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Effect of Autoclaving on Proximate and Anti-Nutritional Factors of Jackbean (*Canavalia ensiformis* L) D.C. FLOUR

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Abstract

Jackbean (*Canavalia ensiformis*) is a legume with high nutritive value but with elongated cooking time, hard seed coat and anti-nutritional factors as limiting factors. There is a dearth of information on the impact of different pre-processing methods on the proximate and anti-nutritional factors of Jackbean flour. Therefore, this study is on the influence of autoclaving on proximate and anti-nutritional factors of Jackbean flour. Jackbean procured from the Genetic Resources Unit of International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State Nigeria were subjected to autoclaving at 121 ^{0}C for 30, 60, 90, 120 mins, dried to 12 % moisture content and milled into flour while flour from untreated seed served as control. The data generated were subjected to statistical analyses using SAS 2.0 and SPSS 20.0 packages. The results revealed that autoclaving at 121 ^{0}C for 120 mins resulted in maximum protein content of 34.97 % and drastic reduction in all anti-nutritional factor most particularly trypsin inhibitor from 27.88 % in raw seed to 17.86 % in autoclaved seed. Autoclaving thus had positive effect on the proximate and anti-nutritional factors of Jackbean.

Keywords: Proximate, anti-nutritional, autoclaving, jackbean

Introduction

Jackbean (*Canavalia ensiformis*) – "Sese nla" (Yoruba), is one of the tropical legumes that do well in Nigeria (Okonkwo and Udedibie, 1991). It is grossly under-utilized in most developing countries of the world most particularly Nigeria. This has really affected the maximum propagation of the crop despite its nutritional qualities. Some people plant the seeds in Nigeria as ornamental plants and in some places, it is believed to be "snake repellant". Jackbean has potential in product development and consumption, but its prolonged period of processing, and tough seed coat have affected the 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), sprouting, roasting, dehulling, microwaving, steam blanching, fermentation and autoclaving (El-Adawy, 2002 and Skulinova et al., 2002). Autoclaving had been exploited to a great extent in food processing and found to be more effective in the reduction of anti-nutritional components of leguminous products (Adegunwa et al., 2012).

Food processing operations not only improves the flavor 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 shown 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 a paucity of information on the effect of autoclaving 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 seeks to investigate the effect of autoclaving on the proximate and anti-nutritional factors of Jackbean flour.xxx.

Materials and Methods

Source of Planting Material and Experimental Location

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 planted at the experimental farm of Osun State Polytechnic, Iree. Osun State. Nigeria. 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.

Preparation of the beans

The pods of Jackbean were harvested after four month of maturity and sundried. The seeds were carefully removed from the pod by shelling and were winnowed thereafter so as to remove chaff.

Production of Flour from Raw and Autoclaved 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.

Two hundred grammes (200g) of the seed samples were autoclaved at 151b pressure (121 ^{0}C) in distilled water in 1:10 ratio (bean: water) for 30, 60, 90 and 120 minutes. The autoclaved seeds were dehulled and oven dried at 55 ^{0}C. The dried seeds were then milled in attrition mill, sieved to 1mm mesh size, and packaged in an air-tight container for further analytical work (Adegunwa *et al*.,2012; Uche *et al.,* 2014).

Effect of Pre-treatments on Proximate Constituents of Jackbean Flour

Moisture content and dry matter

The moisture content was determined using the method described by No. 925.10, AOAC (1990).
The percentage moisture content and dry matter were calculated as follows:

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

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

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).

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

% 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

Five (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 appratus.

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

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).

$$\% \text{ Crude fibre} = \frac{W_1 - W_2}{Sw} \times 100\%$$

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

Effect of Pre-treatment on the Anti-nutritional Factors of the Flour

Phytate content

The phytate content of the flour was determined using the 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 15mins 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 spectrophometer at 519nm.

Tannin content

Tannin content of the samples were 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 pursion 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:

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

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 Autoclaving on Proximate Composition of Jackbean Flour

The result of the effect of autoclaving on proximate composition of Jackbean flour is presented in Table 1.0. Sample T1 showed higher significant effect (P < 0.05) and mean of 8.73 + 0.46 for moisture content compared to other samples and the raw sample. The raw sample and sample T4 showed no significant difference but lower mean value of 8.33 + 0.23 for moisture content. Moisture content was observed to be increasing among the pre-processed samples over the period of autoclaving except for sample T4 when there is drop in the moisture content to 8.33 + 0.11. The moisture content of the samples was within the recommended range of 10-15 for dry products (Adegunwa et al., 2012).

The protein content of samples T3 and T4 displayed higher significant (P< 0.05) effect and mean values of 34.91 + 0.35 and 34.97 + 0.15 respectively compared to the raw sample. The values ranged between 34.97 + 0.15 and 32.02 + 0.08 while the raw sample recorded the least value. The protein content was increasing over the autoclaving period. This might be due to the elimination of some anti-nutritional factors. The values recorded for protein were higher compared to the value reported for autoclaved Beniseed (Adegunwa et al., 2012). This indicated that the sample could be a cheap source of plant protein.

The mean values of 3.80 + 0.10 and 3.72 + 0.08 was recorded for samples T1 and the untreated sample for fat content compared to other samples. There was decrease in fat content as the autoclaving period increased from 3.72 + 0.08 to 3.43 + 0.06. Sample T4 recorded the least value. This might be due to evaporative loss during the process of drying. The ash content showed a significant effect (P < 0.05) and mean value of 4.43 + 0.11 for the untreated sample while other samples present no significant effect and mean values ranging from 4.00 + 0.00 to 4.08 + 0.10. There was reduction in ash content over the autoclaving period. The ash content is relatively lower compared to the value recorded for benniseed (Adegunwa et al., 2012). Similar significant effect (P < 0.05) but mean values of 5.01 + 0.34 and 4.99 + 0.33 were recorded for samples To and T1 which was followed by samples T2 and T3 with mean values of 4.71 + 0.11 and 4.63 + 0.06 respectively while sample T4 recorded the last value of 4.52+0.07. The crude fibre is the amount of indigestible sugar present in a food sample. The values were however higher than the value reported for cooked African Yam Bean (Spherostylis sternocarpa) seed as reported by Nwosu et al. (2011).

Table 1: Effect of Autoclaving on Proximate Composition of Jackbean Flour

Parameters (%)	To	T1	T2	T3	T4
Moisture Content	8.33 + 0.23c	8.73 + 0.46a	8.60 + 0.40b	8.47 + 0.23ab	8.33 + 0.11c
Protein	32.02 + 0.08c	31.42 + 0.02d	33.13 + 0.11b	34.91 + 0.35a	34.97 + 0.15a
Fat	3.80 + 0.10a	3.72 + 0.08a	3.56 + 0.06b	3.48 + 0.03bc	3.43 + 0.06c
Ash	4.43 + 0.11a	4.08 + 0.10b	4.01 + 0.01b	4.00 + 0.00b	4.00 + 0.00b
Crude fibre	5.01 + 0.34a	4.99 + 0.33a	4.71 + 0.11ab	4.63 + 0.06ab	4.52 + 0.07b
Carbohydrate	46.42 + 0.43b	47.02 + 0.22a	45.99 + 0.21b	44.51 + 0.40c	44.62 + 0.10c

Values are means of triplicate determinations. Means with the same superscript are not significantly different (P < 0.05) along the column

TO = Raw Jackbean Flour
T1 = Autoclaved jackbean flour at 121 oC for 30 min
T2 = Autoclaved jackbean flour at 121 oC for 60 min
T3 = Autoclaved jackbean flour at 121 oC for 90 min
T4 = Autoclaved jackbean flour at 121 oC for 120 min

Higher significant effect (P < 0.05) and mean of 47.02 + 0.22 was displayed by sample T1 for carbohydrate content compared to other pre-processed samples and the untreated sample. The value was however found to decrease from 47.02

+ 0.22 to 44.62 + 0.10 over the autoclaving period. Samples T3 and T4 showed the lowest means of 44.51 + 0.40 and 44.62 + 0.10 but similar significant effect for carbohydrate.

The improvement in the proximate constituents of the sample due to the effect of autoclaving supported the views of Adegunwa et al. (2012); Doss et al. (2011) and Nwosu et al. (2011) on biochemical composition, anti-nutritional, proximate, and nutritional values of beniseed, underutilized legume and African yam bean respectively. The reduction in carbohydrate and lipid content in this sample is beneficial for patients of diabetes mellitus, cardiovascular disease and hypercholesterolemia (Myrene, 2013). The carbohydrate content was relatively low compare to the value reported for autoclaved field bean by Myrene (2013).

Effect of Autoclaving on Anti-nutritional Factors of Jackbean Flour

The results of the effect of autoclaving on anti-nutritional factors of Jackbean flour is shown in Table 2. There was higher significant effect (P < 0.05) with a mean value of 1.99 + 0.07 on phenolic compound by raw sample. This was closely followed by sample T1 with a mean value of 1.72 + 0.11 while samples T2 and T3 showed no significant effect but have mean values of 1.47 + 0.06 and 1.42 + 0.02 respectively. Sample T4 recorded the least value of 1.05 + 0.10 for this anti-nutritional factor. There was gradual reduction of phenolic compound as the autoclaving time increased, which corroborate the observations of Udensi et al. (2008) they recorded lower value for phenolic compound for Mucuna utilis and velvet beans respectively.

There was variation in tannin content among all the samples over the autoclaving times. Higher significant effect (P < 0.05) with a mean of 1.99 + 0.07 was recorded by the untreated sample while samples T2 and T3 showed similar significant effect but varied mean values of 1.47 + 0.06 and 1.42 + 0.02 respectively. The least content of 0.73 + 0.06 for tannin was recorded by sample T1. Tannins are astringent and adversely affect feed intake (Microsoft Encarta, 2006). Autoclaving as a form of pre-processing method thus reduced this anti-nutritional factor drastically. The phytate causes reductions in absorption of calcium from the gastrointestinal tract and consequently implicated in the development of ricket (Deshpande and Cheryan, 1984). The value for phytate ranged between 0.52 + 0.04 and 0.14 + 0.03. Higher significant (P<0.05) effect was recorded by sample T4 with a mean value of 0.52 + 0.04 while the raw sample recorded the least value. Autoclaving had an incremental effect on phytate. Higher significant (P<0.05) effect and mean value of 0.12 + 0.01 was recorded for raw sample for oxalate compared to other autoclaved samples.

Table 2: Effect of Autoclaving on Anti-nutritional Factors of Jackbean Flour

Parameters	To	T1	T2	T3	T4
Phenolic (mg/g)	1.99 + 0.07a	1.72 + 0.11b	1.47 + 0.06c	1.42 + 0.02c	1.05 + 0.10d
Tannin (mg/g)	1.99 + 0.07a	0.73 + 0.06d	1.47 + 0.06b	1.42 + 0.02b	1.05 + 0.10c
Phytate (mg/g)	0.14 + 0.03d	0.13 + 0.02e	0.17 + 0.01c	0.37 + 0.01b	0.52 + 0.04a
Oxalate (%)	0.12 + 0.01a	0.10 + 0.01b	0.07 + 0.01c	0.06 + 0.01c	0.05 + 0.01c
Saponin (%)	0.75 + 0.05bc	0.91 + 0.07ab	0.89+ 0.02ab	0.79 + 0.02b	0.68 + 0.02c
Trypsin Inhibitor (%)	27.88 + 0.01a	23.38 + 0.01b	20.42 + 0.03c	19.80 + 0.03d	17.86 + 0.16e

Values are means of triplicate determinations. Means with the same superscript are not significantly different at P<0.05 along the column
TO = Raw jackbean flour
T1 = Autoclaved jackbean flour at 121 oC for 30 min
T2 = Autoclaved jackbean flour at 121 oC for 60 min
T3 = Autoclaved jackbean flour at 121 oC for 90 min
T4 = Autoclaved jackbean flour at 121 oC for 120 min

There was reduction of oxalate content as the autoclaving time increased. The value reduced from 0.10 + 0.01 in sample T1 to 0.05 + 0.01 in sample T4 while samples T2, T3 and T4 showed similar significant effect. This reducing effect is in line with the submission of Nwosu (2010) who reported reduction in oxalate content of Asparagus bean. The content of oxalate in the sample is relatively low compared to other anti-nutritional factors. Significant reduction in saponin was recorded when the sample was autoclaved for 120 min (T4) with a mean value of 0.68 + 0.01. There was a steady reduction in the content of saponin from 0.91 + 0.01 in sample T1 to 0.68 + 0.01 in sample T4 indicating that autoclaving had a reducing effect on this anti-nutritional factor. There is no significant effect recorded for samples T1 and T2 based on saponin content.

There is drastic reduction of trypsin inhibitor from 27.88 + 0.01 in sample T0 to 17.86 + 0.01 in sample T4 due to autoclaving. Sample T4 recorded the least value for trypsin inhibitor while highest value of 27.88 + 0.01 was recorded in the raw sample. Autoclaving, a thermal treatment, thus had a reducing effect on this heat labile anti-nutritional factor thus corroborating the submission of Myrene, (2013); Adegunwa et al. (2012) on the effect of traditional and thermal processing on nutritional and biochemical , antinutritional and functional properties of field bean and beniseed.

Conclusion

The study revealed that autoclaving had significant effects on proximate and anti-nutritional factors. Autoclaving at 120 0C for 120 min resulted in significant reduction of anti-nutritional factors especially trypsin inhibitor and increment in protein and carbohydrate contents associated with low moisture. Autoclaving of Jackbean therefore represents a pre-processing technique with positive effect on proximate and anti-nutritional factors. With this treatment properly applied, Jackbean could be crop of great industrial potentials, thus, its maximum propagation should be encouraged.

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Conflict of Interests

The authors declare that there is no conflict of interest.

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