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Processing Effects on the Nutritional and Anti-Nutritional Contents of African Locust Bean (*Parkia biglobosa* Benth.) Seed

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Abstract: African locust bean (*Parkia biglobosa* Benth.) seeds with thin layers of pulp material were processed by soaking in water for 12 h, washing to depulp the seeds and boiling in water for 8 h to dehull the seeds. The dehulled seeds were boiled again for 30 min to produce the processed substrate which were fermented for 72 h. Mechanically dehulled bean seeds were obtained from depulped bean seeds with a pair of pliers. Proximate compositions of the samples were determined for crude protein, ether extract, ash and total carbohydrate content. The presence and levels of trypsin inhibitors, tannin and phytic acid were determined. Fermentation gave rise to significant increase in crude protein content. The ether extract was significantly increased by soaking and boiling, this was further increased by fermentation at 72 h. The ash content decreased significantly by soaking and boiling. Also the total carbohydrate content decreased significantly by soaking and boiling, this was further decreased by fermentation at 72 h. In all the anti-nutritional factors evaluated, soaking and boiling significantly reduced the levels of anti-nutritional factors, fermentation at 72 h led to further reduction in the levels of anti-nutritional factors. The reduction in content of trypsin inhibitors activity at 72 h fermentation was greater than the other anti-nutritional factors with a total reduction level of 89.0%.

Key words: Anti-nutritional factors, *Parkia biglobosa*, soaking, boiling, fermentation

Introduction

The high cost of animal protein has directed interest towards several leguminous seed proteins as potential sources of vegetable protein for human food and livestock feed. Among the plant species, grain legumes are considered as the major source of dietary proteins. They are consumed world wide, especially in developing and under developed countries where consumption of animal protein may be limited as a result of economic, social, cultural or religious factors.

African locust bean seeds are rich in protein and usually fermented to a tasty food condiment called *dawadawa* which is used as a flavour intensifier for soups and stews and also adds protein to a protein-poor diet (Ikenebomeh and Kok, 1984; Odunfa, 1986; Dike and Odunfa, 2003). However, the use of African locust bean seeds and other legumes as protein source is limited by the presence of anti-nutritional factors which are a diverse range of naturally occurring compounds in many tropical plants. The anti-nutritional factors cause poor protein digestibility in man and animals and are capable of precipitating other deleterious effects. Manifestations of toxicity from the consumption of legumes containing anti-nutritional factors range from severe reduction in food intake and nutrient availability or utilization, to profound neurological effects and even death (Osagie, 1998). To improve the nutritional quality and organoleptic acceptability of leguminous seeds, processing techniques have been employed to reduce or destroy the anti nutrients present in them. 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 fermentation.

Little information is available on the effect of processing on the anti-nutritional factors of African locust bean. This study was therefore undertaken to investigate the effects of processing on some nutrients and anti-nutritional factors in African locust bean seeds and the extent of reduction of these following processing.

Materials and Methods

Collection of African Locust Bean seed (ALB): African locust bean seeds were purchased from retailers in Kawo market, Kaduna state, Nigeria. The dry raw bean seeds were stored at room temperature, 28±2°C until used.

Processing of ALB: The raw bean seeds were processed by depulping, dehulling, hydrating and fermenting by methods adapted from that of Ikenebomeh and Kok (1984). The sample (1.0 kg) was soaked in 5 L of tap water for 12 h to soften the adhering pulpy materials. The pulp was removed by rubbing the seeds between the palms and washing them with water.

The depulped cleaned seeds were dried at room temperature for 48 h and dehulled to free them from the dark brown testae as follows:
Tap water was added to the sample in a flask to give seed: water ratio of 1: 5 (w/v) and boiled on a hot plate for 8 h. The evaporated water was replaced every 2 h in order to keep the seeds covered. The content was allowed to cool to 28±2°C and excess water was drained. The testae were removed by rubbing the seeds between the palms and washing with water. The seeds free of the testae were referred to as dehulled bean seeds. They were hydrated further by boiling with water for 30 min, cooled and drained. The dehulled and hydrated bean seeds were referred to as processed substrate. This was allowed to ferment by solid substrate fermentation which involved weighing 25 g of processed substrate into sterile Petri dish of 100 mm diameter, the bottom of which was lined with a sterile filter paper. The processed substrate was then covered with another sterile filter paper and the Petri dish covered to form the fermentation unit which was incubated at 37°C for 72 h to obtain the fermented bean seeds at 72 h.

Mechanical dehulling of ALB: A pair of pliers were swabbed with 70% alcohol and used for removing the testae from the depulped bean seeds to obtain the mechanically dehulled bean seeds.

Proximate composition analyses of ALB: Crude protein, fat, ash and total carbohydrate contents of the samples were estimated by standard methods (Pearson, 1970; AOAC, 1980).

Determination of trypsin inhibitor activity: Trypsin inhibitor activity of sample was determined by the method of Kakade *et al.* (1974). The digest contained 1.0 g of the sample, 40 µg of trypsin and 2 mg of benzoyl-DL-arginine-P-nitroanilide (BAPA) in Tris buffer. The absorbance of sample was read at 410 nm.

Determination of tannin content: The method of estimation of tannin content in extract by Joslyn (1970) was used for the determination of tannin content in samples. Finely ground sample (0.5 g) was defatted with 5% ethyl ether for 15 min. The tannin in the defatted sample was then extracted with methanol and the absorbance at 760 nm was measured.

Determination of phytic acid: An indirect colorimetric method of Wheeler and Ferrel (1971) was used for phytate determination. This method depends on an iron to phosphorus ratio of 4: 6. Five grams of the test sample was extracted with 3% tri-chloro acetic acid. The phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding sodium hydroxide. The precipitate was dissolved in hot 3.2 N HNO_3 and the colour read immediately at 480 nm. The standard solution was prepared from Fe $(NO_3)_3$ and the iron content was extrapolated from a Fe $(NO_3)_3$ standard curve. The phytate concentration was calculated from the iron results assuming a 4: 6 iron: phosphorus molecular ratio.

Statistical analyses: The data obtained in this work were subjected to statistical analyses using statistical programmes in Microsoft Excel and Statistical Package for the Social Sciences (SPSS 10.0 package). The statistical analyses carried out were mean and standard deviation, analysis of variance (ANOVA), Duncan's Multiple Range (DMR) test and chi-square goodness of fit test (Alder and Roessler, 1977; Ogbeibu, 2005).

Results and Discussion

The proximate composition of samples of African locust bean seed during processing is presented in Table 1. Ash content of the sample decreased significantly ($p<0.001$) from 47.5±3.6 g kg^{-1} in the mechanically dehulled bean seeds to 37.3±2.7 g kg^{-1} in the processed substrate following soaking and boiling. Loss in ash may be due to leaching of soluble inorganic salts into the processing water during the soaking of the sample for 12 h followed by boiling for 8 h. Similar result was reported in Dolichos Lablab Bean seeds (Osman, 2007). The total carbohydrate content of samples also decreased significantly ($p<0.05$) by soaking and boiling from 484.2 g kg^{-1} in mechanically dehulled bean seeds to 425.0 g kg^{-1} in the processed substrate. This was further decreased significantly ($p<0.05$) by fermentation at 72 h fermentation period to 291.6 g kg^{-1} (Table 1). This result was in agreement with results of earlier workers (Addy *et al.*, 1995; Omafuvbe *et al.*, 2004; Osman, 2007). Loss in carbohydrate during soaking and boiling may be due to leaching of soluble carbohydrates like sugars into the soaking and cooking water; while loss in carbohydrate during fermentation may be as a result of the utilization of some of the sugars by fermenting organisms for growth and metabolic activities.
The crude protein content of African locust bean seeds increased significantly ($p<0.001$) by fermentation from 275.3±12.5 g kg^{-1} in the processed substrate to 328.1±4.0 g kg^{-1} in the fermented product at 72 h (Table 1). The increase in protein content obtained in this study during fermentation agreed with other reports on African locust bean seeds (Ikenebomeh, 1986; Omafuvbe *et al.*, 2004). However, higher values were reported by Omafuvbe *et al.* (2004). The difference in the levels of protein content obtained in both studies may be attributed to differences in cultivars of *P. biglobosa* studied and experimental procedures used.

Table 1: Proximate compositions of samples of African locust bean seeds on dry weight basis

			Sample			
Parameter	MDB	PS	FB 24	FB 48	FB 72	p-value
Ash (g kg^{-1})	47.5±3.6^{b}	37.3±2.7^{a*}	34.5±1.0^{a}	33.1±2.1^{a}	33.0±3.0^{a}	p<0.001
Crude protein (N×6.25 g kg^{-1})	270.1±12.3^{a}	275.3±12.5^{a}	315.7±4.7^{b}	319.2±14.5^{b}	328.1±4.0^{b}	p<0.001
Ether extract (g kg^{-1})	198.2±11.0^{a}	262.4±11.5^{b}	265.1±7.6^{b}	281.5±8.1^{b}	347.3±14.5^{c}	p<0.001
Total carbohydrate** (g kg^{-1})	484.2^{a}	425.0^{b}	384.7^{b}	366.2^{b}	291.6^{c}	p<0.05

Note: *Same letters indicate mean values that are not significantly different on the same row; **Total carbohydrate was by difference. Results are expressed as mean±standard deviation of four determinations; MDB = Mechanically dehulled bean seeds; PS = Processed substrate; FB24 = Fermenting bean seeds at 24 h; FB48 = Fermenting bean seeds at 48 h; FB72 = Fermented bean seeds at 72 h

Table 2: Anti-nutritional contents in African locust bean samples

			Sample			
Parameter	MDB	PS	FB24	FB48	FB72	p-value
TIA (mg g^{-1})	2.9±0.08^{c*}	1.1±0.27^{b} (62.1%)**	0.73±0.09^{a} (74.8%)	0.55±0.18^{a} (81.0%)	0.32±0.05^{a} (89.0%)	p<0.001
Tannin (mg g^{-1})	40.0±3.0^{c}	25.1±4.8^{b} (37.3%)	23.2±6.8^{b} (42.0%)	18.3±2.7^{a} (54.3%)	16.1±3.8^{a} (59.8%)	p<0.001
Phytic acid (mg g^{-1})	2.4±0.3^{d}	1.5±0.2^{c} (37.5%)	1.3±0.1^{b} (45.8%)	1.2±0.1^{b} (50.0%)	0.9±0.03^{a} (62.5%)	p<0.001

Note: Results are expressed as mean±standard deviation of four determinations; *Same letters indicate mean values that are not significantly different on the same row; **Values in parentheses refer to the percentage reduction in the levels of anti-nutritional factors; TIA = Trypsin inhibitor activity; MDB = Mechanically dehulled bean seeds; PS = Processed substrate; FB24 = Fermenting bean seeds at 24 h; FB48 = Fermenting bean seeds at 48h; FB72 = Fermented bean seeds at 72 h

Ether extract of samples also increased significantly (p<0.001) by soaking and boiling, which was further increased by fermentation at 72 h. The increase in ether extract obtained in this work is in agreement with the findings of Omafuvbe *et al.* (2004), Addy *et al.* (1995) and Ikenebomeh (1986). Soaking and boiling of the sample might have led to the cleavage of the protein-lipid or carbohydrate-lipid linkages thereby, facilitating the easy extraction of the oil by the extracting solvent.

The increases recorded in the crude protein and ether extract may be due to the reduction in the carbohydrate content and may be regarded as apparent increase in both the protein and fat contents to complement the decrease in carbohydrate. The carbohydrate would have been used by the micro-organisms for metabolic energy causing a decrease.

The results of the effect of processing and fermentation on the levels of some anti-nutritional factors of African locust bean are given in Table 2. In all the anti-nutritional factors examined, soaking, boiling and fermentation resulted in decreased levels of anti-nutritional factors. The level of Trypsin Inhibitor Activity (TIA) reduced significantly (p<0.001) from 2.9±0.08 mg g^{-1} in the mechanically dehulled bean to 0.32±0.05 mg g^{-1} in the fermented product at 72 h fermentation period (FB72). This represents 89% reduction in the TIA content of African locust bean (Table 2). The loss in TIA content during processing can be attributed to leaching during soaking, by heat treatment during boiling and also by the action of micro organisms during fermentation. Similar results were reported by other workers in African locust bean and chick pea (Addy *et al.*, 1995; El-Adawy, 2002; Alonso *et al.*, 1998). However, differences exist in the levels of trypsin inhibitor activity reported in this work and that of other workers. Addy *et al.* (1995) reported 9-42% reduction in trypsin inhibitor activity values in African locust bean (*Parkia filicoidea*) by the processing treatments of acid, alkali and fermentation used in their study. El-Adawy (2002) reported 33.9 to 83.7% reduction in trypsin inhibitor activity values in chick pea (*Cicer arietinum* L.) undergoing different cooking methods of autoclaving, boiling and microwave cooking and germination. The reason for differences in results obtained in this work and those reported by others is unknown but may be explained in terms of differences in analytical methods and also differences in the cultivars of legumes studied.

The tannin content reduced significantly (p<0.001) from 40.0±3.0 mg g^{-1} in mechanically dehulled bean to 16.1±3.8 mg g^{-1} in the fermented product at 72 h representing 59.8% reduction in the tannin content of sample (Table 2). Loss of tannin may be due to its solubility in water and its sensitivity to heat during boiling. Similar reduction in tannin content was recorded during processing of legumes by other workers. Igboeli *et al.* (1997) reported 35.9 to 43.6% reduction in tannin content during the processing of baobab seed (*Adansonia digitata*) by dehulling, cold water, hot water, hot alkali and acid treatments. El-Adawy (2002) reported 48.0 to 50.1% reduction during processing of chick pea (*Cicer arietinum* L.) by different cooking methods. Mbajunwa (1995) observed that during the processing of African oil bean seed (*Pentaclethra macrophylla* Benth.), cooking of the seed led to 51.6% reduction in the tannin content while fermentation further reduced the tannin content to 56.8% total reduction. The phytic acid content of African locust bean samples also reduced significantly (p<0.001) from 2.4±0.3 mg g^{-1} in mechanically dehulled bean to 0.9±0.03 mg g^{-1} in the fermented bean at 72 h; representing 62.5% reduction in

the phytic acid content (Table 2). Mbajunwa (1995) in a study on the effect of processing on some anti-nutritional and toxic components and on the nutritional composition of African oil bean seed *Pentaclethra macrophylla* Benth. reported 11.2 g kg^{-1} in the raw sample and 2.7 g kg^{-1} in the fermented sample; which represented 75.9% decrease in phytic acid in the fermented product.

The various processing methods affected the nutritional value of African locust bean. The higher crude protein and ether extract obtained for the Processed African locust bean are improvement on its nutritional quality. In addition, trypsin inhibition activity, tannin and phytic acid were significantly reduced by the various processing methods. Knowing the health implications of these anti-nutritional factors to man and livestock, the reduction in the levels of anti-nutritional factors during processing of African locust bean is very vital for the safety of the product. Soaking and boiling significantly improved the value of the product; however, the fermentation process achieved better quality that will enhance utilization of the product. Therefore, the fermentation of African locust bean for 72 h will add value to its nutritional quality.

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References

Addy, E.O.H., L.I. Salami, L.C. Igboeli and S.H. Remawa, 1995. Effect of processing on nutrient composition and anti-nutritive substances of African locust bean (*Parkia filicoidea*) and baobab seed (*Adansonia digitata*). Plant Foods Hum. Nutr., 48: 113-117.

Alder, H.L. and E.B. Roessler, 1977. Introduction to Probability and Statistics (6th edition). Freeman W.H., San Francisco, pp: 1-426.

Alonso, R., E. Orue and F. Marzo, 1998. Effects of extrusion and conventional processing methods on protein and anti-nutritional factorcontents in pea seeds. Food Chem., 63: 505-512.

AOAC, 1980. Official Methods of Analysis (13th edition). Association of Official Analytical Chemists, Washington, DC, USA, pp: 211-222.

Dike, E.N. and S.A. Odunfa, 2003. Microbiological and biochemicalevaluation of a fermented soyabean product-soyadawadawa. J. Food Sci. Tech., 40: 606-610.

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

Igboeli, L.C., E.O.H. Addy and L.I. Salami, 1997. Effects of someprocessingtechniques on the anti-nutrient contents of baobabseeds (*Adansonia digitata*). Biores Tech., 59: 29-31.

Ikenebomeh, M.J., 1986. African locust bean fermentation-some physico-chemical parameters. Nig. J. Appl. Sci., 4: 101-108.

Ikenebomeh, M.J. and R. Kok, 1984. Mass balance of the processingandfermentation of the African locust bean (*Parkia filicoidea*Welw.). J. Can. Inst. Food Sci. Tech., 17: 48-50.

Joslyn, N., 1970. Methods in Food Analysis. Physical, Chemical andInstrumental Methods of Analysis (2nd edition). Academic Press, New York, pp: 708-720.

Kakade, M.L., J.J. Rackis, J.E. Mcghee and G. Puski, 1974. Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. Cereal Chem., 51: 376-382.

Mbajunwa, O.K., 1995. Effect of processing on some anti-nutritiveand toxiccomponents and on the nutritional composition of theAfrican oil bean seed (*Pentaclethra macrophylla* Benth.). J. Sci. Food Agric., 68: 153-158.

Odunfa, S.A., 1986. Dawadawa. In: Reddy, N.R., M.D. Pierson and D.K. Salunkhe (Eds.), Legume-Based Fermented Foods. CRS Press, Boca Raton, Florida, pp: 173-189.

Ogbeibu, A.E., 2005. Biostatistics, a Practical Approach to Researchand DataHandling. Mindex Publishing Company Ltd. BeninCity, Nigeria, pp: 264.

Omafuvbe, B.O., O.S. Falade, B.A. Osuntogun and S.R.A. Adewusi, 2004. Chemical and biochemical changes in African locust bean(*Parkia biglobosa*) and melon (*Citrullus vulgaris*) seeds duringfermentation to condiments. Pak. J. Nutr., 3: 140-145.

Osagie, A.U., 1998. Anti-nutritional factors. In: Osagie, A.U. and O.U. Eka (Eds.), Nutritional Quality of Plant Foods. Post-harvest Research Unit, Department of Biochemistry, University of Benin, Benin-City, Nigeria, pp: 221-244.

Osman, M.A., 2007. Effect of different processing methods on nutrient composition, antinutritional factors and *in vitro* protein digestibility of Dolichos lablab bean [*Lablab purpuresus* (L) Sweet]. Pak. J. Nutr., 6: 299-303.

Pearson, D., 1970. The Chemical Analysis of Foods (7th edition). Churchhill Livingstone, Edinburgh, pp: 6-25.

Wheeler, E.L. and R.E. Ferrel, 1971. Method for phytic acid determination in wheat and wheat fractions. Cereal Chem., 48: 312-316.