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BIOCHEMICAL EVALUATION OF RAW AND PROCESSED KIDNEY BEAN (*Phaseolus vulgaris* L.) SEEDS FOR MONOGASTRIC ANIMAL FEED PRODUCTION

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ABSTRACT

The red variety of kidney beans was subjected to various methods of processing to determine the processing method that was most effective in destroying the anti-nutritional factors while preserving the nutrient content of the seeds. The processing methods were sun-drying (raw seeds), boiling, toasting, boiling/fermenting and sprouting. The five processing methods were replicated three times in a completely randomized design. The anti-nutritional factors investigated were trypsin inhibitor, hydrocyanic acid (HCN), tannins, phytic acid, oxalate and haemagglutinins. The effects of processing method on the proximate composition and profile of amino acids were also investigated. The results showed that fermented seeds had significantly ($P<0.01$) higher crude protein content than boiled seeds, which had a higher ($P<0.01$) value than toasted and sprouted seeds. Boiled and fermented kidney beans had similar contents of ether extract which were significantly ($P<0.05$) higher than those of other processing methods. Methionine and tryptophan were present in minute amounts in the raw and processed kidney beans. With the exception of isoleucine and phenylalanine, all the essential amino acids of the raw and processed kidney beans did not differ ($P>0.05$) significantly. Fermented kidney bean seeds had the highest percent destruction of trypsin inhibitor of 97.42, followed by boiling, sprouting and toasting whose values were 94.85, 89.69 and 88.92, respectively. The per cent destruction of HCN was 71.30, 67.48 81.45 and 58.40 for boiled, toasted, fermented and sprouted kidney beans, respectively, and that of tannin was 80.84, 39.62, 82.58 and 84.91 respectively. Each processing method significantly ($P<0.01$) reduced all the anti-nutritional factors relative to the raw seeds. Boiling, toasting or fermentation is recommended for processing kidney beans for use in the diets of monogastric animals.

Key words: kidney beans, processing, anti-nutritional factors

INTRODUCTION

Feed, which accounts for 60 – 80% of the total cost of production of most livestock species, is by far the major factor limiting the growth and expansion of the livestock industry (Ogundipe, 1992; Ikani *et al*., 2001). The high cost of feed has resulted in a continuous increase in the cost of livestock production, causing a phenomenal rise in the unit price of livestock products. Currently, the conventional protein ingredients for monogastric animal feed production such as soyabeans and groundnut cake are often scarce and expensive due to the high demand for them for human consumption. Consequently, animal nutritionists in developing countries, such as Nigeria, have resorted to exploring other potential, and hitherto, neglected feed resources in order for the monogastric animal feed industry to have a wider range of alternatives to choose from. The availability of alternative sources of nutrients will encourage a shift to the sources for which there is less competition from humans as food. Efforts have been made to use other vegetable protein sources such as pigeon pea (Amaefule and Obioha, 2001, Iorgyer *et al*, 2009; Iorgyer, 2010); *Mucuna pruriens* seeds (Emenalom and Udedibie, 1998; Tuleun *et al*., 2011) and Jackbean (Esonu *et al*., 1998) in monogastric diets with encouraging results. There are, however, many other legumes whose seeds can be explored for their nutritional value for monogastric animals (Bawa, 2003). One of such legumes is the kidney beans (*Phaseolus vulgaris* L.).

Kidney bean is a leguminous crop grown in different parts of the world. Several varieties of this crop are currently cultivated in fairly large quantities in Mangu and Bokkos areas on the Jos Plateau, Nigeria. The varieties have crude protein contents ranging from 17 to 28% (Olomu, 2011), and are worth investigating for possible use as a protein ingredient in monogastric animal feed production. However, like other grain legumes, the usefulness of kidney beans as a feed ingredient for monogastric animals may be limited due to the presence of some anti-nutritional factors (Kingsley, 1995). Kidney bean seeds have been reported to have various levels of such anti-nutritional factors as trypsin inhibitor, hydrocyanic acid, tannin, phytic acid, oxalate and haemagglutinin (Olomu, 2011). The presence of these anti-nutritional substances has been associated with growth depression and pancreatic hypertrophy in many species (Birk, 1988; Olomu, 2011). Livers in chickens fed raw kidney bean diets showed marked coagulative necrosis and degeneration of hepatocytes; in addition, severe congestion of glomeruli and distention of the capillary vessels with thrombi in the kidneys have been reported (Emiola *et al*., 2007).

It has been established that heat treatment and other processing methods exert beneficial effects on the nutritional quality of the seeds of grain legumes by destroying the anti-nutritional factors inherent in them (Balogun *et al*., 2001). Some of the anti-nutritional factors are, however, thermostable, requiring different processing methods applied individually or in combination. For instance, the effectiveness of heat treatment in detoxifying tannin, phytate and oxalate in kidney beans has been found to be low (Emiola *et al*., 2007). Other processing methods need to be explored in addition to heat treatment in order to reduce the anti-nutritional factors to tolerable levels in the diets of monogastric animals (Damang, 2015).

The objective of this study was to determine the effect of the various techniques of processing kidney beans on its proximate composition, amino acid profile and concentration of anti-nutritional factors, for incorporation in the diets of monogastric animals.

MATERIALS AND METHODS

Methods of processing kidney bean seeds

The red variety of kidney beans, the most widely cultivated on the Jos Plateau in Nigeria, was selected for this study. A sample of this variety obtained from four locations was subjected to various processing methods with the aim of destroying the anti-nutritional factors. Each processing method was taken as a treatment and each of the four collection points as a replicate in a completely randomized design. The methods of processing employed were as described below:

Sun-drying (raw seeds)

The raw seeds procured from the market were sun-dried for 72 hours to minimize variations in their moisture content and ensure that they were sufficiently dried for feed production (approximately 90% dry matter). All the seeds procured were subjected to this treatment prior to the application of the other processing methods.

Boiling

The boiling of the kidney bean seeds involved heating the water to boiling point (100^{o}C) before turning the seeds into the boiling water as described by Bawa, (2003). Care was taken to ensure that the water was sufficient from the onset to cover the seeds throughout the cooking time. The seeds were then cooked for 60 minutes. The cooking time was taken from the moment the seeds were turned into the boiling water. Thereafter, the cooked seeds were drained of the water and sun-dried to less than 10% moisture.

Toasting

The toasting of the kidney bean seeds involved sprinkling some water onto them to facilitate deeper penetration of heat into the seeds, after which they were turned into an empty pan set on fire. The seeds were stirred continuously to ensure uniform heating, and to avoid charring. The heating continued until the seeds became crispy. They were then evacuated from the pan and sun-dried to less than 10% moisture.

Fermenting

The kidney bean seeds were first of all boiled for 60 minutes as described under 'Boiling' above; thereafter, they were drained of the water, turned into a plastic jar and covered for 72 hours to ferment. The fermented seeds were washed using clean water to remove any fungal growth and/or anti-nutritional factors that may be adhering to the surface of the seeds, and then sun-dried to less than 10% moisture.

Sprouting of the seeds (for 96 hr)

The kidney bean seeds were turned into a plastic jar containing enough water to cover the beans. They were left to soak for 12 hours, after which they were drained of the water, partially spread on the floor and covered with empty bags to induce sprouting. They were washed with clean water every 12 hours to prevent fungal growth, and then covered again on the floor. This was repeated daily for 4 days (96 hr) when over 90% sprouting was observed. The seeds were then sun-dried to less than 10% moisture.

A sample of the kidney beans from each of the treatment replicates was ground and analyzed for its proximate composition, amino acid profile, and anti-nutritional factors, to determine the effect of processing on each of these chemical components.

Proximate analysis of kidney bean seeds

The dry matter was determined based on the weight loss after 24 hours in an oven set at 100^{o}C. The nitrogen content was determined by Kjeldahl method of AOAC (1995) and crude protein calculated as N x 6.25. The ash was determined as the residue remaining after incinerating the sample at 600^{o}C for 3 hours in a muffle furnace. The AOAC (1995) method was also employed for ether extract and crude fibre determinations.

Determination of amino acid profile of kidney bean seeds

Each sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and then loaded into the Technicon Sequential Multisample Amino Acid Analyzer (TSM). The amino acid contents were determined using the method described by Sparkman *et al*., (1958).

Determination of anti-nutritional factors of kidney beans

The anti-nutritional factors studied were those commonly found in legume seeds. They included trypsin inhibitor, hydrogen cyanide, tannins, phytic acid, oxalate and haemagglutinins. The trypsin inhibitor activity of sample extracts was determined according to the method of Kakade *et al*., (1974) with the modification described by Liu and Markakis (1989). The hydrocyanic acid content of raw and processed kidney beans was determined using the procedure of Cooke and Maduagwu (1978) as modified by Ikediobi and Fashagba (1985). Tannic acid was estimated using the method of Earp *et al*., (1981). Phytic acid was determined using the method of Sutardi and Buckle (1985). Oxalate was determined using the procedure of AOAC (2006), and haemagglutinin was determined using the method of Lees (1971).

The data on all the parameters were subjected to analysis of variance using the SAS (1995) procedure, and treatment means separated using the Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULTS

Proximate composition of raw and processed kidney bean seeds

The proximate composition of differently processed kidney bean seeds is as presented in Table 1. The crude protein content of fermented seeds (24.75%) was significantly ($P<0.01$) higher than that of boiled seeds (22.94%), which was significantly ($P<0.01$) higher than that of toasted seeds (22.70%). The protein content of the toasted seeds did not differ significantly from that of sprouted seeds (22.69%), but both were significantly ($P<0.01$) higher than that of the raw seeds (22.15%). The fermented and boiled kidney beans did not differ significantly in their ether extract contents (8.55% and 8.44%, respectively), but had significantly ($P<0.05$) higher values than 7.87%, 5.65%, and 6.65%, for raw, toasted and sprouted kidney beans, respectively. The toasted seeds had the least ether extract and the highest ash content (4.70%) of all the processing methods. Boiled and fermented kidney beans had the least ash content (3.62% and 3.67%, respectively). Toasted kidney beans had a significantly ($P<0.01$) higher crude fibre content (9.10%) than the raw (7.30%) and other processed seeds (7.43%, 7.48% and 7.07%, for boiled, fermented and sprouted, respectively), whose values were statistically similar. The raw seeds had a significantly ($P<0.05$) higher content (56.67%) of nitrogen free extract (NFE) than toasted kidney beans (56.25%), which in turn, had a significantly ($P<0.05$) higher NFE content than 55.59%, 51.47% and 54.72% for boiled, fermented and sprouted kidney beans, respectively. Fermented kidney beans had the least NFE content.

Amino acid profile of raw and processed kidney bean seeds

The amino acid profile of differently processed kidney bean seeds is as presented in Table 2. Methionine and tryptophan were present in minute amounts that could not be quantified in the seeds in the five treatments. The most abundant essential amino acid observed in kidney beans in this study was leucine, with values of 10.31 – 11.52g/100g protein. With the exception of isoleucine and phenylalanine, processing method had no significant ($P>0.05$) effect on all the essential amino acids. The isoleucine contents of boiled, toasted and fermented kidney beans did not differ significantly ($P>0.05$), but were significantly ($P<0.05$) higher than the value for sprouted seeds. The phenylalanine contents of fermented and sprouted kidney beans did not differ significantly ($P>0.05$), but were significantly ($P<0.01$) higher than the values for the raw and toasted seeds.

Table 1: Effect of Processing Method on the Proximate Composition of Kidney Bean Seeds

		Kidney Bean Processing Methods				
Proximate Composition (%)	**Raw**	**Boiled**	**Toasted**	**Fermented**	**Sprouted**	**SEM**
Dry matter (DM)	98.57[a]	97.15[e]	98.24[b]	97.90[c]	97.64[d]	0.06**
Crude protein (CP)	22.15[d]	22.94[b]	22.70[c]	24.75[a]	22.69[c]	0.09**
Ether extract (EE)	7.87[b]	8.44[a]	5.65[d]	8.55[a]	6.65[c]	0.21*
Crude fibre (CF)	7.30[b]	7.43[b]	9.10[a]	7.48[b]	7.07[b]	0.24**
Total ash	3.91[c]	3.62[d]	4.70[a]	3.67[d]	4.19[b]	0.10**
Nitrogen free extract (NFE)	56.67[a]	55.59[c]	56.25[b]	51.47[e]	54.72[d]	0.40*

[abcd] Means within the same row bearing different superscripts are significantly different
SEM = Standard error of mean
* = P<0.05
** = P<0.01

Table 2: Effect of Processing Method on the Essential Amino Acid Profile of Kidney Beans

	Kidney Bean Processing Methods					
†Amino Acid (g/100g Protein)	**Raw**	**Boiled**	**Toasted**	**Fermented**	**Sprouted**	**SEM**
Arginine	8.11	8.28	7.52	8.37	8.17	0.38[NS]
Histidine	3.43	3.33	3.47	3.48	3.29	0.19[NS]
Isoleucine	0.20[bc]	0.23[ab]	0.27[a]	0.23[ab]	0.17[c]	0.02*
Leucine	10.31	10.94	10.62	11.52	10.22	0.34[NS]
Lysine	5.29	5.32	5.70	5.77	5.08	0.26[NS]
Phenylalanine	3.31[c]	3.49[bc]	3.41[c]	4.64[ab]	5.37[a]	0.38**
Threonine	4.17	4.36	4.67	4.81	4.30	0.32[NS]
Valine	4.52	4.99	4.10	5.06	4.47	0.29[NS]

†Methionine and tryptophan were present in minute amounts which could not be quantified in all the processing methods
[abc] Means within the same row bearing different superscripts are significantly different
SEM = Standard error of mean
* = P <0.05
* = P<0.01

Anti-nutritional factors of raw and processed kidney bean seeds

The anti-nutritional factors of the raw and processed kidney beans were as presented in Table 3. Each processing method employed significantly reduced the quantity of each of the anti-nutritional factors relative to the raw seeds. The percent destruction of trypsin inhibitor in the boiled, toasted, fermented and sprouted kidney beans was 94.85, 88.92, 97.42 and 89.69, respectively. Fermentation resulted in the greatest destruction of trypsin inhibitor, followed closely by boiling, and then sprouting. Toasting recorded the least destruction of this anti-nutritional factor. The residual hydrocyanic acid (HCN) contents of boiled, toasted, fermented and sprouted kidney beans did not differ significantly. Their respective percent destructions arising from processing were 71.30, 67.48, 81.45 and 58.40. Fermented seeds recorded the highest percent destruction of HCN, with 81.45%. This was followed by boiled seeds with 71.30%, and toasting with 67.48%. The residual phytic acid contents of the boiled, toasted, fermented and sprouted kidney beans did not differ significantly (P>0.05) from one another. Its percent destruction in the boiled, toasted, fermented and sprouted kidney beans was 91.71, 90.32, 91.66 and 91.64, respectively. The greatest destruction of this anti-nutritional factor occurred in the fermented seeds, in which 91.66% was destroyed.

Table 3: Effects of Processing Method on the Anti-nutritional Factors of Kidney Beans

	Kidney Bean Processing Method					
Parameter	**Raw**	**Boiled**	**Toasted**	**Fermented**	**Sprouted**	**SEM**
Trypsin inhibitor (mg/100g)	3.88^{a}	0.20^{b}	0.43^{b}	0.10^{b}	0.40^{b}	0.06**
Percent destruction	-	94.85	88.92	97.42	89.69	
Hydrocyanic acid (mg/100g)	15.96^{a}	4.58^{b}	5.19^{b}	2.96^{b}	6.64^{b}	0.63**
Percent destruction	-	71.30	67.48	81.45	58.40	
Phytic acid (mg/100g)	23.15^{a}	2.15^{b}	2.24^{b}	1.93^{b}	2.05^{b}	0.10*
Percent destruction	-	90.71	90.32	91.66	91.14	
Tannin (mg/100g)	179.00^{a}	34.30^{c}	108.08^{b}	31.18^{c}	27.02^{c}	3.48**
Percent destruction	-	80.84	39.62	82.58	84.91	
Oxalate (mg/100g)	17.80^{a}	8.70^{b}	7.70^{b}	8.50^{b}	10.40^{b}	0.08*
Percent destruction	-	51.12	56.74	52.25	41.57	
Haemagglutinin (mg/100g)	3.85^{a}	1.29^{c}	2.36^{b}	0.340^{d}	0.18^{d}	0.04**
Percent destruction	-	66.49	38.70	91.17	95.32	

[abcd] Means within the same row bearing different superscripts are significantly different
SEM = Standard error of mean
* = P<0.05
** = P<0.01

DISCUSSION

Effect of processing on the proximate composition of kidney bean seeds

The crude protein content of kidney beans ranged from 22.15 – 24.75%; it is similar to 23.9% and 24.0% reported by Olomu (2011) and Anon. (2012), respectively. It is also similar to 24.67% and 23.77% for cowpea and pigeon pea, respectively (Aduku, 2005). The fact that the fermented kidney bean seeds had the highest crude protein content in this study agrees with the findings of Eka (1979) that fermentation tends to increase the protein content of feedstuffs. This is probably due to the fact that microorganisms involved in fermentation use the carbohydrates in the substrate, with atmospheric nitrogen, to synthesize their own body proteins to enable them to grow and multiply, thereby elevating the overall protein content of the substrate (feedstuff) and lowering its carbohydrate level. Next to the crude protein of fermented seeds was that of boiled kidney beans. This implies that with respect to protein content, fermentation and boiling presented the best results. The relatively high protein content of kidney beans is significant since the crop is intended for use mainly as a protein feedstuff. Boiled and fermented kidney beans had the least ash content. Some of the minerals may have leached out into the boiling water since both processes involved boiling in water (100^{o}C) for 60 minutes after which the seeds for fermentation were covered in a plastic jar for 72 hours to undergo fermentation. The loss of minerals through leaching was also reported by Bawa (2003), who observed that some mineral content of Dolichos lablab (*Lablab purpureus*) seeds were leached out when the seeds were boiled at 100^{o}C for 60 minutes. Ogundipe (1980) had earlier reported that toasting had an advantage over boiling in reducing nutrient loss through leaching. The raw seeds had a significantly ($P<0.05$) higher content of nitrogen free extract (NFE) than toasted kidney beans, which in turn, had a significantly ($P<0.05$) higher NFE content than all other treatments. Fermented kidney beans had the least NFE content. This shows that a significant amount of the soluble carbohydrates was lost during fermentation. This agrees with findings of Damang (2013) in which the NFE content of fermented, decorticated African locust bean seeds was observed to be significantly lower than those of other processing methods. This was attributed to the possible utilization of some of the soluble carbohydrates by the anaerobic microorganisms as sources of energy for their metabolism, or their possible conversion to alcohol.

Effect of processing kidney bean seeds on its amino acid profile

Methionine and tryptophan were observed in minute amounts which could not be quantified in the seeds in the five treatments. This is corroborated by Olomu (2011) who reported that kidney beans are deficient in sulphur amino acids and tryptophan. This makes the supplementation of these two amino acids inevitable in the diets of monogastric animals. The lysine contents obtained ranged from 5.08 – 5.77g/100g protein which were, however, lower than 2.7 and 2.4% given for soyabean meal and full-fat soyabean, respectively (Aduku, 2005).

Effect of processing on the anti-nutritional factors of kidney bean seeds

All the kidney bean processing methods studied significantly reduced all the anti-nutritional factors evaluated in relation to the raw seeds. However, the degree of reduction varied both with the anti-nutritional factors and the processing methods. Out of these anti-nutritional factors, trypsin inhibitor and phytic acid appeared to be more uniformly destroyed by the different processing methods, and on the whole, a greater amount of these anti-nutritional factors were destroyed, with a percent destruction ranging from 88.92 – 97.42% and 90.32 – 91.66, respectively. The processing method that resulted in the greatest destruction of these anti-nutritional factors was fermentation. In the case of trypsin inhibitor, boiling was the next most effective processing method, but in the case of phytic acid sprouting was the next most effective processing method. Toasting recorded the least percent destruction of both trypsin inhibitor and phytic acid, with 88.82 and 90.32%, respectively. The relatively high percent destruction of trypsin inhibitor in boiled kidney beans agrees with the findings of Bawa (2003) who reported a high per cent (82.82%) destruction of trypsin inhibitor in a study during which Dolichos lablab seeds were boiled in water (100^{o}C) for 60 minutes. In a similar study, Damang (2011) also reported 88.11% destruction of trypsin inhibitor when African locust bean seeds were boiled in water (100^{o}C) for 60 minutes. Phytic acid levels were more effectively destroyed by germination than by heating. This agrees with the report of Iyayi and Egharevba (1998). These authors attributed the greater losses with germination to the fact that phytase activity during germination results in the hydrolysis of phytate phosphorus to inositol monophosphate which contributes to the loss of phytic acid. The least percent destruction of phytic acid was recorded on toasted kidney beans with 90.32%. The earlier report by Emiola *et al* (2007) that heat treatment was ineffective in detoxifying phytic acid is contrary to the high per cent destruction of phytic acid recorded in the boiled and toasted kidney bean seeds in the present study. However, the report of Emiola *et al* (2007) on the low destruction of phytic acid by heat agrees with the observation by Damang (2011) that only 44.28% of phytic acid was destroyed when African locust bean seeds were boiled in water (100^{o}C) for 60 minutes. It appears from these observations that the destruction of phytic acid in heat treated legume seeds varies with the leguminous species. The degree of destruction of hydrocyanic acid varied more with the processing methods than that of trypsin inhibitor and phytic acid. The processing method which gave the highest per cent destruction of hydrocyanic acid was fermentation, with 81.45%. This was followed by boiling with a value of 71.30%, and toasting with 67.48% destruction. Sprouting recorded the least destruction of hydrocyanic acid, with 58.40%. These values are similar to 70.25% reported for Dolichos lablab by Bawa (2003) when the seeds were boiled in water for 60 minutes, and higher than 26.74% for African locust bean seeds by Damang (2011). A substantial variation was observed in the destruction of tannin, with the highest percent destruction of 84.91% recorded in sprouted seeds and the lowest value, 39.62% recorded by toasted seeds. Boiling and fermentation recorded 80.84 and 82.58% destruction, respectively. The low per cent destruction of tannin in toasted kidney beans agrees with the report of Emiola *et al* (2007) that tannin was thermostable. However, boiling in water, another form of heat treatment, was found to destroy 80.84% of this anti-nutritional factor in the current study. Udensi *et al*. (2005) had also reported a significant reduction in the tannic acid content of mucuna beans when the latter was boiled in water for 60 minutes. However, Tuleun and Pantrick (2011) reported only 29.70% destruction of tannin in mucuna seeds when the beans were boiled in water for 60 minutes. In the case of oxalate, just about half of its content was destroyed by each of the processing methods employed. The greatest destruction of 56.74% was recorded by toasted kidney beans, followed by 52.25% recorded by fermented seeds and 51.12% by boiling. The lowest value of 41.57% destruction was recorded by sprouting. This result agrees with the report of Emiola *et al* (2007) that heat treatment was ineffective in detoxifying oxalates in kidney bean seeds. Haemagglutinin recorded a similar pattern as tannin in having a wide variation in its destruction among the processing methods. In both anti-nutritional factors, sprouting recorded the highest percent destruction, followed by fermentation, boiling and toasting, in that order. The level of destruction of haemagglutinin in sprouted kidney beans was 95.32%. Fermentation destroyed 91.17%, while boiling destroyed 66.49%. Toasting recorded 38.70%, the least amount destroyed.

CONCLUSION

The relatively high crude protein, ether extract and low crude fibre contents of fermented and boiled kidney bean seeds tend to suggest that these processing methods can be adopted for use by farmers for non-ruminant animal feed production using kidney bean seeds. These processing methods had no negative effects on the essential amino acid profile of kidney bean seeds. Rather, there were net increases in the isoleucine content of the kidney beans. Boiling and fermentation were found to be most effective in destroying the anti-nutritional factors of the kidney beans. With the application of any of these two processing methods, the anti-nutritional factors present in kidney beans may no longer pose any serious danger to monogastric animals since substantial proportions of them were destroyed, rendering the beans safe for use in their diets. Boiling or fermentation is therefore, recommended for processing kidney beans for inclusion in the diets of non-ruminant animals for their effectiveness in destroying the anti-nutritional factors in kidney beans with no negative effects on the nutritional status of the beans.

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