

International Journal of **Life Sciences**

(A peer reviewed International Journal)

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International Journal of Life Sciences, Vol. 2 No. 2, pp. 60-67 2277-193x. 2012

ISSN 2277 – 193x

Article type *Full Length Research Article*

Publication date *09 May 2013*

Article URL <http://www.crdeep.com/category/ijls>

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**Full Length Research Paper****The Antinutritional Properties and Ease of Dehulling on the Proximate Composition of Pigeon pea (*Cajanus cajan*) as Affected by Malting*****Nwosu J. N, Ojukwu M, Ogueke C. C, Ahaotu I, and Owuamanam C. I.***Department of Food Science and Technology, Federal University of Technology, P.M.B. 1526, Owerri, Imo State Nigeria****Corresponding Author: Nwosu J.****Abstract**

The effect of malting and the ease of dehulling on the proximate composition and the anti-nutritional properties of Pigeon Pea (*Cajanus cajan*) was studied. The pigeon peas were steeped for 24 hours and 48 hours respectively and then malted for 4 days after which they were dried, dehulled and ground into flour. The proximate composition decreased under all the malting conditions except for protein that increased from 18.90% to 24.78% as a result of enzymes involved in the degradation of the malted sample, and carbohydrates that also increased from 58.40% to 60.57% as a result of decrease in the other values. Analysis of the antinutritional factors showed a significant decrease ($p \leq 0.05$) for phytate, saponin, HCN, Alkaloids and Tanin. Also, there was no significant difference ($p \geq 0.05$) in phenol and Oxalate as the steeping and germination time increased from 24 hours to 48 hours and 24 hours to 96 hours respectively.

Keywords: Steeping, germination, anti-nutritional factors, malting.**Introduction**

Pigeon Pea (*Cajanus cajan*) belongs to the order of *Fabaceae*. Those used as food are divided into two groups namely; the pulses and oil seeds. Pigeon pea belongs to the group of pulses (peas). The colour of the testa of matured dry seed may be dirty white, cream, brown, dirty grayish white, pink or purplish –black. Pigeon pea originated from India and Asia where it travelled to African countries (Onyebuashi, 1986).

It is an erect woody short perennial shrub which grows in semi-tropical and tropical regions. In Nigeria, it is grown extensively in Enugu, Anambra and Benue States. It is called “Fio-Fio” in Anambra State, “Agbubu” in Enugu State and “Waken Kurawa” or “Otile” in some parts of the Northern States (Enwere, 1998).

Pigeon pea seeds are composed of cotyledon (85%), embryo (1%) and seed coat (14%) (Faris and Sigg, 1990). The composition of matured and dry pigeon pea are water (11.5%), protein 20.4%, fat 1.2%, carbohydrates 63.4%, crude Fibre (4.4%) and Ash 3.5%. The starch and protein are the major constituents of pigeon pea. (Ihekoronye and Ngoddy, 1985).

In Nigeria, dry matured seeds are cooked whole until tender. They are mixed with cooked yam, maize and dried cocoyam grit (Achicha) or freshly cooked cocoyam, sweet potatoes in addition to vegetables, palm oil, salt and ground pepper. It is not often used to prepare steam gel (Moi-Moi) fried cake (Akara) (Enwere, 1998). Pigeon pea is consumed in various forms like whole seed, cooked seed, roasted seed, canned seed, cream seed, sprouted seed, dhal (Splitted seed without seed coat). Pigeon pea seed has a hard coat with slight acid taste (Rachie and Roberts, 1994). They dry pigeon pea seeds are soaked overnight and cooked with salt spices. The boiled whole seeds are sometimes fried with species and eaten with cereals particularly in Africa. Foods such as ‘Bongo’ and Brubus” made with the whole seeds are soaked in water and allowed to sprout. The sprouted seed are eaten raw or cooked (Aykrayel and Doughty, 2002).

Tempeh is prepared in combination with soybean by fermenting, soaking, dehulled and cooked pigeon pea seed with rhizopus mould. Sometimes, the whole seeds are canned. Ketchup (Sauce) is prepared by fermenting pigeon pea in salt solution with *Aspergillus oryze*, *Aspergillus niger* and *Rhizopus specie*. The green seeds of pigeon pea are used as a vegetable. The green immature pods are harvested and cooked like French beans and also used as salads (Faris and Singh, 1990).

Several methods of pre-dehulling processes that have been used include steeping, toasting, boiling, soaking, frying and pressure –steaming. The other processes apart from pre-dehulling are drying, splitting and grinding. The oldest and most common home scale

technique for hulling pigeon pea is to pound them in a mortar with a pestle either by spreading the grains in the sun for few hours or after mixing them with a little water. (Ogunji *et al.*, 2005).

Pigeon pea has some problems that are associated with it. These include long time cooking for matured seeds which takes about 18-24hours before it becomes tender and edible (Enwere, 1998). Pigeon pea contains considerable amounts of several anti-nutritional factors that create problems when ingested.

The husks of pigeon pea are not easily removed. It must undergo long process before the husks are removed. This affects the flavor and odour of the ground pea flour (Kamath and Belavady, 2003).

The objective of this study therefore, is to determine the effect of malting and the ease of dehulling on the proximate composition and the anti-nutritional properties of pigeon pea. It is hoped that this will enhance better acceptability and utilization of pigeon pea in various food formulations.

Materials and Methods

Pigeon pea seeds used for this research work were purchased from a local market (Ekeonunwa) in Owerri, Imo State. The chemicals and equipment used were of analytical grade and were obtained from the Department of Food Science and Technology, Federal University of Technology, Owerri, National Roots Crops Research Institute, Umudike, Umuahia and Gateway Laboratory, Owerri.

Sample preparation

The pigeon pea seeds were sorted to remove dirt and other extraneous materials. About 2kg of the clean seeds were winnowed and thoroughly washed. These seeds were then steeped in water using different containers for 24hours and 48hours respectively, while changing the steep water 6 hourly. The resultant steeped grains were allowed to germinate on an improvised malting bed at room temperature for 4 days. They malted seeds were kilned at a temperature of 54⁰C and cooled. The kilned malts were dehulled and milled into flour with an attrition mill. The control which is the raw seeds were dried, dehulled, and ground into flour.

Proximate composition analysis

This was carried out according to the method of AOAC (1990).

Determination of anti-nutritional factors

Determination of Alkaloids

Alkaloids were determined using the alkaline precipitation gravimetric method (A.O.A.C, 1990) Flour samples of about 5g ethanol were soaked in 100ml of 10% acetic acid solution in ethanol. The mixture was well shaken and allowed to stand for 4 hours before it was filtered. The filtrate was evaporated and the alkaloid in the extract, precipitated by drop wise addition of concentrated ammonium hydroxide until full turbidity was obtained. The precipitate was filtered off and washed with 1% ammonium hydroxide and dried in an oven at 60⁰C for 30 min, cooled and reweighed by weight difference. The weight of alkaloid was determined and expressed as a percentage of the sample.

Determination of Tannins

Flour samples of about 5g each were put inside a volumetric flask and 50ml of distilled water was dispensed inside the volumetric flask, shaken for 30 min and filtered.

About 5ml of the filtrate was measured into 50ml volumetric flask and diluted with 35ml of distilled water. Similarly, 5ml of standard tannic acid solution and 5ml of distilled water were measured with separate flasks to serve as standard and blank respectively. These were also diluted with 35ml of distilled water separately. 1ml of follins-Dennis reagent was added to each of the flask followed by 2.5ml of saturated sodium carbonate solution. The content of each flask was up with distilled water and incubated for 90 min at room temperature. The absorbance of the developed colour was measured at 760nm wavelength with reagent blank at zero. The tannin content was calculated as shown below

$$\% \text{ Tannin} = \frac{100}{W} \times \frac{A_u}{A_s} \times \frac{C}{1000} \times \frac{V_f}{V_a} \times D$$

Where, W = weight of the sample analyzed

A_u = absorbance of the test sample

A_s = absorbance of standard tannic solution

C = concentration of standard in mg/ml

V_f = Total volume of extract

V_a = Volume of filtrate analyzed

D = Dilution factor, where applicable

Determination of phenols

0.2g of the dried flour samples were dispensed into the test tubes. About 10ml of methanol was then added to the sample inside the test tubes and thoroughly shaken. The mixture was left to stand for 5min before being filtered using whatman filter paper. About 1ml of the extract was placed in the test tubes and 1ml of follins reagent was added into the test tube containing the extract with 5ml of distilled water. The colour was allowed to develop for about 3 hours at room temperature. The absorbance of the developed colour was measured at 760nm. The experiment was repeated. The phenol content was calculated as % phenol

$$= \frac{100}{W} \times \frac{Au}{As} \times \frac{C}{100} \times \frac{Vf}{Va} \times D$$

Determination of saponin

According to double solvent extraction gravimetric method. About 5g of the flour samples were mixed with 50ml of 20% aqueous ethanol solution. The mixture was heated with periodic agitation in water bath for 90 min at 55°C. Titration was carried out and the residue collected. The residue was extracted with 50ml of 20% ethanol and both extracts were poured together. The combined extracts were reduced to about 40ml at 90°C 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 and the aqueous layer reserved. Re-extraction by partition was done 3times until the aqueous layer become clear in colour. The saponins were extracted with 60ml of normal butanol. The combined extracts were washed with 5% aqueous NaCl(aq). It was dried at 60°C in the oven and re-weighed. The experiment was repeated thrice and the average taken. The saponin was determined and calculated as a percentage of the original samples.

Determination of Oxalate.

This was carried out according to (A.O.A.C., 1990). About 2.0g of the sample was weighed out and extracted thrice at 55°C stirred for 1hour with 20ml of 0.3N HCl. The combined extract was diluted to 100ml with distilled water and used for total oxalate estimation. For oxalate estimation, about 5ml of extract was made alkaline with 1ml of 5N ammonium hydroxide. About 3 drops of phenolphthalein were added to the extract and acetic acid was added in drops. Also about 1ml of 5% CaCl(aq) was then added to the mixture and allowed to stand for 2hours after which it was centrifuged at 3,000 rpm for 15min. The supernatant was hot water thoroughly mixed and centrifuged each time. In the test tube, 2ml of 3N H₂SO₄ was added and the precipitate was dissolved by warming in water bath at 75°C. The content of the test tube was then titrated with freshly prepared 0.01N KMnO₄ at room temperature until the first pink colour appeared throughout the solution. This was then warmed at 75°C and the titration continued until the pink colour persisted.

$$\% \text{ Oxalate} = \frac{Vt}{Ws} \times \frac{Vme}{V} \times \text{Titre}$$

Where Vt = Total volume of titrate = 100

Ws = Weight of the sample = 2g

Vme = Volume –mass equivalent (ie. 1cm³ of 0.05M KMnO₄ is equivalent to 0.00225g anhydrous oxalic acid is equivalent to 0.00225g anhydrous oxalic acid.)

Determination of Cyanogenic glucoside.

This was carried out according to A.O. A.C (1990). 250ml round bottom flask was washed and dried in an oven. About 1.0g of the sample was weighed into 250ml round bottom flask using electronic balance. Then 200ml of distilled water was added into the flask and allowed to stand for 2hours. Full distillation was then carried out and 160ml of the distillate was collected in 250ml conical flask containing 20ml of 2.5% NaOH. About 1 drop of tannic acid which is an antifoaming agent was added before distillation.

About 8ml of 6N ammonium hydroxide and 2ml of 5% K⁺ was added to the 100ml of the distillate containing cyanogenic glycoside. Then the mixture was mixed and titrated with 0.2N AgNO₃(aq) using a micro burette against a blank background of sample. About 5g of the sample was ground into paste and was dissolved in 50ml of distilled water in a corked conical flask. The cyanide extraction was allowed to stay overnight. The extract was filtered and the filtrate was used for cyanide determination. About 1ml of the sample filtrate was placed in a water bath for 5min. A reddish brown colour was read in the absorbance of the corked test tube in spectrophotometer at 490nm. The absorbance of the blank containing only 1ml of distilled water and 4ml alkaline picrate solution was read.

$$\text{HCN (mg/kg)} = 1000 \times Au \times 0.05 \times W$$

Where Au = absorbance of the test sample As = absorbance of standard solution W = weight of sample.

Determination of phytate

This was carried out according to A.O.A.C. (1990). About 2.0g of the sample was weighed using electronic balance. The sample was extracted using 0.2N HCl(aq). About 0.5ml of the extract was pipetted into a test tube fitted with glass stopper. Then, 1ml of the solution was added in the tube and covered with stopper which is fixed with a clip. The tube was heated in a boiling water bath for 30

min and the tube was covered very well with the stopper for the first 15min. Then the test tube containing the solution was cooled in ice water for 15min and allowed to adjust to room temperature. Then the content of the test tube was mixed very well and centrifuged for 30 min. About 1ml of the supernatant was transferred into another test tube and about 1.5ml of the solution was added. The absorbance at 519nm against distilled water was measured.

$$\% \text{ phytate} = \frac{A_u \times C}{A_s \times W} \times \frac{100}{V_a} \times \frac{V_f}{V_a}$$

A_u = absorbance of test sample.

A_s = Absorbance of standard solution

C = concentration of standard solution

W = Weight of sample used.

V_f = Total volume of extract

V_a = Volume of extract

Results and Discussion

Proximate composition of pigeon pea

The result of the proximate composition of the samples are shown in Table I and graph Ia and Ib.

Table 1: Mean value of the results of the proximate composition of pigeon pea flour

SAMPLES		MOISTTURE	PROTINE	ASH	CRUDE FIBRE	FAT	CARBHYDRATE
CONTROL	A	11.00 ^a	18.90 ^d	3.50 ^g	7.00 ^g	1.20 ^g	58.40 ^b
24/24	B	10.50 ^{ab}	19.88 ^c	3.00 ^g	6.40 ^{ab}	1.10 ^{ab}	59.12 ^b
24/48	C	9.00 ^{bc}	20.58 ^{bc}	2.00 ^b	6.20 ^{bc}	0.95 ^b	61.21 ^a
24/72	D	8.50 ^c	21.28 ^{ab}	1.80 ^b	5.80 ^{bc}	0.85 ^b	61.79 ^a
24/96	E	7.50 ^c	21.98 ^a	2.00 ^b	5.60 ^c	0.70 ^b	61.79 ^a
LSD		1.9987	0.7076	0.6867	0.7089	0.2509	1.3789
			48hours	steeping			
CONTROL	A	11.00 ^g	18.90 ^e	3.50 ^a	7.00 ^a	1.20 ^a	59.40 ^b
48/24	F	10.50 ^a	21.11 ^d	1.70 ^b	6.20 ^b	1.00 ^a	59.49 ^{ab}
48/48	G	8.50 ^b	22.33 ^c	1.60 ^{bc}	6.00 ^{bc}	0.90 ^b	60.67 ^a
48/72	H	7.50 ^{bc}	23.03 ^b	2.00 ^b	3.60 ^{cd}	0.80 ^b	61.07 ^a
48/96	I	7.00 ^c	24.78 ^g	1.50 ^c	5.40 ^d	0.75 ^b	60.57 ^a
LSD		1.4219	0.1963	0.3267	0.5209	0.2509	1.9870

Mean values with the same lower case super scripts on the same column are not significantly different at $P \geq 0.05$

Key note: A = Raw pigeon pea, B = 24h steeping with 24h germination, C = 24h steeping and 48h germination, D = 24h steeping and 72h germination, E = 24h steeping with 90h germination, F = 48h steeping with 24h germination, G = 48h steeping with are 48h germination, H = 48h steeping with 72h germination, I = 48h steeping with 96h germination; h = Hour.

Moisture: The percentage moisture content of the malted samples ranged from 10.30% - 7.50% but for the raw pigeon pea, the moisture content was 11.00%. From the graph, it can be clearly stated that moisture content decreased, with increase in germination time for peas steeped for 24hours. This showed that the absorption of moisture in the malted samples reduce during malting than the raw sample which had higher moisture content. This result is in agreement with the work of Faris and Singh, (1990).

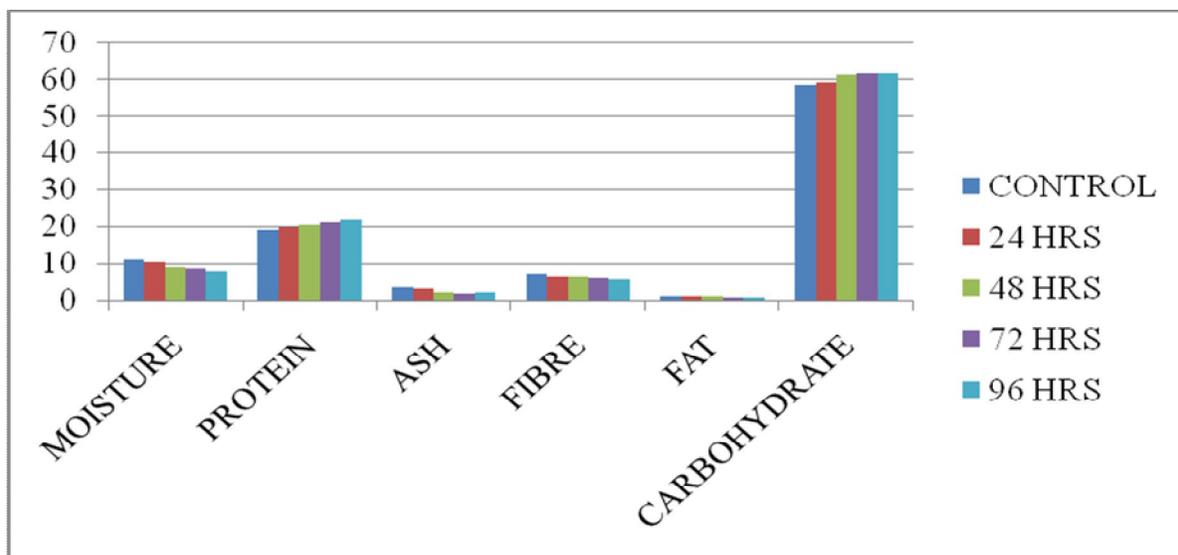
Protein: The percentage protein content of the malted samples ranges from 19.99% to 21.98% and 21.11% to 24.78% for peas steeped for 24hours and 48hours respectively, but for the raw samples, the protein content was 18.9%. This increase could be attributed to the enzymes involved in the degradation of the malted samples (Damardjti and Widowati, 1991) which are also proteins.

Ash: The percentage ash content of the malted samples ranged from 3.00% - 1.80% and 2.00% to 1.50% for malts steeped for 24hours and 48hours respectively, but the ash content of the raw pigeon pea was 3.50%. This implied that the raw pigeon pea sample had more mineral contents than the malted samples because of the processing treatments given, like drying, dehulling and grinding which were done after malting. These treatments could have reduced the mineral contents of the seeds (Ihekoronye and Ngoddy, 1985), as a result of usage during germination.

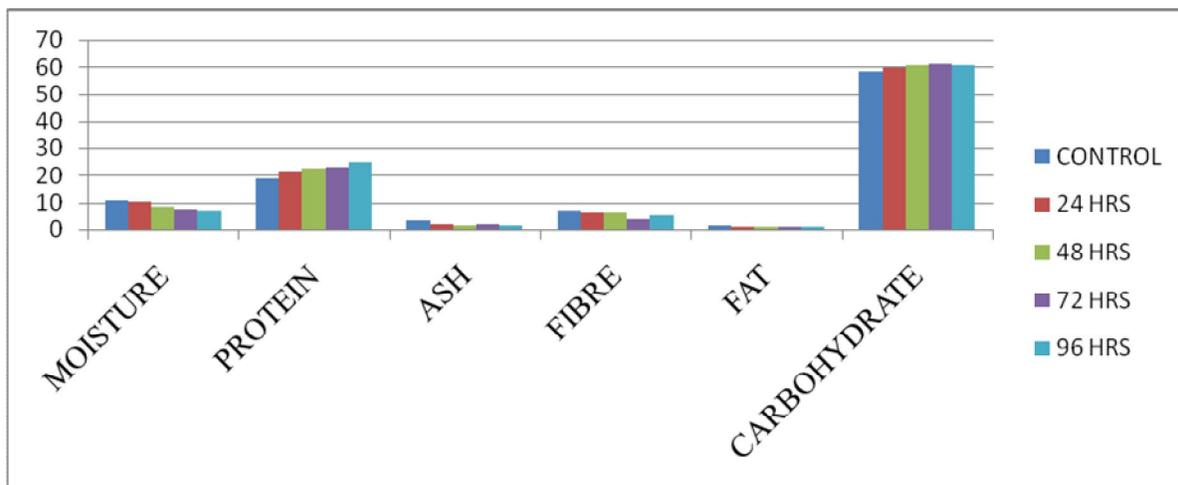
Crude fibre: The percentage crude fibre content of the malted flour samples ranged from 6.40% to 5.60 and 6.20% to 5.40% for samples steeped for 24hours and 48hours respectively. This decrease agree with the report which stated that decreased fibre content of foods decrease incidence of diseases associated with high fibre consumption (Akinola and Whiteman, 1975).

Fat: The percentage fat content of the malted pea flour sample for 24hour and 48hours steeping ranged from 1.10% to 0.70% and 1.00% to 0.98% respectively, while the raw pigeon pea sample was 1.20%. The fat content of the raw sample was higher than the malted samples because of the processes like drying, dehulling and grinding which was carried out after malting. This could have reduced the fat content of this seed (Ihekoronye and Ngoddy, 1985).

Carbohydrate: The percentage carbohydrate content of the malted samples was generally lower than that of the raw samples. This is mainly due to the reduction in the values of other nutrients as malting progressed. There was no significant difference ($p=0.050$) for malt samples that germinated between 48h and 96h



Graph Ia: Comparative graph showing proximate analysis of pigeon pea flour samples steeped for 24 hours.



Graph Ib: Comparative graph showing proximate analysis of pigeon pea flour samples steeped for 48 h.

The anti-nutritional factors of pigeon pea flour

The results of the anti-nutritional properties of the raw and the malted pigeon pea flour samples are shown in table II and depicted in Graph IIa and IIb

Table 2: Mean value of the results of the antinutritional properties of pigeon pea flour

Samples		(%)	(%)	(%)	(mg)	(%)	(%)	(%)
		Phytate	Phenol	Oxalate	HCN	Saponin	Alkaloid	Tannin
Control	A	1.017 ^a	0.160 ^a	0.139 ^a	7.180 ^a	0.466 ^a	0.323 ^a	0.220 ^a
24/24	B	1.005 ^b	0.153 ^a	0.120 ^a	6.847 ^a	0.427 ^b	0.327 ^a	0.213 ^a
24/48	C	0.863 ^c	0.150 ^a	0.105 ^a	0.797 ^a	0.424 ^b	0.333 ^b	0.183 ^a
24/72	D	0.798 ^d	0.150 ^a	0.097 ^a	6.390 ^b	0.407 ^c	0.347 ^b	0.180 ^c
24/96	E	0.698 ^e	0.146 ^a	0.097 ^a	4.657 ^c	0.393 ^d	0.353 ^b	0.180 ^c
LSD		0.0096	0.0432	0.0444	0.3448	0.0068	0.0069	0.0070
48 HOURS STEEPING								
Control	A	1.017 ^a	0.160 ^a	0.139 ^a	7.180 ^a	0.466 ^a	0.323 ^a	0.220 ^a
48/24	F	0.795 ^b	0.150 ^a	0.109 ^a	6.797 ^a	0.420 ^b	0.353 ^b	0.217 ^a
48/48	G	0.816 ^c	0.149 ^a	0.097 ^a	5.370 ^c	0.413 ^{bc}	0.327 ^{bc}	0.180 ^b
48/72	H	0.776 ^d	0.143 ^a	0.094 ^a	4.750 ^d	0.407	0.307 ^b	0.170 ^c
48/96	I	0.640 ^e	0.140 ^a	0.086 ^a	4.083 ^c	4.0387 ^d	0.293 ^d	0.170 ^c
LSD		0.0113	0.0434	0.0456	0.337	0.0101	0.0091	0.0081

Mean values with the same lower case super scripts on the same column are not significantly different at $P \geq 0.05$

Key note: A = Raw pigeon pea sample; B= 24h steeping with 24h germination; C = 24h steeping with 48h germination; D = 24h steeping with 72h germination, E = 24h steeping with 96h germination; F = 48h steeping with 24h germination; G = 48h steeping with 48h germination; H = 48h steeping with 72h germination; I = 48h steeping with 96h germination.

Phytate: The phytate of pigeon pea for the malted samples ranged from 1.005% to 0.698% and 0.795% to 0.640% for samples steeped for 24hours and 48hours respectively. However, the reduction, when compared with the raw sample (1.017%) though negligible was as a result of time of sprouting and germination and also in the seed coat which is dehulled after drying (Prabhavathi and Narasinga, 1995).

Phenols: The phenol of malted pigeon pea range from 0.153% to 0.146% and 0.150% to 0.140% for samples stepped for 24hours and 48hours respectively, while the raw sample was 0.160%. The reduction was very infinitesimal, hence malting may not be the most efficient way to reduce phenols in pigeon pea because a few water soluble components are leached out during germination of pigeon pea as reported by Adeyemi (2011).

Oxalate: The oxalate of pigeon pea steeped for 24hours and 48hours ranged from 0.120% to 0.097% and 0.109% to 0.086% respectively, while the raw sample was 0.139% to 0.086% respectively while the raw sample was 0.139%. This suggests that malting really reduced oxalate but no significant difference occurred even with the reduced Oxalate in pigeon pea because of the minerals which are used during germination. This could help release the minerals which are bond with the Oxalates and make the seeds more nutritional.

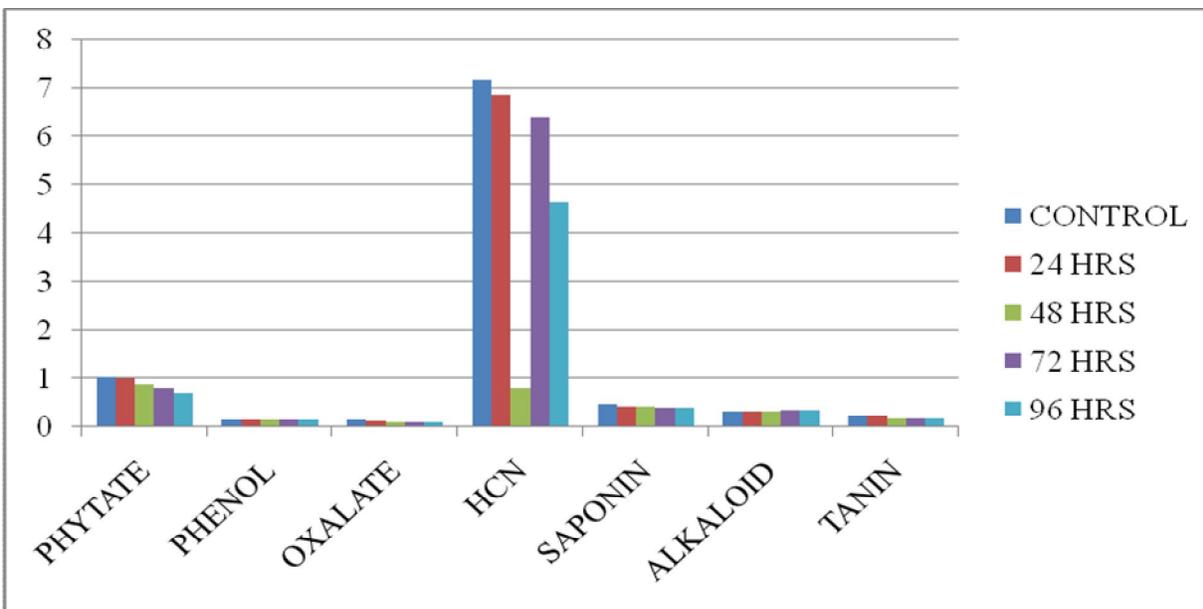
Hydrogen cyanide: the hydrogen cyanide for the various samples showed that malting may not be the best processing method to reduce the HCN content of pigeon pea. However, the little reductions occurred because HCN in the seed coat were lost during dehulling of pigeon pea. (Kumar and Singh, 2002).

Saponin: The saponin contents of pigeon pea samples steeped for 24hours and 48hours ranged from 0.427% to 0.393% and 0.420% to 4.0387%. This showed that there was no significant reduction($p=0.05$) in saponin during malting as expected. This suggests that malting may not be a good method in reducing saponin in pigeon pea.

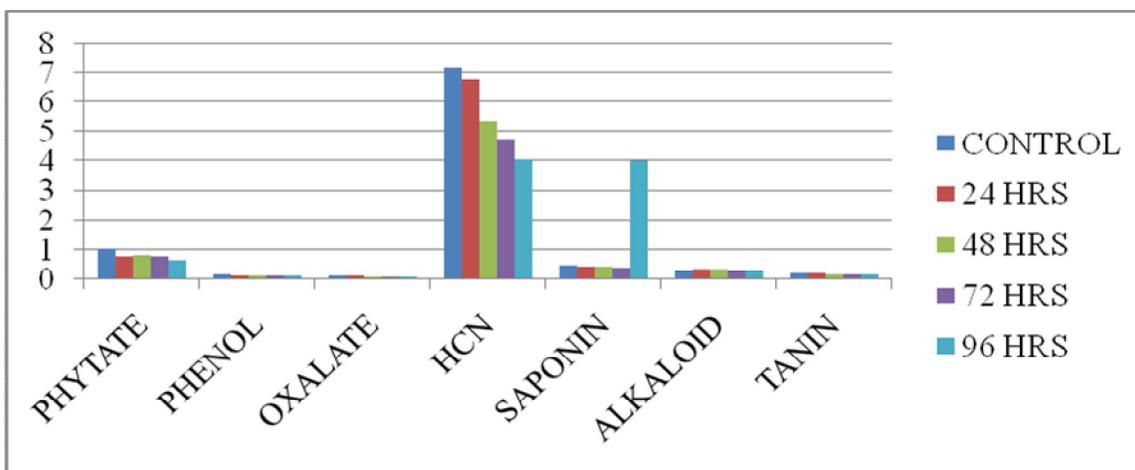
Alkaloid: The alkaloids of pigeon pea steeped for 24hours and 48hours ranged from 0.323% TO 0.293%. This showed some increase in alkaloid content as the malting progress. This could be due to the enzyme which were degraded during germination that caused the increase in the alkaloid content for malted samples than raw samples (Saxena *et al.*, 2010).

Tannins: The results obtained here showed that there was no significant reduction ($P \geq 0.05$) in tannins for all the malted samples when compared with the raw samples. However, the little reduction could be as a result of the bitter taste which reduced on prolonged steeping Adeyemi (2011).

In the dehulling of pigeon pea, the longer the steeping time and germination time, the easier the dehulling process and vice versa. This is because steeping and germination softens the seed coat of the pea for easy dehulling using the hand while the raw sample is dehulled using attrition mill. The steeping helped to loosen the hulls from the cotyledon.



Graph IIa: Comparative graph showing the anti-nutritional properties of pigeon pea flour samples steeped for 24 h.



Graph IIb: Comparative graph showing the anti-nutritional properties of pigeon pea flour samples steeped for 48 h.

Conclusion

The results of this research has shown how malting affect the proximate composition, the ease of dehulling and the anti-nutritional properties of pigeon pea (*Cajanus cajan*) flour. Proximate analysis reveals decrease in the moisture content, crude fibre, fat and ash content during malting as against increase in protein content.

From the results of the anti-nutritional analysis of malted pigeon pea, though there were slight reductions in the anti-nutritional contents, most of the anti nutritional factors were not reduced to a reasonably low level. Malting is not really the best method of detoxifying pigeon pea; rather better processing methods are suggested as means of reducing the anti-nutritional properties of pigeon pea. Dehulling is easily done for pigeon pea when malted, than for unmalted samples

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