Effect of Soaking, Blanching and Cooking on the Anti-nutritional Properties of Asparagus Bean (*Vigna Sesquipedis)* Flour

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ABSTRACT: The effect of soaking, blanching and cooking on the anti-nutritional properties of asparagus bean was investigated. Flour samples were generated by soaking, (for 48 h while changing steep water every four h); blanching (2, 4, 6 and 8 min) and cooking (20, 30, 40, 50 and 60 min). The results obtained showed that the raw sample had higher anti-nutrients than all the other treated samples. The soaking time significantly (P≤ 0.05) affected a reduction in the anti-nutrients studied for instance Typsin reduced from 13.82 (raw sample) to about 9% after 48 h soaking and to 7.82% after 60 min cooking. Tannins also reduced from 0.23 (raw) to 0.09% after 48 h soaking and 0.08% after 60 min cooking. In all, cooking for 60 min gave the least value of these anti-nutrients showing that cooking for this period drastically reduced most of the anti-nutritional components found in asparagus bean. [Nature and Science 2010;8(8):163-167]. (ISSN: 1545-0740).

Key words: soaking; blanching; anti-nutritional properties

1. Introduction

Asparagus bean (*Vigna sesquipedalis*) is an annual crop which belongs to the family *leguminoseae* and sub-family *papilionadeae*. It is also known as "black akidi" in the eastern part of Nigeria. Asparagus bean products, seeds and fruits can be utilized in precisely similar ways (Smart and Hymowitz 1985). It is a nutritious component of both human diet and livestock. The protein content is rich in amino-acids-lysine and tryptophan compared to cereal grains however, it is deficient in sulfur containing amino acids (cystine and methionine) as observed by Smart and Hymowitz (1985). It is used at all stapes of growth as a vegetative crop. The tender green leaves are an important food source in Africa and are prepared as a porridge like spinach (Bressani, 1985). The seeds can be processed into various products such as flour, protein concentrates and isolates, starch and extruded products.

The asparagus bean like other legumes contains significant amount of nutrients, however, they also contain several undesirable components and attributes. These undesirable components are called anti-nutritional stress factors. These are factors which in themselves or in combination with other factors inhibit the functioning of food nutrients. These include inhibitors of Enzymes such as Trypsin inhibitors, Lectins, Phenolic compounds (tannins and phytates) and oligosaccharides (Oyenuga, 1968). It has also been found that it is associated with some problems of beany off-flavor and cookability which reduces its consumption and utilization rates. Most of the antinutritional factors become ineffective by simple measures such as heating, soaking, germination or autoclaving (Nowak and Haslberger 2000).

Consequently, the objective of this research work is to investigate the effect of soaking, blanching and cooking on the anti-nutritional properties of asparagus bean. It is hoped that an enhanced reduction of this antinutritients with this method will improve the utilization of this seed.

2. Materials and Methods

2.1 Sources of Materials

The asparagus bean seeds used for this study were obtained from Ogbaette Market in Enugu Municipal L.G.A of Enugu State. Some selected Chemicals for this study and Equipment used were obtained from National Root Crop Research Institute Umudike in Abia State and Department of Food Science and Technology; Federal University of Technology, Owerri Imo State.

2.2 Preparation of Sample

The asparagus beans were cleaned sorted and weighed. The sample was divided into three portions and given different treatments (soaking, blanching and cooking) for different times. Soaking was done for 4 to 48 hr while changing the steep water every four hour. Blanching was carried out for 2, 4, 6, and 8 min respectively while cooking was done for 20, 30, 40, 50 and 60 min respectively. The resultant samples were then drained off-water, dried at 60^0C for 6hr in a moisture extraction oven, cooled and milled into flour using a attrition mill. It was then packaged in air-tight containers ready for analysis of the anti-nutritional components.

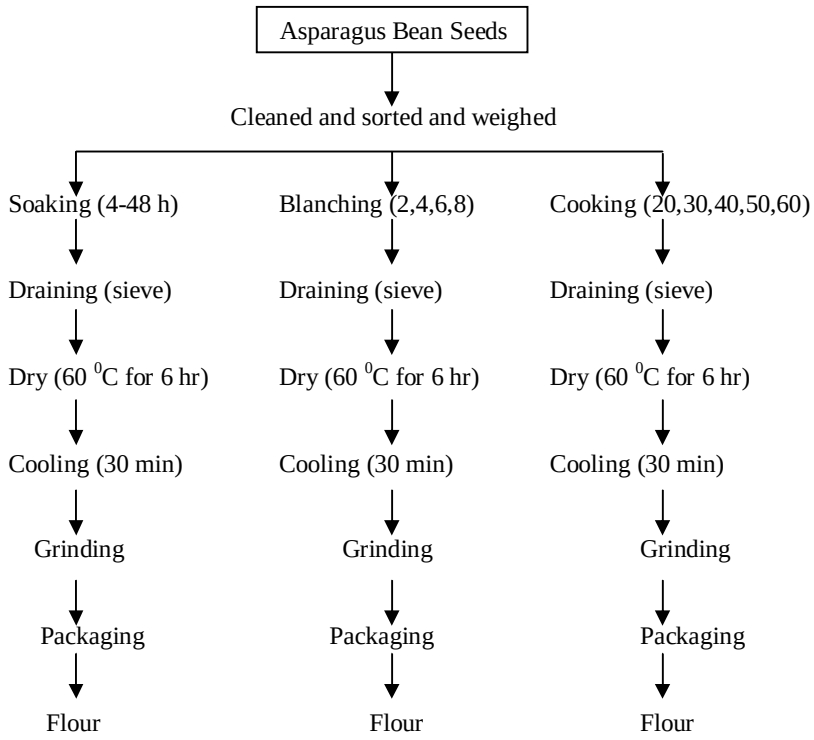

Fig. 1: Flow diagram of flour products from asparagus bean using different processing methods

2.3 Analysis of Anti-nutritional Factors

The solvent extraction gravimetric method (Harbone, 1973) was employed in the saponin content determination of the sample.

Two grammes of the sample was mixed with 50ml of 20% aqueous ethanol solution. The mixture was incubated at 55^0C in a water bath with periodic agitation for 90 minutes. Then it was filtered through Whatman filter paper No. 40. The residue was extracted with the 50ml portion of the 20% aqueous ethanol and both extracts were pooled together. The combined extracts was reduced to about 40ml at 90^0C and transferred to a separating funnel where 40ml of diethyl ether was added and shaken vigorously. The separation was by partition during which the ether layer was discarded while the aqueous layer was reserved. Re-extraction by partition was done repeatedly until the aqueous layer became clear in colour.

The saponin were extracted with 60ml of normal butanol solution and evaporated to dryness in a pre-weighed evaporating dish. It was dried at 60^0C for 30 min in the oven (to remove any residual solvent), cooled and reweighed. The experiment was repeated two more times to get an average. The saponin content was determined by difference and calculated as a percentage of the original sample thus:

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

Where;

W_1 = Weight of evaporating dish

W_2 = Weight of dish + sample

2.3.1 Determination of Tannins

Tannin content of the test sample was determined by the Follins-Dennis spectro photometric method by Pearson (1976). A measured weight of the dry test sample (Ig) was dispensed in 50ml of distilled water and shaken to mix well for 30 min in the shaker. The mixture was filtered and the filtrate was used for the experiment.

Five milliliters of the extract was measured into 50ml volumetric flask and diluted with 35ml of distilled water. Similarly, 5ml of standard tannin solution (tannic acid) and 5ml of distilled water were measured into separation flasks to serve as standard and blank respectively. Both were also diluted with 35ml of distilled water. 1ml of Follin-Dennis reagent was added to each of the flasks followed by 2.5ml of saturated sodium carbonate (Na_2C0_3) solution. The content of each of each flask was made up to mark and incubated for 90min at room temperature.

The absorbance of the developed color was measured at 760nm wavelength with the reagent blank at zero. The experiment was repeated two more times to get an average.

The tannin content was calculated as shown below:

$$\% \text{ Tannin} = \frac{100}{1} \times \frac{Au}{As} \times C \times \frac{Vf}{Va} \times D$$

Where;

Va = Weight of sample analyzed

Au = Absorbance of the test sample

As = Absorbance of standard tannin solution

C = Concentration of standard in mg/ml

Vf = Volume of filtrate analysed
D = Diluted factor where applicable

2.3.2 Determination of Alkaloids

The quantitative determination of alkaloids was carried out by the alkaline precipitation through Gravimetric method described by Harbone (1973). Two grammes 2g of the sample was soaked in 20ml of 10% ethanolic acetic acid. The mixture was allowed to stand for 4 hr at room temperature. Thereafter, the mixture was filtered through Whatman filter paper no. 40. The filtrate (extract) was concentrated by evaporation over a steam bath t0 ¼ of its original volume.

For the alkaloids to be precipitated, concentrated ammonia solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using a previously weighed filter paper. After filtration, the precipitate was washed with 1% ammonia solution and dried in the oven at 60^0C for 30min, cooled in a desiccator and reweighed.

The experiment was repeated two more times and the average was taken. The weight of alkaloids was determined by difference and expressed as a percentage of the weight of the sample analysed as shown.

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

Where;
W_1 = Weight of Filter paper
W_2 = Weight of paper + alkaloid precipitate

2.3.3 Determination of Hydrocyanic Acid

This was determined by the alkaline picrate colorimetric method of Balagopalam, *et al.*, (1988).

A measured weight of each sample (0.5g) was dispensed in 200mls of distilled water in a conical flask and mixed properly. A strip of alkaline picrate paper was suspended over the mixture with the aid of rubber stopper in a way that the paper did not touch the surface of the mixture. The set up was incubated for 18 hours at room temperature and at the end of the incubation period, the picrate paper was carefully removed and eluted in 60ml of distilled water. Meanwhile, a standard cyanide solution (KCN) was prepared and treated as described above.

The absorbance of the elutes from the standard and the samples were measured in a spectrophotometer at 540nm with the reagent blank at zero. The cyanide content (HCN mg/kg) was calculated using the formula below:

$$\text{HCN mg/kg} = \frac{100}{1} \times \frac{Au}{As} \times C \times D$$

Where;
W = Weight of the samples analysed
Au = Absorbance of the sample
As = Absorbance of the standard HCN solution
C = Concentration (mg/ml of standard HCN solution)
D = Dilution factor where applicable

2.3.4 Determination of Phytates

The colorimetric method described by Hang and Lantzseh (1983) was used to determined phytic acid content of the samples.

A measured weight of the sample was extracted with 100mls of 0.2HCI solution. The extraction was done by shaking the mixture at room temperature for 30 min and filtering with Whatman No. 40 filter paper to obtain the extract used for the analysis. 0.5ml of the extract was mixed with 1ml of standard $FeCl_3$ solution in a test tube. Then, the test tube was covered and boiled for 30 minutes and then cooled in ice for 15min before allowing to attain room temperature. Then it was centrifuged at 3000xg (where g = force of gravity) for 30 min. Then, 1ml portion of the supernatant was treated with 1.5ml bipyridine solution. Meanwhile, the standard phytic acid solution was prepared, 1ml of it and 1ml of distilled water were put in separate test tubes and treated as discussed above. The second as the standard and reagent blank respectively. The absorbance of each was measured at 519nm in a spectrophotometer with the reagent blank at zero.

The phytic acid content was calculated using the relationship below.

$$\% \text{ phytic acid} = \frac{100}{W} \times au \times \frac{C}{as} \times \frac{vf}{va} \times D$$

Where;
W = Weight of sample
au = Absorbance of the test sample
as = Absorbance of the standard phytic acid solution
vf = Total extract volume
va = Volume of extract analysed
C = Concentration of the standard phytic acid solution
D = Dilution factor where applicable

2.3.5 Determination of Trypsin Inhibitor

The Arntified *et al*, (1985) method was employed in the determination of trypsin inhibitor activity. 0.5g of the sample was dispensed in 50mls of 0.5m Nacl solution and shaken for 30min at room temperature.

The mixture was centrifuged and the supernatant was used as the extract. Assay for trypsin inhibitor activity involved mixing a portion (1ml) of the extract with 90mls of 0.03% Trypsin substrate (BAPA) in a test tube containing 1ml of 0.6% Trypsin enzyme solution. After mixing, the mixture was allowed to stand for 15min before its absorbance was reached at 410nm in a spectrophotometer.

A control which consist of 1ml enzyme solution in 9mls of Trypsin substrate (BAPA) but no extract was set up as described above and its absorbance was also measured. Trypsin inhibitor activity was calculated using the formula below:

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

Where;
W = Weight of sample

au = Absorbance of sample at 410nm
as = Absorbance of control
vf = Total extract volume
va = Volume of extract analysed

3. Results and Discussion

The results obtained Table 1 showed that the raw sample had the highest anti-nutrients when compared with the treated samples. For soaking alkaloids reduced from 0.34 to 0.28% tannin 0.23 to o.09% saponin 0.42 to 0.24%; HCN 8.63 to 5.68%; phytate 0.18 to 0.09 and trypsin inhibitor 13.82 to 9.41 TlU/100g. This reduction was expected as soaking helped in the removal of the soluble anti-nutrients. This result was in agreement with the report of Philips and Abbey (1989) that steeping hydrates the grain and induces the leaching out of water soluble anti-nutrients such as glycoside, alkaloids, phytates, oligosaccharides and tannins.

For alkaloids, the raw and 4hr soaked samples were statistically equivalent (P=0.05); also no different existed between soaking for 12 to 24 hr. Most of the leaching effects occurred from 28 to 48hr and this was statistically different ($p \leq 0.05$). For tannins significant variation was observed from 28hr soaking which increased at 36 to 40hr and then 44 to 48hr respectively. From the table I it showed that the length of soaking time actually decreased the anti-nutrients statistically at p≤0.0.5.

Also the effect of heat on the reduction of other anti-nutrients was noticed. For Trypsin inhibitor there was significant reduction ($p \leq 0.05$) from the raw (13.82 to 13.08TlU/100g) in the 8min blanched samples and 9.19 (20 min cooked samples) to 7.82TIU/100g for 60min cooked sample which showed a high reduction rate. There was no significant (p=0.05) change in the tannin with raw and blanched sample. Significant reduction ($p \geq 0.05$) was only observed during cooking. The same phenomenon as for tannins goes for alkaloids, saponins, phytates and HCN. This shows that in order to reduce these anti-nutrients drastically, cooking using longer periods of time has to be employed as recommended by Ebge and Akinyele (1990). Tannins were reduced from 0.23 (raw) to 0.08%, alkaloids from 0.34 to 0.24%, saponin from o.42 to 0.28%, phytates from 0.18 to 0.14% and HCN from 8.63 to 3.99% for raw and 60min cooked samples respectively. Blanching had little or no effect on the reduction of these anti-nutrients as the time lag was too short for them to have hydrated and induced leaching (Egbe and Akinyele 1990).

It has been deduced that from the preprocessing treatments given (soaking, blanching and cooking); cooking was able it reduce most of this anti-nutrients more than soaking while soaking to a longer extent also had more reduction than blanching. For instance soaking reduced alkaloids significantly ($p \geq 0.05$) from 0.34 to 0.28% while blanching only reduced it from o.34 to 0.32% while cooking reduced it to 0.24%. Also foods that need further processing could be blanched or soaked to still achieve high level of reduction.

Table 1. Mean values of the anti-nutritional properties of asparagus bean, flour as affected by soaking, blanching and cooking.

Processing Method	Time	TIU/100g	Tannin%	Alkaloid%	Saponin%	Phytate%	HCN %
Raw	NA	13.82^{a}	0.230^{a}	0.34^{a}	0.42^{a}	0.18^{a}	8.63^{a}
Blanching	2	13.62^{b}	0.229^{a}	0.34^{a}	0.40^{a}	0.18^{a}	8.47^{a}
	4	13.60^{b}	0.229^{a}	0.34^{a}	0.40^{a}	0.17^{a}	8.47^{a}
	6	13.50^{b}	0.227^{a}	0.32^{a}	0.36^{a}	0.17^{a}	8.22^{a}
	8	13.08^{b}	0.226^{a}	0.32^{a}	0.36^{a}	0.17^{a}	8.22^{a}
LSD		**0.201**	**0.002**	**0.020**	**0.010**	**0.011**	**0.023**
Cooking min	20	9.19^{c}	0.134^{b}	0.28^{b}	0.32^{b}	0.15^{b}	6.35^{b}
	30	8.62^{c}	0.121^{b}	0.26^{b}	0.30^{b}	0.15^{b}	6.06^{b}
	40	8.44^{c}	0.111^{b}	0.26^{b}	0.30^{b}	0.15^{b}	5.31^{c}
	50	8.14^{c}	0.099^{c}	0.24^{b}	0.28^{b}	0.14^{b}	5.07^{c}
	60	7.82^{d}	0.087^{c}	0.24^{b}	0.28^{b}	0.14^{b}	3.99^{d}
LSD		**2.05**	**0.10**	**0.20**	**0.12**	**0.11**	**2.12**
Soaking Time	Na	13.82^{a}	0.23^{a}	0.34^{a}	0.42^{a}	0.18^{a}	8.63^{a}
	4	13.82^{a}	0.23^{a}	0.34^{a}	0.42^{a}	0.18^{a}	8.09^{c}
	8	13.82^{a}	0.23^{a}	0.34^{a}	0.40^{b}	0.18^{a}	8.22^{b}
	12	13.80^{a}	0.23^{a}	0.32^{ab}	0.38^{c}	0.18^{a}	8.22^{b}
	16	12.99^{c}	0.22^{a}	0.32^{ab}	0.36^{d}	0.18^{a}	7.85^{d}
	20	12.67^{c}	0.22^{a}	0.32^{ab}	0.32^{c}	0.18^{a}	7.22^{c}
	24	11.83^{c}	0.22^{a}	0.32^{ab}	0.28^{f}	0.15^{b}	6.76^{f}
	28	11.65^{d}	0.16^{b}	0.30^{bc}	0.28^{f}	0.13b^{c}	6.56g
	32	11.46^{d}	0.15b	0.28^{c}	0.26^{g}	0.14^{b}	6.18^{h}
	36	11.40^{d}	0.12^{c}	0.30^{bc}	0.26^{g}	0.11^{c}	5.98^{i}
	40	11.21^{e}	0.12^{c}	0.28^{c}	0.24^{h}	0.11^{c}	5.98^{i}
	44	10.08^{f}	0.10^{d}	0.28^{c}	0.24^{h}	0.11^{c}	5.89^{j}
	48	9.41^{k}	0.0943^{d}	0.28^{c}	0.24^{h}	0.09^{c}	5.68
$LSD_{0.05}$		**0.0180**	**0.0174**	**0.0203**	**0.0161**	**0.0223**	**0.0169**

4. Conclusion

This research work has indicated the effects of some processing treatments (soaking, blanching and cooking) on the anti-nutritional properties of asparagus bean. From the results obtained it is indicative that this seed can be more utilized since some of the ways these anti stress factors can be reduced has been found. Also the result has shown that some of the treatments reduced these anti-nutrients more than the others. So depending on the use the seed has to be put into, will determine the type of preprocessing treatment that will be given.

Acknowledgement:

FST FUTO, Akubuiro Adaku for the processing of the work into the appropriate format.

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7/21/2010