juice's quality during short-term storage is indicated by this stability period, which makes it extremely important [41]. On Day 14, sample B , D and E showed a significant drop in TTA. Comparing these treatments to the control group revealed significant differences ($p < 0.05$). The antibacterial qualities of the additional spices and herbs may have prevented the microbial activity that normally generates organic acids, which would have resulted in a drop in acidity [42]. cloves contain a large amount of eugenol, which has potent antibacterial effects, the treatments containing cloves in particular demonstrated a more pronounced impact [38]. By Day 21, TTA for all treatment showed a significant decline, falling below 0.31. This decline points to a significant change in the juice's chemical composition over time, as a result of the preservatives' that extended antibacterial activity, which inhibits microbial fermentation and acid generation [43,44]. Clove-containing treatments B and D maintained the lowest TTA values, demonstrating that cloves are useful for preserving juice stability by lowering the populations of microorganisms that produce acid [45-47].

At day 0, the vitamin C concentration of the untreated control sample A was 64.12 ± 1.39 . This figure is similar to the amount of vitamin C found in samples that were preserved using natural preservatives. At 70.56 ± 0.14, sample C had the greatest initial vitamin C concentration. Sample D came in second with 67.12 ± 0.16. These results imply that the vitamin C concentration was not negatively impacted by the treatment of natural preservatives. By day 21, the vitamin C content had considerably dropped in every sample. There was a decrease in control sample A to 57.46 ± 0.09. Comparable trends were seen in samples B, D, and E, which were 55.57 ± 0.96, 56.04 ± 0.76 and 51.87 ± 0.65, respectively. At 65.84 ± 0.99, Sample C continued to be the highest, indicating that the combination of garlic and moringa seed was the most successful in maintaining vitamin C during the storage period. According to studies by Benkeblia [48-50], garlic has potent antioxidant qualities that support the preservation of vitamin C. Similarly, it was shown by Anwar et al. [51-53] that extracts from moringa seeds have strong antioxidant activity, which helps to preserve vitamin C. According to Anwar et al. [51] fruit juices' acidity and stability two factors that are essential for the preservation of vitamin C were successfully preserved by natural preservatives like ginger and cloves.

Day 0: 3.20 ± 0.19 was the pH of the untreated control sample A. Sample B, had the lowest pH of 3.13 ± 0.01 among the treated samples (B to E), with the other samples having somewhat lower or comparable pH values. This initial acidity is normal for fresh pineapple juice and shows that the pH was not dramatically changed right away by the addition of natural preservatives. All samples showed a significant increase in pH on the 21st day, with the control sample A reaching 4.72 ± 0.01. The pH values of the treated samples B, C, D, and E were also high; they ranged from 4.56 ± 0.01 to 4.63 ± 0.01. The observed pattern indicates a noteworthy decrease in acidity, maybe ascribed to the decomposition of organic acids and microbial spoiling throughout the prolonged storage duration. Fruit juices pH tends to rise while they are stored because of microbial activity and the degradation of organic acids, according to Barth et al. [54-56] observed comparable patterns, emphasizing that fruit juices that have been preserved may have an increase in pH due to microbial deterioration. According to Ahmed et al. [57] natural preservatives like ginger and cloves may affect how stable the pH is in fruit juices, causing the pH to rise more slowly than in samples that were not treated.

At Day 0: 13.23 ± 0.04 was the TSS of the untreated control sample A. Sample B had the highest TSS at 13.85 ± 0.12. The TSS values for the treated samples B to E were either slightly higher or relatively equivalent. This shows that the TSS was not markedly changed immediately by the addition of the natural preservatives. But at say 21, all samples showed a significant drop in TSS with the control sample A having the lowest TSS at 12.48 ± 0.34. The TSS values of the treated samples B, C, D, and E were also low, ranging from 9.76 ± 0.33 to 10.68 ± 0.06. This pattern points to a considerable drop in sugar concentration, as a result of spoilage and microbial activity during the storage time. According to Anwar et al. [51,58], fruit juices treated with natural preservatives such as ginger and cloves may have a different TSS stability than untreated samples, resulting in a slower TSS decline. This is in line with our findings, which indicated that, particularly in the early storage stages, treated samples displayed a less pronounced drop in TSS when compared to the control. Because moringa seed extracts have potent antioxidant and antibacterial properties, [51,59,60] observed that their application contributes to the preservation of fruit juice quality and TSS. This is consistent with our observations that samples containing moringa seed had higher TSS stability.

4. CONCLUSION

The results show that the natural preservatives have antibacterial activity on the microorganisms found in pineapple fruit. The use of cloves, garlic, ginger, and moringa seeds as natural antimicrobial agents in pineapple juice is effective in controlling microbial growth, thus enhancing the safety and shelf life of the juice. These treatments also influence the chemical and sensory properties of the juice, contributing to consumer acceptability. The overall findings support the potential use of these natural preservatives in the food industry to improve the quality and longevity of fruit juices. Given the effectiveness of cloves, garlic, ginger, and moringa seeds in controlling microbial growth and enhancing the safety and shelf life of pineapple juice, it is recommended that the food industry consider incorporating these natural antimicrobial agents in the production of fruit juices.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Obe Theresa Onyeka, Amove Julius and Eke Mike Ojotu hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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