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EFFECTS OF DIFFERENT HEAT PROCESSING METHODS ON THE ANTINUTRITIONAL FACTORS (ANFS) LEVEL OF *P. reticulatum* SEED MEAL

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ABSTRACTS
The antinutritional factors (ANFs) in raw and differently heat processed P. reticulatum seed meal were evaluated. The seed was processed as raw seed (T1), 30 minutes boiled (T2), 60 minutes boiled (T3), traditionally boiled (T4), 40 minutes toasted (T5), 80 minutes toasted (T6), 120 minutes toasted (T7) and traditionally roasted seed (T8). The seed meal was evaluated for saponin, tannin, cyanide, oxalate, phytate and phenols. The result showed that heat processing significantly (P < 0.05) affected the ANFs in the seed meal. Highest value of saponin (0.07mg/100g) was recorded in T1, while zero level of saponin was recorded in T4. The highest value (0.26mg/100g) of tannin was recorded in T1, while T4 recorded the least value (0.05mg/100g). Cyanide highest value of (0.17mg/100g) was observed in T1, while T4 recorded the least value (0.02mg/100g). The highest oxalate level was observed in T1 (0.04mg/100g) and zero level was recorded in T3, T4 and T8. Phytate highest level (0.14mg/100g) was recorded in T1, and zero level of phytate was recorded in T4. The highest phenol level was observed in T1 (0.06mg/100g) while zero level of phenol was recorded in T4. T4 is the most effective processing method that reduced all the ANFs with 100% reduction in saponin, oxalate, phytate and phenols. The processing methods used reduced the seed ANFs and therefore, indicated the possible utilization of the seed in animal feed formulation (fish inclusive).
Keywords: Antinutritional factors, heat, P. reticulatum, processing, seed.

INTRODUCTION

P. reticulatum is a shrub or small tree 5-10m high having a bushy spherical canopy, with a trunk that is often twisted, and possessing a dark brown, fibrous and deeply longitudinally fissured outer bark and pink to red inner side of the bark. The leaves are bilobed (split in half in cattle hoof shape) and covered below by a reddish pubescence and are 7.5 – 16cm long and 10-18cm broad. The flowers are usually white and fragrant. The flowers are clustered in short hairy, axillary racemes 4-5cm. The petals of the flower are white with pink stripes, while the pods are woody, flat, straight, undiluted or twisted, hard, glabrous or sparsely pubescent, indehiscent and persisting, many – seeded, up to 25cm long x 5cm wide and dark brown when ripe (Adjanohoun *et al.,* 1991; Assi and Guinko, 1991 ; Vodouhe *et al.,* 2010). The pod contains the seed. The plant fibre is strong; the wood is heavy and hard. The wood is liable to attacks by termites and borers. The wood is good source of fire wood, because it does not burn fast. Synonyms to *P. reticulatum* in the past includes *Bauhinia thonningii* (Schumach.), *Bauhinia reticulate* (DC), *Bauhinia abyssinica* (A. Rich.), *Bauhinia pyrrhocarpa* (Hochst) and *Piliostigma pyrrhocarpum* (Hochst) (Watt and Breyer – Brandwijk, 1962).

The local Nigerian names of *P. reticulatum* are Kargo (Or Kalgo) in Hausa (Northern Nigeria), Abafe in Yoruba language (Western Nigeria) and Okpo-atu in Igbo (Eastern Nigeria) (Dalziel, 1937; Burkill, 1995). Other common names include camel food (English); pied de chameau, semallier (France), Musacanca (Portugal) (Vodouhe *et al.,* 2010). The plant is also known as Barkee – hi in fulfulde by Fulani in Nigeria (Roger and Mallam, 2006).

The members of the genus *Piliostigma* occur in tropical Africa and Indo-Malaya. The two African species *P. reticulatum* (DC.) Hochst and *P. thonningii* (Schum.) milne – Redhead, inhibit dry and moist savannahs, respectively (Allen and Allen, 1981).

Although there is limited information on the use of *P. reticulatum* seed as ingredients in livestock feed formulation. Akin-Osanaiye (2009) conducted a research on the nutritional potentials of *P. reticulatum* with a view of exploitation of plant seeds as an alternative plant protein source for human and livestock feed formulation.

Processing feedstuff make it safe for consumption. The factors that affect nutrients content resulting from feedstuff processing are; sensitivity of the nutrients to light, heat, oxygen (Morris *et al*., 2004).

The different ways of feed stuff processing may affect significantly the concentration and availability of minerals, vitamins and other essential compounds in the feedstuff. To improve the nutritional quality and to provide effective utilization of legumes for animal feed, it is important to establish a processing method (s) that will remove or reduce the anti-nutritional factors. Various processing methods have been used in legume seeds (Farran *et al*, 2001; Barbour *et al.,* 2001). The aim of the study was to determine the processing method that will reduce the antinutritional factors in the seed for animal (fish inclusive) feed formulation.

MATERIAL AND METHODS

Seed Samples Collection and Identification

Dry pods of *Piliostigma reticulatum* were obtained from Damfamin –Tofa, Ganduje Village, of Dawakin- Tofa local government area and new campus Bayero University Kano, all in Kano state. Kano is in the Sudan Savannah of Nigeria. It lies within latitude $11^{o}30^{''}$ N and longitude $8^{o}30'$ E and at an altitude of about 400 m above sea level (Olofin, 2008). The pods were treated using motor and pestle and winnowed on a tray by blowing air through to remove the chaff in order to have a clear seeds for the research.

The plant and the Pods Samples were identified in the department of biological scienses, Ahmadu Bello University, (ABU) Zaria.

Seed Processing

The collected seed samples were divided into eight (8) portions and processed differently, a modification of Anwa *et al.* (2007) Method as follows-

Treatment one (T1)= raw *P. reticulatum* seed (RPRS), Treatment two (T2)= 30 minutes boiled *P. reticulatum* seed ($BPRS_{30}$) Treatment three (T3)= 60 minute boiled *P. reticulatum* seed ($BPRS_{60}$) , Treatment four (T4)= traditionally boiled *P. reticulatum Seed (*$BPRS_T$*),* Treatment five (T5)= 40 minutes toasted *P.* reticulatum seed ($TPRS_{40}$), Treatment six (T6)= 80 minutes toasted. *P. reticulatum* Seed ($TPRS_{80}$), Treatment seven (T7)=120 minutes toasted *P. reticulatum S*eed ($TPRS_{120}$) and Treatment eight (T8)= Traditionally roasted *P. reticulatum* Seed ($RPRS_T$).

The raw *P. reticulatum* seed samples were boiled with clean bore hole water using one part of the raw seeds to ten (10) parts of the bore hole water 1:10 (W/V) at the ratio of 5kg: 10litres (Vadivel and Pugalanthi, 2007) in an aluminum pot of 15litres capacity on a stable flame gas cooker for the respective treatment periods that has to do with boiling. The water boiled at temperature of 100^{0}C before the seeds were poured into the pot. Time of the boiling was monitored using stop watch. Traditional boiling was carried out using bigger aluminum pot on fire wood as source of heat with excess water to seed ratio. The boiling was continued until the seeds were very soft to allow for hand de-hulling. Dense ox- brown exudates were observed during boiling and it became more pronounced as the duration of boiling increased. Toasting was done using an electric oven (DHG – 9101 model) set at 75^{0}C for each processing that had to do with toasting. Traditional roasting was done by constantly stirring the raw seed to prevent charring in a dry 5 minutes pre-heated open metal pan (traditionally made) using fire wood heat until browned The boiled seed samples were spread and allowed to cool on clean trays, while the roasted and toasted seeds were exposed to air on trays in the laboratory to hasten cooling. The processed seed samples were milled separately into a fine powdered form and were then stored in air tight containers separately in a cool place for chemical analysis.

The experimental design used was complete randomize design (CRD) and the treatments were in triplicates.

Determination of the Seed Anti-Nutritional Factors

Antinutritional factors of the seed were determined using diverse standard methods viz: saponin was estimated using Hudson and El-Difrawi (1979) method, tannin (AOAC, 1980), cyanide (AOAC, 1984), oxalate (Abeza *et al*., 1968), phytate (Reddy *et al*., 1982) and phenols by Obadoni and Ochuko (2001).

Statistical Analysis

The data generated during the seed antinutritional factors analysis was analysed using One-way Analysis of Variance (ANOVA) and the differences among the means were tested for significance using Duncan Multiple Range Test (Duncan, 1955) at 95% level of probability. The statistical packages used were SAS package version 9 (SAS, 2002) of SAS Institute Inc. Cary. NC. USA

RESULTS

The antinutritional factors levels in raw and differently heat processed *P. reticulatum* seed meal (DHPPRSM) (mg/100g) are presented in Table 1.

There was significant difference in levels of antinutional factors between the raw and the differently heat processed *P. reticulatum* seed meal (DHPPRSM) except saponin and oxalate in T5 (40 minutes toasted seed) ($P<0.05$) (Table 1).

The level of saponin showed no significant difference ($P> 0.05$) between T2, T5, T6 and T7 and also between T 3 and T 8. Treatment 1 had the highest saponin level and treatment 4 recorded zero level of saponin (Table 1). Among the treated seed, treatment 5 and 6, 2 and 7 and treatment 3 and 8 showed no significant difference in tannin level ($P>0.05$). Treatment 3 had the least mean tannin values after T4. There was no significant difference between treatment 3, 4, 7 and 8 in cyanide level at ($P>0.05$) (Table 1). Oxalate showed no significant difference between treatments 3, 4 and 8 all with zero level ($P>.0.05$). Treatment 1 had the highest oxalate level (Table 1).Phytate levels showed no significant difference between treatments 2, 5 and 6 and also between treatments 3, 4, 7 and 8 ($P>0.05$), however, treatment 4 recorded zero levels while treatment 1 has the highest values of phytate (Table 1). The level of phenols showed no significant difference ($P>0.05$) between treatments 3, 7 and 8 and also so did for treatment 2, 5 and 6 (Table 1).The percentage reduction of the anti-nutritional factors in raw and differently heat processed *P. reticulatum* seed meal are presented in Table 2.

The best processing method in terms of anti nutritional factors percent reduction among the processing methods used was T4 then followed by T3 and T8. T5 was the least in the percentage reduction for all the anti-nutritional factors analyzed with exception of phytate (Table 2). Total elimination of the anti-nutritional factors analyzed was observed in T4 with exception of tannin and cyanide (Table 2).

Table 1: Antinutritional Factors Levels in Raw and Differently Heat Processed *Piliostigma reticulatum* Seed Meal (DHPPRSM) (mg/100g)

Anti-nutritional factors (mg/100 g)	TREATMENT								MSE
	T_1 Raw Seed	T2 30mins. Boiled	T3 60mins. Boiled	T4 TRAD* Boiled	T5 40mins. Toasted	T6 80mins. Toasted	T7 120mins. Toasted	T8 TRAD* Roasted	
SAPONIN	0.07^{a}	0.04^{bc}	0.03^{c}	NIL ^{d}	0.05^{ab}	0.04^{bc}	0.03^{bc}	0.03^{c}	0.0135
TANNIN	0.26^{a}	0.17^{c}	0.11^{d}	0.05^{e}	0.23^{b}	0.22^{b}	0.14^{c}	0.13^{d}	0.0130
CYANIDE	0.17^{a}	0.11^{bc}	0.04^{d}	0.02^{d}	0.13^{b}	0.11^{c}	0.03^{d}	0.04^{d}	0.0145
OXALATE	0.04^{a}	0.02^{bc}	NIL^{d}	NIL^{d}	0.03^{ab}	0.02^{bc}	0.02^{c}	NIL^{d}	0.0073
PHYTATE	0.14^{a}	0.05^{bc}	0.03^{cd}	NIL^{d}	0.06^{bc}	0.09^{b}	0.04^{cd}	0.02^{cd}	0.0236
PHENOLS	0.06^{a}	0.03^{bc}	0.02^{c}	NIL^{d}	0.04^{b}	0.04^{b}	0.02^{c}	0.02^{c}	0.0073

Means with the same letter within a raw are not significantly different (P>0.05) with DMRT at 5% level of probability.
*TRAD=Traditionally

Table 2: Percentage of Anti-nutritional Factors Reduction in Raw and Differently Heat Processed *P. retuculatum* Seed Meal

Anti-nutritional factors (%)	TREATMENT							
	T1 Raw Seed	T2 30mins. Boiled	T3 60mins. Boiled	T4 *TRAD Boiled	T5 40mins. Toasted	T6 80mins. Toasted	T7 120mins. Toasted	T8 TRAD Roasted
SAPONIN	0.00	42.86	57.14	100.00	28.57	42.86	57.14	57.14
TANNIN	0.00	34.62	57.67	80.77	11.54	15.38	48.15	50.00
CYANIDE	0.00	35.29	76.47	88.24	23.53	35.29	82.35	76.47
OXALATE	0.00	50.00	100.00	100.00	25.00	50.00	50.00	100.00
PHYTATE	0.00	64.29	78.57	100.00	57.14	35.71	71.43	85.71
PHENOLS	0.00	50.00	66.67	100.00	33.33	33.33	66.67	66.67

Means with the same letter within a raw are not significantly different (P>0.05) with DMRT at 5% level of probability.

*TRAD = Traditionally

DISCUSSION

The antinutritional factors (ANFs) analysis indicated the presence of saponins, tannins, cyanides, oxalates, phytates and phenols. Saponins, phenolics, cyanides were reported earlier by Jimoh and Oladiji (2005) in a related plant species (*Piliostigma thonningii* seed). Awe and Omojasola (2009) reported the presence of tannins, alkaloids, phenolics, triterpenses, phiobatinins in the barck extract of *P. reticulatum* Plant.

The antinutritional factors results showed that the seed meal have more tannins followed by cyanides, phytates, with moderate level of saponins. Oxalates recorded the least followed by phenols. There was significant difference ($P<0.05$) in the level of antinutritional factors (ANFs) between the raw and the differently heat processed *P. reticulatum* seed meal (DHPPRSM) except saponins and oxalates in 40 minutes toasted (T5). Balogun (2011) reported similar trend on *Bauhinia* seeds.

Generally the ANFs levels reduce with increase in heat treatment period in this work, more especially in wet/moist heat treatments. The same findings were reported by Nwonsu (2011) on Oze seeds. T4 the recorded the best in term of percentage ANFs reduction in this study. This is in accordance with Khattab and Arntfield (2009) reports.

The level of saponin (0.07mg/100g) of raw *P. reticulatum* seed meal was found to be lower when compared to earlier reports on *A. Lebbeck* seed meal (18%) (8000mg/100g) (Auta and Anwa, 2007); Mango seed meal (14.21mg/g) (1421mg/100g) (Alatise, 2011) and *B. monandra* seed meal (2.74mg/100g) (Balogun, 2011). The differences observed might be due to differences in species and processing techniques.

The percentage reduction range of 28.57 -100% of saponin recorded in this study was found higher when compared to 11.11% - 77.78% recorded by Auta and Anwa (2007) using different types of processing methods (boiling, toasting and roasting) on *A. labbeck* seed meal.

The level of tannins recorded in this study (0.26mg/100g) in the raw seed was higher than the level of (0.002mg/g) (0.20mg/100g) recorded by Auta and Anwa (2007) in the raw seed meal of *Albizzia labbeck*.

The percentage reduction range of 21.33-34.43% reported by Effiong and Umoren (2011) using multi-processing methods on *Mucuna urens* was low compared to this study with of about (11.54-80.77%).

The least value of percentage reduction (11.54%) in tannins was recorded in the toasted seed in this study (Table 2). Similar result was reported by Auta and Anwa (2007) on the toasted seed of *Albezzia lebbeck*.

The value of hydrogen cyanide recorded in this study for the raw seed of (0.17mg/100g) was found to be lower when compared to the hydrogen cyanide content of certain common legume raw seeds such as vegetable cow pea *(Sesquipedalis)* (3.20mg/kg) (0.32mg/100g) (Udensi *et al*., 2007); *Cajanus cajan* (396.60mg/kg) (39.66g/100g) (Iorgyer *et al.,* 2009) and *Mucuna urens (*56.30 mg/100g) (Effiong and Umoren, 2011).

The hydrogen cyanide peorcentage reduction range of 23.53-88.24 in this study was found to be higher when compared to the percentage reduction of some common legume seeds using different heat processing methods such legume seeds include vegetable cowpea (12.50% - 87.58%) (Udensi *et al.,* 2007*) Cajanus cajan* (53.43% - 70.05%) (Iorgyer *et al.,* 2009*)* and *Mucuna urens (*12.54% - 73.00%) *(*Effiong and Umoren, 2011).

The highest and lowest percentage reduction recorded at T4 (Traditionally boiled seed) and T5 (40 minutes toasted seed) respectively in this work is on accordance with Balogun (2011) reports that the levels of antinutrients in the boiled samples were more reduced to a considerable level than in the toasted samples.

More higher values of oxalate of raw legume seed anti-nutritional factors (oxalate) were reported compared to the value (0.04mg/100g) recorded in this study by many other workers 280mg/100g (Auta and Anwa 2007) on *Albizzian lebbeck* seed meal; 0.83% (830mg/100g) (Iorgyer *et al*., 2009*)* on *Pigean pea* seed meals; 12.08mg/100g (Balogun, 2011) on *B. monandra* seed meal and 33.00mg/100g (Effiong and Umorens, 2011) on *Mucuna urens* seed meal. These differences might be due to species variation, seed condition (wet or dry), geographical location, climate, processing methods among others.

The values of percentage reduction of oxalate (25.00% -100%) recorded in this study were higher when compared to those of other various researchers who reported reduction range of oxalate with similar processing method on legume seeds (Auta and Anwa, 2007; Iorgyer *et al*., 2009 and Balogun, 2011).

The value of phytate (0.14mg/100g) of the raw seed recorded in this study was low compared to the findings of various researchers who reported on the phytate contents of some legume raw seeds. *A. lebbeck* seed meal (0.26mg/g) (26mg/100g) (Auta and Anwa 2007*), Lathyrus sativus* L. (352.04mg/100g) (Gashaw*,* 2010) and soybean (345.00mg/100g) (Ari *et al.,* 2012).

The percentage reduction range values of (35.71-100%) obtained in this study for phytate were higher than the values obtained by Auta and Anwa (2007) for *A. Lebbeck* seed (25.00-62.50%); Udensi *et al.* (2007) for vegetable cow pea (7.06-68.34%); Gashaw (2010) for *Lathyrus sativus* L. (29.76-74.32%*)* and Ari *et al.* (2012*)* for soybean (48.12-71.71%).

The highest reduction of phytic acid (phytate) in the boiled seed T4 (Traditionally boiled seed) is in accordance of Udensi *et al.* (2007) reports on vegetable cow pea (*Sesquipedalis)* seed, using thermal processing methods (boiling, roasting and autoclaving). The lowest percentage reduction recorded at T5 (40 minutes toasted seed) agreed with Ari *et al.* (2012) findings on soybean seed who reported that toasted soybean gave the least reduction in phytic acid (48.12%). The values of phenols (0.06mg/100g) recorded in the raw seed of this study was more closer

to the values of 0.41mg/100g reported by Fila *et al.* (2012*)* for dry rambutan seed.The percentage reduction of 33.33 – 100.00% of phenol observed in this study was higher than 53.00 – 78.00% recorded by Doss *et al.* (2011) using soaking, cooking and autoclaving on Jack bean seed. In the present study, T4 (Traditionally boiled seed) recorded zero phenol level, therefore chosen as the best method for the phenols reduction in this study.

CONCLUSION

This study has showed the importance of head processing on the studied seed, since it reduced the ANFs level of the seed. Among the processing methods adopted in this study, traditionally boiling method appeared to be the best with zero level in some of the ANFs studied. Thus the study showed the need of processing feed ingredients in any animal feed formulation in order to reduce the effects of ANFs in the feed.

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