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Effect of salt treatment on fungi spoilage, proximate and mineral compositions of fermented African locust bean (*Parkia biglobosa*) seeds during ambient storage

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ABSTRACT

This study was undertaken to investigate effect of salt treatment on fungi spoilage, proximate and mineral compositions of fermented locust beans during storage. In a completely randomized experimental set up, 20g of fresh African locust beans were separately weighed and distributed into clean transparent small plastic containers. Two grams (2g) of common salt were then added and the containers covered before storage at 28+20C and at75+5%RH. Unsalted samples served as control. By day 5 of storage, *Rhizopus* sp, *Aspergillus niger* and A. *flavus* were the fungi isolates found associated with the deterioration of the unsalted locust bean as evident by discolouration and mycelia appearance. Interestingly, salted samples did not show any sign of deterioration even by day 28 of storage. Results also showed significant decrease (except in moisture contents) in the proximate and mineral compositions of the unsalted samples when compared with the salted.

INTRODUCTION

The prevailing population pressure in Nigeria as in other less-developed countries has resulted in an increasing demand for wild under-exploited nutritious plant products with aesthetic and organoleptic appeal in the daily diet (Enujiugha 2005). The common edible portions of most under-utilized plants are the seeds which in some cases are cooked or roasted and eaten directly as snack foods e.g. conophor nut and bambara groundnut while some are cooked and fermented for use as soup and sauce ingredients e.g. African oil bean, locust bean, castor bean and melon. There are various plant seeds that are fermented and used as food in some rural parts of Nigeria among which are 'iru' from African locust bean (Parkia biglobosa) 'ogiri' from castor bean (Ricinus communis), 'okpei' from mesquite seed (Prosopis africana) and 'Ugba' from African oil bean (Pentaclethra macrophylla) (Eze et al. 2014).

African locust bean (Parkia biglobosa) is a popular food condiment in West African countries which is widely consumed for its high nutritional value to millions of Nigeria both in rural and urban areas (Fagbemi 1989). The fermented locust bean known as 'iru' and 'dawadawa' among the Yoruba and Hausa people of Nigeria respectively is basically used among these people as food seasoning and condiment (Ntui et al. 2012). Apart from the flavouring attribute of the fermented locust bean, it also contributes significantly to the intake of protein, carbohydrate calcium, phosphate, iron and essential fatty acids particularly vitamin B riboflavin and vitamin A (Odebunmi et al. 2010).

Nevertheless, its production in rural areas is discouraged by the way it rapidly spoils or deteriorates and consequently leads to loss of produce andto farmersbecause storage of fermented locust beans without anyform of preservation such as salting or refrigeration is about 48hours due to inherent high moisture content (>55%) and vulnerability toperoxidation due to high fat content (>37%) (Oni 1997). Hence, this study was undertaken to investigate effect of salt treatment on fungi spoilage, proximate and mineral compositions of fermented locust beans during storage. Besides,there is adearth of information on fungal species causing deterioration oflocust beans especially during storage in Southern Nigeria.

MATERIALS AND METHODS

Source of samples

Fermented locust bean samples were obtained from Irese Market Akure Ondo State, Nigeria. The samples were immediately brought to the research laboratory of the Department of Biology Federal University of Technology Akure for salt treatment and subsequent storage.

Salt Treatment and Storage

In a completely randomized experimental set up, 20g of fresh African locust beans were separately weighed and distributed into clean and transparent small plastic containers. Two grams (2g) of common salt were then added and the containers covered before storage at 28+20Cand at75+5%relative humidity. Unsalted (fresh)samples served as control. The set up was observed daily for any fungal appearance or rottenness. In another experimental set up, the same procedure was repeated except that the set up was checked for proximate and mineral compositions. Each set up was in three replicates.

Isolation of fungi from rottened locust bean samples

Malt extract agar (MEA) was used for isolating spoilage moulds from the rottened locust bean samples. Diseased portion of the sample was removed and plated on sterilized and solidified MEA. It was then incubated at 28+20C for 72 hours. Subculturing of the fungi isolate was then made severally in order to obtain pure cultures. Identification of the pure isolate was carried out using cotton in-blue lactophenol method, then examined under a binocular microscope at x40 objective magnification andidentifiedusing its colonial characteristics and microscopic morphologies according to the texts of Odunfa & Oyewole (1981), Alexopolous et al. (1996)and Nagamani et al. (2006).

Determination of proximate and mineral compositions

Proximate compositions of both unsalted fresh samples andsalted fresh samples during storage were carried out according to the method of AOAC (2000) while mineral compositions were determined using standard methods obtained from AOAC (1990). Data were analyzed using SPSS version 21 windows(SPSS USA) software using complete randomized design (CRD). New Duncan's Multiple Range Test was used to determine the significant of differences between groups. All the results were expressed as mean ± SE and significant difference were expressed a significant level of $p \leq 0.05$.

RESULTS

Fungi associated with the spoilage of African locust bean

Unsalted locust bean samples started showing signs of spoilage as evident by discolourations and presence of fungi mycelia by day 5 of storage. The various fungi identified to be associated with the spoilage of the unsalted fresh samples as reported in Table 1 were *Aspergillus niger*, A. flavusand *Rhizopus* sp. A. *niger* appeared colourless when young on MEA and later turned black with age due to the production of black conidia. Microscopically the conidia were globose to elliptical in shape and short chains of two or more spores on erect hyphae while A. flavus formed greenish colonies on MEA and microscopically, the conidia were globose and short chains of two or more spores on erect hyphae . The hyphae were branched and septate. Meanwhile, *Rhizopus* sp. appeared whitish, fluffy and cottony in texture on MEA. Microscopically, the sporangiophores were erect and the sporangia were oval and angular in shape . However, salted samples did not show any sign of deterioration even by day 28 of storage.

Proximate compositions of unsalted and salted locust bean samples during storage

Table 2 showed the proximate compositions of unsalted and salted locust bean samples during storage. The values of carbohydrate (18.08%), protein (9.80%), crude fibre (16.50%), fat (26.50%) and ash (4.80%) contents in salted locust bean samples by day 7 of storage were significantly higher (P>0.05) comparably with proximate of unsalted samples having values of carbohydrate (12.14%) , protein (3.22%) , crude fibre (11.83%), fat (7.44%) and total ash (1.78%) contents. However, there was a significant reduction in the moisture content of the unsalted locust bean samples from 63.50 to 24.50% in the salted samples.

As storage duration progressed to day 28, proximate compositions of the salted bean samples had further increased although non significantly to 19.50% (carbohydrate),10.44% (protein), 5.32% (total ash) and 17.20% (crude fibre). Nevertheless, the moisture and fat contents had decreased to 22.52and 24.18% respectively.

Mineral compositions of unsalted and salted locust bean samples during storage

Mineral compositions of both unsalted locust beans and salted samples during storage were reported in Table 3. Results showed that there was a significant increase ($P<0.05$) in the concentrations (mg/L) of sodium (1498.99), potassium (297.32) and calcium (178.39) of salted samples by day 7 in storage when compared with the concentrations (mg/L) of sodium (1112.21), potassium (137.64)and calcium (108.61) in unsalted samples. Similarly, concentrations (mg/L) of iron (1.99), zinc (8.94), magnesium (108.24) and copper (4.96) in the salted samples by day 7 in storage were significantly higher when compared with the concentrations of iron (0.18), zinc (6.00),magnesium (95.21) and copper (2.98) in unsalted samples. However, phosphorous, lead, nickel and cadmium were not detected in both unsalted and salted samples.

As storage duration progressed to day 28, concentrations of sodium, potassium and calcium had further increased significantly to 1500.91, 463.64and 363.64mg/L respectively (Table 3) while respective concentrations of iron, zinc, magnesium and copper also recorded further significant increase to 4.02, 15.10, 138.60 and 8.26 mg/L.

DISCUSSION

In this study, Rhizopus sp, Aspergillus niger and A. flavuswere the fungi isolates found associated with the deterioration of the unsalted (fresh) locust bean samples as evident by discolouration and mycelia appearanceas fromday 5 of storage. This was in agreement with the work of Ogaraku (2010) who reported that Aspergillus and Rhizopus cause discolouration and mouldy smell of locust bean seeds during storage. The emergence of these isolates on the deteriorating bean samples suggest possible contamination by spores in the air. The light spores of these isolates might be present in the surrounding air of the production unit where the samples were processed and eventually settled on the preparation. This was supported by Ajayi (2014) who reported that the production of African locust bean is basically by traditional household level fermentation technology laden with food-borne hazards at virtually all points during processing.

The seeds usually come with a substantial microbial load comprising spores of aerobic spore-forming bacteria and mould spores, most of which are drastically reduced during the prolonged boiling to soften the test a as obtainable in related fermentations (Gernah et al. 2007). Cooling is there after done in open air and this may introduce particulate and microbial contaminants coupled with the nature of local calabash and similar utensils used.

Besides, the presence of these fungal isolates on the deteriorating bean samples was supported by the work of Ogbonnaet al. (2015) who reported genera of Aspergillus, Mucor, Penicillium and Rhizopusas mycoflora of stored locust bean seeds from markets in Jos Nigeriaand these isolates have been implicated in post-harvest rot of some fruits and vegetables in storage (Adaskaveg et al. 2002). In fact, the significance of Rhizopus sp, Aspergillus niger and A. flavus on stored foodstuffs stems not only from their spoilage potentials but also from their potentials to produce a variety of mycotoxins. These mycotoxins are poisonous even in minute concentrations and pose a threat to the health of consumers taking such contaminated samples. For instance,A. flavus, one of the fungal isolates is capable of producing mycotoxins known as aflatoxin which causes aflatoxicosis.

However, the non-deterioration of the salted locust bean samples by day 28 of storage cannot but be connected with the salt treatment. The salt drew water out of the bean sample thereby lowering its moisture content to a level that inhibited fungal growth. The low water content is desirable according to the report of Qayyum et al.(2012) that high moisture content encourages growth of microorganisms which could reduce stability and shelf storage capability. Also, the salt treatment could have probably created hypertonic environment to any attacking moulds on the samples thus generating osmotic imbalance that eventually resulted to the destruction of the attacking moulds. This is in consonance with the report of Ademola et al.(2011) that salting is one of the oldest methods of preserving food and is used because most bacteria, fungi and other potentially pathogenic organisms cannot survive in a highly salty environment, due to the hypertonic nature of salt and that common salt is probably the oldest known antimicrobial agent. Consequently, this further confirmed the report of Elemo et al. (2011) that dehydration, salting and packaging of home produced food items such as locust beans (iru) in simple polyethylene bags is desirable to extend their shelf life.

Also, results from this study showed significant percent decrease (except in moisture contents) in the proximate compositions of the unsalted deteriorated locust bean samples when compared with the salted non-deteriorated samples. This was supported by the report of Ogbonna et al. (2015) that colonisation of the locust bean seeds by the storage fungi reduced significantly the nutrient composition of the seeds. This reduction in the nutrient values of the locust bean seeds is an evidence of utilisation of the nutrient contents of the seeds by the fungi. However, the increase in the moisture content of the unsalted deteriorated locust bean samples could be attributed to maceration of cellulose and pectin components of the beans cell wall by the fungal isolates. Itcould also be as a result of the biodeterioration activities of the spoilage fungi with the resultant elaboration of their metabolites into the surrounding bean seeds and the dissolution of the beans tissues into rot thereby increasing the moisture level of the bean seeds (Ogbonna et al. 2015). As storage duration extended to day 28,the carbohydrate, protein, crude fibre and total ash contents had further increased in the salted samples though insignificantly while the moisture and fat contents had also further reduced insignificantly. The further reduction in the moisture content probably accounted for further increase in the carbohydrate, protein, crude fibre and total ash contents of the salted samples because Adegunloye (2012) reported that lower moisture content causes reduction in solubility of nutrients in substrate while decrease in the fat contents could be as a result of oxidative activities (Zia-ur-Rehman et al.2003).

Meanwhile, the relative significant increase in the concentrations of Na, K, Ca, Fe, Zn, Mg and Cu in the salted non deteriorated samples throughout the storage period when compared with the unsalted samples that deteriorated by day 5 of storageis in agreement with the observation of Ademola et al. (2011) who reported increase in concentrations of mineral elements in locust bean preserved and stored for up to 4weeks. Addition of salt must have also contributed to the increase in the mineral concentrations particularly sodium and calcium. Besides, the lower concentrations recorded in the unsalted samples could not but be connected with the fact that the spoilage fungi that deteriorated the unsalted samples probably used these minerals as growth factors and this consequently resulted in their significant decrease in the unsalted samples.

Table 1: Storage moulds found associated with the spoilage of African Locust Bean during storage at 28±2°C and 75+5% RH

Storage moulds	Samples: unsalted locust bean by day 5 of storage	Samples: salted locust bean by day 28 of storage
Aspergillus niger	+	-
Aspergillus flavus	+	-
Rhizopus sp.	+	-

Note: + = present, - = absent, RH = relative humidity

Table 2: Proximate compositions of unsalted and salted African locust Bean during storage

Samples	Proximate parameters (Mean ± S.E) in %					
	Moisture	CHO	Crude Fibre	Protein	Fat	Total ash
Unsalted locust beans by day 7 of storage	63.50 ±0.25[b]	12.14 ± 0.24[a]	11.83 ± 0.28[a]	3.22 ± 0.30[a]	7.44 ± 0.06[a]	1.78 ± 0.20[a]
Salted locust beans by day 7 of storage	24.50 ± 0.14[a]	18.08 ± 0.55[b]	16.50 ± 0.55[b]	9.80 ± 0.09[a]	26.50 ± 0.16[b]	4.80 ± 0.44[b]
Salted locust beans by day 28 of storage	22.52 ± 0.16[a]	19.50 ± 0.30[b]	17.20 ± 0.60[b]	10.44 ± 0.10[a]	24.18 ± 0.10[b]	5.32 ± 0.22[b]

Note: Each value is a mean ± standard error of three replicates and means followed by the same letter are not significantly different (p> 0.05) using New Duncan's Multiple Range Test.
CHO - Carbohydrate

Table 3: Mineral compositions of unsalted and salted African locust beans during storage

Samples	Mineral concentrations (mg/L)						
	Na	K	Ca	Fe	Zn	Mg	Cu
Unsalted locust bean by day 7 of storage	1112.21 ± 0.01[a]	137.64 ± 0.03[a]	108.61 ± 0.40[a]	0.18± 0.01[a]	6.00± 0.03[a]	95.21 ± 0.02[a]	2.98 ± 0.03[a]
Salted locust bean by day 7 of storage	1498.99 ± 0.19[b]	297.32 ± 0.21[b]	178.39 ± 0.08[b]	1.99 ± 0.01[b]	8.94 ± 0.01[a]	108.24± 0.01[a]	4.96 ± 0.00[a]
Salted locust bean by day 28 of storage	1500.91 ± 0.15[c]	463.64 ± 0.60[c]	363.64 ± 0.66[c]	4.02 ± 0.53[c]	15.10 ± 0.60[c]	95.45 ± 0.04[a]	8.26 ± 0.20[c]

Note: Each value is a mean ± standard error of three replicates and means followed by the same letter are not significantly different (p> 0.05) using New Duncan's Multiple Range Test.
Na – Sodium, K – Potassium,Ca – Calcium, Fe – Iron,Zn – Zinc, Mg –Magnesium and Cu – copper.

CONCLUSION

This study showed that African locust bean (Parkia bioglobosa) seeds can be preserved and stored for 28days under ambient conditions after treatment with appropriate gram of common salt as experimented in the study without showing any evidence of deterioration or significant reduction in proximate and mineral values during storage. Consequently, salt treatment is hereby recommended for extending shelf life of locust bean seeds after harvest because salting was able to preserve the processed locust bean seed for 28days while unsalted seeds deteriorated fast by day 5 of storage.

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