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The Impact of Boiling Periods on The Proximate Composition and Level of Some Anti-Nutritional Factors In Pigeon Pea (*Cajanus Cajan)* Seeds

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Abstract

This study was conducted to determine the impact of boiling periods on the proximate composition and level of some anti-nutritional factors in pigeon pea seeds. Pigeon pea seeds were subjected to boiling for 30, 40 and 60 minutes. For each batch, water was first brought to boil in a big aluminium pot, then the seeds were poured into the boiling water and allowed to come back to boil before timing commenced. The seeds were thoroughly dried and representative sample for each boiling period was taken to the laboratory for proximate analysis and determination of anti- nutritional factors. The raw seeds served as control. Each batch of seed was replicated thrice. Data obtained were subjected to one way analysis of variance. Results obtained showed that boiling pigeon pea seeds for 30, 40 and 60 minutes significantly (P<0.05) reduced crude protein by 5.84, 8.45 and 10.86%, crude fibre by 4.74, 5.99 and 12.98% and ash by 5.92, 7.42 and 11.84% respectively. Nitrogen free extract values in seeds were significantly (P<0.05) increased by 3.61, 4.46 and 5.30% by 30, 40 and 60 minutes boiling respectively. Boiling however did not have significant effect on dry matter and crude fat levels of seeds. Results of anti-nutritional factor determination showed that apart from phytate, all the anti-nutritional factors determined were significantly (P<0.05) reduced by boiling. The reductions increased with increased in boiling period with 60 minutes resulting in the greatest reductions (100% trypsin inhibitor, 47% tannin, 20.48% oxalate, 70.05% hydrocyanic acid, and 17.97% saponin). It is concluded that boiling of pigeon pea seeds for up to 60 minutes is beneficial.

Keywords: *Boiling period, pigeon pea, proximate composition, anti-nutritional factors.*

INTRODUCTION

One of the major problems facing the livestock industry in Nigeria is the increasing unavailability and high cost of conventional feedstuffs like maize, groundnut, soyabeans etc (Bawa *et al,* 2007). This has been attributed to the high level competition between livestock and man for the same ingredients. The overall effect of this competition is shortage of animal protein production and hence, shortage of animal protein intake due to scarcity and high cost of animal products (Akinmutimi *et al*, 2007). The need therefore to source for alternative feedstuff cannot be over emphasized. Such alternatives should be cheap, available and less competed for by man and industry. Pigeon pea can be one of such alternative feedstuff.

The pigeon pea is produced in large quantities in South Eastern Nigeria (Ikeorgu *et al,* 1992), Southern parts of Nigeria (Adeparasi and Balogun, 1999) and in Central parts of Nigeria particularly Benue state, but has very low human food preference (as it takes longer to cook and is not as palatable as cowpea) and has no industrial use now (Amaefule and Obioha, (2001). Pigeon pea is truly a multipurpose nitrogen fixing plant that provides food, fuel wood, fodder and shelter material for subsistence farmers. Pigeon pea is nutritionally important as it contains high level of proteins ranging from 17.9-30.0% (Obioha, 1992; Aduku, 1993; Amaefule and Obioha, 2001; Onu and Okongwu, 2006). Aduku, (1993) gave the nutrient composition of pigeon pea seeds as: Crude protein- 23.77%, Fat- 1.1%, Crude fibre- 7.49%, Calcium- 0.13%, Phosporus- 0.26%, Lysine- 1.66%, Methionine- 0.29%, Cystein- 0.29%, Arginine- 1.59% and Tryptophan- 0.11%.

The use of pigeon pea in monogastricõs diets may be encumbered by the presence of anti-nutritional factors like trypsin inhibitor, chymotrypsin inhibitor, amylase inhibitor, hemagglutinin, tannins (polyphenols), saponins, cyanide, phytic acid, oxalate, etc (Farris and Singh, 1990; Dõmello, 1995; Duhan *et al,* 2001; Chisowa, 2002; Onu and Okongwu, 2006). Farris and Singh (1990) however reported that pigeon pea seeds contain lower amounts of protein inhibitors than soyabeans. Trypsin and chymotrypsin inhibitors affect the digestibility of legume protein, while other anti-nutritional factors like tannins, phytates, cyanide and hemagglutinins impart bitter or unacceptable taste to the legumes, causing decreased protein digestibility and absorption of divalent metal ions such as F^{2+} , Zn^{2+} in the intestine (Abdu *et al,* 2008). Removal of these undesirable components is essential in order to effectively utilize their full potential as feedstuff.

It has been established that cooking and other processing methods exert beneficial effect by destroying the anti-nutritional factors inherent in legume grains (Balogun *et al,* 2001). Various researchers have boiled or cooked pigeon pea seeds for variable length of time. Adeparasi (1994) pressure cooked pigeon pea seeds at 100^0C for 45 minutes while Amaefule and Obioha (2001) boiled the seeds at the same temperature but for 30 minutes. Onu and Okongwu (2006) on the other hand boiled the seeds for 60 minutes at 100^0C. Elias *et al* (1973) reported that boiling pigeon pea seeds beyond 30 minutes at 100^0C reduced its nutritive value while Farris and Singh (1990) reported that cooking pigeon pea seeds for between 40-60 minutes reduced or eliminated the anti-nutritional factors. Since the discovery of the beneficial effects of heat treatment on the nutritional value of raw legume grains, the challenge has been to determine the appropriate level of heat treatment for optimum utilization.

The objective of this study, therefore, was to evaluate the effects of three boiling periods (30, 40 and 60 minutes) on the proximate composition and levels of some anti-nutritional factors in pigeon pea seed.

MATERIALS AND METHODS

Acquisition and preparation of test material

The pigeon pea seeds used in this study were acquired from Ugbokolo in Okpokwu Local Government Area of Benue State. After cleaning, the seeds were subjected to boiling for 30, 40 and 60 minutes. For each batch, water was first brought to boil in a big aluminium pot, then the seeds were poured into the boiling water and allowed to come back to boil before timing commenced. The boiled seeds were spread on a concrete floor and allowed to dry thoroughly. The raw seeds served as control. The samples were milled and sent to the laboratory for analysis.

Determination of proximate composition and anti-nutritional factors

The proximate components of both raw and boiled pigeon pea seeds were determined according to AOAC (1990). Each batch of seed was replicated three times.

Trypsin inhibitor was determined according to Kakade *et al* (1974). Hydrogen cyanide was determined by the spectrophotometric method as outlined by Bradbary *et al* (1999), while saponin was analyzed by the spectrophotometric method of Brunner (1984). Tannin and phytic acid were determined according to Sutardi and Buckle (1985) and Earp *et al* (1981) respectively.

Data analysis

Data collected were subjected to one-way analysis of variance and means were separated using Hsuœs MCB (Multiple Comparison with the Best) method (MINITAB, 1991).

RESULTS AND DISCUSSION

Proximate components

The impart of boiling periods on proximate composition of raw and boiled pigeon pea seeds is shown in Table 1. Proximate analysis revealed that raw pigeon pea seeds contained 88.35% dry matter (DM), 21.90% crude protein (CP), 1.13% crude fat (Cfat), 8.01% crude fibre (CF), and 64.20% nitrogen free extract (NFE). The CP content of raw seeds used in this study (21.90%) is similar to the 23.34% that Skermam *et al* (1988) reported, higher than the 17.90% reported by Geervani (1980) but lower than the 27.15% reported by Onu and Okongwu (2006). The CP content of pigeon pea seeds ranged from 21.90% (raw seeds) to 19.52% (60 minutes boiled seeds). This range qualifies pigeon pea seeds as a protein source. The 1.13% Cfat analyzed in the raw seeds in this study is similar to the 1.10% recorded by Aduku (1993) but lower than the 2.56% and 2.10% reported by Adeparasi (1994) and Amaefule *et al* (2006) respectively. The NFE in the raw seeds in this study (64.20%) is higher than the 55.02%, 55.63% and 61.40% reported by Etuk and Udedibie (2006), Adeparasi (1994) and Amaefule and Obioha (2001). The reason for these variations may be due to varietal differences. Salunkhe *et al* (1985) stated that a wide variability exists in the composition of pigeon pea seeds depending on the geographical location cultivar and growth conditions.

Boiling did not have significant ($P>0.05$) effect on DM and Cfat content of pigeon pea seeds. There was however significant ($P<0.05$) reductions in CP, CF and Ash content of seeds with boiling. The reductions increased with increase in boiling period. Boiling also caused significant ($P<0.05$) increase in NFE content of seeds with 60 minutes boiling effecting the greatest increase. The decrease in proximate components with boiling could be due to leaching of nutrients into the boiling water.

The non significant ($P>0.05$) effect of boiling on DM content of seeds means the seeds were equally dried as it is the level of dryness that can affect the DM of seeds. This result conforms

with findings of Tuleun and Patrick (2007) who cooked *Mucuna utilis* seeds for 0, 20, 30 and 60 minutes and reported non-significant ($P>0.05$) effect of cooking on DM content of seeds.

Boiling for 30, 40 and 60 minutes effected a 5.84, 8.45 and 10.86% reduction respectively in the CP content of seeds. This reduction became significant ($P<0.05$) when boiling exceeded 30 minutes. The decrease in CP content of seeds with boiling could be as a result of leaching of soluble or proteineous part of the seeds into the boiling water. Farris and Singh (1990) reported similar reductions when pigeon pea seeds were cooked at different temperatures. The result of this trial is in agreement with findings of Adeparasi (1994) and Onu and Okongwu (2006) who reported decrease in CP content of pigeon pea seeds with boiling. Tuleun and Patrick (2007) also reported significant ($P<0.05$) decrease in CP content of *Mucuna utilis* seeds with increased cooking time.

The slight non-significant ($P>0.05$) increase in Cfat with increase in boiling period is in variance with results of Akinmutimi (2007) who reported significant increase in ether extract of *Mucuna pruriens* with increased cooking time.

Boiling significantly ($P<0.05$) reduced the CF levels of pigeon pea seeds with 60 minutes boiling effecting the highest reduction of 12.98%. This result is in consonance with Aletor and Ojo (1989) who stated that cooking generally reduce CF content of legumes. Decrease in CF with increase in duration of cooking agrees with Akinmutimi

(2004, 2007) who worked on mucuna species. The decrease in CF content of seeds with boiling is an advantage to monogastrics that lack the ability to utilize high fibre.
Ash content of pigeon pea seeds was reduced by 5.92, 7.42 and 11.84% with 30, 40 and 60 minutes boiling respectively. The significant ($P<0.05$) reduction in ash content of seeds with increased boiling period is in agreement with results of Onu and Okongwu (2006) who recorded decreased in ash from 5.31% in the raw seeds to 4.21% in the boiled seeds. Amaefule *et al* (2006) also recorded decrease in ash content of pigeon pea seeds from 5.50% (raw seeds) to 4.00% (30 minutes boiled seeds). Adeparasi (1994) however observed increase in ash content of pigeon pea seeds from 3.67% in the raw seeds to 5.40% in the cooked seeds.

Boiling resulted in gradual increase in NFE from 64.20% in the raw seeds to 67.60% in the 60 minutes boiled seeds. The NFE values of raw and boiled seeds were all significantly ($P<0.05$) different from each other. 30, 40 and 60 minutes boiling caused 3.61, 4.46 and 5.30% increase respectively in NFE values. The significant ($P<0.05$) increase in NFE values with boiling agrees with reports of Adeparasi (1994) and Onu and Okongwu (2006). The increase in NFE with boiling implies higher total digestible nutrient (Akinmutimi, 2004).

Anti-nutritional Factors

The level of anti-nutritional factors (ANFs) as influenced by boiling periods is in Table 2. Results of ANFs determination have revealed that pigeon pea seeds contained trypsin inhibitor, tannin, oxalate, hydrocyanic acid, saponin and phytate. This finding confirm the reports of DõMello (1995), Duhan *et al* (2001), Chisowa (2002), Udedibie and Carlini (2002) and Onu and Okongwu (2006) that pigeon pea contain these ANFs.
Boiling resulted in reduction of all the ANFs analysed in this study in agreement with Farris and Singh (1990) and Balogun *et al* (2001), who reported that most ANFs in legume seeds can be reduced by proper application of heat. The reduction increased as boiling period increased. This trend may be due to higher ability of hydrolyzing the ANFs as boiling period increased.
Boiling significantly (P<0.050 reduced the trypsin inhibitor units (TIU) in the pigeon pea seeds with 81.97% destroyed just by boiling for 30 minutes. The reduction of TIU increased with increase in boiling period with 60 minutes boiling eliminating it completely (100%) from
the seeds. Onu and Okongwu (2006) recorded 97.84% reduction in trypsin inhibitor activity (TIA) in 60 minutes boiled pigeon pea seeds. Akinmutini (2007) witnessed 100% destruction of trypsin inhibitor when *mucuna pruriens* seeds were cooked for 60 minutes. Bawa *et al* (2003, 2007), reported 83.00 and 83.76% TIA destruction (the highest) when lablab and locust bean seeds were cooked for 60 minutes respectively.

Trypsin inhibitor is the most heat labile of all the ANFs evaluated in this study. The thermo liability of trypsin inhibitor implies that protein digestibility will not be hampered when boiled pigeon pea seeds are fed to livestock especially monogastrics. The problem of hypertrophy also is eliminated.

There was significant (P<0.05) destruction of tannin with boiling in this study. 15.29, 23.53 and 47.06% tannin destruction was realized with 30, 40 and 60 minutes boiling respectively. Several authors have also observed significant (P<0.05) reduction in tannin content with cooking of: *Lablab purpurens* beans (Abeke *et al,* 2008), Locust bean seeds (Bawa *et al,* 2007) and Soyabeans (Kaankuka *et al*, 1996). Tannin in this study is fairly heat stable in conformity to findings of Akinmutimi (2007) and Abeke *et al* (2008). The thermo-stability of tannin has been attributed to by Akinmutimi (2004) to the existence of intra molecular force within tannin. The 47.06% tannin destruction with 60 minutes boiling recorded in this study, is lower than the 75.41% recorded by Bawa *et al* (2007) when they cooked locust bean seeds for 60 minutes, but higher than the 17.50% reported by Akinmutimi (2007) with 60 minutes cooked *Mucuna prupriens.*

Oxalate content of pigeon pea seeds in this study was influenced by boiling. Oxalate was reduced from 0.83% in the raw seeds to 0.66% in the 60 minutes boiled seeds. Boiling for 30, 40 and 60 minutes significantly (P<0.05) reduced the oxalate content of pigeon pea seeds by 6.02, 16.86 and 20.48% respectively. Percent reduction increased as boiling period increased. Oxalate is another ANF in this study that is heat stable.

The level of hydrocyanic acid (HCN) was reduced significantly (P<0.05) by boiling. The reduction of 53.43, 69.34 and 70.05% were recorded when pigeon pea seeds were boiled for 30, 40 and 60 minutes respectively. Values for boiled seeds were not significantly (P<0.05) different from each other but were significantly lower than the value in the raw seeds. HCN in this study is the second most heat labile ANF as 70.05% was eliminated by boiling for 60 minutes. Abeke *et al (*2008) recorded 60% HCN destruction after cooking lablab seeds for 20 minutes and Akinmutimi (2007) reported 66.45% reduction in HCN when mucuna seeds were cooked for 60 minutes. The significant reduction of HCN in boiled seeds is due to the volatile nature of HCN which has a boiling point of 26^{o}C (Oke, 1996). The destruction of HCN with boiling implies that less methionine will be required when cooked pigeon pea seeds are used for feed formulation for detoxification of HCN (Rhodenase pathway) (Akinmutimi, 2007).

There was reduction in saponin content of pigeon pea seeds with boiling. 30 minutes boiling didnőt effect a significant (P>0.05) reduction in saponin content, but 40 and 60 minutes boiling reduced saponin level from 0.89% in the raw seeds to 0.77 and 0.73% respectively which are significantly (P<0.05) lower than the levels in the raw and 30 minutes boiled seeds. Saponin is fairly heat stable in agreement with findings of Duhan *et al* (2001) who reported 38% as maximum reduction of saponin content of pressure cooked soaked and dehulled pigeon pea seeds.

Boiling did not produce any significant (P>0.05) effect on phytate content of pigeon pea seeds. There was slight insignificant reduction in phytate content of seeds with increase in boiling period. The non- significant effect of boiling on phytate level of the seeds signify that phytate is heat stable. The heat stability of phytate could be due to strong covalent linkage between oxygen atoms and the phosphate structure (De Boland *et al,* 1975). Bawa *et al* (2003) also reported non-significant reduction in phytate when lablab seeds were cooked for 30 minutes.

CONCLUSION

From the results obtained in this trial, it can be concluded that boiling pigeon pea seeds is beneficial and can be done for 60 minutes. Reasons being that the reduction of anti-nutritional factors by boiling far exceeds the reduction of the proximate components of the seeds. The benefits that will be derived from the reduction of these anti-nutritional factors will outweigh the disadvantages that will be associated with the reduction of proximate components of the seeds.

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Table 1: The Proximate Composition of Raw and Boiled Pigeon Pea Seeds Boiling Periods (mins)

Nutrients	0	30	40	60	SEM
Dry matter (%)	88.38	88.4	88.43	88.49	8.72
Crude protein (%)	21.90[a]	20.62[ab]	20.05[bc]	19.52[c]	0.20
% CP reduction	-	5.84	8.45	10.86	
Crude fat (%)	1.13	1.27	1.33	1.51	3.14
% increase in Cfat	-	12.39	17.70	33.63	
Crude fibre (%)	8.01[a]	7.63[b]	7.53[b]	7.04[c]	1.50
% CF reduction	-	4.74	5.99	12.98	
Ash (%)	4.56[a]	4.29[b]	4.22[bc]	4.02[c]	1.89
% Ash reduction	-	5.92	7.45	11.84	
NFE (%)	64.20[d]	66.52[c]	67.06[b]	67.60[a]	1.35
% increase in NFE	-	3.61	4.46	5.30	

a, b, c means on the same row with different superscript are significantly different ($p<0.05$).
Nitrogen free extract (NFE) obtained by difference. NFE=100-(%CP +%fat+ %CF+ %As*h)*

Table 2: Level of Some Anti-nutrients in Raw and Boiled Pigeon Pea Seeds

	Boiling periods				
Anti-nutrients	0	30	40	60	SEM
Trypsin inhibitor (TIU/mg protein)	28.95[a]	5.22[b]	3.64[b]	0.00[c]	1.92
% TIU destruction	-	81.97	87.43	100.00	
Tannin (%)	0.085[a]	0.072[b]	0.065[c]	0.040[d]	1.51
% Tannin destruction	-	15.29	23.53	47.06	
Oxalate (%)	0.83[a]	0.78[b]	0.69[c]	0.66[c]	1.23
% Oxalate destruction	-	6.02	16.86	20.48	
Hydrogen cyanide (mg/kg)	396.60[a]	184.66[b]	121.57[b]	118.77[b]	1.83
% Hydrogen cyanide destruction	-	53.43	69.34	70.05	
Saponin (%)	0.89[a]	0.85[a]	0.77[b]	0.73[b]	1.32
% Saponin destruction	-	4.49	13.48	17.97	
Phytate (%)	1.25	1.23	1.20	1.60	2.46
% Phytate destruction	-	1.60	4.00	7.20	

a, b, c means on the same row with different superscript are significantly different ($p<0.05$).