Effect of duration of cooking on the levels of some antinutritional factors of nine varieties of *Lablab purpureus* beans

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Abstract

The objective of this study was to determine the effect of duration of cooking of various varieties of *Lablab purpureus* beans at 100°C on their antinutritional factor compositions. Nine different varieties of *Lablab purpureus* beans were subjected to cooking for 0.0, 10.0, 20.0, 30.0, 40.0, 50.0 and 60.0 minutes respectively at 100°C. For each batch of cooking, 4 litres of water was first brought to boiling in a 10 litre aluminium cooking pot and about 50g of the seed was poured into the boiling water, covered and allowed to cook for each specified time period. The seeds were sun dried for 3 days before milling. A representative sample for each cooking time and for each variety was taken to the laboratory for the determination of antinutritional factors to include trypsin inhibitors, phytic acid, tannin and hydrocyanic acid. Results obtained indicate that duration of cooking had a significant ($P < 0.05$) effect on the antinutritional composition of the various varieties of *lablab* beans. The levels of antinutritional factors in the beans were found to decrease significantly ($P < 0.05$) as the duration of cooking increased across the various varieties. However percent destructions of trypsin inhibitor activity, phytic acid and hydrocyanic acid were very rapid at about 30 minutes of cooking.

Keywords: Antinutritional factors, cooking duration, varieties, *lablab* beans, poultry diets.

Introduction

It is believed that most farmers would prefer to feed raw *Lablab purpureus* beans to chickens in order to eliminate the cost of transportation of the raw beans to the processor and back to the farm as well as the heating and other handling costs. This has not been the case because it has been known that proteins in raw legumes have low nutritive value due to some anti-nutritional factors, which hinder their effective breakdown and utilization of their stored protein by the monogastrics. Cooking improves the nutritive value and brings the value close to that of meat and milk products by destroying most of the anti-nutritional factors present (Ogundipe, 1980;

Amaefule and Obioha, 2001).

These anti-nutritional factors such as trypsin inhibitors, haemagglutinins, lectins, phytic acid, goitrogens, etc. result in poor growth when raw legume seed diets are fed to monogastrics. Processing also improves the utilization of proteins and energy contained in the legumes (Omeje, 1999 and Kaankuka *et al.*, 2000). Like other legume seeds, *lablab* seeds have many antinutritional factors, which interfere with their utilization by monogastrics. These antinutritional factors can be eliminated or reduced to the barest minimum through adequate heating of the beans.

The use of unconventional legume seed has been limited owing to lack of, or inadequate in-

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formation about their chemical constituents or processing method that will bring them to a state where they could be considered safe for incorporation into poultry diets.

The need therefore to provide base line information about the common varieties of lablab in Nigeria in terms of the effect of duration of cooking on their antinutritional factors becomes very important. This study was therefore conducted to determine the effect of duration of cooking at 100°C on the antinutritional factors in various varieties of *Lablab purpureus* beans.

Materials and Methods

Nine varieties of *Lablab purpureus* beans were used for this experiment. They were obtained from the ILRI experimental farm in Zaria. The varieties are Rongai (Milky white), PI164302 (dark); NAPRI 2 (dark brown); NAPRI 3 (light brown); PI 401553 (black); PI 345608 (dark brown); ILRI 147 (dark); TLN 7 (black); and PI 19851 (light brown). The beans were processed by boiling in water. About 4 litres of water was first brought to boiling in a 10-litre aluminium cooking pot and about 50 grammes of the seed was poured into the boiling water and covered. From the moment the beans were poured into the water, the specified time of cooking was taken. The beans were cooked for 10, 20, 30, 40, 50 and 60 minutes respectively. They were sun-dried for 3 days before milling. A representative sample of the milled lablab seed from each of the cooking time along with a raw sample of each variety and groundnut cake were taken to the laboratory for the determination of the antinutritional factors. The inclusion of groundnut cake was for the purpose of comparison to ascertain if groundnut cake which is popularly used as a protein ingredient in poultry feed contain some of the antinutritional factors being analysed for.

Determination of Trypsin inhibitor activity

Ten grammes each of ground groundnut cake, raw and cooked lablab seeds were extracted for three hours with petroleum ether (b.p 60-80°C) at room temperature. The defatted material was sieved through a 40mm mesh screen. One gramme of each sample was suspended in 20ml of 0.05N HCL and kept over night at 4°C in a refrigerator. The insoluble matter was removed by centrifugation at 800 rpm for 20 minutes in a unipan centrifuge model No.310. The supernatant was used as trypsin inhibitor (TI) extract and diluted 1:1000 with water before use. The trypsin inhibitor activity (TIA) on sample extract was assayed according to the method of Kakade *et al* (1974) with modification described by Liu and Markakis (1989).

Determination of phytic acid

Ten grammes each of defatted finely ground groundnut cake, raw and cooked lablab seed samples were soaked in 200ml of 2% HCL solution for 3 hours and then filtered through two layers of hardened filter paper. Fifty (50) ml of the filtrate was placed in a 400ml beaker and 110ml distilled water was added to give it proper acidity and thereafter titrated with a standard ferric chloride solution containing 19.5mg Fe/ml until a brownish yellow colour persisted for 5 minutes. Phytic acid was thereafter determined using the method of Sutardi and Buckle (1985).

Determination of Tannin

Ten grammes each of the groundnut cake meal, the raw and the cooked samples of the lablab seeds were defatted and later extracted with 5% ethyl ether and ethanol. Tannin was estimated using the method of Earp *et al*. (1981). The method employed the Vanillin-HCL procedure based on acid catalysed addition of vanillin to flavonols and their polymers as well as other phenolic compounds such as dihydrocalcone and flavones. These reactions were determined colometrically

at 500nm using a spectrometer (spectronic 20).

Determination of Hydrocyanic acid

The hydrocyanic acid of groundnut cake, raw and cooked lablab seeds were determined using the procedure of Cooke and Maduagwu (1978) as modified by Ikediobi and Fashagba (1985). Two grammes of each sample was extracted with 15ml of 0.1m sodium phosphate buffer pH 6.8 for 30 minutes using a shaker. The mixture was centrifuged at 100rpm and the supernatant obtained was decanted into a 50ml volumetric flask. Into a test tube 0.2 millilitres of the supernatant was pipetted and the volume made up to 2ml with 0.1m phosphate buffer pH 6.8. Four millilitres of alkaline picrate was added and then mixed thoroughly. The mixture was incubated at 95°C in a water bath for 10 minutes. It was allowed to cool and absorbance was read at 490nm. Total cyanide content was obtained by extrapolation from a standard curve of potassium cyanide.

All data generated were subjected to the analysis of variance using the SAS (1985) general model procedure while treatment means were separated using the Duncan's Multiple Range Test (Steel and Torrie 1980)

Results and Discussion

The results of the antinutritional analysis are presented in tables 1, 2, 3, and 4 respectively.

The duration of cooking of the lablab seeds had a significant ($P < 0.05$) effect on the levels of antinutritional factors present in the seeds. The levels of trypsin inhibitor activity, phytic acid, tannin and hydrocyanic acid were found to decrease as the duration of cooking increased. When compared across the various varieties trypsin inhibitors activity was found to be the most heat labile followed by hydrocyanic acid. About 75% of the trypsin inhibitor activity and over 60% of the hydrocyanic acid across the vari-

Table 1: Analysed trypsin inhibitor activity in different varieties of *Lablab purpureus* beans cooked at 100°C for different duration of time and their percent destructions (TIA mg/100g)

Cooking time (minutes)	Rongai (milky-white)	PI-164302 (Dark)	NAPRI-2 (Dark brown)	NAPRI-3 (Light-brown)	PI-401553 (Black)	PI345608 (Dark brown)	ILRI 147 (Dark)	TLN7 (Black)	PI-195851 (Light brown)	GNC
0.00	579.82 ^a	684.21 ^a	736.11 ^a	631.42 ^a	635.82 ^a	682.95 ^a	894.00 ^a	842.35 ^a	789.00 ^a	132.41
10.00	368.12 ^b	420.88 ^b	532.22 ^b	460.24 ^b	480.10 ^b	468.92 ^b	592.01 ^b	576.42 ^b	562.08 ^b	
% Loss	36.51	38.49	27.70	27.11	24.49	31.34	33.78	31.57	28.76	
20.00	147.28 ^b	150.50 ^b	185.20 ^b	138.40 ^b	142.22 ^b	158.50 ^b	216.55 ^b	210.48 ^b	189.06 ^b	
% Loss	74.60	78.00	74.84	78.08	77.63	76.79	75.78	75.01	76.04	
30.00	136.38 ^c	110.07 ^c	128.28 ^c	126.10 ^c	136.44 ^c	139.22 ^c	192.28 ^c	170.86 ^c	160.82 ^c	
% Loss	76.48	83.91	82.57	80.03	78.54	79.61	78.49	79.72	79.62	
40.00	132.56 ^c	105.02 ^c	120.30 ^d	122.60 ^c	128.28 ^d	137.41 ^c	186.61 ^c	168.25 ^c	158.11 ^c	
% Loss	77.14	84.65	83.66	80.58	79.82	79.88	79.13	80.03	79.96	
50.00	119.28 ^d	108.46 ^d	115.44 ^d	120.88 ^d	124.48 ^d	136.80 ^c	160.46 ^d	150.46 ^d	138.68 ^d	
% Loss	79.28	84.69	83.74	79.85	79.68	78.46	81.28	81.20	80.48	
60.00	111.46 ^c	102.62 ^d	111.20 ^d	119.68 ^d	122.41 ^d	135.70 ^c	150.44 ^d	149.22 ^d	137.25 ^d	
% Loss	80.78	85.00	84.89	81.05	80.75	80.13	83.32	82.29	82.60	
SEM	3.42	4.18	3.98	2.46	3.32	3.48	5.22	4.44	5.46	
L	*	*	*	*	*	*	*	*	*	

^{a,b,c,d} Means within each column with different superscripts are significantly ($P < 0.05$) different.

SEM=Standard error of the means.

L=Polynomial analysis showing linear relationship within cultivars of *lablab purpureus* beans.

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Table 2: Analysed phytic acid content of different varieties of *Lablab purpureus* beans cooked at 100°C for different duration of time and their percent destruction (mg/100g)

Cooking time (minutes)	Rongai (milky-white)	PI-164302 (Dark)	NAPRI-2 (Dark brown)	NAPRI-3 (Light brown)	PI-401553 (Black)	PI345608 (Dark brown)	ILRI 147 (Dark)	TLN7 (Black)	PI-195851 (Light brown)	GNC
0.00	6.46 ^a	6.00 ^a	14.31 ^a	14.08 ^a	6.46 ^a	12.69 ^a	12.46 ^a	8.54 ^a	14.31 ^a	3.90
10.00	5.28 ^b	4.72 ^b	9.56 ^b	8.92 ^b	4.98 ^b	10.88 ^b	8.92 ^b	7.02 ^b	10.59 ^b	
%Loss	18.27	21.33	33.19	36.64	22.91	14.26	28.41	17.80	25.99	
20.00	4.20 ^c	3.92 ^c	9.23 ^b	8.31 ^b	4.62 ^b	10.38 ^b	8.77 ^b	6.92 ^b	10.38 ^b	
% Loss	34.98	34.67	35.50	40.98	28.48	18.20	29.61	18.97	27.46	
30.00	1.88 ^d	1.79 ^d	4.18 ^c	3.08 ^c	2.06 ^c	3.94 ^c	3.90 ^c	2.56 ^c	3.07 ^c	
%Loss	70.82	68.57	70.02	76.46	68.47	69.01	75.31	69.88	76.08	
40.00	1.80 ^d	1.77 ^d	4.15 ^c	3.00 ^c	2.01 ^c	3.92 ^c	3.00 ^c	2.54 ^c	3.00 ^c	
% Loss	72.14	70.50	70.84	78.69	68.89	69.11	75.92	70.26	79.04	
50.00	1.58 ^d	1.76 ^d	3.26 ^d	2.79 ^c	1.56 ^d	3.28 ^d	2.60 ^c	2.38 ^c	2.49 ^c	
%Loss	75.22	70.12	75.82	79.84	74.28	74.02	76.29	72.21	81.48	
60.00	1.50 ^d	1.74 ^d	3.23 ^d	2.77 ^c	1.54 ^d	3.23 ^d	2.54 ^c	2.31 ^c	2.31 ^d	
% Loss	76.78	71.00	77.30	80.33	76.16	74.55	79.61	72.95	83.86	
SEM	0.26	0.24	0.36	0.42	0.21	0.16	0.56	0.44	0.28	
L	*	*	*	*	*	*	*	*	*	

^{a,b,c,d} Means within each column with different superscripts are significantly (P<0.05) different

SEM= Standard error of the means.

L = Polynomial analysis showing linear relationship within cultivars of *lablab purpureus* beans.

Table 3: Analysed tannin content of different varieties of *Lablab purpureus* beans cooked at 100°C for different duration of time and their percent destruction (TA mg/100g)

Cooking time (minutes)	Rongai (milky-white)	PI-164302 (Dark)	NAPRI-2 (Dark brown)	NAPRI-3 (Light brown)	PI-401553 (Black)	PI345608 (Dark brown)	ILRI 147 (Dark)	TLN7 (Black)	PI-195851 (Light brown)	GNC
0.00	0.24 ^a	0.27 ^a	0.44 ^a	0.57 ^a	0.35 ^a	0.48 ^a	0.42 ^a	0.23 ^a	0.41 ^a	0.08
10.00	0.23 ^b	0.26 ^a	0.43 ^b	0.56 ^a	0.34 ^b	0.47 ^b	0.40 ^b	0.22 ^b	0.40 ^b	
%Loss	4.17	3.70	2.27	1.75	2.86	2.08	4.76	4.35	2.44	
20.00	0.21 ^c	0.23 ^b	0.40 ^c	0.49 ^b	0.31 ^c	0.42 ^c	0.37 ^a	0.20 ^c	0.36 ^c	
% Loss	12.50	14.81	9.09	14.04	11.43	12.50	11.90	13.04	12.19	
30.00	0.14 ^d	0.14 ^c	0.18 ^d	0.27 ^c	0.18 ^d	0.24 ^d	0.22 ^d	0.14 ^d	0.20 ^d	
%Loss	53.69	54.42	62.48	54.11	54.26	53.92	51.49	51.98	52.75	
40.00	0.11 ^c	0.12 ^d	0.16 ^c	0.26 ^c	0.16 ^c	0.22 ^c	0.20 ^c	0.11 ^c	0.19 ^c	
% Loss	54.17	55.56	63.64	54.39	54.29	54.17	52.38	52.17	53.66	
50.00	0.10 ^f	0.11 ^d	0.16 ^c	0.26 ^d	0.14 ^f	0.19 ^f	0.15 ^f	0.12 ^f	0.17 ^f	
%Loss	60.11	64.89	64.92	55.44	64.78	62.93	62.38	55.32	61.48	
60.00	0.09 ^g	0.09 ^c	0.15 ^f	0.25 ^d	0.12 ^g	0.17 ^g	0.15 ^f	0.10 ^g	0.15 ^g	
% Loss	62.50	66.67	65.91	56.14	65.71	64.58	64.29	56.52	63.41	
SEM	0.002	0.005	0.002	0.003	0.001	0.002	0.002	0.004	0.002	
L	*	*	*	*	*	*	*	*	*	

^{a,b,c,d,e,f,g} Means within each column with different superscripts are significantly (p<0.05) different.

SEM= Standard error of the means.

L = Polynomial analysis showing linear relationship within cultivars of *lablab purpureus* beans.

Table 4: Analysed hydrocyanic acid content of different varieties of *lablab purpureus* beans cooked at 100°C for different duration of time and their percent destruction (HCN mg/100g)

Cooking time (minutes)	Rongai (milky-white)	PI-164302 (Dark)	NAPRI-2 (Dark brown)	NAPRI-3 (Light-brown)	PI-401553 (Black)	PI345608 (Dark brown)	ILRI 147 (Dark)	TLN7 (Black)	PI-195851 (Light brown)	GNC
0.00	2.13 ^a	2.02 ^a	1.79 ^a	2.01 ^a	2.35 ^a	2.03 ^a	2.13 ^a	3.36 ^a	3.47 ^a	0.72
10.00	0.88 ^b	0.89 ^b	0.72 ^b	0.87 ^b	1.11 ^b	0.87 ^b	0.95 ^b	1.48 ^b	1.45 ^b	
%Loss	58.69	55.94	59.78	56.72	52.77	57.14	55.40	55.95	58.21	
20.00	0.85 ^b	0.80 ^b	0.69 ^b	0.80 ^b	0.91 ^b	0.79 ^b	0.86 ^c	1.35 ^b	1.35 ^c	
% Loss	60.09	60.40	61.45	60.20	61.28	61.08	59.62	59.82	61.09	
30.00	0.66 ^c	0.62 ^c	0.47 ^c	0.46 ^c	0.65 ^c	0.60 ^c	0.63 ^d	0.98 ^c	1.09 ^d	
%Loss	69.01	69.31	73.74	77.11	72.34	70.44	70.42	70.83	68.59	
40.00	0.63 ^c	0.60 ^c	0.46 ^c	0.45 ^c	0.63 ^c	0.58 ^c	0.62 ^d	0.96 ^c	1.08 ^d	
% Loss	70.42	70.30	74.30	77.61	73.19	71.43	70.89	71.43	70.61	
50.00	0.62 ^c	0.56 ^c	0.46 ^c	0.45 ^c	0.63 ^c	0.49 ^d	0.58 ^d	0.96 ^c	1.07 ^d	
%Loss	70.11	72.32	74.28	77.82	72.79	75.86	73.98	70.88	70.62	
60.00	0.60 ^c	0.52 ^c	0.42 ^c	0.44 ^c	0.61 ^c	0.48 ^d	0.51 ^e	0.91 ^c	0.98 ^e	
% Loss	71.83	74.26	76.54	78.11	74.04	76.35	76.06	72.92	71.76	
SEM	0.04	0.08	0.05	0.04	0.06	0.03	0.02	0.07	0.04	
L	*	*	*	*	*	*	*	*	*	

ab,c,d,e Means within each column with different superscripts are significantly (P<0.05) different. SEM= Standard error of the means.

L = Polynomial analysis showing linear relationship within cultivars of *lablab purpureus* beans

ous varieties were destroyed after cooking the beans for only 20 minutes at 100°C. Phytic acid and tannin were found to be more heat stable as only about 18-40% and 9-14% loss in activity respectively were observed after cooking the beans for 20 minutes at 100°C. It was however observed in this study that between 50 and 80 percent of all the antinutritional factors present in the various varieties of *lablab* were lost after cooking the seeds for at least 30 minutes. This result is similar to the report of Balogun *et al* (2001) who observed that most antinutritional factors in legume seeds are lost after cooking the seeds for a minimum of 30 minutes. Bawa *et al* (2003) also reported similar results when *lablab* seeds were subjected to boiling at 100°C for 30 minutes. Udedibe and Igwe (1989) had earlier reported that before legume seeds can be incorporated into poultry diets, they should be well processed preferably by cooking for at least 30 minutes to en-

sure that most of the anti-nutritional factors have been destroyed.

Legumes generally contain antinutritional factors but the levels of these antinutrients differ from legume to legume. Different processing methods such as heating (dry or aqueous), fermentation, autoclaving and soaking with chemicals are adopted to reduce or eliminate these antinutritional factors but the level of destruction of these antinutritional factors depends to a large extent on the processing method. However there is always a residual amount of some antinutrients no matter the processing method adopted. According to Omeje (1999) antinutritional factors interferes with digestion and utilization of nutrients particularly in monogastric animals. This is because these animals do not possess microbes that are capable of breaking down these substances. Antinutrients interfere with nutrient utilization by forming complexes with

the substrate at the site of digestion. Therefore to efficiently utilize the nutrient content of legumes by monogastric animals these antinutrients must be reduced to the barest minimum and one way of doing this is to cook the seeds for at least 30 minutes (Ogundipe *et al* 2003; Abeke *et al* 2003).

Conclusion

The results obtained in this study indicate that between 50-80% of most of the antinutrients in lablab seeds can be destroyed by cooking the seeds for at least 30 minutes at 100°C. This will render the seeds relatively safe for incorporation into poultry diets.

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