

## Effects of cooking duration on nutrient composition and levels of some anti-nutritional factors of lablab (*Lablab purpureus* C.V. Rongai) seeds

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### Abstract

This study investigated the effects of varying the duration of cooking on the nutrient composition and levels of some antinutritional factors in lablab seeds. Raw lablab seeds were subjected to four durations of cooking, viz: 15, 30, 45 or 60 minutes respectively, in a drum of boiling water. The samples were dried and assayed for their proximate compositions, mineral contents, presence and levels of trypsin inhibitors (TIA), phytic acid, tannin and hydrocyanic acid (HCN). The raw lablab seeds contained 94.03% dry matter (DM), 26.12% crude protein (CP), 7.86% crude fibre (CF), 2.02% ether extract (EE), 4.43% ash and 59.57% nitrogen free extract (NFE). The proximate composition was not significantly ( $P > 0.05$ ) affected by duration of cooking. Potassium (15.66g/Kg DM) and iron (245.17g/Kg DM) were the most abundant macro and micro mineral elements, respectively in the raw lablab seeds while sodium (0.06g/Kg DM) and copper (53.62 mg/kg DM) were the least for macro and micro minerals, respectively. Except for calcium and sodium, all the minerals assayed were leached significantly ( $P < 0.05$ ) during cooking. Cooking lablab seeds at 100°C for forty-five minutes decreased trypsin inhibitor activity (TIA) from 593.87 mg/100g to 132.00 mg/100g; phytic acid from 5.65 mg/100g to 0.10 mg/100g; tannin from 0.22 mg/100g to 0.10 mg/100g and hydrocyanic acid (HCN) from 1.58 mg/100g to 0.45 mg/100g, respectively. These values correspond to 77.77, 78.93, 54.55 and 71.52 percent reductions in TIA, phytic acid, tannin and hydrocyanic acid, respectively. Cooking lablab seeds for 45 minutes appear to be the optimum for elimination of these antinutrients from lablab seeds.

Key words: Lablab seeds, cooking time, toxic factors, proximate compositions, minerals.

### Introduction

High cost and scarcity of the conventional protein concentrates like soyabean and groundnut cake have been major constraints to increased commercial livestock production in Nigeria. There is the need therefore to expand the raw material base for livestock feed formulation to accommodate unconventional feed ingredients. One of such unconventional feed resource is lablab seed. Lablab seeds have low human preference for food and unlike soyabean and groundnut cake, its

nutritive value in livestock feeding has not been fully investigated. Like other tropical legume seeds, lablab seed contains some antinutritional factors, which limit its use in animal feeding (Souza *et al.* 1992 and Chau-Chifai, 1997).

A precise control of the heating process is critical to the preparation of legume grains for optimal nutritional value. Several methods of heat treatment capable of reducing the antinutritional factors to a threshold level have been reported (Marty

and Chavez, 1993; Balogun *et al.*, 2001), but facilities for many of such heat treatments described are rare in developing countries, including Nigeria. However, a simple on-farm technology for processing lablab seed will enhance its nutritive value as an alternative source of protein.

The objective of this study was to investigate the effect of duration of cooking on the proximate, mineral composition and levels of some antinutritional factors in lablab seeds.

## **Materials and methods**

### **Seed processing**

To process the seeds, batches of 25kg lablab seeds were subjected to cooking durations of 0, 15, 30, 45 and 60 minutes. Each cooking time represented a treatment. For each cooking time, 50 litres of water was first brought to boiling point in a 200-litre drum container. A batch of 25kg lablab seed was then poured into the boiling water, from this point, the specified time for cooking was taken. At the end of the period of cooking, excess water was drained off. The cooked seeds were then sun-dried for 5 days, milled and samples taken for laboratory analysis.

### **Proximate analysis**

The dry matter (DM) content was determined based on the weight loss after 24 hours in an oven at 100°C. Nitrogen (N) content was determined by the macro Kjeldahl method of A.O.A.C. (1990) and crude protein (CP) calculated as  $N \times 6.25$ . The ash content was determined as the residue remaining after incinerating the sample at 600 °C for 3 hrs in a muffle furnace. The A.O.A.C. (1990) methods were employed for ether extracts (EE), crude fibre (CF) and all other proximate components determinations.

### **Mineral analysis**

The mineral profile was determined using

0.5g wet digested samples of soyabean meal, raw lablab seeds and the cooked lablab seeds as described by A.O.A.C. (1990). Potassium and sodium were determined by flame photometry using the flame photometer at 967 and 589 nm, respectively. Calcium, copper, iron, magnesium, manganese and zinc were determined using the Perkin-Elmer (model 403) Atomic Absorption Spectrophotometer (AAS). Calcium was determined by first treating the digest with 1% lanthanum solution before using the appropriate lamp. Phosphorus was estimated by the automated procedure which utilizes the reaction between phosphorus and molybdovanadate to form phosphomolybdovanadate complex which was measured colorimetrically at 450nm using Technicon Autoanalyser All (Pearson, 1976). The analyses for minerals were done in triplicate for each of the samples.

### **Anti-nutritional factor assay**

Trypsin inhibitor activity (TIA) on sample extracts was assayed according to the method of Kakade *et al.* (1974). The phytin content was determined using the method of Sutardi and Buckle (1985) and tannin content estimated using the method of Earp *et al.* (1981). The hydrocyanic acid (HCN) content of the lablab seeds were determined using the procedure of Cooke and Maduagwu (1978) as modified by Ikediobi and Fashagba (1985). Assays for the antinutritional factors were carried out in triplicate for each of the samples.

### **Statistical analysis**

The data obtained from this study were subjected to the analysis of variance and where statistical significance were observed, the means were compared using the Duncan's Multiple Range Test according to SAS (1995).

**Table 1:** Chemical composition of soyabean meal, raw lablab seeds and lablab seeds cooked at 100°C for different durations, % DM basis

Parameters	Duration of Cooking Lablab Seeds (Minutes)						SEM
	SBM	0	15	30	45	60	
Dry matter (DM), %	93.71	94.03	94.69	94.53	94.72	94.61	0.70
Crude protein (C)P, %	46.83 <sup>a</sup>	26.12 <sup>b</sup>	25.36 <sup>b</sup>	25.45 <sup>b</sup>	23.50 <sup>b</sup>	25.54 <sup>b</sup>	0.15
Crude fibre (CF), %	6.14	7.86	7.73	7.65	7.72	7.73	0.07
Ether extract (EE), %	8.83 <sup>a</sup>	2.02 <sup>b</sup>	1.84 <sup>b</sup>	1.61 <sup>b</sup>	1.27 <sup>b</sup>	1.07 <sup>b</sup>	0.05
Ash, %	5.24	4.43	4.48	3.89	3.71	3.41	0.13
Nitrogen free extract (NFE), %	32.96 <sup>b</sup>	59.57 <sup>a</sup>	60.69 <sup>a</sup>	60.40 <sup>a</sup>	63.80 <sup>a</sup>	62.25 <sup>a</sup>	0.43

<sup>a, b</sup> Means within the same row bearing different superscripts differ significantly ( $P < 0.05$ ).

Each value represents the mean of three determinations

SBM - Soyabean meal

SEM - Standard error of mean.

## Results

### *Effect of cooking time on the proximate and mineral composition of lablab seeds*

Duration of cooking did not have significant ( $P > 0.05$ ) effect on the proximate composition of lablab seeds (Table 1). However, the analysed CP, EE and NFE values for the raw and cooked lablab seeds were significantly different ( $P < 0.05$ ) from that of soyabean meal.

According to Table 2, there was a significant ( $P < 0.05$ ) decrease in the levels of potassium and phosphorus with increased duration of cooking of lablab seeds. The sodium contents were

generally low. Calcium and sodium contents of the lablab seeds were not affected ( $P > 0.05$ ) by duration of cooking. Potassium was the most abundant macro mineral in lablab seeds and was the most leached into water as duration of cooking increased. Comparatively, the soyabean meal had higher levels of all the major minerals than the raw or cooked lablab seeds. Duration of cooking caused a significant ( $P < 0.05$ ) reduction in levels of all the micro minerals assayed with iron and copper being the most affected (Table 3).

**Table 2:** Effects of Duration of cooking on some macro mineral levels of Lablab seeds (g/kg DM)

Mineral	Duration of Cooking Lablab Seeds (Minutes)						SEM
	SBM	0	15	30	45	60	
Potassium (K)	21.56 <sup>a</sup>	15.66 <sup>b</sup>	14.50 <sup>b</sup>	14.33 <sup>b</sup>	13.84 <sup>b</sup>	12.88 <sup>c</sup>	0.70
Phosphorus (P)	3.98 <sup>a</sup>	3.66 <sup>b</sup>	3.32 <sup>b</sup>	3.54 <sup>b</sup>	3.23 <sup>b</sup>	2.99 <sup>b</sup>	0.01
Calcium, (Ca)	3.05 <sup>a</sup>	1.84 <sup>b</sup>	1.97 <sup>b</sup>	1.93 <sup>b</sup>	1.89 <sup>b</sup>	1.84 <sup>b</sup>	0.03
Sodium (Na)	0.10 <sup>a</sup>	0.06 <sup>b</sup>	0.06 <sup>b</sup>	0.06 <sup>b</sup>	0.06 <sup>b</sup>	0.06 <sup>b</sup>	0.05

<sup>a, b</sup> Means within the same row bearing different superscripts differ significantly ( $P < 0.05$ ).

SBM - Soyabean meal

SEM - Standard error of mean.

The most abundant trace mineral in raw lablab seed was iron (245.17 Mg/kg DM) while manganese was the least (40.86 mg/kg DM).

### *Effect of duration of cooking on the levels of some antinutritional factors in lablab seeds*

Heat treatment of raw lablab seeds resulted in significant ( $P < 0.05$ ) reduction in the

*Effects of cooking duration on nutrient composition and anti-nutritional factors of lablab seeds*

**Table 3: The effects of Duration of Cooking on some Micromineral levels of lablab seeds (mg/kg DM)**

Mineral	SBM	Duration of Cooking Lablab Seeds (Minutes)					SEM
		0	15	30	45	60	
Iron (Fe)	175.00 <sup>c</sup>	245.17 <sup>a</sup>	220.68 <sup>b</sup>	201.46 <sup>c</sup>	164.42 <sup>d</sup>	144.66 <sup>f</sup>	3.03
Zinc (Zn)	87.500 <sup>a</sup>	47.67 <sup>b</sup>	41.08 <sup>c</sup>	40.41 <sup>c</sup>	40.14 <sup>c</sup>	38.53 <sup>d</sup>	0.08
Manganese (Mn)	46.88 <sup>a</sup>	40.86 <sup>b</sup>	41.10 <sup>b</sup>	42.92 <sup>b</sup>	39.41 <sup>c</sup>	38.33 <sup>c</sup>	0.42
Copper (Cu)	31.25 <sup>c</sup>	53.62 <sup>a</sup>	47.29 <sup>b</sup>	32.88 <sup>c</sup>	28.65 <sup>c</sup>	15.33 <sup>d</sup>	1.65

<sup>a, b, c, d, e, f</sup> Means in the same row bearing different superscripts differ significantly (P<0.05).

SBM - Soyabean meal

SEM - Standard error of means.

levels of the antinutritional factors (Table 4). There was a significant decrease (P<0.05) in trypsin inhibitor activity as the duration of cooking increased. The value of 593.87 mg TIA per 100g sample obtained for the raw seeds was significantly reduced to 149.74, 141.65, 132.00 and 101.78mg per 100g sample at 15, 30, 45 or 60 minutes of cooking respectively.

As the duration of cooking increased, the concentration of phytic acid in the raw seeds (5.65, mg/100g) was significantly (P<0.05) reduced to 4.66, 3.88, 1.19 and 1.17 mg/100g at 15, 30, 45 and 60 minutes of cooking respectively. Unlike the trypsin inhibitor activity, the rate of destruction of phytic acid was very slow initially with only 17.52 and 31.33% destruction

achieved after 15 or 30 minutes of cooking, respectively.

Thereafter, the rate of destruction was very rapid, reaching 79% when cooking time increased to 45 or 60 minutes.

A significant (P<0.05) reduction in the concentration of tannic acid was also observed with increase in the duration of cooking. The rates of destruction of 54.45% and 59.09% were attained after 45 and 60 minutes of cooking respectively. Lablab seed contained hydrocyanic acid and its rate of destruction followed the same trend as that of trypsin inhibitor activity. A destruction of about 65% of HCN was achieved after 15 minutes of cooking. The rate of HCN destruction reached the peak of 71.52% after 45 minutes of cooking.

**Table 4: Antinutritional factor content of soyabean meal, raw lablab seeds and lablab seeds cooked at 100 °C for varying time periods and their percent destructions**

Parameters	SBM	Duration of Cooking Lablab Seeds (Minutes)					SEM
		0	15	30	45	60	
TIA, mg/100g	129.00 <sup>c</sup>	593.87 <sup>a</sup>	149.74 <sup>b</sup>	141.65 <sup>b</sup>	132.00 <sup>c</sup>	101.78 <sup>d</sup>	2.549
Percent destruction of TIA (%)	-	-	74.79	76.15	77.77	82.82	-
Phytic acid, mg/100g	4.30 <sup>b</sup>	5.65 <sup>a</sup>	4.66 <sup>b</sup>	3.88 <sup>c</sup>	1.19 <sup>d</sup>	1.17	0.007
Percent destruction of phytic acid (%)	-	-	17.52	31.32	78.93	79.29	-
Tannin, mg/100g	0.09 <sup>b</sup>	0.22 <sup>a</sup>	0.200 <sup>a</sup>	0.12 <sup>b</sup>	0.10 <sup>b</sup>	0.08 <sup>b</sup>	8.58
Percent destruction of tannin (%)	-	-	9.09	45.45	54.55	59.09	-
Hydrocyanic acid (HCN), mg/100g	0.60 <sup>b</sup>	1.58 <sup>a</sup>	0.56 <sup>b</sup>	0.47 <sup>c</sup>	0.45 <sup>c</sup>	0.47 <sup>c</sup>	0.01
Percent destruction of hydrocyanic acid (HCN, %)	-	-	64.56	70.25	71.52	70.25	-

<sup>a, b, c, d</sup> Means in the same row bearing different superscripts differ significantly (P<0.05).

SBM - Soyabean meal

SEM - Standard error of means

TIA - Trypsin inhibitor activity.

## Discussion

The percent dry matter of the raw lablab seeds (94.03%) is similar to those reported for soyabean (93.80%, Kaankuka et al., 1996), *Mucuna utilis* (90.23%, Iyayi and Egharevba, 1998) and cowpea, (93.10%, Oke et al., 1996). The percent crude protein value (26.12) obtained for the raw lablab seeds was within the range of 22.40 to 31.30% reported by Deka and Sarka (1990); 20-28% reported by Purseglove (1968), 21-29% reported by Kay (1979) and similar to 26% crude protein value obtained by Seno et al. (1996). Variations in the percentage crude protein for lablab seeds reported by various workers could be due to the variety of lablab seeds used as well as the method and the accuracy of the laboratory analyses employed. Kaankuka et al. (1996) observed a slight increase in crude protein as the duration of cooking of soyabeans increased. The authors explained that the loss of more of the non-protein fractions during cooking relative to the amount of proteins lost may account for the slight increase in the crude protein as the duration of cooking increased. Aletor and Ojo (1989) reported that cooking, roasting and autoclaving generally reduced the crude fibre levels in legume seeds. Such a reduction in the crude fibre value was not observed in the present study.

The value of EE (2.02%) obtained for the raw lablab seeds is significantly lower compared to 8.83% obtained for the soyabean meal. The EE of the raw lablab seed however compared favourably with the oil content of most underutilized legume grains (Oke et al., 1996 and Amaefule and Obioha, 2001). This is expected since lablab is not an oil seed. The slight decrease in the oil content of the cooked lablab seeds could be due to leaching of the oil into the processing water. Total ash declined slightly as the duration of

cooking increased. Loss in ash content could be due to leaching of the soluble inorganic salts into the processing water. The duration of cooking of the lablab seeds had no significant effect on the NFE content. The NFE of the lablab seeds however was significantly higher than that of the soyabean meal.

There was a general decrease in the levels of potassium and phosphorus with increase in duration of cooking. This observation agreed with the findings of Ologhobo (1980) who reported a decrease in the levels of P, Ca, Mg and Na in Ife brown, Aduki and Far V-13 varieties of cowpea with cooking. Aletor and Ojo (1989) and Iyayi and Egbarevba (1998) also reported a decrease in the K, Mg, Na and Ca contents of samples of cooked soyabean, lima beans and *mucuna utilis* seeds.

The rate of reduction in the activity of the trypsin inhibitors observed in this study as the duration of cooking raw lablab seeds increased supported the widely held view that protein inhibitors are easily denatured by heat (Liener and Kakade, 1980 and Aykroyd and Doughty, 1982). The fact that about 75% destruction of the trypsin inhibitor activity was achieved after 15 minutes of cooking the lablab seeds at 100 °C supported the claim that most of the protease inhibitors (including trypsin and chymotrypsin inhibitors) are heat labile (Ologhobo, 1987). Liener and Kakade (1980) reported that heat treatment of legume seeds induced some denaturation in the trypsin inhibitor molecules thereby destroying the active sites, making the amino acid residues inaccessible, through the formation of trypsin-inhibitor complex. Manjunath and Devaraj (1995) reported that lablab seeds cooked for 30 minutes at 100 °C had 83% reduction in trypsin inhibitor activity. Kaankuka et al. (1996), in a similar study with full fat soyabean also

reported 90% destruction of trypsin inhibitor activity after 30 minutes of cooking at 100 °C. In the present study however, about 76% destruction of trypsin inhibitor activity was achieved after 30 minutes of cooking at 100 °C. Cooking of the seeds for 60 minutes only resulted in 83% loss of the trypsin inhibitor activity. Although, the highest destruction of trypsin inhibitor activity (83%) was achieved at 60 minutes of cooking, this length of cooking time may affect the bioavailability of some of the essential nutrients in lablab seeds. Chau-chifai *et al.* (1997) reported that cooking lablab seeds for 60 minutes resulted in a reduction of all the essential amino acids except leucine, histidine, lysine and threonine. Since the essential amino acids are very important in the synthesis of protein in monogastrics, cooking the lablab seeds for only 45 minutes at 100 °C which resulted in 78% destruction of the trypsin inhibitor activity could be considered adequate for processing of lablab seeds.

The phytic acid content of the raw lablab seeds was significantly reduced with 45 and 60 minutes cooking. This observation agree with the results of Ologhobo and Fetuga (1984) and Sutardi and Buckle (1985). The loss of phytic acid according to these authors was due to its solubility in processing water during cooking. Endogenous phytase activity in the intestinal mucosa of monogastric animals is extremely low (Davies and Flett, 1978 and Moore and Veum, 1983). The degree of reduction in the levels of phytic acid obtained after 45 minutes of cooking lablab seeds as observed in the present study will increase the availability of the minerals in the digestive tracts as the chelating capacity of phytic acid is greatly reduced (Khan *et al.*, 1991 and Sharma *et al.*, 1996). Only 31% of the phytic acid was eliminated after

30 minutes of cooking, in this trial. This observation depicts phytic acid as being heat stable which may be due to a strong covalent linkage between the oxygen atoms and the phosphate radicals within the phosphate structure (De Boland *et al.*, 1975 and O'Dell and De Boland, 1976).

The lablab seeds contain tannin which were significantly reduced by cooking. This is in agreement with the reports of other workers who observed that cooking was effective to significantly reduce the tannin content of winged bean (Tan *et al.*, 1984); soyabean (Bressani *et al.*, 1982 and Kaankuka *et al.* 1996); common beans (Elias *et al.*, 1979); cowpea (Oke *et al.*, 1996) and lima beans (Ologhobo, 1980). The loss of tannin with increased cooking time could be due to its solubility in water as tannins are known to be water soluble (Kingsley, 1995). In this study, about 55% destruction of tannic acid was achieved after 45 minutes of cooking. At this same duration of cooking, about 78% trypsin inhibitor activity; 79% phytic acid and 72% hydrocyanic acid were eliminated. These observations suggest that tannic acid is heat resistant. This could be due to the formations of linkages of tannic acid, like other phenolic compounds with protein and other macromolecule. In addition, the intra-molecular force that exists within tannic acid structure would require much heat energy to overcome (Bate-Smith, 1973), thus reducing the rate of molecular thermo-disintegration and thereby making elimination difficult.

Cooking of lablab seeds resulted in an appreciable reduction in the cyanide (HCN) level. With 15 minutes cooking about 65% of the HCN was destroyed and reached the optimum of 71.5% at 45 minutes cooking. Significant losses in the cyanide level according to Oke *et al.*, (1996) could be due to the volatile nature of hydrocyanic acid as it has a boiling point of 26 oC. It is also

possible that the degree of cooking applied resulted in destruction of the enzyme rhodanase which is responsible for the release of HCN. The 65-71% loss in hydrocyanic acid level observed in the present study fell within the range reported by other workers. Eka (1986) reported that boiling of raw cassava peels for 30 minutes

resulted in the reduction of 65-85% of the HCN content whereas Siddhuraju et al. (1996) reported 67 and 78% reduction in the levels of HCN in *Mucunna pruriens* when dry heated and autoclaved, respectively.

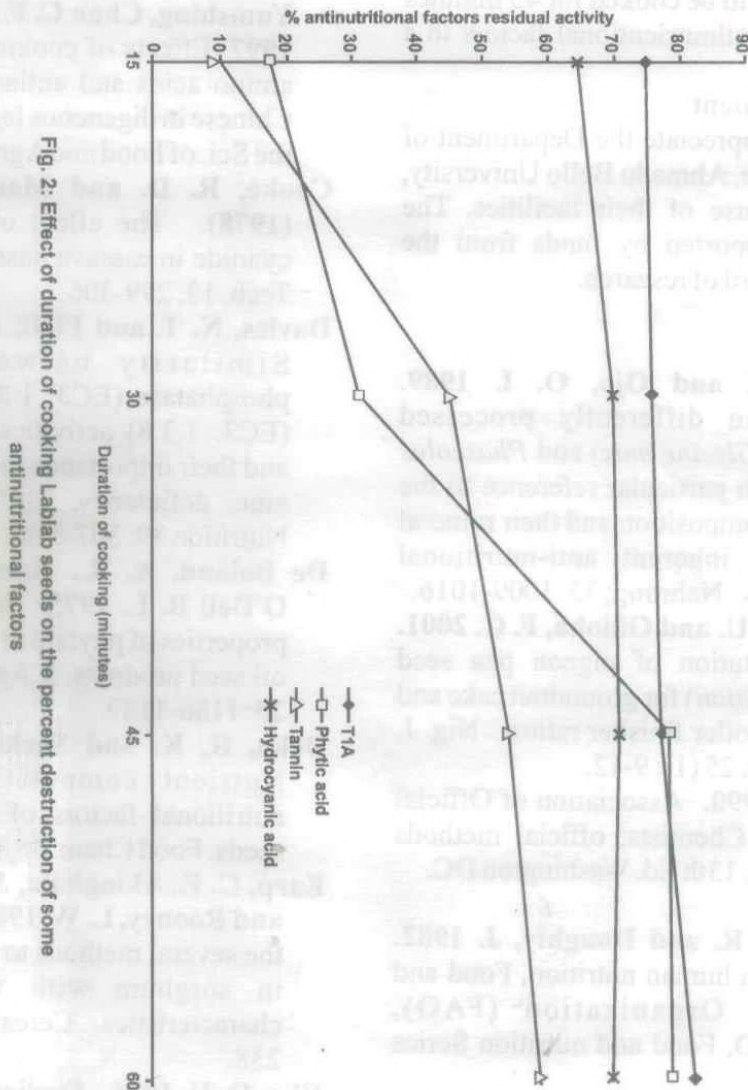


Fig. 2: Effect of duration of cooking Lablab seeds on the percent destruction of some antinutritional factors

### Conclusion

The nutritional profile of lablab seeds showed that it has a potential feeding value for livestock. Its proximate components are similar to that of most under utilized legumes. It contains P, Ca, K, Na, Mn, Fe, Cu and Zn, some of which were significantly reduced during cooking mainly due to leaching. For optimum utilization in livestock feeding however, the raw seeds have to be cooked for 45 minutes to reduce the antinutritional factors to a threshold level.

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