Influence of Sprouting on Proximate and Anti-nutritional Factors of Jacbean (*Canavalia ensiformis*) Flour

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ABSTRACT: The influence of sprouting on proximate and anti-nutritional factors of Jackbean flour was investigated. Jackbean was procured from the Genetic Resources Unit of International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State Nigeria. The seeds were carefully sorted and sprouted for 10 days. The sprouted and unprocessed (control) bean were then milled into flour and thereafter assessed for proximate and anti-nutritional factors. The data generated were subjected to statistical analyses using SAS 2.0 and SPSS 20.0 packages. The results of influence of sprouting on proximate constituents and anti-nutritional factors revealed that sprouting for nine days (S_5) resulted in flour sample with highest protein content and significant reduction in anti-nutritional factors most especially trypsin inhibitor from 32.08 ± 0.13; 29.18 ± 0.46 in control to 39.50 ± 0.06; 18.33 ± 0.03 in the sprouted samples respectively. Sprouting thus had positive effect on proximate and anti-nutritional factors of Jackbean flour.

Jackbean (*Canavalia ensiformis*) referred to as "Sese nla" in Yoruba, is amongst the tropical legumes that thrives well in Nigeria (Okonkwo and Udedibie, 1991, Odedeji, *et al*., 2019). Jackbean is grossly under-utilized in most developing countries of the world most importantly Nigeria. This has seriously affected the maximum propagation and utilization of the crop despite its nutritional qualities. Those planting the seeds in Nigeria plant them as ornamental plants and in some places, the plant is believed to be "snake expellant" (Odedeji, *et al*., 2019). The legume has potential in product development and consumption, but its extensive period of processing, and tough seed coat have affected the pasting properties of the flour (Akande *et al*., 2013). Quality parameters of food products developed from legume flour samples generally affect the functional and pasting properties influenced by the processing conditions employed (Ezeocha and Onwuka, 2010). Various pre-processing techniques had been employed to improve the processing, nutritional quality, organoleptic acceptability, reduction in the oligosaccharides and other anti-nutritional factors. Some of the commonly used pre-processing techniques included soaking, boiling at high temperatures (in water, alkaline or acidic solutions), fermentation, autoclaving, roasting, dehulling, microwaving, steam blanching and sprouting (El-Adawy, 2002 and Skulinova *et al.,* 2002). Sprouting of leguminous seeds improves the protein and other nutritional contents and reduces the anti-nutritional compositions (Mahando, 2004; Harper and Zandi, 2008 and Srilakshmi, 2008). Plant proteins are major sources of human's nutrition since animal protein are expensive for the poor (Owuamanam *et al*., 2014). Food processing operations not only improves flavour and palatability of foods but also increase the bioavailability of nutrients by inactivating some anti-nutritional factors and growth inhibitors (Chau *et al.,* 1997). Studies had revealed that pre-treatments such as sprouting, soaking/fermentation, autoclaving, blanching or roasting could enhance the quality of seeds and grain products (Ade- Omowaye *et al.,* 2003; Kirbaslar and Erkmen, 2003 and Carmelia *et al.,* 2007). Sprouting of soybean had been reported to increase the ascorbic acid content, riboflavin and niacin (Iwe, 2003). Reported studies on the beans are majorly on its utilization in animal feed formulations (Udebibie, 1997 and Anyanwu *et al*., 2011). There is paucity of information on the effect of sprouting on the proximate and anti-nutritional factors of Jackbean grown in developing countries, which is responsible for its in-extensive use (Ikegwu *et al.,* 2010). This work therefore investigated the effect of sprouting on

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the proximate and anti-nutritional factors of Jackbean flour.

MATERIALS AND METHODS

Materials: The seeds of Jackbean (*Canavalia ensiformis*) with Accession Number: TCe4 were collected from the Genetic Resources Unit of International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State Nigeria. The seeds were cultivated at the experimental farm of Osun State Polytechnic, Iree. Chemicals (Analytical Grade) and equipment were obtained from the Department of Food Science and Engineering, Ladoke Akintola University of Technology, Ogbomoso and Department of Food Technology, Osun State Polytechnic, Iree, Osun State Nigeria.

Preparation of the beans: The fruits (pods) of Jackbean were harvested after maturity (four months) and sundried. The seeds were removed from the pod by shelling and were thereafter winnowed to remove chaff.

Production of Flour from Raw and Treated Jackbean: Matured Jackbean seeds, after four months of planting, were thoroughly cleaned and sorted to remove defective ones. The seeds were thereafter graded according to their sizes. The cleaned seeds were soaked in warm water to soften the hull and ease its removal. The hulls were removed by repeated working between the palms and were removed leaving behind dehulled seeds. The dehulled seeds were thereafter drained and dried to reduce the moisture content and facilitate grinding into flour. The modified method of Hassan *et al.* (2006) was used for sprouting. 200 g of beans were steeped in 1500 ml of water for 24 hours at room temperature. The steeped beans were drained, each portion was allowed to malt for 1, 2, 3, 4, and 5 days respectively. The samples were wetted and turned twice daily to keep the temperature below 19 ^{0}C. The samples were sprayed with 2% formaldehyde solution to prevent the growth of mold. The sprouted beans were dried in a cabinet dryer at 45 $\pm$ 2 ^{0}C for 6hrs to moisture content of 12%. The plumules were removed before processing into flour. The dried seeds were pulverized to fine particle size and sieved to obtain fine powder (1 mm mesh size) for the control sample. The flour samples were packaged using high density polyethylene material for further analytical work (Uche *et al.,* 2014).

Effect of Sprouting on the Proximate Constituents of Jackbean

Moisture content and dry matter: The moisture content was determined using method described by No. 925.10, AOAC (1990).

The percentage moisture content and dry matter were calculated as follows:

$$\% \text{ Dry matter} = \frac{W_2 - W_0 \times 100}{W_1 - W_0}$$

$$\% \text{ Moisture content} = \frac{(W_2 - (W_3) \times 100}{(W_2 - W_1)}$$

W_0 = Weight of crucible; W_1 = Weight of crucible; W_2 = Weight of crucible + dry sample; W_3 = Weight of crucible + dry sample with constant weight.

Crude Protein: The crude protein content was determined using kjedhal method as described by AOAC, (2000)

$$\%N = \frac{1.401 \times (cm^3 HCI \text{ sample} - cm^3 HCI \text{ titre of blank}) MHCI}{Sw}$$

% protein =% N x 6.25; Sw = Sample weight; N = Nitrogen

Ash Content: The ash content was determined using AOAC, (1990) method.

$$\% \text{ Ash content} = \frac{W_2 - W_0}{W_1 - W_0} \times 100\%$$

W_0 = Weight of the crucible; W_1 = Weight of the crucible + sample before ashing; W_2 = Weight of the crucible + sample after ashing

Crude Fat: 5 g of the sample was measured into a set of three free thimbles after it has been dried free of moisture (AOAC, 2000) using soxhlet apparatus.

$$\% \text{ Crude fat (ether extract)} = \frac{W_1 - W_2 \times 100\%}{Sw}$$

W_1 = Weight of the flask; W_2 = Weight of the flask and the oil; Sw = Sample Weight

Crude Fibre: Crude fibre was determined through the documented method of AOAC, (2000).

$$\% \text{ Ash content} = \frac{W_2 - W_0}{W_1 - W_0} \times 100\%$$

W_1 = Weight before incineration: W_2 = Weight after incineration; Sw = Sample Weight

Effect of Sprouting on the Anti-nutritional Factors of the Flour

Phytate content: The phytate content of the flour was determined using the modified method of Maga (1982). 2g of each finely ground flour sample was soaked in 20 ml of 0.2N HCl filtered, 0.5 ml filtrate was mixed with 1 ml Ferric ammonium sulphate solution, boiled for 30 min , cooled in ice for 15 min. and centrifuged for 15 min. One milliliter of the supernatant was mixed with 1.5 ml of 2.2 – pyridine solution and the absorbance measured in a spectrophotometer at 519 nm.

Tannin content: Tannin content of the samples was determined using the method of Makkar and Goodchild, (1996).

Oxalate Content: Titration method described by Day and Underwood (1986) was used to determine the oxalate content.

Polyphenols Content: The total polyphenols in the sample was determined using purssion blue spectrophotometric method with tannic acid as the standard as described by Oladele *et al.* (2009).

Trypsin Inhibitor: The modified method of Arntified *et al.* (1985) was used in the determination of trypsin inhibitor which involves the use of spectrophotometer to measure the absorbance of supernatant of centrifuged mixture of 0.5g of the sample with 50 mls of 0.5m NaCl solution The absorbance for the control was measured. Trypsin inhibitor activity was calculated using the formula:

$$\text{TuI/g} \; = \; \frac{1}{W} \; \times \; \frac{au - as}{0.01} \; \times \; \frac{vf}{va}$$

Where: w = Weight of sample; au = Absorbance of sample at 410nm; as =Absorbance of control; vf = Total extract volume; va = Volume of extract analyzed

Saponin Content: The solvent extraction gravimetric method described by Nwosu, (2010) was used for the determination of saponin content of the sample.

$$\% \text{ Saponin} \; = \; \frac{W_2 - W_1}{\text{Wt of sample}} \; \times \; \frac{100}{1}$$

Where: W_1 = Weight of evaporating dish; W_2 = Weight of dish of sample

Data Analysis: Data collected were subjected to analysis of variance (ANOVA) using SPSS version 20.0 and SAS 2.0 package. The means were separated by Duncan's multiple range test at 5% level of probability, while Pearson's correlation co-efficient was also done to establish relationship among the preprocessing methods used in this research.

RESULTS AND DISCUSSION

Effect of Sprouting on Proximate Composition of Jackbean Flour: The result of influence of sprouting on proximate constituents of jackbean flour is presented in table 1. The moisture content of sample S_4 showed higher significant effect (P < 0.05) with a mean value of 8.73 ± 0.11 compared to all other samples and the raw. Sample S_1 displayed the least value of 8.27 ± 0.31 for moisture content. The values for this parameter ranged between 8.73 ± 0.11 and 8.27 ± 0.31, which fall within the acceptable limit of 0 – 13%, which has been reported to be suitable for storage and processing of flours without microorganism degradation of the triglyceride (James, 1995). The moisture content of the samples were lower compared to the one reported for sprouted field bean by Myrene, (2013). There was increment in moisture content over the sprouting period until in sample S_5 where there was slight reduction to 8.60 ± 0.35. The slight increment in moisture content over the sprouting period might be due to moisture uptake by the germinating seed as reported by Nonogaki *et al.,* (2010). Sample S_5 showed higher significant effect (P < 0.05) with a mean value of 39.50 ± 0.06 for protein compared to the raw sample. It was observed that there was sudden drop from 32.08 ± 0.13 in protein in the raw sample to 30.53 ± 0.23 in sample S_1. This quickly increased on the second day to 34.30 ± 0.55 and throughout the sprouting period compared to the raw sample. The highest value of 39.50 ± 0.06 was recorded by sample S_5. The lowest value of 33.43 ± 1.22 was recorded by sample S_4. The significant increase in protein content as observed in the sprouted samples might be attributed to increased water activity as a result of induction of hydrolytic enzymes (Bau *et al.,* 1997), hormonal changes (Nonogaki *et al.,* 2010) or a compositional change following the degradation of other constituents. Similar increase in protein content was reported for African oil bean, mungbean and Australian sweet lupin upon germination (Enujiugha *et al.,* 2003; Mubarak, 2005; Rumiyati *et al.,* 2012).

The fat content of raw sample displayed higher significant (P<0.05) effect and mean of 3.80 ± 0.10 compared to other pre-processed samples. It was observed that as the sprouting time was on the increase, the fat content was reducing. The value reduced from 3.60 ± 0.20 in sample S_1 to 3.13 ± 0.06 in sample S_5. This might be as a result of evaporative loss during drying of the flour sample. This equally

makes the product healthier as fat has been implicated in coronary heart disease (NSRL, 2002). The ash content, an indication of mineral content displayed higher significant effect ($P < 0.05$) for the untreated sample. The ash content was on the decrease over the sprouting period. The ash content decreased from 3.52 ± 0.03 in sample S_1 to 2.77 ± 0.60 in sample S_5. The values reported were higher than the value reported for field bean by Myrene, (2013). The reduction in ash content might be due to the leaching out of both macro and micro elements (Myrene, 2013). The crude fibre displayed similar significant effect ($P < 0.05$) with varied mean values 5.42 ± 0.03, 5.41 ± 0.01 and 5.40 ± 0.03 for samples S_1, S_2 and S_3 compared to the raw sample. This parameter displayed steady reduction from 5.42 ± 0.03 to 4.83 ± 0.02 over the sprouting period. The raw sample, S_4 and S_5 showed similar but lower significant effect for this parameter. The crude fibre value recorded were however relatively higher than the value reported for kidney bean by Audu and Aremu (2011). Sample S_1 showed higher significant effect ($P < 0.05$) with mean of 48.66 ± 0.43 for carbohydrate compared to the untreated sample. Samples S_3, S_4 and S_5 showed no significant difference for carbohydrate. The carbohydrate content was found to be reducing from 44.76 ± 2.73 in sample S_2 to 41.50 ± 0.06 for sample S_5 compared to the raw sample and S_1. The low carbohydrate and fat content due to sprouting is of dietary significance to diabetic patients, and for people suffering from cardiovascular disease and hyper-cholesterolemia (Myrene, 2013).

Effect of Sprouting on Anti-nutritional Factors of Jackbean Flour: Table 2 presents the results of the effect of sprouting on anti-nutritional factors of jackbean flour. Sprouting is partial germination of produce. It initiates three main types of chemical changes in the seed; the breakdown of certain materials, transport of materials from one part of the seed to another, especially from the endosperm to the embryo or from the cotyledons to the growing parts and finally the synthesis of new materials from the breakdown products formed (Esonu, *et al.,* 1998; Shipard, 2005). The phenolic compound is higher in samples S_1 with a value of 2.29 ± 0.10 mg/kg but with no significant effect ($p<0.05$) between sample S_2 and the untreated sample with a value of 2.23 ± 0.06 and 2.26 ± 0.03 mg/kg respectively. The values for this anti-nutritional factor ranged between 2.29 ± 0.10 and 1.86 ± 0.02 mg/g. It was noted that this anti-nutritional factor was decreasing as the sprouting day increased. Sample S_5, which represented sample sprouted for 5 days after the first sign of germination, recorded the least value. The least value recorded due to sprouting is in line with the submission of Owuamanam, *et al.* (2014) who worked on the use of seed sprouting in modification of food nutrients and reduction of anti-nutrients.

Similar trend was observed for tannin but with relatively lower mean value in samples S_o, S_1, and S_2, showing similar significant effect ($p<0.05$) but with slightly varied tannin content of 1.00 ± 0.02, 1.02 ± 0.06 and 0.99 ± 0.03 respectively. Sample S_5 recorded the least value of 0.80 ± 0.01. There was steady reduction in this anti-nutritional factor as sprouting day increased, which corroborated the view of Megat and Azrina, (2012) who reported reduction in tannin content of soybean and peanut.

Table 1: Effect of Sprouting on Proximate Constituents of Jackbean Flour

Parameters	So	S₁	S₂	S₃	S₄	S₅
Moisture Content	8.33 ± 0.23^d	8.27 ± 0.31^e	8.53 ± 0.11^c	8.67 ± 0.23^b	8.73 ± 0.11^a	8.60 ± 0.35^{bc}
Protein	32.08 ± 0.13^e	$30.53 \pm 0.23f$	34.30 ± 0.55^c	37.80 ± 1.44^b	33.43 ± 1.22^d	39.50 ± 0.06^a
Fat	3.80 ± 0.10^a	3.60 ± 0.20^{ab}	3.50 ± 0.10^{bc}	3.37 ± 0.11^{cd}	3.27 ± 0.03^{de}	3.13 ± 0.06^e
Ash	4.41 ± 0.14^a	3.52 ± 0.03^b	3.50 ± 0.01^b	3.46 ± 0.01^b	3.41 ± 0.01^b	2.77 ± 0.60^c
Crude Fibre	5.00 ± 0.35^b	5.42 ± 0.03^a	5.41 ± 0.01^a	5.40 ± 0.01^a	4.83 ± 0.03^b	4.83 ± 0.02^b
Carbohydrate	46.38 ± 0.41^{ab}	48.66 ± 0.43^a	44.76 ± 2.73^c	41.27 ± 1.72^c	41.33 ± 1.33^c	41.50 ± 0.06^c

Values are means of triplicate determinations; Means with the same superscript are not significantly different at P<0.05 along the column; S_0 = Raw jackbean flour; S_1 = Sprouted jackbean flour Day 5; S_2 = Sprouted jackbean flour Day 6; S_3 = Sprouted jackbean flour Day 7; S_4 = Sprouted jackbean flour Day 8; S_5 = Sprouted Jackbean Flour Day 9

Table 2. Effect of Sprouting on Anti-nutritional Factors of Jackbean Flour

Parameters	So	S₁	S₂	S₃	S₄	S₅
Phenolic (Mg/g)	2.26 ± 0.03^a	2.29 ± 0.10^a	2.23 ± 0.06^a	1.99 ± 0.05^b	1.93 ± 0.07^{bc}	1.86 ± 0.02^c
Tannin (Mg/g)	1.00 ± 0.02^a	1.02 ± 0.06^a	0.99 ± 0.03^a	0.86 ± 0.02^b	0.83 ± 0.03^{bc}	0.80 ± 0.01^c
Phytate (Mg/g)	0.13 ± 0.01^d	0.23 ± 0.03^c	0.36 ± 0.01^a	0.29 ± 0.03^b	0.35 ± 0.04^a	0.22 ± 0.03^c
Oxalate (%)	0.13 ± 0.01^a	0.11 ± 0.01^b	0.11 ± 0.02^b	0.10 ± 0.01^b	0.10 ± 0.01^b	0.09 ± 0.01^b
Saponin (%)	0.78 ± 0.03^a	0.77 ± 0.07^a	0.71 ± 0.05^b	0.71 ± 0.04^b	0.61 ± 0.01^c	0.60 ± 0.03^c
Trypsin Inhibitor (%)	29.18 ± 0.46^a	28.67 ± 0.15^{ab}	23.46 ± 0.07^c	23.96 ± 0.09^c	28.23 ± 0.53^b	18.33 ± 0.03^d

Values are means of triplicate determinations; Means with the same superscript are not significantly different at P<0.05 along the column; S_0 = Raw jackbean flour; S_1 = Sprouted jackbean flour Day 5; S_2 = Sprouted jackbean flour Day 6; S_3 = Sprouted jackbean flour Day 7; S_4 = Sprouted jackbean flour Day 8; S_5 = Sprouted jackbean flour Day 9

Phytate had higher significant effect and value of 0.36 ± 0.04 in sample S_2 compared to other pre-processed sample and the raw that recorded the least value of 0.13 ± 0.01. Sample S_4 recorded the next higher value of 0.35 ± 0.04 but with no significant effect with sample S_2. It was observed that there was increase in phytate content over the sprouting period compared to the raw sample, a view that deviated from the finding of Megat and Azrina, (2012). The values recorded for all the samples however, were less than one. Oxalate recorded higher significant effect (P<0.05) and a mean value of 0.13 ± 0.01 for the raw sample compared to other pre-processed samples. It was observed that there was steady reduction in oxalate content as the sprouting period increased. It reduced from 0.11 ± 0.01 in sample S_1 to 0.09 ± 0.01 in sample S_5. This supported the findings of Megat and Azrina (2012); Owuamanam, *et al.* (2014). Relatively, oxalate was the least anti-nutritional factor in the sample. The saponin content was found to decrease steadily over the sprouting time. It decreased from 0.77 ± 0.01 in sample S_1 to 0.60 ± 0.03 in sample S_5. Higher and similar significant effect (P<0.05) was recorded for samples S_0 and S_1 but with values of 0.78 ± 0.03 and 0.77 ± 0.07 respectively. Samples S_2 and S_3 equally gave similar mean value of 0.71 ± 0.05 and the effect is significant. Sample S_5 recorded the least value for this anti-nutritional factor but with similar significant effect with sample S_4.

This reduction in saponin content supports the submission of Owuamanam *et al.* (2014) who work on the use of seed sprouting in modification of food nutrients and pasting profile profile of tropical legume flours. Trypsin inhibitor showed higher significant (P < 0.05) effect and mean value of 29.18 ± 0.46 for raw sample. There was decrease in the trypsin inhibitor over the sprouting period from 28.67 ± 0.15 in sample S_1 to 18.33 ± 0.03 in sample S_5. Samples S_2 and S_3 showed no significant effect with mean values of 23.46 ± 0.07 and 23.96 ± 0.09 respectively. The reduction in trypsin inhibitor due to sprouting corroborated the submission of Owuamanam *et al.* (2014) report on the use of seed sprouting in modification of food nutrients and pasting profile of tropical legume flours.

Conclusion: The study revealed that sprouting of Jackbean had significant effects on proximate and anti-nutritional factors. Sprouting for nine days resulted in sample with highest protein content and greatest reduction in anti-nutritional factors. This pre-processing method therefore suggests that Jackbean could be crop of great industrial potentials, thus, its maximum propagation should be encouraged.

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