

Effect of Domestic Processing on Antinutrients and Availability of Protein and Minerals of Lupin (*Lupinus termis*) Seeds

Amro B. Hassan, Gammaa A. Osman and Elfadil E. Babiker

Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Khartoum North, P.O. Box 32, Shambat, Sudan

Abstract: Two lupin cultivars namely Dongola and Golo were used to investigate the effect of domestic processing methods on antinutritional factors and protein and minerals availability. Processing methods were observed to reduce both tannin and phytic acid contents. However, phytic acid was greatly reduced when the seeds were soaked in distilled water compared to other processing methods. On the other hand, soaking of seeds followed by cooking or dehulling greatly reduced tannin content. Most domestic processing methods of lupin seeds were observed to improve protein digestibility and mineral availability for both cultivars. Soaking of seeds followed by dehulling of Golo cultivar was observed to increase protein digestibility to 92%, compared to the same treatment applied to Dongola cultivar (83.9%). Soaking of the seeds followed by cooking and dehulling of Dongola cultivar significantly ($P = 0.05$) increased iron availability to 78.2% compared to the same treatment applied to Golo cultivar (31.9%). Soaking of whole seeds significantly ($P = 0.05$) increased Ca and Co availability of cultivar Golo to 74.6% and 79.6%, compared to the same treatment applied to Dongola cultivar by 50% and 32.2%, respectively.

Key words: Domestic, Processing, Antinutrient, Protein, Minerals

Introduction

Lupins are one of several plants protein sources that have been shown to provide sound nutritional value to a range of aquaculture species. Compared to other plants protein sources, the potential of lupin is similar to soybean meals, which are presently widely accepted and used as rich sources of protein (New and Csavas, 1993). Lupin seeds are widely utilized in many countries around the world, its food products range from whole beans to mashed beans, yoghurt and milk substitutes. Flours and meals derived from these products could be expected to contain alkaloids. Lupin bean meal is often used as a substitute for soybean meal (Petterson, 1998). Full fat sweet lupin flour, which is rich in protein (39.7%) and has a fairly high concentration of the indispensable amino acids: lysine, leucine and threonine which were used to fortify bread at 7 and 10 level on flour basis (El dash and Sgarbieri, 1980). Lupins are typically low in antinutritional factors, though a range of various substances have been reported. Traditionally, lupins are not considered a viable feed grain because of inherently high alkaloid levels in the grain. Other potential antinutritional factors present in lupins include oligosaccharides, phytate, saponins, tannins and protease inhibitors, though notably most of these are usually at levels not considered influential. Lectins have not been detected in lupins (Petterson *et al.*, 1997). Lupin seeds are generally considered as low tannin content seeds that they are unlikely to cause any antinutritional effect (Petterson 2000). Also they have low levels (500 mg/100g) of phytate, similar to that found in peas and soybean meal and considerably less than that in rapeseed/canola meal (Carter and Hauler, 1999). Petterson, (2000) reported that the key mineral in lupin seeds include calcium, magnesium, phosphorus, potassium, sodium and sulphur. Mineral from plant sources, particularly those from plant seeds are less bioaccessible than those from animals sources due in part to phytic acid, tannins and fibre content (Moeljopawiro *et al.*, 1998 and WHO, 1998). These antinutritional factors chelate dietary mineral in the gastrointestinal tract reducing their bioaccessibility and bioavailability (Frolich, 1995). Processing techniques such as soaking, cooking, germination and fermentation have been found to reduce significantly the levels of phytates and tannins by exogenous and endogenous enzymes formed during processing (Mosha and Savanberg, 1990; Iorri and Savberg, 1995 and WHO, 1998). In this study we would like to investigate the effect of different domestic processing methods on antinutritional factors and availability of protein and minerals of lupin seeds.

Materials and Methods

Materials: Seeds of two lupin cultivars, (Dongola and Golo), were obtained from the local market, Khartoum North. Seeds were cleaned and freed from foreign materials and broken seeds. After processing the seeds were ground to pass a 0.4 mm mesh. Unless otherwise stated all reagents used in this study were reagent grade.

Processing Dehulling: Lupin seeds hulls were removed manually.

Soaking: Seeds were soaked in distilled water for 3 days with water changing every 8 h. The soaked seeds were dried in an oven at 60 °C.

Cooking: Seeds after soaking were boiled in distilled water for 30 min.

Methods

Crude Protein Determination: Total nitrogen content of raw and processed samples was estimated using the semi-mikrokjeldahl digestion and distillation method as described by the Official Methods of Analysis (AOAC, 1970). Crude protein content was calculated by multiplying the percent nitrogen by protein conversion factor ($N\% \times 6.25$).

In Vitro Protein Digestibility Determination: In vitro protein digestibility of raw and processed samples was measured according to the method of Saunders *et al.* (1973). About 250 mg sample was suspended in 15 ml of 0.1 N HCl containing 1.5 mg pepsin (1:10,000) in a 100 ml conical flask. The mixture was incubated at 37 °C for 3 hours only. The mixture was then neutralized with 0.5 N NaOH and treated with 4 mg pancreatin (Grade VI porcine) in 7.5 ml of 0.2 M phosphate buffer (pH 8.0), containing 0.005 M sodium azide. The mixture solution was incubated at 37 °C for 24 hours. Ten milliliters of 10% trichloro acetic acid (TCA) were added to the mixture to stop the reaction. The mixture was then centrifuged at 5000 rpm for 5 minutes. Five milliliters aliquots from the supernatant were pipetted and analyzed for nitrogen content.

Calculations:

$$\text{Protein digestibility \%} = \frac{\text{N in supernatant-enzyme N}}{\text{N in sample}} \times 100$$

Determination of Tannins Content: Quantitative estimation of tannin for each sample was carried out using modified vanillin-HCl in methanol method as described by Price *et al.* (1978). About 0.2 g of the ground sample was placed in a 100 ml conical flask. Ten milliliters of 1% HCl in methanol (v/v) were added, shaken for 20 min. and centrifuged at 2500 rpm for 5 min. One milliliter of the supernatant was pipetted into a test tube and 5 ml of vanillin-HCl reagent were added. The optical density was read using a colorimeter (Lab System Analyzer – 9 filters, J. Mitra and Bros. Pvt. Ltd) at 500 nm after 20 minutes incubation at 30 °C. A standard curve was prepared expressing the results as catechin equivalents, i.e. amount of catechin (mg per ml) which gives a colour intensity equivalent to that given by tannins after correcting for blank.

Determination of Phytic Acid Content: Phytate of raw and processed samples was determined according to the method described by Wheeler and Ferrel (1971). One gramme of finely ground sample was weighed into a 125 ml conical flask, extracted with 50 ml 3% TCA (w/v) for 3 hours with mechanical shaking. Then the suspension was centrifuged at 3000 rpm. Ten milliliters aliquots of the supernatant were transferred into 50 ml boiling tubes. Then, 4 ml of FeCl_3 (2 mg ferric iron per ml 3% TCA), centrifuged at 3000 rpm for 15 minutes and the clear supernatant was decanted carefully. The precipitate was then washed twice by dispersing well into 25 ml 3% TCA and heated in a boiling water bath for 5–10 minutes and centrifuged. The precipitate was cautiously dispersed in a few ml distilled water enriched with 3 ml 1.5 N NaOH with mixing. The volume was made approximately to 30 ml with distilled water and heated in a boiling water bath for 30 minutes. The contents of the tube were filtered hot (quantitatively) through filter paper (Whatman No. 1) and the filtrate was discarded. The precipitate was dissolved in 40 ml of 3.2 N HNO_3 (hot) into a 100 ml volumetric flask. The precipitate was washed with distilled water, the washings were collected in the same flask. The contents of the flask were cooled to room temperature (28 – 32 °C) and diluted to volume with distilled water. Five milliliters aliquots were transferred to another 100 ml volumetric flask and diluted to approximately 70 ml with distilled water. Then, 20 ml of 1.5 M KSCN (potassium thiocyanate) were added, to complete the volume up to mark. The intensity of the colour was immediately assessed (within one minute) using colorimeter (Lab System Analyzer – 9 filters, J. Mitra and Bros. Pvt. Ltd.) at 480 nm. A blank probe was run with each set of sample. A standard curve of different $\text{Fe}(\text{NO}_3)_3$ concentrations was plotted to calculate the ferric ion concentration. The phytate phosphorous from the ferric ion concentration assuming 4: 6 iron: phosphorous molar ratio.

Determination of Mineral Content: Mineral of raw and processed samples were extracted according to Pearson's method (1981). Each sample was burnt in a muffle furnace at 550 °C and placed in a sand bath for 10 minutes after addition of 5 ml of 5N HCl. Then the solution was carefully filtered in a 100 ml volumetric flask and finally distilled water was added to make up to the mark. The extracts were stores in bottles for further analysis. Minerals Fe, Mn, Co and Zn were determined using atomic absorption spectrophotometer. Calcium content was carried out according to Chapman and Pratt (1968) method. Potassium and sodium contents were determined according to

AOAC (1984) using Flame photometer (Corning 400). Phosphorous content was determined according to the method of Chapman and Pratt (1961).

HCl -Extractability of Mineral (In Vitro Availability): Mineral in the samples were extracted by the method described by Chauhan and Majan (1988). One gramme of the sample was shaken with 10 ml of 0.03 M HCl for 3 h at 37 °C and then filtered. The clear extract obtained was oven-dried at 100 °C and then acid-digested. The amount of the extractable minerals was determined by the methods described above. Thereafter, the extractable mineral was determined as a percentage of the individual minerals.

Results and Discussion

Effect of Domestic Processing Methods on Tannin and Phytic Acid Contents of Lupin Seeds: Fig. 1 (a and b) shows tannin and phytic acid contents of treated and untreated lupin cultivars (Dongola and Golo). Tannin content of Dongola cultivar was found to be 95 mg/100g. (Fig.1a), while that of Golo was 92.3 mg/100g (Fig.1b). Processing of lupin seeds significantly ($P = 0.05$) reduced tannin content for both cultivars. Soaking of seeds followed by cooking and dehulling significantly decreased tannin content for both Dongola and Golo cultivars and was found to be 10.4 and 21 mg/100g, respectively. Results obtained were lower than those reported by Rahma and Roa (1984). Alonoso *et al.* (2000) reported that tannin content of faba bean seeds was reduced by 47.7 % after 12 h soaking in double deionized water. Higher reduction in tannin content of presoaked cooked lupin seeds may be due to the fact that cooking of seeds after soaking removed significant amount of tannin as observed by Kataria *et al.* (1988). Abdulrahim *et al.* (2004) reported that dehulling of faba bean seeds significantly ($P = 0.05$) reduced tannin content. Since most tannin are located in the testa, its physical removal reduced tannin content. Phytic acid content of Dongola cultivar was found to be 124 mg/100g (Fig.1a) while that of Golo was 209 mg/100g (Fig.1b). Processing of the seeds significantly ($P = 0.05$) decreased phytic acid content for both cultivars. Soaking of the whole seeds significantly decreased phytic acid for both cultivars compared to other methods and was found to be 58 and 70.3 mg/100g for Dongola and Golo, respectively. The results obtained are within the range of those reported by Carter and Hauler (1999). Ologhbo and Fetuga (1984) reported that soaking of 10 cowpea varieties for three days significantly decreased phytic acid. Reduction of phytate and tannin contents of legumes during soaking may be attributed to leaching out in the soaking water (Kataria *et al.*, 1988 and Kataria *et al.*, 1989).

Effect of Domestic Processing on Protein Content and Availability: Fig. 2 (a and b) summarizes the protein content and in vitro protein digestibility (IVPD) of treated and untreated lupin seeds of Dongola and Golo cultivars. The protein content of Dongola cultivar was found to be 44.7% (Fig.2a) while that of Golo was 41.6% (Fig. 2b). Processing methods of lupin seeds were found to increase the protein content for both cultivars. Soaking of seeds followed by dehulling significantly ($P = 0.05$) increased the protein content for both cultivars compared to other processing methods. The increment in protein content of the processed sample may be due to quantitative reduction of the antinutritional factors (tannin and phytic acid) and other water soluble constituents. Results obtained agreed with those reported by Elamin (1996) who found that debittering of lupin increased the protein content of the seeds. *In vitro* protein digestibility (IVPD) of Dongola cultivars was found to be 72.6 % (Fig.2a), while that of Golo was 74.8 % (Fig.2b). Processing of lupin seeds was observed to increase IVPD for both cultivars. Soaking of whole seeds significantly ($P = 0.05$) increased IVPD of cultivar Dongola IVPD of Golo cultivar was significantly ($P = 0.05$) increased (89.4%) (Fig. 2a), after soaking and dehulling of seeds (Fig.2b). The increment in IVPD after dehulling may be attributed to the removal of tannin and fibre which are mainly located in the seed coat (Alonso *et al.*, 2000 and Bressani *et al.*, 1984). The increment in IVPD of faba bean seeds after soaking was reported by Babiker and El Tinay (1993). Kataria *et al.* (1989) reported that soaking of seeds followed by cooking in distilled water improved IVPD of mung bean seeds.

Effect of Domestic Processing on Total and Available Some Major Mineral of Lupin Seeds: Figs 3 and 4 show total and available major mineral, of treated and untreated lupin seeds of the cultivars Dongola and Golo. Calcium (Ca) content of Dongola cultivar was found to be 105.3 mg/100g (Fig.3a) and out of this amount about 75% was found to be available (Fig.3b). Soaking of the whole seeds and soaking followed by dehulling of the seeds was significantly ($P = 0.05$) increased Ca content of cultivar Dongola and was found to be 158.9 and 155.9 mg/100g respectively, while other processing methods gradually decreased the total Ca which was ranged between 78.2 and 104.7 mg/100g (Fig.3a). Processing of lupin seeds significantly ($P = 0.05$) decreased the available Ca of Dongola cultivar and was ranged between 50 and 75% (Fig.3b). Total Ca of Golo cultivar was found to be 79.8 mg/100g (Fig.4a) and out of this amount about 66.7% was available (Fig.4b). Processing methods of lupin seeds significantly ($P = 0.05$) increased Ca content of Golo cultivar. Soaking of the whole seeds followed by cooking was significantly ($P = 0.05$) increased Ca content of Golo cultivar and was found to be 132.3 mg/100g, compared to other processing methods. Soaking of whole seeds and Soaking followed by cooking and dehulling significantly

($P = 0.05$) increased the available Ca of Golo cultivar and was found to be 75 and 74.6% (Fig.4b), while other processing methods caused a reduction in Ca availability to 50-60% (Fig.4b). For Dongola cultivar reduction in total Ca may be attributed to washing out of minerals during soaking. Moreover, cooking expected to concentrate Ca of the seeds. Therefore, the amount of Ca is expected to increase. The available Ca for both cultivars fluctuated. Some processing methods caused an increment while others decreased the available Ca. Increment in Ca availability maybe due to qualitative as well as quantitative differences between Ca obtained after treatment. Reduction in available Ca is likely to be due to complexation of it with other food constituents. Results obtained of total Ca were lower than those reported by Hove (1974) who found that Ca content was ranged between 250 and 770 mg/100g for whole seeds and kernel for *L. angustifolious*, while the available Ca on the present work was higher than that obtained by Sahuquillo *et al.* (2003) who found that the available Ca of Lentils was 46.6%. Bioavailability of Ca enhanced significantly when pigeon pea seeds were processed and cooked (Duhan *et al.*,1999). Sodium (Na) content of Dongola Cultivar was found to be 1.1 mg/100g (Fig.3a), out of this amount about 68.8% was found to be available (Fig.3b). Soaking of the whole seeds significantly ($P = 0.05$) decreased Na content of the cultivar Dongola and was found to be 0.9 mg/100g, while other processing methods increased it and was ranged between 1.1 and 1.7 mg/100g (Fig.3a). Soaking of Seeds followed by dehulling significantly ($P = 0.05$) increased Na availability of the cultivar to 72.1%, while other processing methods decreased it and was ranged between 28.6 and 58.5%(Fig3b). Fig. 4 (a and b) shows the total and available Na of Golo Cultivar, Na content of the cultivar, was 0.5 mg/100g and out of this amount about 38.95 was found to be available. Soaking of the whole seeds followed by cooking reduced total Na and was found to be 0.4 mg/100g, while other processing methods significantly ($P = 0.05$) increased it in the ranged of 0.9 and 1.6 mg/100g (Fig.4a). Processing of the seeds gradually increased the available Na of the cultivar. However, soaking followed by cooking and dehulling significantly ($P = 0.05$) decreased Na availability to 33.6% (Fig. 4b). Reduction in total Na may be attributed to washing out of the mineral during soaking. Moreover, cooking is expected to concentrate Na of the seeds. Therefore, the amount of Na is expected to increase. Increment in Na availability maybe due to qualitative as well as quantitative differences between Na obtained after treatment. Reduction in available Na is likely to be due to complexation of it with other food constituents. Results obtained were lower than those reported by Malik *et al.* (2004) who reported that total and available Na of grapefruit was 15.2 mg/100g and 86.2%. Kalil (2001) reported that soaking and cooking of guar and faba bean significantly ($P = 0.05$) decreased the content of Na. Potassium (K) content of Dongola cultivar was found to be 18.2 mg/100g (Fig.3a) and about 30.8% was found to be available (Fig.3b). Soaking of whole seed followed by cooking slightly decreased K content of the cultivar and was found to be 17 mg/100g, while other processing methods significantly ($P = 0.05$) increased total K in the range between 19.6 and 27.1 mg/100g (Fig.3a). Processing of the seeds significantly ($P = 0.05$) decreased the available K of Dongola cultivar in the range between 16.6 and 30.7 (Fig.3b). Potassium (K) content of Golo cultivar was found to be 17.0 mg/100g (Fig.4a) and out of this amount about 31.3% was found to be available (Fig.4b). Soaking of seeds followed by dehulling significantly ($P = 0.05$) increased K content, while soaking followed by cooking or dehulling significantly ($P = 0.05$) decreased total K of the cultivar to 4.0 and 2.6 mg/100g (Fig.4a). Processing of the seeds significantly ($P = 0.05$) decreased the available K in the range between 13.9 and 30.9 % (Fig.4b). Results obtained agree with Kalil (2001) who reported that soaking and cooking of guar and faba bean significantly ($P = 0.05$) decreased the K content. Reduction in K might be attributed to the leaching of it in soaking water. Results of total K obtained were slightly higher than those obtained by Malik *et al.* (2004) who found that the available K of grapefruit and orange was 16.9 and 16.7 % respectively. Phosphorous (P) content of Dongola cultivar was found to be 3.8 mg/100g (Fig.3a) and about 20.5% of this amount was found to be available (Fig.3b), while that of Golo was 4.6 mg/100g (Fig.4a) and out of this amount about 14.2% was available (Fig.4b). Processing of the seeds gradually increased P content, while available P for both cultivars was significantly ($P = 0.05$) decreased. Soaking of seeds followed by dehulling significantly ($P = 0.05$) increased P content for both cultivars to 8.4 mg/100g (Fig.3a) and 11.1 mg/100g (Fig.4a) compared to other processing methods. Soaking of seeds followed by cooking or dehulling significantly ($P = 0.05$) decreased P availability for both cultivars to 5.4 % (Fig.3b) and 5.8% (Fig.4b). These results were lower than those obtained by Hove (1974) who found that total P of whole seed and kernel of legumes was 15 and 16 mg/100g. Saharan *et al.* (2001) reported that dehulling and soaking of faba bean seeds decreased P content. Duhan *et al.* (2002) reported that availability of P improved significantly when pigeon pea seeds were soaked in water. Malik *et al.* (2004) found that the available P of grapefruit and orange was 15.9 and 19.0%, respectively.

Effect of Domestic Processing on Total and Available Some Trace Mineral of Lupin Seeds: Fig. 5 and 6 show total and available trace mineral of treated and untreated lupin seeds. Manganese (Mn) content of Dongola cultivar was found to be 57.7 mg/100g (Fig.5a) and out of this amount about 8.8% was found to be available (Fig.5b). Soaking

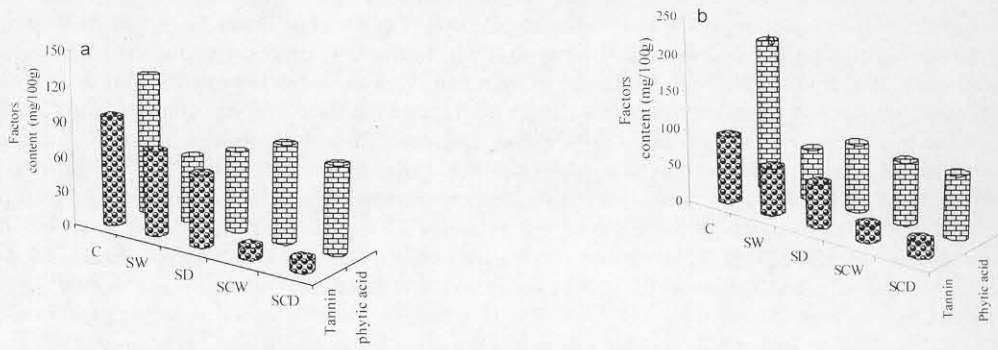


Fig. 1: Tannin and phytic acid contents (mg/100g) of a. Dongola and b. Golo cultivars.

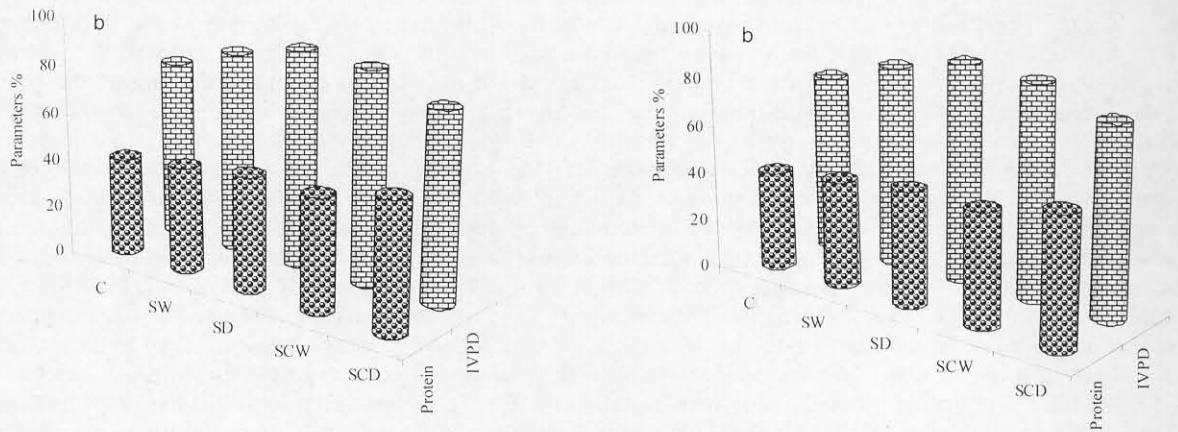


Fig.2: Protein content and in vitro protein digestibility (IVPD) of a. Dongola and b. Golo cultivars.

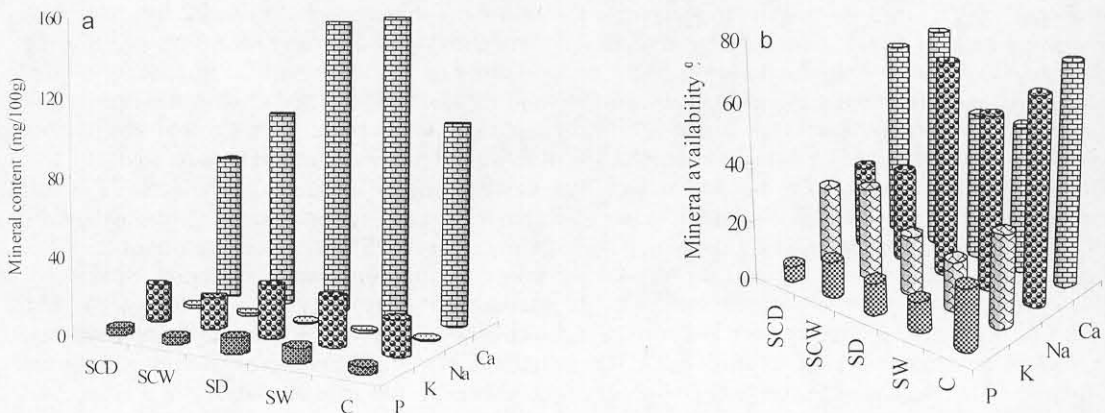


Fig. 3. Major minerals a. content (mg/100g) and b. availability (%) of Dongola cultivar.

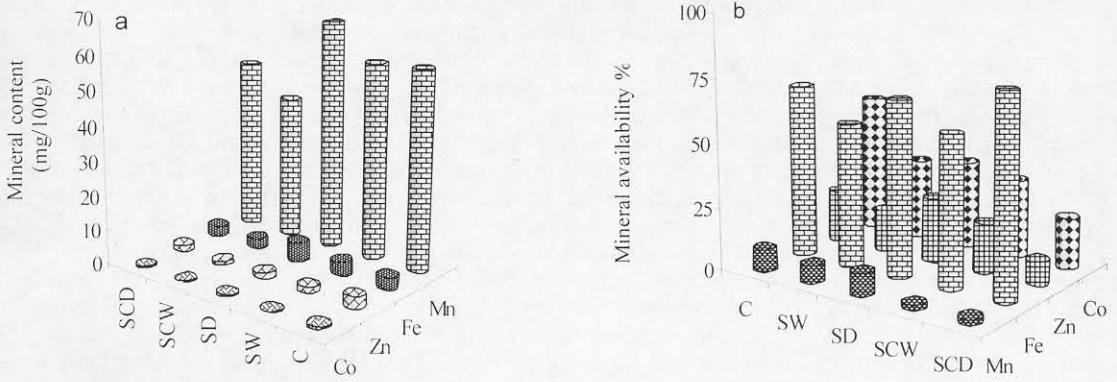


Fig. 4: Major minerals a. content (mg/100g) and b. availability (%) of Golo cultivar.

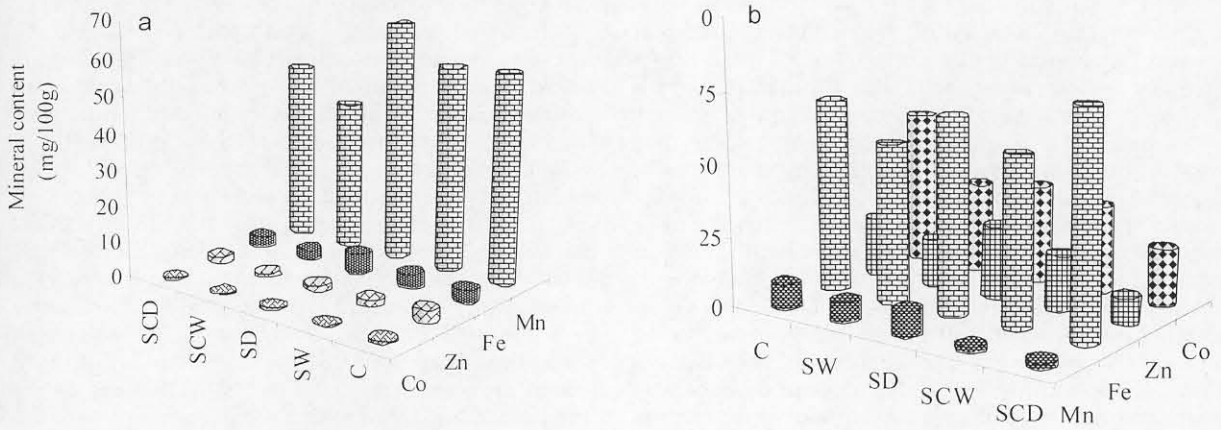


Fig. 5: Minor minerals a. content (mg/100g) and b. availability (%) of Dongola cultivar.

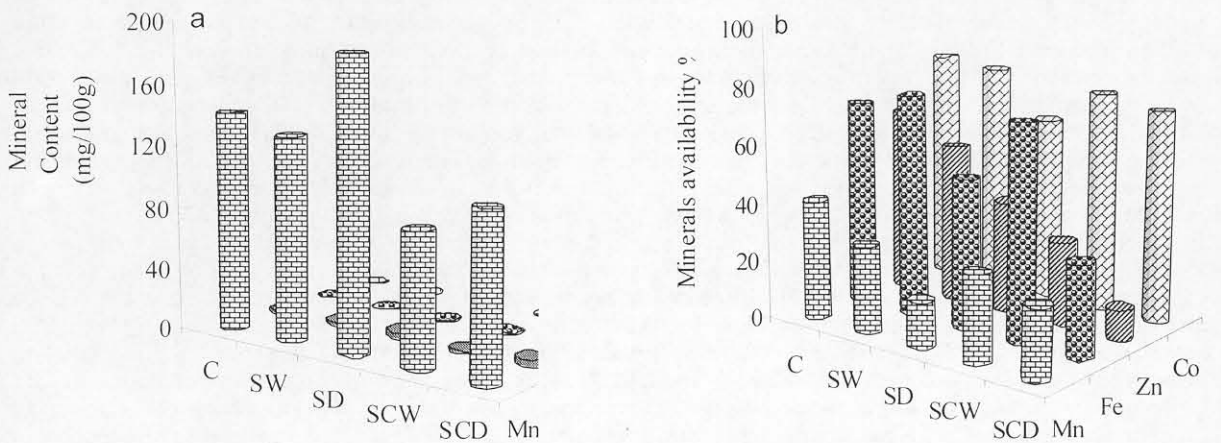


Fig. 6: Minor minerals a. content (mg/100g) and b. availability (%) of Golo cultivar.

Appreviations:

C = Control whole seeds, SW = Soaked whole seeds, SD = Soaked dehulled seeds, SCW = Soaked cooked whole seeds and SCD = Soaked cooked dehulled seeds.

of seeds followed by dehulling significantly ($P = 0.05$) increased the total and available Mn of the cultivar to 66.6 mg/100g (Fig.5a) and 9.1% (Fig.5b), while other processing methods significantly reduced total and available Mn in the range between 41.6 and 56.9 mg/100g (Fig.5a) and 2.1 and 7.3% (Fig.5b), respectively. Mn content of Golo cultivar was found to be 142.7 mg/100g (Fig.6a) and about 41.4 % of this amount was found to be available (Fig.6b). Soaking of the seeds followed by dehulling significantly ($P = 0.05$) increased Mn content to 188 mg/100g, compared to other processing methods which were significantly decreased Mn content of the cultivar (86.6 and 132.3 mg/100g). Processing of the seeds gradually decreased the available Mn of Golo cultivar (Fig.6b). For both cultivars reduction in total Mn may be attributed to washing out of the mineral during soaking. Reduction in available Mn is likely to be due to complexation with other food constituents. Soaking and cooking of guar and faba bean seeds significantly decreased the content of Mn (Khalil, 2001). Khetarpaul and Chauhan (1989) reported that germination and fermentation significantly increased the available Mn. Results obtained for Mn content of the present work were higher than those obtained by Malik *et al.* (2004) who found that Mn content of grapefruit and orange was 0.19 and 0.23 mg/100g, while the available Mn of the fruits were higher than those of lupin seeds. Iron (Fe) content of Dongola cultivar was found to be 3.6 mg/100g (Fig.5a) with available amount of 68.4% (Fig.5b). Soaking of whole seeds and soaking of seeds followed by dehulling significantly ($P = 0.05$) increased Fe content and was found to be 4.0 and 5.6 mg/100g, respectively. Soaking of whole seeds followed by cooking and dehulling significantly decreased the total Fe and was found to be 3.2 mg/100g (Fig.5a). And significantly ($P = 0.05$) increased Fe availability of the cultivar to 78.2% (Fig.5b). Fe content of Golo cultivar was found to be 1.8 mg/100g (Fig.6a) and about 71.5 % was found to be available (Fig.6b). Processing of the seeds significantly ($P = 0.05$) increased total Fe of the cultivar. Soaking of seeds followed by cooking and dehulling significantly increased Fe content of the cultivar to 5.1 mg/100g (Fig.6a). Soaking of whole seeds and soaking of seeds followed by cooking significantly ($P = 0.05$) increased the available Fe to 76.9 and 74.1% respectively, while other processing methods significantly decreased the available Fe of the cultivar. Reduction in total Fe of the cultivar Dongola may be attributed to washing out of the mineral during soaking. The available Fe for both cultivars fluctuated. Some processing methods caused an increment while other decreased the available Fe. Reduction in available Fe is likely to be due to complexation of it with other food constituents. Soaking and cooking of guar and faba bean seeds significantly decreased the content of Fe (Khalil, 2001). Khetarpaul and Chauhan (1989) reported that germination and fermentation significantly increased the available Fe. Duhan *et al.* (1999) reported that domestic processing significantly ($P = 0.05$) increased the available Fe of pigeon pea seeds. Dehulling and soaking of rice bean and faba bean enhanced the available Fe, while there was a significant loss of total Fe (Saharan *et al.* 2001). Zinc (Zn) content of Dongola cultivar was found to be 3.3 mg/100g (Fig.5a) and about 20.8% was found to be available (Fig.5b). Processing methods of lupin seeds significantly ($P = 0.05$) decreased total Zn of the cultivar. Soaking of whole seeds followed by cooking decreased Zn content to 0.95 mg/100g (Fig.5a) of the cultivar compared to other processing methods. Soaking of seeds followed by dehulling significantly ($P = 0.05$) increased available Zn of the cultivar to 25.8%, while other processing methods caused reduction of available Zn (Fig.5b). Zn content of Golo cultivar was found to be 0.7 mg/100g (Fig.6a) and about 64.9% was found to be available (Fig.6b). Soaking of whole seeds decreased Zn content and was found to be 0.5mg/100g, while other processing methods significantly ($P = 0.05$) increased Zn content of the cultivar (Fig.6a). Soaking of seeds followed by cooking and dehulling decreased the available Zn of the cultivar to 10.8% compared to other processing methods (Fig.6b). For both cultivars reduction in total Zn may be attributed to washing out of the minerals during soaking. Reduction in available Zn is likely to be due to complexation of it with other food constituents. Results obtained of total Zn were lower than those reported by Hove (1974) who found that Zn content of legumes was 3.8 mg/100g for whole seeds and kernel, respectively. Kalil (2001) reported that soaking and cooking slightly decreased Zn content of guar and faba bean seeds. Kaur and Kawatra (2002) reported that dehulling, soaking and pressure cooking decreased Zn content of rice bean, while soaking and dehulling enhanced Zn availability. Cobalt (Co) content of Dongola cultivar was found to be 0.8 mg/100g (Fig.5a) and about 55.2% was found to be available (Fig.5b), while that of Golo was 0.5 mg/100g (Fig.6a) about 81.9% was found to be available (Fig.6b). Processing methods of lupin seeds significantly ($P = 0.05$) decreased Co content and the available Co for both cultivars. Soaking of whole seeds significantly ($P = 0.05$) decreased Co content for the cultivars compared to other processing methods and was found to be 0.2 mg/100g (Fig.5a) and 0.3 mg/100g (Fig.6a), for Dongola and Golo cultivars respectively. Soaking of seeds followed by cooking and dehulling significantly ($P = 0.05$) decreased Co availability to be 20.3% (Fig.5.b) of Dongola cultivar. For both cultivars reduction in total Co may be attributed to washing out of the minerals during soaking. Moreover, cooking is expected to concentrate Co of the seeds. Therefore, the amount of Co is expected to increase. Reduction in available Co is likely to be due to complexation of it with other food constituents. These results were lower than those obtained by Malik *et al.* (2004) who found that Co content of Grapefruit and Orange was 0.68 and 0.65 mg/100, while the available Co was higher than that of the fruits.

In Conclusion, domestic processing methods commonly applied to prepare lupin seeds meal, resulted in reduction of antinutritional factors such as tannin and phytic acid for the cultivars. Soaking of seeds in water greatly reduced phytic acid, while soaking of seeds followed by cooking or dehulling reduced tannin content. Quantitative reduction of tannin and phytic acid was found to be accompanied by improvement in protein quantity and availability as

evidenced by increase of IVPD for both cultivars. Most domestic processing methods improve minerals availability of lupin seeds for both cultivars. Due to high mineral and protein contents as well as high availability of such constituents after processing we recommended addition of treated lupin to both food and weaning foods to improve the nutritional quality.

References

- AOAC, 1970. Association of Official Agriculture Chemists. Official Methods of Analysis, 14th edn. Washington D.C.
- AOAC, 1984. Association of Official Agriculture Chemists. Official Methods of Analysis, 16th edn. Washington D.C.
- Abdulrahim, S. I. Elfadil, E. Babiker and A. H. El Tinay, 2004. Effect of domestic processing methods on antinutritional factor and in vitro protein digestibility of faba bean cultivars. Industrial Research J., in press.
- Alonos, R., A. Aguirre and F. Marzo, 2000. Effect of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. Food Chem., 68: 159 - 165
- Babiker, E. E. and A. H. Eltinay, 1993. Effect of reconstitution and Na₂CO₃ on Tannin content and in vitro protein digestibility of faba bean cultivars. Plant Food, for Human Nutrition, 44: 119 - 130
- Bressani, R., L. G. Elias and J. E. Braham, 1984. Efecto del descascado sorbe el valor proteinico y comolementario del frijol comun. INCAP in Forme Annual, 62 - 63
- Carter, C. G. and R. C. Hauler, 1999. Evaluation of phytase in Atlantic Salmon, Salmo salar L., Feeds containing fish meal and different plant meal. In fish meal replacement in Aquaculture feeds for Atlantic salmon. project 93/120. Fisheries research and Development Corporation, Canberra, Australia, 34 - 45
- Champman, H. D. and F. P. Pratt, 1961. Calcium and magnesium by titration methods" methods of analysis for soil, plants and water- California Univ., Public Division of Agric. Sci., 20
- Champman, H. D. and F. P. Pratt, 1968. Ammonium molybdate - Ammonium vanadate method for determination of phosphorous" methods of analysis for soil, plants and water- California Univ., Public Division of Agric. Sci., 169- 170
- Chauhan, B. M. and H. Mahjan, 1988. Effect of natural fermentation on the extractability of minerals from pearl millete flour. J. Food Sci., 53: 1576-1577
- Cook, J. D., M. B. Reddy, J. Burri, M. A. Juillerate, R. F. Hurrel, 1997. The influence of different cereal grains on iron absorption from infant cereal foods. Amjclin Nutr., 65: 964-969
- Duhan, A., N. Khetarpaul and S. Bishnoi, 2002. Changes in phytates and HCl-extractability of calcium iron and phosphorus of soaked, dehulled, cooked and sprouted pigeon pea cultivars (UPAS-120). Plant Food Hum Nutr., 57: 275-284
- Eldash, A. A. and V. C. Sgarbieri, 1980. Sweet lupin-fortified bread: nutritional value and amino acid content. Cereal Chemistry, 57: 9-11
- Frolish, W., 1995. Bioavailability of micronutrient in a biber rich diet, especially related to mineral. Eur., 49: 116-122
- Hove, E. L., 1974. Composition and protein quality and toxin of seeds of the grain legumes glycine max. Lupinus spp., Phaseolus spp., Pisumstaiyum spp. and Vicia faba. New Z. J. Agric., 21: 457-462
- Iorri, W. and U. Svanberg, 1995. An overview of the use of fermented food for child feeding in Tanzania. Eco. Food Nutr. 34:65-81
- Kalil, M. M., 2001. Effect of soaking, germination, autoclaving and cooking on chemical and biological value of Guar compared with faba bean. Nahrung /Food, 4: 246-250
- Kataria, A., B. M. Chauhan and D. Puria, 1988. Effect of domestic processing and cooking on the anti-nutrients of blackgram. Food Chem., 30: 149- 156
- Kataria, A., B. M. Chauhan and D. Puria, 1989. Anti-nutrients and protein digestibility (in vitro) of Mung bean as affected by domestic processing and cooking. Food Chem., 32: 9-17
- Kaur, M. and B. L. Kawatra, 2002. Effect of domestic processing on zinc bioavailability from ricebean (Vigna umbellate) diets. Plant Food Hum. Nutr., 3: 307-318
- Khetarpaul, N. and B. M. Chauhan, 1989. Effect of Germination and pure culture fermentation on HCl-extractability of minerals of millet (Pennisetum Typhoideum). J. Food Sci. and Technol., 24: 327-331
- Malik, I. O., E. Elfadil Babiker, E. Nabila Yousif and H. Abdullahi, El tinay, 2004. In vitro availability of minerals of some tropical and citrus fruits as influenced by antinutritional factors. Nahrung/food, 48: 65-68
- Moelijopawiro, S., Mt. Fields and D. Gordon, 1998. Bioavailability of zinc in temented sayabeans. J. Food Sci., 53: 460-463
- Mosha, Ac. and U. Svanberg, 1990. The acceptance and food intake of bulk reduced weaning. The liganga village study. Food Nutr. Bulletin, 12: 69-74
- New, M. B. and I. Csavas, 1993. A summary of information on aquafeed production in eleven Asian Countries, 397-419
- Ologhobo, D. A. and L. B. Fetuga, 1984. Effect of processing on the trypsin inhibitors haemagglutinin, tannic acid and phytic acid contents of seeds of 10 cowpea varieties. Tropical Agric. (Trinidad) 61: 261-264
- Pearson, D., 1981. Pearson's chemical analysis of foods-H. Egan, R.S. Kirk. And R. Sawyer (eds) 18th ed., London, New York
- Petterson, D. S., 2000. The use of lupins in feeding system-Review. Australian J. Animal Sci., 13: 86 - 88
- Petterson, D. S., 1998. Composition and food uses of lupin. In : Lupins as crop plants; Biology, Production and Utilisation (Glasdstones, JS. Atkins, CA and Hamblin, J Eds.) CAB International, 353 - 384
- Petterson, D. S., S. Sipsa and J. B. Mackintosh, 1997. The chemical composition and nutritional value of Australian pluses. Grains research and development corporation, Canbrrre, Australia, 65
- Price, M. L., S. Van Socoyoc and L. G. Butter, 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghumgrain, Agric. Food. Chem., 26: 1214-1218
- Saharan, K., N. Khetarpaul and S. Bishnoi, 2001. Processing of newly released ricebean and faba bean cultivars changes in total and available calcium, iron and phosphorus. Int. J. Food Sci. Nutr., 52: 413-418
- Sahuquillo, A., Reyes Barbera and Rosaura Farre, 2003. Bioaccessibility of calcium, iron and zinc from three legume samples. Nahrung/food, 47: 438-441
- Sauders, R. M., M. A. Conner, A. N. Booth, E. M. Bickott and G. O. Kohler, 1973. Measurements of digestibility of alfalfa proteins concentrate by in vivo and in vitro methods, J. Nutr., 103: 530-535
- Wheeler, E. I. and R. E. Ferrel, 1971. Methods for phytic acid determination in wheat and wheat fractions. Cereal Chem., 48: 312-320
- WHO, 1998. Complementary feeding of young children in developing countries: A review of current scientific knowledge, Geneva, 432-543