Full Paper

FUNCTIONAL AND SENSORY PROPERTIES OF FERMENTED LOCUST BEAN SEEDS USING OPTIMIZED PRE-FERMENTATION CONDITIONS

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ABSTRACT

This study is a continuation of previous work set to establish optimal processing conditions for the production of fermented locust bean seeds. The effect of employing these already determined conditions (First cooking at 50 kPa for 54 min per 100 g of seed and second cooking time of 15 min per 90 g of seed) on anti-nutritional factors, functional and sensory properties of the product was determined. The functional properties investigated were water and oil absorption capacity, emulsion capacity and solubility while tannin, phytate and oxalate were the anti-nutritional factors determined. Water absorption capacity and emulsion capacity decreased with dehulling but were increased by fermentation and drying while the oil absorption capacity and solubility decreased with processing. There was significant reduction in all anti-nutritional factors investigated. The consumer preference for taste and mouth-feel of both the traditionally and optimally processed *Iru* were significantly comparable ($p<0.05$). There was a significant difference in the preference for colour, aroma, texture, and overall acceptability. The study concluded that processing locust bean seeds under optimized conditions yields a product with acceptable nutritional benefits.

Keywords: *Fermented Locust Bean, Functional and Sensory Properties, Anti-nutrient*

1. INTRODUCTION

Locust Bean seeds provide an alternative source of protein in local food systems especially in West Africa. The fermented seed is prominently used in the preparation of stews and soups as condiment to enhance flavour and nutritional values. This condiment is referred to as *iru* in Yoruba, *dawadawa* in Hausa and *ogiri-igala* in Igbo. It is also referred to as *kinda* in Sierra-Leone and *Kpalugu* in Ghana (Odunfa, 1981).

Processing techniques may be employed to reduce or destroy the anti-nutrients present in them and to improve the nutritional quality or organoleptic acceptability of leguminous seeds. Some of the commonly used processing techniques include soaking in water, boiling at high temperatures in water, alkaline or acidic solutions, sprouting, autoclaving, roasting, dehulling, microwave treatment, steam blanching and fermentations (Christiana and Marcel, 2008). Omafuvbe *et al.*, (2004) reported an increase in the moisture content of "*dawadawa*" when compared with the unprocessed seed and this may probably be due to boiling and subsequent soaking in water. It may also be as a result of metabolic activities of microorganism during fermentation period which gave out moisture as one of their end products. Several other researchers have come up with appropriate fermentation conditions such as pH, temperature and humidity (Odunfa and Adewuyi, 1985; Obeta, 1985; Ogbadu and Okagbue, 1988) for *iru* processing but there is dearth of information on the effects of optimization of pre-processing conditions such as dehulling and re-cooking on the functionality of the seeds.

Seed proteins are required to possess the essential requisite functional properties for successful utilization in various food products. These functional properties are intrinsic physico-chemical characteristics which affect the behaviour of properties in food systems during processing, manufacturing, storage and preparation. The properties include emulsion capacity, activities and stability. It also includes foam capacity and stability. Other paramount functionalities are proteins solubility, water and fat absorption capacity, organoleptic properties and bulk density (Aremu *et al.*, 2007).

The quality and technological usability of food proteins are determined by their nutritional and functional properties (Ulloa *et al.*, 2011). When protein is not considered as the main food component but as one of many constituents, its functionality can be an even more important evaluation criterion than its nutritional value. Owen (1996) reports that the sensory attributes of foods are achieved by complex interactions among various functional ingredients and since proteins possess a multitude of physical and chemical properties, it is difficult to delineate the role of each of these properties with respect to a given functional property. deMan (1999) agrees that in low and intermediate moisture foods, such as bakery and comminuted meat products, the ability of proteins to bind water is critical to the acceptability of these foods.

In a previous work by Koledoye and Akanbi (2013), optimum conditions for cooking and dehulling locust bean seeds prior to fermentation was established. But the study did not investigate the effect of employing these conditions on the anti-nutritional factors, functionality and sensory properties of the fermented product. This study therefore seeks to use the established conditions to achieve this purpose.

2. EXPERIMENTAL PROCEDURE

2.1. Sample preparation

Fresh locust bean seeds were processed using optimal conditions for dehulling and re-cooking established by Koledoye and Akanbi (2013). About 100 g of seeds were cooked in 4 L of water at 50 kPa (using a Prestige Pressure Cooker) for 54 min and re-cooking 90 g of seeds in 3 L of water for 15 min. Dehulling was carried out using a hand-held Russell Hobbs mixer set at a determined speed. The dehulled seeds were fermented following a modified method of Abiose *et al.* (1986). The seeds were transferred hot into calabashes lined with banana leaves and incubating at 30 °C for 48 h. A portion of the fermented seeds was dried in a hot air oven at 65 °C for 24 h. The dried seeds were packaged as such in polythene bags.

2.2. Determination of Functional Properties

Sample solubility, emulsifying capacity, water and oil absorption capacity determinations were carried out on both the raw and dried fermented locust bean seeds. Solubility of the products was carried out using the method of Beuchat (1977), Emulsifying capacity was determined using the method of Neto *et al.* (2001). Water and oil absorption capacity was carried according to the method of Hayta and Naysar (2002).

2.3. Determination of Anti-nutritional factors

The anti-nutritional factors investigated in this study were Tannin, Phytate and Oxalate. The tannin content was determined using the method of Makkar and Goodchild (1996), Phytate content was determined using the method described by Haugh and Lantzsch (1993) while determination of oxalic acid content was according to the method of Oke (1966).

2.4. Sensory Evaluation of the Optimally Processed *Iru*

The processed *Iru* was assessed by presenting both traditionally and optimally processed *Iru* samples at random to a 10 member panellists who are familiar with the product and asking them to score the following attributes: colour, taste, aroma, mouth feel, texture and overall acceptability using a 7-point Hedonic scale.

2.5. Statistical Analyses

The data collected were expressed as Mean ± standard deviation of three experiments, and subjected to statistical analysis using one-way analysis of variance to determine significant differences between means (α = 0.05). Tukey's Least Significant Difference test was used to compare means. All statistical procedures were carried out using PlotIT 3.2 software.

3. RESULTS AND DISCUSSION

3.1. Effect of Processing on the Functional Properties of the Raw and Fermented Locust Bean Seeds

The functional properties of the raw and fermented locust beans seeds are shown in Table 1. The water absorption capacity of the dehulled seeds was lower than that of the raw seeds but it significantly increased with fermentation. The reduction in the water absorption capacity of the dehulled seed may be due to the loss of insoluble fibre (Cadden, 1987) but the water absorption capacity increased with fermentation and dehydration. The water absorption capacity for the dehydrated *Iru* was significantly higher than that of the wet *Iru*. Choonhahirun (2010) reports that water absorption capacity describes the flour-water association ability under limited water supply and it is also used as an indication of performance in several food formulations (Circle and Smith, 1972). Therefore, the dehydrated seeds when ground to flour will be of great use in soups and gravies as a thickener.

Abbey and Ibeh (1988), reported that heat processing increases water absorption by about 52%. According to Eke and Akobundu (1993); Narayana and Narasigan Rao (1984); during heat processing gelatinization of the carbohydrate and swelling of the crude fibre occur which could also lead to increased water absorption. The higher water absorption capacity may be due to the presence of high polar amino acid residues of proteins having affinity for water molecules (Yusuf *et al.*, 2008). The major chemical components that enhance the water absorption capacity of flours are proteins and carbohydrates, since they contain hydrophilic parts such as polar or charged side chains (Lawal and Adebowale, 2004).

The oil absorption capacity also decreased with processing i.e. fermentation and dehydration but the dehydrated product had a higher oil absorption capacity than the wet fermented product. Oil absorption is an important property in food formulations because oil improves the satiety, flavour and mouth feel of foods (Kinsella, 1976). The high values of oil absorption capacity agree with the findings of Ibrahim *et al.* (2011) for roasted and defatted cashew nut flour. Obatolu *et al.* (1995) reported that oil absorption was attributed to the physical entrapment of oil which is related to the number of non-polar side chains in the protein that bind hydrocarbon chains of the fatty acid. Lawal (2003) attributed this behaviour to the presence of non-covalent bonds, such as hydrophobic, electrostatic and hydrogen bonding forces that are involved in lipid-protein interactions. The major chemical component affecting oil absorption capacity is protein which is composed of both hydrophilic and hydrophobic parts. Non-polar amino acid side chains can form hydrophobic interactions with hydrocarbon chains of lipid (Jitngarmkusol *et al.*, 2008). In addition, Adebowale and Lawal (2004) reported that variations in the presence of non-polar side chains, which might bind the hydrocarbon side chains of oil among the flour explains difference in the oil binding capacity.

The solubility of the seeds after dehulling increased with fermentation and dehydration by 28% and 73%, respectively. This implies that the fermented seed is capable of going into solution in the dehydrated form much more than the wet form.

The high emulsion activity suggests that the product can be useful as an additive for stabilization of fat emulsions in food formulations. The results agree with Jitngarmkusol *et al.*, (2008)'s report that processing contributes to the emulsifying capacity of protein. According to Vioque *et al.* (2000), limited hydrolysis improves the emulsifying properties of protein owing to the exposure of hydrophobic amino acid residue that may interact with the oil while the hydrophilic residues interact with water. This thus explains the improved emulsifying properties observed in the dehydrated product. Obatolu *et al.* (1995) reported that no observable emulsion activity in fermented locust bean seeds and attributed it to the length of time in boiling before fermentation which led to protein denaturation. It then suffices to say that the short cooking time employed in this study enhanced emulsion activity in the product.

3.2. Effect of Processing on Anti-Nutrient Activity of the Raw and Fermented Locust Bean Seeds

The anti-nutrient activity levels of the raw and fermented seeds are shown in Table 2. There was a significant reduction in the tannin content from the raw state to the fermented state by 78% while there was a 54% decrease in the tannin content of the dried fermented seeds as compared with the raw. This reduction is similar to that obtained in a similar study by Esenwah and Ikenebomeh (2008) who reported a 59% reduction in hydrolysable tannin content. Helsper *et al.* (1993) reported that condensed tannins were responsible for the testa-bound trypsin inhibitor activity of faba beans. They also have the ability to form complexes with vit B_{12} (Liener, 1980). A reduction in tannin content of legumes due to processing has been reported by several authors. A 48.0 to 50.1% reduction was reported by El-Adawy (2002) during the processing of chickpea using different cooking methods. Esenwah and Ikenebomeh (2008) also asserted that the loss of tannin may be due to its solubility in water and its sensitivity to heat during processing.

The phytic acid content also reduced significantly by 92% in the wet fermented seeds and 88% in the dehydrated seeds. This reduction also agrees with a similar study by Mbajunwa (1995) although on another variety of locust beans. Phytic acid has been reported by Hendricks and Bailey (1989) to chelate metal ions such as calcium, magnesium, zinc, copper, iron and molybdenum to form insoluble complexes that are not readily absorbed from the gastro-intestinal tract. It also inhibits the action of some gastro-intestinal enzymes such as tyrosinase, trypsin, pepsin, lipase and α-amylase. Martinez (1977) also reported that in oilseeds which contain little or no endosperm, the phytates are distributed throughout the kernel found within the subcellular inclusions called aleurone grains or protein bodies.

A 30% decrease was observed in the oxalic acid content of the dried fermented seeds while the decrease in the wet seeds was just 50%. These observations imply that fermentation and heat processing reduce the anti-nutrient content of locust beans. According to Olomu (1995), oxalic acid binds calcium and forms calcium oxalate which is insoluble. Calcium oxalate adversely affects the absorption and utilization of calcium in the animal body.

The significant decrease in all anti-nutrient levels checked makes the product safe for consumption either in the raw form or as a condiment in soups.

3.3. Sensory Evaluation

As shown in Table 3, the optimally and traditionally processed *Iru* samples had comparable consumer preference for taste and mouthfeel while there was a significant difference in the preference for colour, aroma, texture and overall acceptability.

The colour of the optimally processed sample was brown and wasn't different from the colour of the unfermented seeds unlike the traditionally processed sample which was black as a result of prolonged cooking and soaking that allowed the bleaching of the colour of the hull into the cotyledon. This agrees with Sadiku (2010), who reported that the cooking of seeds in water, especially pre-dehulling cooking has a relationship with changes in colour of seeds.

The fermentation period of the optimally processed sample was longer than the traditionally processed one and this resulted in it having a slightly marshy texture thus agreeing with the report of Omafuvbe *et al.* (2004) that the amount of water absorbed by the cotyledons during cooking primarily determines how marshy the product will be while the bacterial (mainly Bacillus and Staphylococcus) activities during fermentation enhance it.

The significant difference in overall acceptability can be attributed to the consumers being accustomed to the conventional fermented locust bean found in the market.

Table 1: Functional Properties of the Raw and Fermented Locust Bean Seeds

	Water Absorption Capacity (%)	Oil Absorption Capacity (%)	Solubility (%)	Emulsion Capacity (%)
Raw Seeds	145.5 ± 0.68^{c}	117.25 ± 0.43^{d}	48.47 ± 0.18^{c}	55.56 ± 0.49^{c}
Dehulled Seeds	97 ± 1.52^{a}	85.06 ± 0.21^{a}	12.04 ± 0.20^{a}	24.85 ± 0.63^{a}
Fermented Seeds	119.5 ± 0.65^{b}	73 ± 1.44^{b}	16.71 ± 0.14^{b}	39.04 ± 1.18^{b}
Fermented and Dehydrated Seeds	191 ± 0.50^{d}	101.75 ± 0.09^{c}	39.80 ± 0.11^{c}	60.09 ± 0.51^{d}

**Means within the same column having the same superscript are not significantly different at 5% level*

Table 2: Anti-nutrient Composition of the Raw and Fermented Locust Bean Seeds

	%Oxalate	%Phytate	% Tannin
Raw Seeds	0.086^{d}	0.57^{d}	0.065^{d}
Dehulled Seeds	0.078^{c}	0.250^{c}	0.055^{c}
Fermented Seeds	0.043^{a}	0.048^{a}	0.014^{a}
Fermented and Dehydrated	0.060^{b}	0.070^{b}	0.030^{b}

**Means within the same column having the same superscript are not significantly different at 5% level*

4. CONCLUSIONS

The study revealed that processing locust bean seeds under optimized conditions yields a product with acceptable nutritional benefits. The study also shows that reducing cooking time significantly causes a reduction in anti-nutrient activity which could have otherwise been a major draw-back in the utilization of *iru* as a food condiment. Conclusively, the conditions used in this study can be adapted in mechanizing the entire process of *iru* production without altering the functionality and consumer acceptability of the product.

Table 3: Sensory Qualities of the Optimally Processed and Traditionally Processed Iru

	Sensory Attributes*					
	Colour	Aroma	Taste	Mouthfeel	Texture	Overall Acceptability
TPI	6.8^{a}	6.4^{a}	6.4^{a}	5.8^{a}	6.6^{a}	6.6^{a}
OPI	5.6^{b}	5.2^{b}	5.6^{a}	4.6^{b}	4.8^{b}	4.6^{b}

**Means within the same column having the same superscript are not significantly different at 5% level*

TPI = Traditionally Processed Iru

OPI = Optimally Processed Iru

REFERENCES

Abbey, B. W. and Ibeh, G. O., Functional properties of raw and heat processed cowpea (*Vigna unguiculata*) flour, *Journal of Food Science*, 53: 1775 – 1991, 1988.

Abiose, S. H., Talabi, T. A., and Ajayi, L. O., Fermentation of African Locust Beans: Microbiological and Biochemical studies, *Nigerian Journal of Biological Science*. 1(2): 109 – 117, 1986.

Adebowale, K. O. and Lawal, O. S., Comparative study of the functional properties of Bambara groundnut (*Voandezia subterranean*), jack bean (*Canavalia ensiformins*) and mucuna bean (*Mucuna pruriens*) flour, *Food Research International*, 37(4): 355-365, 2004.

Amza, T., Amadou, I., Kamara, M. T., Zhu, K., and Zhou, H., Chemical and Nutrient Analysis of Gingerbread Plum, *Advance Journal of Food Science and Technology* 2(4):191 – 195, 2010.

Aremu, M. O., Olaofe, O. and Akintayo, E. T., Functional properties of some Nigerian varieties of legume seed flours and flour concentration effect on foaming and gelation properties, *Journal of Food Technology*, 5 (2): 109 – 115, 2007.

Beuchat, L. R., Functional and electrophoretic characteristic of succinylated peanut flour protein, *Journal of Agriculture and Food Chemistry*, 25: 258-261, 1977.

Cadden, A. M., Comparative effects of particle size reduction on physical structure and water binding properties of several plant fibers, *Journal of Food Science*, 52: 1595 – 1599, 1987.

Choonhahirun, A., Proximate composition and functional properties of (*Elateriospermun tapos* Blume) seed flour, *African Journal of Biotechnology*, 9(36): 5946-5949, 2010.

Christiana, N. E. and Marcel, J. I., Processing Effects of the Nutritional and Anti-nutritional content of African Locust Bean (*Parkia biglobosa* benth) Seed, *Pakistan Journal of Nutrition* 7: 214 -217, 2008.

Circle, S. J. and Smith, A. K., Functional properties of commercial edible soybean protein products, In: Seed Proteins. Inglet G.E (ed). Avi, Westport, CT. pp. 242 – 254, 1972.

Eke, O. S. and Akobundu, E. N. T., Functional properties of African yam bean (*Sphenostylis stenocorpa*) seed flour as affected by processing, *Food Chemistry*, 48: 337 – 340, 1993.

El-Adawy, T. A., Nutritional composition and anti-nutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination, *Plant Foods and Human Nutrition*, 57: 83-97, 2002.

Esenwah, C. N. and Ikenebomeh, M. J., Processing Effects on the Nutritional and Anti-Nutritional Contents of African Locust Bean (*Parkia biglobosa* Benth.) Seed, *Pakistan Journal of Nutrition* 7 (2): 214-217, 2008.

Haugh, W. and Lantzch, H. J., Sensitive method for determination of phytate in cereals and cereals product, *Plant Foods for Human Nutrition* 44: 261 – 266, 1993.

Hayta, M. and Naysar, A., Effect of drying method on functional properties of Tarhana, Wheat flour, Yoghurt mixture. *Journal of Food Science*, 67(2): 740-744, 2002.

Helsper, J. P., Hoogendyk, J. M., Van Novel, A. and Burger-Meyer, K., Antinutritional factors in faba beans (*Vicia faba* L.) as affected by breeding toward the absence of condensed tannins, *Journal of Agriculture and Food Chemistry*, 41: 1058 – 1061, 1993.

Hendricks, J. D. and Bailey, G. S., "Adventitious toxins". In: Fish Nutrition. 2nd edition, Halver, J. E. (ed.). Academic Press inc., New York, USA. pp: 606-651, 1989.

Ibrahim, T. A., Omosuli, V., Oloye, D. A., Aladekoyi, G. and Ogundowole, O., Functional properties of roasted and defatted cashew nut (*Anarcadium occidentale*) flour, *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 10(4): 2135 – 2138, 2011.

Jitngarmkusol, S., Hongsuwankul, J. and Tananuwong, K., Chemical composition, functional properties, and microstructure of defatted macademice flours, *Food Chemistry*, 110: 23 – 30, 2008.

Kinsella, J. E., Functional properties of food proteins: A review. CRC Crit. Rev. *Food Sci. Nutr.* 7:219 – 280, 1976.

Koledoye, A. O. and Akanbi, C. T., Optimisation of process conditions for dehulling and softening of locust bean (*Parkia biglobosa*, Jacq. Benth) seeds prior to fermentation in *Iru* production, *International Journal of Postharvest Technology and Innovation*, 3: 123 - 139, 2013.

Lawal, O. S., "Functionality of African locust bean (*Parkia biglobosa*) protein isolate: effects of pH, ionic strength and various protein concentrations", *Food Chemistry*, 86: 345–355, 2003.

Lawal, O. S. and Adebowale, K. O., Effect of acetylation and succinylation on solubility profile, water absorption capacity, oil absorption capacity and emulsifying properties of muncuna bean (*Mucuna prueins*) protein concentrate. *Nahrung/Food*, 48(2): 129-136, 2004.

Liener, I. E., "Heat labile antinutrient factors". In: Advanced Legume Science, (Editor: R. J. Sumerfield and A. H. Bunting), Kew London, Royal Botanical Gardens, pp: 157-170, 1980.

Makkar, A. O. S. and Goodchild, A. V., "Qualification of Tannins", A laboratory manual. International Centre for Agricultural Research in the Dry Areas (ICARDA) Aleppo, Syria. 25p, 1996.

Martinez, W. H., "Evaluation of Proteins for Humans", (Editor, C.E. Bodwell). Publishing Co. Inc., Westport, CT. 203p, 1977.

Mbajunwa, O. K., Effect of processing on some anti-nutritive and toxic components and on the nutritional composition of the African oil bean seed (*Pentaclethra macrophylla* Benth.), *Journal of the Science of Food and Agriculture*, 68: 153-158, 1995.

Narayana, K. and Narasinga Rao, M. S., Effect of partial proteolysis on the functional properties of sunflower meal product, *Journal of Food Science*, 49: 944 – 947, 1984.

Neto, V. Q., Narain, N., Silva, J. B., and Bora, P.S., Functional properties of raw and heat processed cashew nut (*anarcadian occidentale,* L.) kernel protein isolate, *Nahrung/Food*, 45, 258 – 262, 2001.

Obatolu, V. A., Osho, S. M. and Uwaegbute A. C., Comparative physicochemical properties of fermented soybean and locust bean, Post-harvest technology and commodity marketing. Proceedings of a post-harves conference. Nov 2nd - Dec 1st 1995, Accra Ghana.

Obeta, J. A. N., "A note on microorganism associated with the fermentation of African locust bean (*Parkia filicoidea*) during Iru preparation, *Plant Foods Human Nutrition* 5: 245 – 250, 1985.

Odunfa, S. A and Adewuyi, E. Y., Optimization of process conditions for the fermentation of locust bean (*Parkia biglobosa*). Effects of time, temperature and humidity, *Food Chemistry and Microbiology Technology* 9: 6 – 10, 1985.

Odunfa, S. A., Microorganisms associated with fermentation of African locust bean (*Parkia filicoidea*) during iru preparation, *Journal of Plant Foods* 3, 245 – 250, 1981.

Ogbadu, L. J and Okagbue, R. R., Fermentation of African locust bean (*Parkia biglobosa*). Involvement of different species of Bacillus species, *Journal of Food Microbiology* 5: 195 – 199, 1988.

Oke, O. L., Chemical studies on some Nigerian Vegetables, *Tropical Science*, 8(3): 128-132, 1966.

Okeoma, J. and Oyeleke, K., Nutritional consideration in the choice of flavour enhancer in Nigerian soups, *Nigerian Journal of Nutritional Sciences*, 9 (2): 87-95, 1988.

Oladunmoye, M. K., Effects of fermentation on nutrient enrichment of locust beans (*Parkia biglobosa*, Robert bam), *Research Journal of Microbiology*, 2: 185-189, 2007.

Olomu, J. M., Monogastric animal nutrition, principles and practice. Jachem publication, 320p, 1995.

Omafuvbe, B. O., Falade, O. S., Osuntogun, B. A. and Adewusi, S. R. A., Chemical and Biochemical Changes in African Locust Bean (*Parkia biglobosa*) and Melon (*Citrullus vulgaris*) Seeds During Fermentation to Condiments, *Pakistan Journal of Nutrition*, 3 (3): 140-145, 2004.

Sadiku, O. A., Processing Method Influence the Quality of Fermented African Locust Bean (iru/ogiri/dadawa), *Parkia biglobosa*, *Journal of Applied Sciences Research*, 6(11):1656-1661, 2010.

Ulloa, J. A., Rosas-Ulloa, P. And Ulloa-Rangel B. E., Physicochemical and functional properties of a protein isolate produced from safflower (*Carthamus tinctorius* L.) meal by ultrafiltration, *Journal of Science, Food and Agriculture*, 91(3): 572-577, 2011

Vioque, J., Sanchez-Vioque, R., Clemente, A., Pedroche, J., Bautista, J. and Millan, F., Partially hydrolysed rapeseed protein isolates with improved functional properties, *Journal of American Oil Chemist's Society*, 77; 447 – 450, 2000.

Yusuf, A. A, Ayedun, H. and Sanni, L. O., Chemical composition and functional properties of raw and roasted Nigerian benniseed (*Sesamum indicum*) and Bambara groundnut (*Vigna subterranean*)-, *Food Chemistry*, 111: 277-282, 2008.