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Efficacy of Some Chemical Preservatives in Prolonging the Shelf Life of Fermented Locust Beans '*iru*'

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Abstract

In Nigeria most foods are preserved by adding salt. The efficacy of this and some other chemical preservatives in improving the storage quality of fermented locust beans was the focus of this study. Samples of fermented locust beans were separately treated with table salt (10% w/w), brine (10 and 20% solution), vinegar (4 and 6%) and lactic acid (10 and 20%). After treatment, all the samples were stored at ambient temperature and periodically analysed for sensory and microbiological properties. Samples treated with dry salt became slippery, changed in color and developed bad odor within 1, 4 and 14 days respectively. Except for the softening observed, no significant ($p < 0.5$) change occurred with the color and odor of the other samples during storage. A total of 29 bacterial and 6 fungal isolates were obtained. The dominant bacteria were members of the genera *Bacillus*, *Staphylococcus*, *Pseudomonas*, *Micrococcus*, *Citrobacter*, *Microbacterium*, *Enterobacter*, *Leuconostoc*, *Arthrobacter*, and *Corynebacterium* while *Saccharomyces cerevisae*, *Aspergillus niger* and other *Aspergillus* spp., dominated the fungal population. Other fungi were *Rhizopus stolonifer*, *Alternaria alternata*, and *Candida albicans*. The microbial load and diversity reduced significantly ($p < 0.5$) after treatment and during storage. Among the bacterial isolates, *Bacillus cereus*, *B. pumilus*, *B. lentus*, *B. licheniformis*, *Staphylococcus epidermidis*, *S. saprophyticus Citrobacter freundii*, *Arthrobacter ureafaciens*, *Corynebacterium poinsetiae*, *Micrococcus denitrificans* and *Microbacterium* sp. were inhibited by lactic acid, vinegar and brine with diameter zone of inhibition ranging from 11.0 to 24.0mm; 15.0 to 20.5mm and 12.5 to 22.5 mm respectively. This study has shown that lactic acid, vinegar and brine have some preservative effects on fermented locust beans and can therefore be used to prolong its shelf life.

Keywords: brine, fermented locust beans, lactic acid, preservatives, vinegar

INTRODUCTION

Fermented locust beans (FLB) known variously as dawadawa (Hausa), iru (Yoruba), Ogiri (Igbo) in Nigeria, and as Soumbala in Burkina Faso, Mali, Cote de Voire and Guinea is produced from the seeds of *Parkia biglobosa*, a perennial tree legumes grown in the savannah region of West Africa up to the southern edge of the Sahel zone (Campbell-platt, 1980; Orwa *et al.*, 2009). It is used in soups and dishes as condiments for its flavoring and aroma. It serves as supplements of protein (Ojewumi *et al.*, 2017), Vitamin B, riboflavin and Vitamin A in foods (Odunfa and Adewuyi, 1985; Gernah *et al.*, 2007). The fermented locust beans is reportedly higher in fat and protein content than the raw seeds (Ikenebomeh *et al.*, 1986; Oladunjoye, 2007) and is of rich nutritional value constituting 1.4% of the daily calorie and 5% of the total protein intake of many families in Nigeria (Odunfa, 1985).

African locust bean is inedible until processed to remove toxins and anti-nutrients (Olaoye, 2010). Processing involve harvesting, decorticating, removal of pulp and drying to obtain the locust bean seeds that represent the major raw material from plant (Olaoye, 2010). Seeds are separated from the endocarp and boiled for about 12-24 hours or until they are tender, this is followed by de-hulling by gentle pounding in a mortal or by rubbing the seed between the palms or trampling under foot and sand or other abrasive agents are used to facilitate de-hulling. The cotyledons that result are again boiled for 3 to 4 hours, after which they are packed in large calabash and covered tightly with broad leaves such as those of banana plants, to allow fermentation at room temperature for 2-3 days (Zannou *et al.*, 2018).

In making the soft fermented locust beans ground sunflower seeds is usually added as softening agent during fermentation. A chemical softening agent comprising of sodium carbonate and sodium bicarbonate may also be added during the second boiling to aid softening of the cotyledons. Often, the calabash is covered with jute bags to create the warm environment required for fermentation.

One major factor militating against the acceptance of fermented locust beans is the associated foul smell which is attributable to uncontrolled fermentation leading to the breakdown of amino acids to produce ammonia and hydrogen sulphide. Salting and drying are age long methods used by peasants to preserve the sensory quality of fermented locust beans in Nigeria. Often, dry table salt (sodium chloride) is sprinkled on the fermented seeds which are then spread in open airspace to sundry. The problem with this is the lack of hygiene in the process which also affects acceptance. A more hygienic drying method which uses the oven that combines heat, low humidity and air current to facilitate efficient drying will increase the overhead and affect the economy of the process. This work therefore focused on the efficacy of salt both in the dry form and brine, as well as some other chemical preservatives in improving the storage quality of fermented locust beans.

MATERIALS AND METHODS

Fermented locust beans samples

Fresh samples of fermented locust beans were purchased from a producer in Ilorin, Nigeria. Samples were divided into 5g portions and transferred into 20ml sterile sample bottles. The bottles were grouped into eight and labelled TS, BR10, BR20 VG4, VG6, LA10, LA20 and CT. Each group consist of eight bottles. Table salt, 10% w/w was added to the fermented locust beans in the bottles labelled TS and properly mixed; bottles labelled BR10 and BR20 separately received 10ml of brine at 10 and 20% concentrations respectively.Vinegar (4 and 6%) and lactic acid (10 and 20%) were added respectively to VG4, VG6, LA10 and LA20 bottles while the CT bottles which served as the control received no treatment. All sample bottles were covered and stored in the cabinet at 28±2^{o}C (room temperature). Samples were withdrawn for analysis immediately after preparation, at 24 hours of storage and subsequently every 48 hours until signs of spoilage was observed.

Organoleptic analysis

The organoleptic study was conducted on three hedonic scale using a panel of five volunteers. Panelists were given score card to rate the quality of the samples based on the odor, color and texture.

Isolation, enumeration and characterization of microorganisms

Organisms were isolated on Nutrient Agar and Potato Dextrose Agar and identified using standard laboratory techniques. Enumeration was by colony count.

Antibacterial sensitivity tests

All the bacteria were tested individually for their sensitivity to each of the chemical preservatives using agar well diffusion method. Wells measuring 6mm were made in seeded Mueller Hinton Agar plates. Aliquots, 0.1ml each of the preservatives were loaded into appropriately labelled wells and plates incubated at 37^{o}C for 24 hours. Plates were withdrawn from incubator and observed for growth of organisms and clearance around loaded wells. Diameter zone of clearance was measured in three planes and the mean with standard deviation was taken as the inhibition zone.

RESULTS

Organoleptic properties

The fermented locust beans that was treated with vinegar maintained a very high odor quality throughout the two weeks of storage. Quality was also preserved with table salt and brine for 9 and 5 days respectively, while the odor had started deteriorating by the 5th day of storage in samples treated with lactic acid. However, when compared to the untreated sample, all preservatives significantly ($p < 0.05$) improved odor quality of the beans during storage (Table 1). In general, there was no significant difference ($p < 0.5$) in the color of the treated samples during storage, whereas the color of the untreated deteriorated by the 5th day (Table 2). The texture of samples treated with brine was preserved throughout the storage period. Samples were significantly softened by the 3rd day for those treated with table salt while the change was mild with the other treated sample. The texture also deteriorated by the 7th day for the untreated samples (Table 3). Variation in the concentration of the chemicals used had no effect on the preservative activities. Overall acceptance which was obtained by taking the mean of all evaluations category by category is presented in Figure 1.

Table 1: Effect of Vinegar, Lactic acid and Salt on the Odor of Fermented Locust Beans during Storage

STP (Days)	Sensory evaluation							
	V4	V6	LA10	LA20	BR10	BR20	TBS	CTR
0	2.67±0.58^{a}	2.67±0.58^{a}	2.67±0.58^{b}	2.67±0.58^{bc}	2.67±0.58^{c}	3.00±0.00^{d}	2.67±0.58^{c}	3.00±0.00^{c}
1	3.00±0.00^{a}	3.00±0.00^{a}	2.67±0.58^{b}	3.00±0.00^{c}	3.00±0.00^{c}	2.67±0.58^{c}	3.00±0.00^{c}	2.67±0.58^{c}
3	2.67±0.58^{a}	3.00±0.00^{a}	2.67±0.58^{b}	2.67±0.58^{bc}	2.67±0.58^{c}	3.00±0.00^{d}	3.00±0.00^{c}	2.33±0.58^{bc}
5	3.00±0.00^{a}	2.67±0.58^{a}	2.00±0.00^{ab}	2.00±0.00^{ab}	2.67±0.58^{c}	2.67±0.58^{c}	2.67±0.58^{c}	1.33±0.58^{b}
7	2.67±0.58^{a}	3.00±0.00^{a}	2.00±0.00^{ab}	2.00±0.00^{ab}	2.00±0.00^{bc}	2.00±0.00^{b}	3.00±0.00^{c}	1.00±0.00^{b}
9	3.00±0.00^{a}	3.00±0.00^{a}	2.33±0.58^{ab}	1.67±0.58^{a}	2.00±0.00^{bc}	2.33±0.58^{c}	2.67±0.58^{c}	1.00±0.00^{b}
11	2.67±0.58^{a}	2.67±0.58^{a}	2.00±0.00^{ab}	2.00±0.00^{ab}	1.33±0.58^{ab}	1.33±0.58^{a}	2.00±0.00^{b}	0.00±0.00^{a}
13	3.00±0.00^{a}	3.00±0.00^{a}	1.67±0.58^{ab}	2.33±0.58^{abc}	1.00±0.00^{a}	1.33±0.58^{a}	1.00±0.00^{a}	0.00±0.00^{a}

Values are mean of five independent evaluations ± standard deviation. Homogenous superscripts within same column, are insignificantly different ($p \leq 0.05$). STP denotes storage period, V4 - Vinegar at 4%, V6 - Vinegar at 6%, LA10 - 10% Lactic acid, LA20 - 20% Lactic acid, BR10 - 10% Brine, BR20 - 20% Brine, TBS - Table salt, CTR - Control

Table 2: Effect of Vinegar, Lactic acid and Salt on the Color of Fermented Locust Beans during Storage

STP (Days)	Sensory evaluation							
	V4	V6	LA10	LA20	BR10	BR20	TBS	CTR
0	2.67±0.58^{b}	2.67±0.58^{b}	2.67±0.58^{b}	2.67±0.58^{b}	2.67±0.58^{ab}	3.00±0.00^{b}	2.67±0.58^{ab}	3.00±0.00^{c}
1	3.00±0.00^{b}	3.00±0.00^{b}	2.67±0.58^{b}	3.00±0.00^{c}	3.00±0.00^{b}	2.67±0.58^{b}	3.00±0.00^{b}	2.67±0.58^{c}
3	2.67±0.58^{b}	3.00±0.00^{a}	2.67±0.58^{b}	2.67±0.58^{b}	2.67±0.58^{ab}	3.00±0.00^{b}	3.00±0.00^{ab}	3.00±0.00^{c}
5	3.00±0.00^{b}	2.67±0.58^{b}	2.00±0.00^{ab}	2.00±0.00^{a}	2.67±0.58^{ab}	2.67±0.58^{b}	2.67±0.58^{b}	1.00±0.00^{b}
7	2.67±0.58^{b}	3.00±0.00^{b}	2.00±0.00^{ab}	2.00±0.00^{a}	2.00±0.00^{a}	2.00±0.00^{b}	3.00±0.00^{b}	1.33±0.58^{b}
9	3.00±0.00^{b}	3.00±0.00^{b}	2.33±0.58^{ab}	1.67±0.58^{a}	2.00±0.00^{a}	2.33±0.58^{b}	2.67±0.58^{ab}	1.00±0.00^{b}
11	2.00±0.00^{a}	2.33±0.58^{a}	2.00±0.00^{ab}	2.00±0.00^{a}	2.67±0.58^{ab}	2.33±0.58^{b}	2.00±0.00^{a}	0.00±0.00^{a}
13	2.33±0.58^{a}	2.33±0.58^{a}	1.67±0.58^{a}	2.33±0.58^{ab}	3.00±0.00^{b}	1.67±0.58^{a}	2.00±0.00^{a}	0.33±0.58^{a}

Values are mean of five independent evaluations ± standard deviation. Homogenous superscripts within same column, are insignificantly different ($p \leq 0.05$). STP denotes storage period, V4 - Vinegar at 4%, V6 - Vinegar at 6%, LA10 - 10% Lactic acid, LA20 - 20% Lactic acid, BR10 - 10% Brine, BR20 - 20% Brine, TBS - Table salt, CTR - Control

Table 3: Effect of Vinegar, Lactic acid and Salt on the Texture of Fermented Locust Beans during Storage

STP (Days)	Sensory evaluation							
	V4	V6	LA10	LA20	BR10	BR20	TBS	CTR
0	2.67±0.58^{bc}	2.67±0.58^{c}	2.67±0.58^{b}	2.67±0.58^{c}	2.67±0.58^{a}	3.00±0.00^{a}	2.67±0.58^{b}	3.00±0.00^{b}
1	3.00±0.00^{c}	3.00±0.00^{c}	2.67±0.58^{b}	3.00±0.00^{c}	3.00±0.00^{a}	2.67±0.58^{a}	1.33±0.58^{a}	2.67±0.58^{b}
3	2.33±0.58^{a}	3.00±0.00^{c}	2.00±0.00^{ab}	2.33±0.58^{ab}	2.67±0.58^{a}	3.00±0.00^{a}	1.33±0.58^{a}	3.00±0.00^{b}
5	2.00±0.00^{a}	2.33±0.58^{b}	1.67±0.58^{ab}	2.33±0.58^{ab}	2.67±0.58^{a}	2.67±0.58^{a}	1.67±0.58^{ab}	2.67±0.58^{b}
7	2.33±0.58^{a}	2.00±0.00^{a}	2.00±0.00^{a}	1.67±0.58^{a}	2.67±0.58^{a}	2.67±0.58^{a}	1.00±0.00^{a}	1.33±0.58^{a}
9	2.33±0.58^{a}	2.33±0.58^{b}	1.67±0.58^{a}	1.67±0.58^{a}	2.67±0.58^{a}	3.00±0.00^{a}	1.33±0.58^{a}	1.00±0.00^{a}
11	2.00±0.00^{a}	2.33±0.58^{b}	2.33±0.58^{ab}	2.00±0.00^{ab}	2.67±0.58^{a}	2.67±0.58^{a}	1.33±0.58^{a}	0.67±0.58^{a}
13	1.67±0.58^{a}	1.33±0.58^{a}	1.67±0.58^{a}	1.67±0.58^{a}	3.00±0.00^{a}	2.67±0.58^{a}	1.00±0.00^{a}	1.00±0.00^{a}

Values are mean of five independent evaluations ± standard deviation. Homogenous superscripts within same column, are insignificantly different ($p \leq 0.05$). STP denotes storage period, V4 - Vinegar at 4%, V6 - Vinegar at 6%, LA10 - 10% Lactic acid, LA20 - 20% Lactic acid, BR10 - 10% Brine, BR20 - 20% Brine, TBS - Table salt, CTR - Control

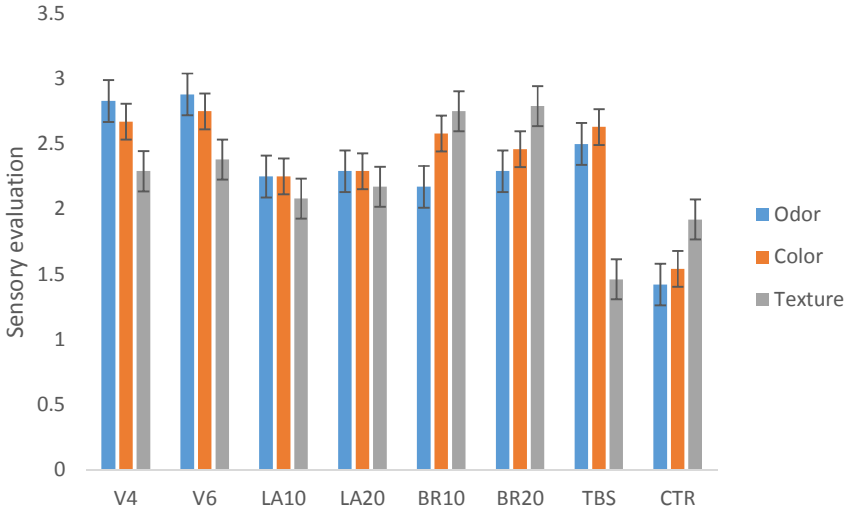

Figure 1: Effect of Vinegar, Lactic acid and Salt on the acceptance profile of fermented locust beans samples during storage.
Legend: Values are mean of evaluations for the total period of storage ± standard error. V4 - Vinegar at 4%, V6 - Vinegar at 6%, LA10 - 10% Lactic acid, LA20 - 20% Lactic acid, BR10 - 10% Brine, BR20 - 20% Brine, TBS - Table salt, CTR - Control

Microbial load and diversity

No organism was isolated from the samples that were treated with 20% lactic acid throughout the period of storage while a wide variation of microbial load was obtained from the other samples including those treated with 10% lactic acid. Except for samples treated with vinegar in which there was an initial increase, the microbial population of all treated samples was significantly reduced during storage and were comparatively lower than the untreated (Table 4).

A total of 35 microorganisms including 29 bacteria and 6 fungi were isolated. *Bacillus cereus*, *Citrobacter freundii* and *Staphylococcus aureus* occurred in all the samples while many of the others were isolated from only one sample. All the fungal isolates were from the samples treated with lactic acid (Table 5) at 10% concentration.

Sensitivity of bacterial isolates to the chemical preservatives

All the bacterial isolates tested, except *Staphylococcus aureus* and *Micrococcus luteus* showed sensitivity to the respective preservatives. *Bacillus cereus* which is one of the most predominant bacterial isolate was inhibited by all the preservatives used. Highest sensitivity occurred with vinegar against *Serratia marcescens* (Table 6).

Table 4: Effect of Some Chemical Preservatives on the Microbial Load of Fermented Locust Beans Samples during Storage.

STP (Days)	**Microbial load (log cfu/ml)**							
	V4	V6	LA10	LA20	BR10	BR20	TBS	CTR
0	$6.7938\pm0.03^{g,h}$	$6.8222\pm0.12^{d,f}$	$6.3930\pm0.02^{c,c}$	$0.0000\pm0.00^{a,a}$	$6.2625\pm0.05^{d,c}$	$6.1553\pm0.02^{b,b}$	$6.2765\pm0.01^{b,c}$	$6.6542\pm0.01^{b,e}$
1	$6.7412\pm0.04^{f,f}$	$6.5635\pm0.04^{c,f}$	6.4314±0.02d,e	$0.0000\pm0.00^{a,a}$	$6.1430\pm0.03^{b,d}$	$6.1206\pm0.01^{a,c}$	$6.2330\pm0.06^{a,b}$	$6.6085\pm0.06^{a,g}$
3	$6.7364\pm0.02^{f,e}$	$6.5079\pm0.03^{b,d}$	$6.2856\pm0.04^{a,c}$	$0.0000\pm0.00^{a,a}$	$6.5078\pm0.02^{f,d}$	$6.5478\pm0.03^{e,e}$	$6.0492\pm0.02^{a,b}$	$6.9154\pm0.15^{e,g}$
5	$6.4771\pm0.01^{a,c}$	$6.5065\pm0.08^{b,d}$	$6.4346\pm0.02^{d,b}$	$0.0000\pm0.00^{a,a}$	$6.5211\pm0.08^{g,d}$	$6.4200\pm0.06^{d,b}$	$6.6513\pm0.11^{d,e}$	$7.1116\pm0.12^{f,f}$
7	$6.5478\pm0.03^{c,d}$	$6.5145\pm0.31^{b,c}$	$6.5705\pm0.12^{e,c}$	$0.0000\pm0.00^{a,a}$	$6.5328\pm0.06^{e,c}$	$6.4031\pm0.06^{d,b}$	$6.6128\pm0.06^{c,e}$	$6.8129\pm0.10^{d,f}$
9	$6.5119\pm0.07^{b,e}$	$6.3096\pm0.06^{a,c}$	$6.3075\pm0.06^{b,c}$	$0.0000\pm0.00^{a,a}$	$6.2330\pm0.05^{c,b}$	$6.1847\pm0.15^{b,b}$	$6.7218\pm0.35^{e,e}$	$6.7803\pm0.06^{c,f}$
11	$6.6085\pm0.08^{d,d}$	$6.4914\pm0.10^{b,e}$	$6.4330\pm0.03^{d,d}$	$0.0000\pm0.00^{a,a}$	$6.0128\pm0.06^{a,b}$	$6.3159\pm0.06^{c,c}$	$6.7612\pm0.15^{f,f}$	$6.7903\pm0.15^{c,g}$
13	$6.6243\pm0.01^{e,f}$	$6.5065\pm0.06^{b,e}$	$6.4031\pm0.06^{c,d}$	$0.0000\pm0.00^{a,a}$	$6.0453\pm0.01^{a,b}$	$6.1173\pm0.02^{a,c}$	$6.7875\pm0.25^{g,g}$	$6.8129\pm0.10^{d,h}$

Values are mean of three independent readings ± standard deviation. Homogenous superscripts within same column and row respectively, are insignificantly different ($p \leq 0.05$). STP denotes storage period, V4 - Vinegar at 4%, V6 - Vinegar at 6%, LA10 - 10% Lactic acid, LA20 - 20% Lactic acid, BR10 - 10% Brine, BR20 - 20% Brine, TBS - Table salt, CTR - Control

Table 5:Effect of some chemical preservatives on the diversity of microorganisms growing in fermented locust beans samples during Storage

S/N	Organism	VINEGAR	LACTIC ACID	BRINE	TABLE SALT
1	*Arthrobacter ureafaciens*	-	+	-	-
2	*Bacillus cereus*	+++	+++	++++	+++
3	*Bacillus pumilus*	-	-	+	-
4	*Bacillus lentus*	-	-	++++	-
5	*Bacillus licheniformis*	-	-	++	-
6	*Citrobacter freundii*	+	+	+	+
7	*Citrobacter koseri*	-	-	+	-
8	*Corynbacterium kutsceri*	-	-	+++	+
9	*Corynbacterium xerosis*	-	-	-	+
10	*Corynebacterium poinsetiae*	-	+	-	-
11	*Enterobacter aerogenes*	-	-	+	-
12	*Enterobacter intermedius*	-	-	++	+
13	*Leuconostoc citrovarum,*	-	+	-	-
14	*Leuconostoc dextranicus*	-	-	+	+
15	*Leuconostoc mesenteroides*	-	-	+	+
16	*Microbacterium lacticum*	-	-	+	+
17	*Microbacterium* sp.	+	-	-	+
18	*Micrococcus albus*	-	-	-	+
19	*Micrococcus candidus*	-	-	+	-
20	*Micrococcus denitrificans,*		+	-	-
21	*Micrococcus luteus*	++	-	++++	+
22	*Micrococcus varians*	-	-	+++	-
23	*Pediococcus spp,*	-	+	-	-
24	*Pseudomonas aeruginosa*	-	-	+	+
25	*Serratia marsecens*	++	-	-	-
26	*Staphylococcus aureus*	+++	+	+++	+++
27	*Staphylococcus capitis*	-	-	+	-
28	*Staphylococcus epidermidis,*	-	+	+	-
29	*Staphylococcus saprophyticus*	+	-	++	+
30	*Aspergillus niger*	-	+	-	-
31	*Aspergillus* spp.	-	+	-	-
32	*Rhizopus stolonifer*	-	+	-	-
33	*Alternaria alternate*	-	+	-	-
34	*Candida albicans*	-	+	-	-
35	*Saccharomyces cerevisae*	-	+	-	-

Legend: organisms that were isolated from the treated samples are indicated with the + sign while those that were not isolated were indicated with – sign. Load of the individual isolate is indicated by the number of + sign thus (+ = x 10^3; ++ = x 10^4; +++ = x 10^5; ++++ = x 10^6)

Table 6: Antibacterial activity of the chemical preservatives on the bacterial isolates of fermented locust beans

Organism	Inhibition zone (mm)								
	V4	V6	LA10	LA20	BR10	BR20	TBS	CTR	
Staphylococcus aureus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacillus cereus	15.50±1.08	15.00±1.08	0.00	9.5±2.08	11.25±2.91	0.00	0.00	2.75±0.9	0.00
Citrobacter freundii	17.50±1.22	21.00±1.14	0.00	7.25±1.71	7.50±1.92	0.00	0.00	0.00	0.00
Microbacterium species	17.50±1.13	20.50±1.21	0.00	NA	NA	NA	NA	NA	NA
Serratia marsecens	19.50±1.15	20.50±1.13	0.00	NA	NA	NA	NA	NA	NA
Micrococcus luteus	0.00	0.00	0.00	NA	NA	NA	0.00	0.00	0.00
Staphylococcus saprophyticus	0.00	0.00	0.00	NA	NA	NA	0.00	1.75±1.0	0.00
Staphylococcus epidermidis	NA	NA	NA	7.75±2.21	9.75±3.5	0.00	0.00	0.00	0.00
Citrobacter freundii	NA	NA	NA	7.25±1.71	7.50±1.92	0.00	NA	NA	NA
Arthrobacter ureafaciens	NA	NA	NA	5.50±1.29	7.25±2.22	0.00	NA	NA	NA
Corynebacterium poinsetiae	NA	NA	NA	6.75±1.71	8.75±3.3	0.00	NA	NA	NA
Micrococcus denitrificans	NA	NA	NA	9.25±1.71	10.50±2.08	0.00	NA	NA	NA
Bacillus lentus	NA	NA	NA	NA	NA	NA	1.50 ±0.6	2.25±0.5	0.00
Bacillus pumilus	NA	NA	NA	NA	NA	NA	1.25±0.5	2.25±1.2	0.00
Bacillus licheniformis	NA	NA	NA	NA	NA	NA	0.00	2.25±1.9	0.00

LEGEND: Values are means of three independent readings ± standard deviation. NA denotes "NOT APPLICABLE" since the organisms were not isolated from samples treated with the respective chemical preservative. Sensitivity to individual organism was not determined for table salt because the other preservatives were in liquid form and a solution of salt in water gives brine which was already included. V4 - Vinegar at 4%, V6 - Vinegar at 6%, LA10 - 10% Lactic acid, LA20 - 20% Lactic acid, BR10 - 10% Brine, BR20 - 20% Brine, TBS - Table salt, CTR - Control

DISCUSSION

Vinegar is used as food additives mainly for its well-known aroma compound (Cerezo *et al.*, 2008) and this explains the high odor quality observed throughout storage period in the fermented locust beans samples that were treated with vinegar. Organisms that produced unpleasant odor in the samples treated with table salt and brine may have been inhibited accounting for maintenance of good odor up to the 9^{th} and 5^{th}day of storage respectively. The deterioration that occurred afterwards may also be a result of regrowth of the organisms.

Result has shown that microbial growth contributes to the color change of fermented locust beans in storage. This is because all the chemicals used were able to preserve the color of the samples throughout storage as against the untreated samples which exhibited significant ($p \leq 0.05$) color change by the 5th day. The texture of the treated fermented locust beans samples were not affected except for samples treated with table salt. The softening that occurred in the samples are likely due to the effect of direct contact with table salt which may have exerted osmotic pressure on the sample.

As presented on figure 1, samples that received vinegar treatment were evaluated highest in odor and color while texture evaluation was highest in brine treated samples. However, except for the texture of the samples treated with table salt, all received higher evaluation compared to the untreated samples. This implies a positive effect of the chemical preservatives on the fermented locust beans samples. Microbial growth was inhibited completely throughout the period of storage in fermented locust beans samples that were treated with 20% lactic acid whereas some organisms were able to grow at 10%. This is an indication that lactic acid has cidal effect at high concentration while the effect is static at lower concentration. Vinegar on the other hand exerted only a mild inhibition of growth and this may account for the slight deterioration of texture in the samples which also corroborates earlier assertion that the good odor obtained could be due mostly to the aroma quality rather than antimicrobial effects. However, when compared with the untreated samples, some antimicrobial activities can also be attributed to vinegar.

Fermented locust beans have high nutritional composition which may be responsible for the high microbial load and diversity obtained. A number of *Bacillus* spp. including *B. cereus*, *B. pumilus; Staphylococcus* spp. and many Lactic Acid Bacteria which were isolated in this study have been reported severally (Antai and Ibrahim 1986; Odunfa and Oyewole, 1986; Barber *et al.*, 1988;Ogbadu and Nokagbue, 1988; Diawara *et al*. 2000;Enujiugha, 2009; Okanlawon, 2010; Ademola *et al.,* 2012; Kabuo *et al.*, 2013; Rabi *et al.,* 2013; Egbebi *et al.*, 2016; Zannou *et al.,* 2018) to play major roles in the production of fermented locust beans. Only six species of fungi were isolated and all were from the samples treated with lactic acid, an indication that the samples may have been contaminated during processing. Very few fungi had been isolated from fermented locust beans due probably to the high moisture content which may be above the water activity of fungi. Members of the genera *Aspergillus*, *Rhizopus* and *Candida* isolated in this work are among other fungi found as major spoilage organisms of fermented locust beans (Nwadiaro *et al.*, 2015). Apart from the untreated samples, the fungi were found only in those treated with lactic acid, an indication that the acidulant may not be effective against them.

In spite of the high microbial load obtained from samples treated with vinegar, a large number of isolated bacteria were found to be sensitive to the preservative (Table 6). Baldas and Altuner, (2018) also obtained high antimicrobial activity from vinegar against some standard strains as well as organisms isolated from foods. This implies that the few organisms that were not sensitive to its antimicrobial effects may have been responsible for the high microbial load and consequent deterioration in the texture of the locust beans. *Bacillus cereus*, a Gram positive spore former which plays major role in the fermentation of locust beans was found to be sensitive to all the preservatives with highest inhibition obtained with vinegar. The other *Bacillus* spp. isolated were also inhibited by the respective preservative. Inhibition of *B. subtilis* by vinegar was also reported earlier (Baldas and Altuner, 2018). This implies that the preservatives have the capacity to avert the growth of these organisms after production thereby preventing undesirable metabolites that may cause the spoilage of the product. Lactic acid is a natural preservative that maintains the color, flavor, texture and nutritional values of fermented foods, and also effectively control microorganisms. In this wise, all the bacteria that were isolated from samples treated with lactic acid were inhibited except *S. aureus* which was recovered in low quantity from the samples treated with 10% lactic acid. The low occurrence of *S. aureus* may however account for its absence in the samples treated with 20% lactic acid. In addition, the preservative effects of lactic acid were demonstrated by the high color, flavor and texture quality of the treated fermented locust beans. Lactic acid as acidulant, lowers the pH of foods thereby affecting the environment of microorganisms to bring about their inhibition. Its effect is however more pronounced with Gram negative organisms (De Vuyst and Leroy, 2007) over the Gram positives such as *S. aureus*, as obtained in this study.

Vinegar, lactic acid and table salt were shown to exert preservative effects on fermented locust beans in this work. These chemicals can therefore be used to prolong its shelf life. However, a toxicity study to ascertain the safety as well as the tolerance level of the chemicals will provide more baseline information necessary for their recommendation as preservatives at commercial level.

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