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Changes in Nutrient Composition, Trypsin Inhibitor, Phytate, Tannins and Protein Digestibility of Dolichos Lablab Seeds [Lablab Putrpuresus (L) Sweet] Occurring During Germination

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Abstract: The changes in nutrient composition, trypsin inhibitors, phytic acid, tannins and in-vitro digestibility of protein of lablab bean during 5 days of germination were studied. The crude protein contents was significantly increased, whereas lipid and carbohydrate contents decreased. Antinutritional study revealed that trypsin inhibitor activity and phytic acid content decreased as germination period increased. In contrast, there was a progressive increase in tannins content with increase in germination time. In-vitro protein digestibility markedly increased with germination time, with a significant increase in day 5.

Key words: Dolichos lablab, trypsin inhibitot, phytic acid, tannins, *in vitro* protein digestibility

INTRODUCTION

Plants are important sources of energy and dietary proteins and play a significant role in human nutrition, particularly in the developing world. Among the plant sources, dry legumes and their products are among the richest sources of food protein. They are generally well adapted to a wide range of climates and environmental conditions. Dolichos lablab bean is an indigenous legume of the arid and semi-arid area of the South and East Asia where, it is traditionally consumed by the natives. Lablab bean plant produced well with limited moisture and is tolerant to temperature stress. It is regarded as potential food crop for arid lands of Africa, Middle East and South America. Lablab bean contains 22.4-31.3% protein and about 46.0-63.3%, 55% carbohydrates (Al-Othman, 1999; Deka and Sarkar, 1990; Ahamad and Nour, 1990; Salimath and Tharanathan, 1982). The bean can be consumed fresh, germinated, boiled steamed or fried. As in other legumes, Dolichos lablab bean contains some antinutritional components such as trypsin inhibitors, phytic acid and tannins (Vijayakumaris *et al*., 1995; Devarj and Manjunath, 1995; Shastry and John, 1991). The nutritional quality and digestibility of plant protein is affected by presence of these antinutritional factors which may limit the use of plants as a source of protein. Several methods have been employed to improve the nutritional quality of legumes. The processing technologies employed include, soaking (Desphande and Sheryan,1983; Vijayakumari *et al*., 2007; Alonso *et al*., 1998), heat treatment (Osman *et al*., 2002; Di Pitro and Liener, 1989; Kadam and Smithard, 1987), fermentation (Reddy and Salunke, 1980; Osman, 2004) and irradiation (Abu Tarboush, 1998; Sidduraju *et al*., 2002).

In the Orient and Far East Asia, germination process is used widely and routinely used as treatment to grain legumes. Germination, of the dry bean is reported to decrease the level of trypsin inhibitors (Frias *et al*., 1995; Sathe *et al*., 1983; Alonso *et al*., 2000), phytic acid (Kataria *et al*., 1989; Vidal-Valverde *et al*., 1994; Sangronis and Machado, 2007) and tannins (Reddy *et al*., 1985; El-Awady, 2002; Mubarak, 2005). Germination is also thought to increase *in-vitro* protein digestibility (Kataria *et al*., 1989; Chau and Cheung, 1997) and essential nutrients (Ghorpade and Kadam, 1989). Contradictory reports on antinutritional factors in germinated seed are also available. Shastry and John (1991) observed and increase in trypsin inhibitors, phytic acid and tannins level during germination of Dolichos lablab. The sprouting, of sorghum, also produced an increase in tannins content (Ahamad *et al*., 1996; Al-Jasser, 2005). This controversy as to whether the germination increase or decreases antinutritional factors seemed arisen because of different types of legumes or different germination conditions used in the studies. The objective of this study was to have a better understanding on the effect of germination on nutritive value, antinutritional factor level and in-vitro protein digestibility of Dolichos lablab.

MATERIALS AND METHODS

Materials: Dolichos Lablab beans [(*Lablab purpureus cl.*) sweet] were obtained from The Agricultural Experiment Station, College of Agriculture, King Saud University, Saudi Arabia. The Beans were cleaned manually and ground in a hammer mill to pass through No 30. Sieve.

Germination: The germinated beans were obtained by soaking the beans in water for 20 h. The soaked beans were spread on metal box, the lower side of which was a mesh covered with a piece of jute sack, another piece of jute sack was used to cover the box. The box was kept at roam temperature in the dark. The beans were watered once a day. Germination was carried out for 5 days.

Sampling, drying and grinding: Samples of germinated beans were taken daily for subsequent analysis. Drying of the germinated beans was carried out at roam temp. The dried germinated beans, were ground usinga hammer mill, to pass through 30 mesh screen size.

Proximate composition: The protein, fat, ash, crude fiber and moisture content of the bean samples were estimated according to the method described by the AOAC (1984). Total carbohydrates were calculated by difference.

Trypsin Inhibitor Activity (TIA): Trypsin inhibitor activity was assayed according to Kakade *et al.* (1969) using BAPA N-benzoyl-DL-arginine-P-nitroanilide hydrochloride and trypsin type III from bovine pancreas. TIA expressed as trypsin inhibitor unit per milligram of sample (TIU mg^{-1} sample) was calculated from the absorbance reading against a blank in spectrophotometer. One trypsin unit is defined as an increase of 0.01 in absorbance reading at 410 nm per 10 mL of the reaction mixture.

Phytic acid: Phytic acid analysis was performed according to the method of Latta and Eskin (1980). Duplicate fat free dry samples weighing 5 g were extracted in 100 mL of 0.667 N HCL for 1 h at room temp. The extract was filtered under vacuum. The phytate content in sample extract was determined by ion exchange method. Phytic acid in elute was determined by a colorimetric method.

Tannin: The modified vanillin-HCl method of Price *et al.* (1978) was followed with minor modification. One gram of sample was extracted with 10 mL 1% HCl in methanol for 24 h at room temperature then centrifuged at 5000 rpm. Vanillin HCl reagent was prepared by mixing prior to use equal volumes of 8% HCl in methanol with 2% vanillin in methanol. One mililiter of supernatant was mixed with 5 mL of vanillin Hcl reagent. The absorbance was read at 500 nm after 20 min incubation at room temperature.

***In vitro* protein digestibility:** *In vitro* digestibility was determined following Hsu *et al.* (1977) as modified by Satterlee *et al.* (1979). The drop of pH of casien (control) and the sample after 20 min hydrolysis by proteolytic enzymes was measured using an Orion pH meter. The enzymes used were trypsin type IX from porcine pancrease, chymotrypsin type II from bovine pancreas, peptidase type III from porcine intestine and protease type VI from streptomyces griseus. All enzymes were supplied by Sigma chemical company (St. Louis, Mo. USA). The *in vitro* digestibility was calculated according to Satterlee *et al.* (1979) equation.

$$\% \textit{ In-vitro} \text{ digestibility} = 234.84\text{-}22.56\ X$$

Where, X = the pH of suspension after 20 min hydrolysis of protein.

Statistical analysis: The data were analyzed used one way ANOVA with mean separated by Least Significance Differences (LSD) at $p<0.05$ (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Proximate composition: Table 1 Shows the changes in proximate composition during the germination period of lablab beans. There was a gradual increase in moisture with the time of germination, with 5th day showing the highest moisture content. The moisture content in lablab bean increased from 6.41-12.93% during germination period. The increase in moisture content is related to increase in water activity during germination due to hydrolytic enzymes. Protein content of germinated bean showed significant decrease in the beginning and then gradual increase was observed. At the end of germination protein content significantly increased. This increase, attributed to the utilization of carbohydrates as source of energy during germination process. The results of this work were in agreement with those obtained with winged bean (King and Putwastien, 1987), Nigerian cowpea (Akpapunam and Achinewhu, 1985), Tepary bean (Idouraine *et al.*, 1989), faba and kidney bean (Alonso *et al.*, 2000) and mung bean (Mubarak, 2005). Fat content of lablab bean decreased significantly by approximately 37% following germination. The reduction was probably caused by break-down of fat by beta oxidation with fat being used for energy purposes in embryo development. These observations, are in agreement with those reported by Idouraine *et al.* (1989), Alonso *et al.* (1998), El-Awady (2002) and Mubarak (2005) and Ghavidel and Prakash (2007). Crude ash content did not vary significantly between the raw and germinated samples. There was a significant increase in total carbohydrates in lablab bean germinated for 48 h, then a decrease occurred after this period. The reduction in total carbohydrates was attributed to the increase in amylotic

Table 1: Proximate composition of raw and germinated seed of the lablab bean on dry weight basis

	Moisture	Protein	Fat	Ash	Carbohydrates
Raw	6.41±0.03^{D}	26.86±0.3^{B}	1.9±0.01^{A}	3.96±0.04^{BC}	67.23±0.30^{CD}
First day	7.79±0.07^{BC}	25.80±0.13^{DC}	0.87±0.13^{D}	3.46±0.04^{BC}	69.82±0.23^{A}
Second day	7.93±0.02^{B}	25.07±0.97^{DEF}	0.86±0.04^{D}	3.70±0.01^{BCD}	70.38±1.00^{A}
Third day	7.80±0.0.08^{BC}	26.60±0.32^{BC}	0.86±0.13^{D}	3.69±0.02^{BCD}	68.46±0.42^{B}
Fourth day	7.60±0.80^{BC}	27.08±0.47^{B}	0.97±0.03^{DC}	3.66±0.01^{BCD}	68.27±0.42^{CD}
Fifth day	12.95±0.80^{A}	28.55±0.21^{A}	1.19±0.08^{BC}	3.83±0.03^{ABC}	66.40±0.14^{D}

All values are means of 3 replicates±SD, Values with same letters (a, b, c, d, e, f within columns) are not significantly different at $p<0.05$

Table 2: Change in antinutrional factors level and protein digestibility during germination of the lablab bean

	Trypsin inhibitor (TIU mg^{-1})	Phytic acid (mg 100 gm^{-1})	Tannins (%catechin equivalent)	IVPD
Raw	28.96±0.30^{A}	605.39±0.39^{A}	0.42±0.01^{F}	88.17±1.70^{BC}
First day	26.85±0.23^{B}	406.69±1.66^{B}	0.56±0.02^{DE}	88.27±0.00^{BC}
Second day	25.07±0.30^{C}	393.24±3.84^{C}	0.57±0.01^{BDE}	88.43±0.00^{BC}
Third day	24.61±0.31^{D}	358.87±4.06^{D}	0.60±0.014^{C}	90.36±0.56^{AB}
Fourth day	23.51±0.99^{E}	317.72±5.01^{E}	1.15±0.01^{B}	89.33±1.73^{AC}
Fifth day	23.35±0.81^{E}	309.10±5.35^{E}	1.27±0.01^{A}	92.27±1.83^{A}

All values are means of 3 replicates±SD, Values with same letters (a, b, c, d, e, f within columns) are not significantly different at $p<0.05$

enzyme activity that hydrolyzes starch into simple absorbable sugar to the developing embryo. Similar results were reported by Ologhobo and Fetuga (1986) for germinated cowpea, Mbithi-Mwikya *et al.* (2000) for germinated finger millet, El-Adway (2002) for germinated chickpea and Mubarak (2005) for germinated mung bean.

Trypsin inhibitor: The change in Trypsin Inhibitor Activity (TIA), Phytic Acid (PA) and tannins during germination is presented in Table 2. A significant ($p<0.05$) decrease in TIA level was observed at the 1st day of germination and further significant reduction following the increase of germination time. During 5 days germination period the TIA progressively decreased from 28.96-23.35 representing reduction of 19.4% at the end of the 5th day. The results obtained in this research agree with those reported for Kidney bean (El-Hag *et al*., 1978), navy bean Great Northern bean (Sathe *et al*., 1983), lima bean (Ologhbo and Fetuga,1983; mung bean (Mubarak, 2005) lentil (EL-Mahdy *et al*., 1983; Frias *et al*., 1995; Valverde *et al*., 1994), soybean (Collin and Sandars, 1976), pigeon bean (Torres *et al*., 2007 ;Sangronis and Machado, 2007) and faba bean and kidney bean (Alonso *et al*., 2000). Such pattern were also observed in other plant like sorghum (Al-Jasser, 2005), finger millet (Mbithi-Mwikya *et al*., 2000), small radish, radish, white mustard and rapeseed (Frias *et al*., 2005) and breadnut, cashew nut and fluted pumpkin (Fagbemi *et al*., 2005). Our results with germinated lablab bean were not exactly the same as those reported earlier by Shastry and John (1991) which indicated TIA increase during the progressive of germination. Similarly, Chang and Herrold (1988) observed an increase in TIA in pinto bean during germination.

Phytic acid: There was a significant reduction in phytic acid content with the increase of germination time of the Dolichos lablab beans (Table 2). The percent of decrease in phytic acid content after 5 days germination was 49.9%. This finding agree with that of Sathe *et al*. (1983), who reported 57.8 reduction in PA content of the Great Northern bean after 5 days of germination. Many workers noticed significant reduction in PA content of legumes. Alonso *et al*. (2000) reported 61 and 30% reduction in PA content in faba bean and kidney bean respectively after germination. Sangronis and Machado (2007) found that germination reduced PA content in white bean, black bean and pengion pea by 44.7, 52.9 and 40.7, respectively. Similarly, Ghavidel and Parkash (2007) observed 18, 20, 21.1 and 20.8% reduction PA 24 h germination for green gram, cowpea, lentil and chickpea, respectively. In contrast germination of Dolichos lablab for 8 days increased phytic acid content (Shastry and John, 1991). The phytate reduction caused by germination was probably due the phytase activity that hydrolyzed phytates.

Tannins: Data on the effect of germination on tannins content of Dolichos lablab, is shown in Table 2. In contrast to trypsin inhibitor and phytic acid, the tannins content significantly increased as period of germination increased. Similar observations were reported by many investigators. Shastry and John (1991) observed significant increase in tannin in Dolichos lablab after 8 day germination. Similarly Valverde *et al*. (1994) reported increase in tannins content in lentil after 6 days of germination. An increase in tannins content has also been reported for sorghum when sprouted for 5 days (Abawi *et al*., 1986; Ahamad *et al*., 1996; Al-Jaseer, 2005). However, several investigators have observed decrease in tannins content on various legumes due to germination. Reddy *et al*. (1985) found that germination for 2 days after soaking reduced the tannins content by 50% in a wide variety of legumes. Alonso *et al*. (2000) also reported a marked reduction in tannins after 72 h germination of

faba bean and kidney bean. Similarly, Shimelis and Rakshit (2006) reported reduction in tannins content in sprouted kidney bean. More recently Ghavidel and Praksh (2007) reported decrease in tannins content in green bean, cowpea, lentil and chickpea germinated for 24 h.

***In vitro* Protein Digestibility (IVPD):** There was a progressive increase in the protein digestibility as the period of germination increase (Table 2). The values for digestibility after 5 days were significantly ($p>0.05$) higher than those after the first three days. These results indicate that the tannins content had no significant effect on *in-vitro* digestibility. The improvement in IVPD of Dolichos lablab during germination may be attributed to the modification and degradation of storage proteins. Germination has been reported to increase protein digestibility of Dolichos lablab (Shastry and John, 1991), of green gram, cowpea, lentil and chickpea (Ghavidel and Prakash, 2007), faba bean and kidney bean (Alonso *et al*., 2000), white bean, black bean and pigeon bean (Sangronis and Machado, 2007), sorghum cultivars (ELmaki *et al*., 1999; Al-Jasser, 2005), mungbean (Kataria *et al*., 1989), Kidney bean (Shimelis and Rakshit, 2006).

CONCLUSION

Germination resulted in increased level of the protein and reduced contents of the lipid and carbohydrates of Dolichos lablab. Germination was found to be effective in reducing trypsin inhibitor and phytic acid content. However, it was found to increase tannins content. Protein *in-vitro* digestibility values increased as germination period increase. Therefore, germination of lablab seed holds a good potential for improving nutritional value of the bean by reduction in antinutritional and increasing protein in-vitro digestibility and thereby enhancing its utilization.

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