



Physical characteristics, proximate composition and anti-nutritional factors in grains of lablab bean (*Lablab purpureus*) genotypes from Kenya

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ABSTRACT

Objectives: The objectives of this study were to determine the physical characteristics, nutritional composition and anti-nutritional factors of grains from three lablab bean varieties with different colours and size developed by The Kenya Agricultural and Livestock Research Organisation (KALRO)

Methodology and results: The length, thickness and width were determined using Vernier callipers. Dry matter, protein, fat and ash were determined using standard association of official analytical chemists (AOAC) methods. Phytates were determined using high performance liquid chromatography (HPLC), while the tannins and trypsin inhibition were determined by UV spectrophotometry. The mean 100 seed weight was significantly different among the three varieties, and ranged from 26.1 g to 29.2 g. The length, thickness and width ranged from 9.7 for KAT/DL-3 to 10.7 mm for KAT/DL-1, 5.2 (KAT/DL-1) to 5.6 mm (KAT/DL-2) and 6.8 (KAT/DL-3) to 7.4 mm (KAT/DL-1), respectively. The hydration and swelling coefficients of the beans ranged from 130.7 in KAT/DL-2 to 147.2 in KAT/DL-1, and 125.1 in KAT/DL-2 to 153.3 (KAT/DL-1), respectively. The mean crude protein and energy contents varied from 22.5(KAT/DL-3) to 25.4 %,(KAT/DL-1) and 347.4 (KAT/DL-3) to 353.7 KCal/100g in KAT/DL-1, respectively, and differed significantly ($p < 0.05$) among the varieties. There were significant differences ($p < 0.05$) in the phytic acid and tannin levels, where KAT/DL-2 recorded the highest levels of 723.6 mg/100 g, and 0.33%CE, respectively.

Conclusion and application of findings: These results indicate that KAT/DL-1 has the best physical characteristics in respect to cooking and nutrition quality. This variety had the highest protein and energy content with low anti-nutritional factors, thus could offer better nutritive value in comparison to KATDL-2 and KAT/DL-3

Keywords: Lablab beans, varieties, proximate composition, physical parameters, anti-nutritional factors

INTRODUCTION

The global challenges of hunger, malnutrition, poverty are likely to become worse due to climate change, particularly in sub-Sahara Africa. This necessitates exploring the potential of

underutilized grain legumes for mitigation of food and nutrition insecurity. The Lablab bean, (*Lablab purpureus*) also referred to as Dolichos lablab is one such legume. It belongs to a species of beans

in the family *Fabaceae* that is native to Africa and was in existence in 1500 BC (Maass *et al.*, 2010). It is a drought tolerant legume, and is part of the staple foods for some communities in Africa and Asia (Kimani *et al.*, 2012). For the low income communities, such legume grains are an important source of relatively cheap dietary proteins, in comparison to animal sources (Tharanathan & Mahadevamma, 2003; Vohra *et al.*, 2015). Apart from using the grain, the immature pods can be used as vegetable while the crop residue after harvest serves as manure (National Research Council, 2006). In Australia and other places it is used as a forage and feed crop (Abeke *et al.*, 2008; Maass *et al.*, 2010). Furthermore legume seeds have been reported to be useful in the prevention and management of common metabolic diseases such as diabetes mellitus and coronary heart diseases (Mathers, 2002; Morris, 2003; Singhal, *et al.*, 2014). The Lablab crop has been documented to exhibit diverse agro-morphological characteristics and to be one of the most versatile legumes in the tropics (Maass *et al.*, 2010). It is one of the most drought tolerant legumes (Amole *et al.*, 2013), with the ability to extract soil water from a depth of two metres even in heavy-textured soils (Cook *et al.*, 2005; Muchow, 1985), hence its adaptability in arid and semi-arid areas. Despite its importance in the food and farming systems, particularly among communities in arid and semi-arid lands, the lablab bean has remained neglected and underutilized in many African countries, (National Research Council 2006). Indeed Maass *et al.*, (2010) classified it among the lost crops of Africa. Though it has been reported to be of high nutritional value (Foyer *et al.*, 2016; Hossain *et al.*, 2016), it is of limited economic importance in the global market, consequently attracting minimal attention from researchers and the food industry (Foyer *et al.*, 2016). In Kenya, it

is mainly grown in the semi-arid areas at subsistence level hence there are no proper records regarding its production. The limited research work on the bean has mostly focused on its genetic and agronomic aspects (Karachi *et al.*, 2003). The Kenya Agricultural and Livestock Research Organisation (KALRO) has developed and released three lablab bean varieties (Nyamongo *et al.*, 2015). These are KAT/DL-1 (brown in colour), KAT/DL-2 (black) and KAT/DL-3 (cream). Though these varieties are different in appearance, there is no information on their nutritional, anti-nutritional factors and other physical characteristics. Other studies have shown that lablab bean varieties and accessions may differ significantly in their physical and nutritional characteristics. Apart from the nutrients, the lablab beans, as is the case with other legume grains, may also contain varying amounts of anti-nutritional factors such as tannins, phytic acid and trypsin inhibitors. These anti-nutrients reduce the absorption and bioavailability of nutrients in legume grains (Chavan *et al.*, 1979; Makokha *et al.*, 2002; Shaahu *et al.*, 2015). The physical characteristics, including size, and hydration characteristics of the legume seeds influence consumer acceptability of these grains. They also determine the cooking and processing characteristics, of these beans (Ojo & Ademowaye, 2015). The physical properties may also influence the design of equipment for harvesting, transporting, cleaning, separating, packing and storing the beans (Altuntas and Demirtola, 2007). The objectives of this study were therefore to determine the physical characteristics, proximate composition and the anti-nutritional factors of the three-lablab bean varieties developed by Kenya Agricultural Livestock and Research Organization (KALRO) in Kenya with a goal of selecting the best variety for processing and nutritive value.

MATERIALS AND METHODS

Samples and preparation : Three freshly harvested varieties of lablab bean (*Lablab purpureus*) -KAT/DL-1, KAT/DL-2 and KAT/DL-3, were obtained from Kenya Agricultural and Livestock Research Organization (KALRO), Katumani Dry land Research Station. The

samples were screened to remove foreign materials and immature seeds. About 100 grams of each sample was ground into powder using a grinder, sieved to comparable particle size and packed in airtight containers for proximate composition analysis.

Seed weight: A hundred seeds were hand counted and weighed in triplicate for each variety of lablab beans and the average weight recorded as the 100 seed weight.

Length, width and thickness: The length width and thickness of 10 seeds were measured using a Vanier calliper reading to 0.01 mm in triplicate. The mean length, width and thickness of one seed was then determined.

Seed density: The seed density was obtained by liquid displacement. Seeds were weighed and then immersed in distilled water in a volumetric flask. The volume of the displaced water was determined. This was also the volume of the seeds. Seed density (g/cm³) was determined by dividing the weight of the seeds (g) by the volume of the displaced water (cm³):

$$\frac{\text{weight of seeds (g)}}{\text{volume of displaced water (cm}^3\text{)}}$$

Hydration coefficient: The hydration coefficient was determined by soaking 10 g of bean seeds in 50 ml deionised water (ratio of 1:5) at 25 °C in an incubator. After 12 hours, the lablab beans were removed from the soaking water. This was followed by free water removal by using a blotting paper. The seeds were then weighed again. The hydration coefficient was determined as:

$$= \frac{\text{weight of bean seeds after soaking}}{\text{weight of bean seeds before soaking}} * 100$$

(Nasar-Abbas *et al.*, 2008)

Swelling coefficient: The volume of raw bean seeds before and after soaking in deionised water for 12 hours at room temperature was determined by measuring the volume of water displaced using a graduated cylinder. The swelling coefficient was determined by dividing the volume of the beans after soaking by the volume before soaking:

$$\frac{\text{volume of bean seeds after soaking}}{\text{volume of bean seeds before soaking}} * 100$$

Proximate composition analysis: The proximate composition was determined according to the procedures of Association of Official Analytical Chemists (AOAC, 2005) on dry matter basis. The moisture content was determined by the dry oven method (AOAC, 2005- 925.09). Crude protein was

determined by the semi Kjeldahl method, multiplying the nitrogen content by the factor of 6.25 (AOAC, 2005-920.152). The crude lipid content was determined using Soxhlet apparatus as described in the AOAC, 2005-920.85. Total ash determination was as per the AOAC, 2005-923.3, where about 2g of the sample in silica dishes was combusted in a muffle furnace at 600°C. The carbohydrates in the samples were estimated by subtracting the sum of dry matter percentage of crude protein, crude fat, crude ash and moisture from 100. To obtain energy values [in kcal/g], the Atwater specific factors (WHO/FAO, 2002) were used. The protein percent in the lablab beans was multiplied by 3.47, fats multiplied by 8.37 and carbohydrates by 4.07.

Determination of phytic acid: Phytic acid was determined using HPLC according to the method of Camire and Clydesdale (1982). About 0.5g of the sample was extracted with 25 mL of 3% H₂SO₄ in Erlenmeyer flasks for 30 min. The solution was filtered and the filtrate heated in a boiling water bath for 5 minutes. 3 mL of FeCl₃ solution was added to the filtrate and heated for 45 min. The cooled solution was centrifuged for 10 min at 2500 rpm and the supernatant discarded. The sample was then heated in 1:9 parts of 1.5N NaOH and distilled water for another 30 min before centrifugation. The supernatant was made to 50ml and micro-filtered into HPLC vials for separation on a C 18 Column of HPLC using a Refractive Index Detector.

Determination of tannins: The tannins in the lablab beans were extracted and quantified using the Vanillin-hydrochloric acid method as described by Prince and Scoyoc (1978). Approximately 0.25 g of the samples was extracted twice with 10ml of 4% HCl and 5 mL of 1% HCl in methanol for 20 min in a shaker, respectively. The samples were then centrifuged for 10min at 4500 rpm. The supernatant aliquots were transferred to 25mL volumetric flasks. The aliquots volume was made up to 25mL. Freshly prepared vanillin-HCl reagent was added to 1mL of the aliquots in a test tube then absorbance read at 500nm after 20 min incubation at room temperature. The tannin content was calculated in percentage catechin equivalent (CE) Trypsin inhibitor activity: The trypsin inhibitor activity was determined according to the Kakade *et al.* (1974) method, with modifications by Mbithi-Mwikya *et al.* (2000). One gram of the sample flour was extracted with 50 mL of 0.01 mol/L NaOH for 1.5 h. Portions (0, 0.6, 1.0, 1.4 and 1.8 mL) of the suspension were pipetted into duplicate sets of test tubes and adjusted to 2.0 mL with water. The tubes were placed in a water

bath at 37°C after adding 2 mL of trypsin solution (4 mg trypsin in 200 mL 0.001 mol/L HCl). Then 5 mL of BAPA solution at 37 °C was added to each test-tube. The reaction was terminated after ten minutes by adding 1 mL of acetic acid (30 mL acetic acid in 100 mL distilled water). After thorough mixing, the contents were filtered and the absorbance of the solution measured at 410 nm against reagent blank. Trypsin inhibitor activity (TIA) was then determined to be = $[(2.632 \times D \times A_1)/S]$ mg trypsin inhibition/g sample.

Where A_1 = change in absorbance due to trypsin inhibition/mL diluted sample extract,
 D = dilution factor and
 S = weight of sample (g).
 TIA is expressed as trypsin inhibitor unit per milligram of sample (TIUmg⁻¹sample). One trypsin inhibitor unit causes an increase of 0.01 absorbance reading per 10 ml of the reaction mixture.
Statistical analysis: The experimental data were subjected to Analysis of Variance (ANOVA) and Duncan's multiple range test for mean separation at $P \leq 0.05$ in GenStat software Version 12.

RESULTS

In appearance, the KAT/DL-1, KAT/DL-2 and KAT/DL-3 seeds were brown, black and cream in colour, respectively (Figure 1). The 100 seed weight was significantly ($p < 0.05$) different among the three varieties, and it ranged from 26.1g in KAT/DL-1 to 29.2 g in KAT/DL-2 (Table 1). The length, thickness and

width ranged from 9.7 to 10.7 mm, 5.2 to 5.6 mm and 6.8 to 7.4 mm, respectively. KAT/DL-1 had significantly greater length and width than the other two varieties. However, its thickness was significantly less than that of the other varieties.



Figure 1: Three lablab bean varieties KAT /DL-1, KAT/DL-2 and KAT/DL-3

Table 1: Physical characteristics of three lablab bean varieties

Physical parameters	Seed Colour	100seed weight (g)	Length (mm)	Thickness (mm)	Width (mm)
KAT/DL-1	Brown	26.1 ^a ±0.16 a	10.7 ^b ±0.98	5.2 ^a ±0.05	7.4 ^b ±0.18
KAT/DL-2	Black	29.2 ^c ±0.46	10.3 ^{ab} ±0.69	5.6 ^b ±0.08	7.2 ^{ab} ±0.14
KAT/DL-3	Cream white	28.3 ^b ±0.39	9.7 ^a ±0.24	5.5 ^b ±0.25	6.8 ^a ±0.51

Means with the same letters in the same column are not significantly different ($p < 0.05$)

The hydration coefficient and swelling coefficient of the three Lablab beans varieties ranged from 130.7(KAT/DL-2) to 147.2(KAT/DL-1), and 125.1 (KAT/DL2) to 153.3(KAT/DL-1), respectively (Table 2).

The coefficients of KAT/DL-1 were significantly higher than those of the other two varieties. However, there was no significant difference in the seed density among the three varieties.

Table 2: Hydration and swelling coefficients of three lablab bean varieties

Parameters	Hydration coefficient	Swelling coefficient	Seed density (g/cm ³)
KAT/DL-1	147.2 ^c ±0.18	153.3 ^c ±0.21	1.1 ^a ±0.01
KAT/DL-2	130.7 ^a ±0.09	125.1 ^a ±0.24	1.2 ^a ±0.11
KAT/DL-3	132.4 ^b ±0.12	133.3 ^b ±0.12	1.1 ^a ±0.04

Means with the same letters in the same column are not significantly different (p<0.05)

For the proximate composition, the moisture content in the three-lablab varieties ranged from 8.1% to 9.8% (Table 3). KAT/DL-1 had significantly lower dry matter content than the other varieties. There were also significant (p<0.05) differences in the protein content among the three varieties, with KAT/DL-1 having considerably higher protein content (25.4%) than the other two varieties on dry matter basis. Similarly, there was significant difference in ash content, as KAT-DL-3

had significantly (p<0.05) higher ash content (4.4%) than the other two varieties. The carbohydrate and fat content ranged between 59.8 to 60.8% and 2.6 to 2.7%, respectively. There were no significant (p<0.05) differences among the three varieties in the carbohydrate and fat content. The energy content of the three varieties ranged from 347.4 to 353.7 Kcal/100 g. KAT-DL-3 had significantly (p<0.05) lower energy content than the other two varieties.

Table 3: Proximate composition of three lablab bean varieties (%)

Variety	Moisture	Crude Protein	Ash	Fats	CHO	Energy kcal/100g
KAT/DL-1	8.3 ^a ±0.1	25.4 ^c ±0.5	4.0 ^a ±0.1	2.6 ^a ±0.1	59.8 ^a ±0.3	353.0 ^b ±0.6
KAT/DL-2	8.1 ^a ±0.1	24.4 ^b ±0.2	4.1 ^{ab} ±0.2	2.6 ^a ±0.1	60.8 ^a ±0.2	353.7 ^b ±0.6
KAT/DL-3	9.8 ^b ±0.2	22.5 ^a ±0.5	4.4 ^b ±0.1	2.7 ^a ±0.04	60.8 ^a ±0.6	347.4 ^a ±0.5

The means in the same column with different letters are significantly different (p<0.05).

Composition of protein, ash, fat and CHO is on dry matter basis.

For the anti-nutritional factors, there was significant (p<0.05) difference in the phytic acid levels in the samples, which ranged from 533.4 in KAT/DL-3 to 723.6 mg/100 g in KAT/DL-2 as indicated in **Error! Reference source not found.**, The tannin levels also varied

significantly (p<0.05) among the samples with a range of 0.23% catechin equivalent in KAT/DL-3 to 0.33% CE in KAT/DL-2. There was no significant difference in the Trypsin inhibitor activity for all the three samples (11.5 in KAT/DL-1 to 13.1 mg⁻¹sample KAT/DL-2).

Table 4: Anti-nutrient content in lablab beans

Lablab variety	Phytic acid mg_100g	Trypsin inhibitor (TIUmg ⁻¹)	Tannins (%CE)
KAT/DL-1	687.8 ^b ±5.46	11.5 ^a ±0.08	0.266 ^b ±0.73
KAT/DL-2	723.6 ^c ±7.01	13.1 ^a ±0.09	0.330 ^c ±0.82
KAT/DL-3	533.4 ^a ±1.65	12.5 ^a ±0.10	0.233 ^a ±0.49

TIU –Trypsin inhibitory unit, CE – Catechin equivalents, Values with similar letters in the same column are not significantly different (p<0.05)

DISCUSSION

The seeds from the three-lablab varieties were significantly different in weight, length, thickness and width. The mean and range of the seed width and length were similar to those reported in other studies (Deka & Sarkar, 1990; Subagio, 2006). However, the mean seed thickness was observed in this study was higher than that reported by Subagio (2006) at 4.0 mm. The KAT/DL-1 variety had the least seed weight (26.1 g/ 100 grains) among the three bean varieties, but it also had the highest length (10.7), width (7.4), swelling

coefficient (153.3) and hydration coefficient (147.2). On the other hand, the black KAT/DL-2 had the highest seed weight (29.2 g/ 100 grains) and true density (1.2) but the least hydration and swelling coefficient. Mortuza observed higher seed density, hydration and swelling coefficient of lablab seeds in Bangladesh. The swelling and hydration coefficients were also significantly different among the varieties. Since hydration process aids softening and starch gelatinization, this implies that lablab variety KAT/DL-1 would have better cooking

characteristics compared to the other two varieties. There is also the potential to select such varieties for their good cooking characteristics. However, the hydration and swelling coefficients observed in this study were lower compared to those obtained by Mortuza and Tzen (2009). The moisture content was similar to results obtained by Kamatchi *et al.* (2010) for lablab beans but low compared to that of various common bean varieties (12-14%) obtained by Barros and Prudencio (2016). However, the moisture content is mainly dependent on drying and storage conditions, but not due to variety. It can also be used to predict the shelf life of the seeds. The total ash content in the three varieties was between 4.0 and 4.4%. These values are in agreement with those reported in other studies (Deka & Sarkar, 1990; Kamatchi *et al.*, 2010; Barros & Prudencio, 2016). However, they are higher in comparison to those observed by Hossain *et al.*, (2016). The ash content is an indicator of the mineral content in the beans. The amount of lipids in the three samples was low (2.6%), and in agreement with other observations for lablab beans (Deka & Sarkar, 1990; Chau *et al.*, 1998; Mortuza & Tzen, 2009; Shaahu *et al.*, 2015), and other commonly consumed legume grains (Sridhar & Seena, 2006; Oluwole & Olayinka, 2011; Barros & Prudencio, 2016; Marimuthu, *et al.*, 2016). Significant difference in the protein content was also observed among the three varieties, where KAT/DL-1 had significantly higher protein than the other two varieties. The mean protein content was similar to what has been reported in other studies elsewhere (Chau *et al.*, 1998; Myrene, 2013; Osman, 2007). A black variety of lablab beans analysed by Hossain *et al.*, (2016) in Bangladesh also had similar protein content to that obtained in this study. However, other studies reported higher protein content in the lablab beans than the mean protein range observed in this study. These include Mortuza & Tzen (2009) and Deka & Sarkar (1990) who reported protein content of 29% in lablab bean cultivars grown in Bangladesh and Assam Agricultural University. On the other hand, (Kalpanadevi & Mohan, 2013b) reported lower protein content of 20% in brown lablab beans grown in Tamil Nadu, India. Where the lablab beans are the main source of protein in the diet, there is therefore good potential to select and promote the production and utilization of high protein lablab varieties. This applies to many communities in developing countries who cannot easily afford animal sources of dietary protein. The relatively high carbohydrates content and energy values of the lablab seeds observed in this study are similar to those

observed in other studies (Asif *et al.*, 2013; Nakitto *et al.*, 2015; Nazni & Devi, 2016). They are also within the range of the other most commonly consumed legume grains (Sánchez-Chino *et al.*, 2015). Apart from the content, the carbohydrate quality is also reported to have a low glycaemic index, as observed by Reddy *et al.* (2013), who reported that lablab bean seeds can be used as therapeutic diets for diabetes. This is attributed to their low post prandial glucose response and slow starch digestibility (Hedges & Lister, 2008). There was significant difference in the phytic acid levels among the varieties. The phytic acid content obtained in this study was similar to the levels of 800 mg/100 g reported by Ashok *et al.*, (2016). On the other hand, Deka & Sarkar (1990) and Wang *et al.* (2010) found relatively higher levels (1300 mg/100 g) of phytic acid in lablab bean varieties obtained from Assam Agricultural University, India. Yet in another study, lablab seeds from the National Seed Project-Bengaluru, India had lower levels (501 mg/100 g) of phytic acid relative to results obtained in this study (Myrna, 2013). Studies on other legume grains have indicated that phytic acid in those grains may occur in even higher amounts than what is found in lablab beans (Vijayakumari, 1997; Kakati *et al.*, 2010; Thapliyal *et al.*, 2014). There was also significant difference in the tannin levels among the three varieties. The black variety (KAT/DL-2) had the highest amounts (0.33% CE) while variety KAT/DL-3 had lower level. These results are in agreement with those obtained by Kamatchi *et al.* (2010) who observed that tannin content may vary significantly among varieties as was the case for five lablab beans sourced from Tamil Nadu Agricultural University- India. However, the tannin levels in this study are relatively higher than those reported by Kalpanadevi & Mohan (2013a), which ranged between 0.17-0.19 % CE. Nakitto *et al.* (2015) in their study conducted on common beans, *Phaseolus vulgaris*, reported even higher levels of tannins (931 mg/100 g) while Luo and Xie (2013) reported 6.45 mg^g on *Vicia faba* beans. The results showed no significant differences in the trypsin inhibitory activity across all the bean varieties studied. Ramakrishna *et al.* (2006) and Kalpanadevi & Mohan (2013) reported similar levels of trypsin inhibitory activity in lablab samples obtained from Andhra Pradesh, India. On the other hand Shaahuet *et al.*, (2015) reported higher levels (29 mg⁻¹). Studies on other legumes have achieved varying quantities of trypsin inhibitory activity. Pious & Veerabahu, (2013) obtained up to 46 TIU mg⁻¹ in *Mucuna* legume while Padmashree *et al.* (2016) attained 17 Trypsin inhibitory unit mg⁻¹ in *Phaseolus*

aureus. It has been observed that anti-nutritional factors' content in legume grains may differ as a result of species, cultivars, climatic conditions, soils, locations, seasons and seed germination (Sridhar & Seena, 2006). The importance attached to these anti-nutritional factors is their ability to reduce absorption and bioavailability of nutrients in the human body (Ashok et al, 2016; Nwafor et al, 2017). However, different processing methods

such as soaking, germination and fermentation have shown significant reduction of these anti-nutrients making the beans safe for human consumption as indicated in different studies (Mcgrath et al., 1982; Dahiya & Kapoor, 1992; Makokha et al, 2002; Soetan & Oyewole, 2009; Abou-Arab & Abu-Salem, 2010; Fernandes et al., 2010; Doss et al, 2011; Okoth et al, 2011).

CONCLUSION

Based on the results obtained, it can be concluded that KAT/DL-1 had the best physical characteristics desirable for easy cooking. The same variety also had the highest protein and energy levels important for selection of varieties with best nutritive values. Interestingly, KAT/DL-1 also had the least anti-nutritional levels. This variety therefore presents the

best option among the three varieties when selecting a lablab bean variety to shorten the time of cooking and meet the nutritional and energy requirements. Based on the different physical and nutritional characteristics, there is potential to select for lablab varieties with good cooking and nutritional characteristics among the varieties grown in Kenya.

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