Effect of Detoxification Treatment on the Physicochemical Properties of Linseed Proteins

K. T. Madhusudhan* and Narendra Singh

The physicochemical properties and in vitro digestibility of proteins of raw and detoxified linseed meal were compared. There was a 30% decrease in available lysine content and a 38% increase in in vitro digestibility. There was no significant change in the amino acid composition on detoxification. The high molecular weight protein from the detoxified meal was dissociated.

Linseed is one of the five major oilseed crops of India. The commercial deoiled linseed meal contains 25–35% protein. In spite of acceptable amino acid composition, the utilization of linseed meal is limited to livestock feed due to the presence of antinutritional factors (Madhusudhan, 1984). The nutritional and protein quality of linseed meal tested on rats and chicks has been reported (Mandokhot and Singh, 1979, 1983a, 1983b). Different detoxification treatments such as water treatment or dry or wet autoclaving and acid treatment followed by wet autoclaving have been reported (McGinnis and Polis, 1946; Kratzer, 1947; Singh and Punia, 1979; Mandokhot and Singh, 1983a). In an earlier paper, we reported the preparation of defatted linseed meal, which involves demucilaging of water-soaked linseed by a fruit pulper followed by flaking the demucilaged seed and separation of the hulls from the cotyledons after oil extraction. Also, the physicochemical properties of proteins of raw linseed meal were reported (Madhusudhan and Singh, 1983). We have also reported detoxification of linseed meal by a water boiling method and the functional properties of raw and detoxified meals have been determined (Madhusudhan and Singh, 1985). Since the functional properties are influenced by the physicochemical properties of proteins, the physicochemical properties of linseed proteins of raw and detoxified meal have been determined and are reported here.

MATERIALS AND METHODS

Linseed, Khategaon variety, was purchased from M/s Flour and Foods Ltd., Indore, India. Defatted linseed meal was prepared as reported earlier (Madhusudhan and Singh, 1983).

Water-boiled linseed meal was prepared as follows: Defatted linseed meal (30 mesh) was added to boiling water at 5 times the weight of meal, and boiling was continued for 15 min. After cooling the slurry to about 50 °C, extra water was added at 15 times the weight of meal taken, amounting to a total ratio of meal to water of 1:20. The slurry was centrifuged (Westfalia, W. Germany) at 12000 rpm. The resultant wet sludge was dried at 40 °C in a vacuum shelf drier (F. J. Stokes Machinery Co.) and passed through 60-mesh (BSS) sieve.

Analyses. Crude protein (N × 6.25), crude fat, ash, and crude fiber contents of meal samples were estimated by AOAC (1980) methods.

Available Lysine Estimation. The available lysine content of the meal samples was estimated by the corrected straight acid procedure of Carpenter (1960) using 2,4-dinitro-1-fluorobenzene (Fluka). DNP–lysine was used as the standard for the assay.

Amino Acid Analysis. The meal samples were hydrolyzed in 6 N HCl containing 0.1% phenol and 0.01% β-mercaptoethanol at 110 °C in vacuo for 24, 48, and 72 h according to Moore and Stein (1963). The acid was evaporated under reduced pressure, and amino acid analysis was done with a Yanagimoto LC-5S automatic amino acid analyzer. The results are the average of duplicate determinations.

In Vitro Digestibility. The method of Villegas et al. (1968) using the pepsin–pancreatin enzyme system was used. The digested protein, correlated to the total protein, was expressed as percent digestibility value.

Protein Concentration. This was determined by measuring the absorbance of the protein solution at 280 nm for raw linseed meal and at 272 nm for water-boiled linseed meal. $E^{1\%}_{1cm}$ of the proteins was established by plotting the absorbance at 280 or 272 nm vs. crude protein concentration, determined by micro Kjeldahl method. A factor of 6.25 was used to convert the nitrogen to protein content. The protein solution was prepared by extracting the meal with 1 M NaCl and extensively dialyzing the extract against the same solution.

Gel Filtration. Sepharose 6B (Pharmacia Fine Chemicals) that had been equilibrated with 1 M NaCl was packed into a column (1.8 × 90 cm). The proteins, extracted with 1 M NaCl and dialyzed against 1 M NaCl, were loaded on the column and eluted at a flow rate of 21 mL/h. Fractions of 3 mL were collected in an automatic fraction collector, and the absorbance of the fractions was monitored at 280 or 272 nm.

Ion-Exchange Chromatography. DEAE Sephadex A50 (Sigma Chemical Co.) gel was packed in a column (2.2 × 25 cm) and was equilibrated with 0.01 M phosphate buffer of pH 7.5. The proteins, extracted with 1 M NaCl and dialyzed against phosphate buffer, were loaded on the column and eluted with a linear gradient of 0–0.8 M NaCl in buffer. Fractions of 4 mL were collected in an automatic fraction collector, and the absorbance of the fractions was monitored at 280 or 272 nm. The NaCl concentration was estimated by Mohr's method (Kolthoff and Stenger, 1947).

Polyacrylamide Gel Electrophoresis (PAGE). These experiments were done in 7.5% gels in 0.05 M phosphate buffer of pH 7.6. Gels were stained by Coomassie Brilliant Blue R 250 and scanned in a Joyce Loebl Chromoscan 200. The sample was prepared by extracting linseed meal in 1 M NaCl and extensively dialyzing the protein extract against phosphate buffer.

Ultracentrifugation. The sedimentation velocity experiment was performed with 1.5% protein solution in 1 M NaCl solution at 27 °C at 59780 rpm in a Spinco Model E analytical ultracentrifuge equipped with rotor temperature indicator and control (RTIC) system and phase plate schlieren optics. $S_{20,w}$ values were calculated from the photographs by the standard procedure (Schachman, 1959).

Subunit Composition. The subunit composition of linseed proteins was determined by sodium dodecyl sulfate (SDS) PAGE by the method of Weber and Osborn (1969)

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0021-8561/85/1433-1219$01.50/0

Table I. Proximate Composition of Raw and Water-Boiled Linseed Meal[a]

constituent, %	raw[b]	water-boiled[b]
crude protein (N × 6.25)	52.7 ± 0.608	54.7 ± 0.608
crude fat	0.32 ± 0.017	0.15 ± 0.020
crude fiber	11.9 ± 0.264	17.5 ± 1.044
ash	6.7 ± 0.264	7.6 ± 0.100
carbohydrate (by diff)	28.3	20.05

[a] On moisture-free basis. [b] Results are expressed as means ± standard deviation.

Table II. Amino Acid Composition of Raw and Water-Boiled Linseed Meal[a]

	raw		water-boiled	
amino acid	mol %	g/16 g of N	mol %	g/16 g of N
Asp[b]	9.4	11.2	9.7	9.9
Thr[c]	4.3	3.9	4.6	4.2
Ser[c]	6.4	5.1	6.6	4.9
Glu[d]	18.0	19.8	16.7	19.5
Pro	4.9	4.6	4.8	4.3
Gly	11.5	4.8	10.7	5.5
Ala	6.9	4.3	7.1	4.7
Val	5.9	5.6	6.4	5.7
$^1/_2$ Cys[c]	0.9	1.4	0.7	1.4
Met[c]	1.3	1.7	1.8	1.9
Ile	4.4	4.6	4.6	4.7
Leu	6.3	5.8	6.5	6.7
Tyr	1.9	3.3	2.3	3.4
Phe	4.0	5.9	4.0	5.2
Lys	3.8	4.1	4.0	4.5
His	1.9	2.5	1.9	2.3
Arg	8.3	11.5	7.7	10.9

[a] Average of duplicate determinations. [b] Includes aspargine. [c] Values at 0 h of hydrolysis by extrapolating 24, 48, and 72 h of hydrolysis values. [d] Includes glutamine.

using 10% gels. The gels were stained by Coomassie Brilliant Blue R 250 and scanned in a Jayce Loebl Chromoscan 200.

Absorption and Fluorescence Spectra. The absorption and fluorescence spectra were recorded in a Perkin-Elmer double-beam recording spectrophotometer, Model 124, and a Perkin-Elmer fluorescence spectrophotometer, Model 203, respectively, at 28 °C in 1 M NaCl solution.

RESULTS AND DISCUSSION

The proximate composition of raw (LM) and water-boiled linseed meal (WB) is shown in Table I. The values reported for raw meal are in the range of literature values (Peterson, 1958; Mandokhot, 1974). There was not much difference in the protein content of the two samples. An increase in crude fiber and a decrease in carbohydrate content of WB may be due to leaching of soluble constituents. The slight reduction of crude fat may be due to the protein–lipid interaction during heat processing leading to nonextractable complexes.

The amino acid composition of LM and WB is shown in Table II. Again, there was not much difference in the amino acid composition of the two samples but for a small decrease in the leucine content of WB. The values reported here for LM are generally in the range of reported values (VanEtten et al., 1967; Sarwar and Sosulski, 1973; Mandokhot, 1974). The higher content of phenylalanine, 5.9 g/16 g of N, as against the reported values 2.5–4.9 g/16 g of N may be due to varietal variation.

The available lysine content of LM and WB was 3.0 and 2.1 g/100 g of protein, respectively, showing that the available lysine was reduced by 30% due to heat processing. This could be due to the reaction of ϵ-amino groups of lysine with the aldehyde groups of carbohydrates

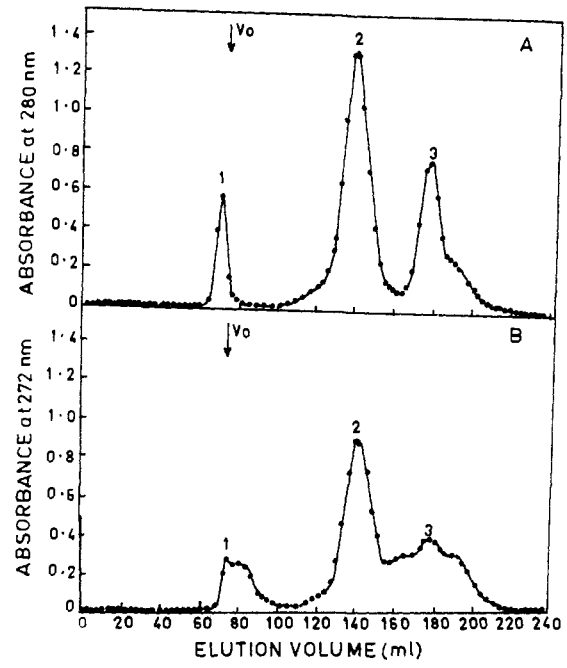

Figure 1. Gel filtration pattern of linseed proteins on Sepharose 6B-100: A, raw linseed meal; B, water-boiled linseed meal.

(Hodge, 1953). A reduction in available lysine content due to heat treatment has been reported in a number of proteins (Carpenter and Booth, 1973; Hurrel and Carpenter, 1974).

The in vitro digestibility measurements gave values of 61% and 84% for LM and WB, respectively. The increase in digestibility of 38% may be due to heat denaturation of proteins. This increase may also be due to the leaching or inactivation of antinutritional factors such as trypsin inhibitor or HCN present in LM (Madhusudhan and Singh, 1983). Several workers have reported an increase in the digestibility due to the inactivation of trypsin inhibitors in legumes or oilseeds (Ekpenyong and Borchers, 1979; Liener, 1976, 1979; Liener and Thompson, 1980).

The gel filtration pattern of LM and WB proteins is shown in Figure 1 (parts A and B). The pattern of LM proteins consisted of three peaks eluting at a V_e/V_o of 1.01, 1.89, and 2.40, and their proportions were 3, 67, and 30%, respectively. This is similar to the pattern reported earlier (Madhusudhan and Singh, 1983). The pattern of WB was similar to that of LM, and there was no significant change in the proportion of the peaks compared to LM proteins. However, the shape of peak 3 of WB proteins was considerably changed in comparison to LM pattern. Peak 3 of LM consists of low molecular weight proteins; LM showed a single symmetrical peak with a hump, whereas this peak of WB showed two humps on either side, indicating the dissociation of the high molecular weight proteins. There was no change in the absorption maximum of peaks 1 and 2 of WB proteins, relative to LM proteins. But, the third peak of WB had an absorption maximum at 272 nm and that of LM at 280 nm.

The DEAE Sephadex chromatographic pattern of WB proteins is shown in Figure 2 (parts A and B). Although both patterns consisted of four peaks, there were minor differences between the two proteins. The major peak of WB proteins eluted at the same salt concentration as that of LM proteins. In the pattern of LM proteins, the low molecular weight basic proteins corresponded to peaks 2 and 3, whereas the high molecular weight protein complex corresponded to peak 1 (Madhusudhan and Singh, 1983). The reduction in the proportion of peak 3 (of LM protein pattern) in the WB proteins may be due to nonextracta-

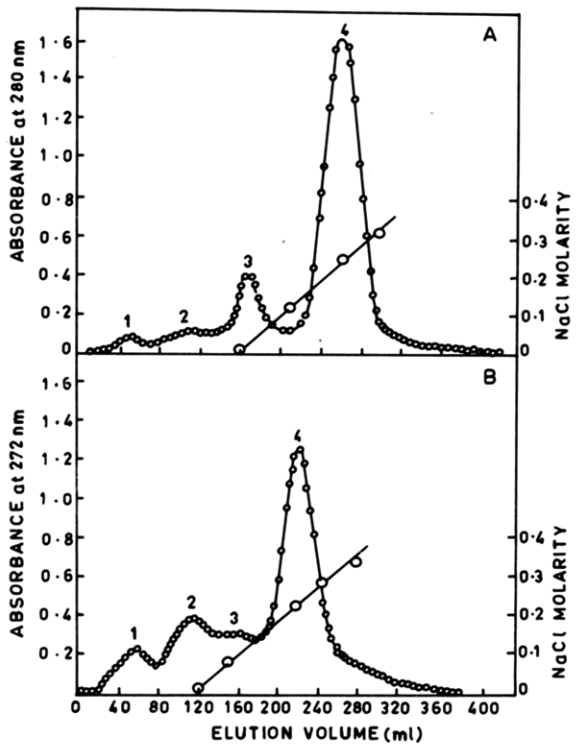

Figure 2. DEAE Sephadex chromatographic pattern of linseed proteins: A, raw linseed meal; B, water-boiled linseed meal.

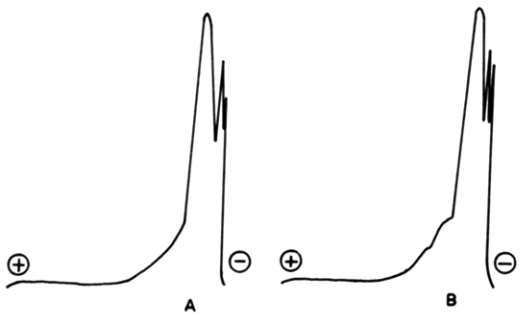

Figure 3. Densitometric scan tracings of PAGE pattern of linseed proteins in 0.05 M phosphate buffer: A, raw linseed meal; B, water-boiled linseed meal.

bility of this component due to heat denaturation or leaching of this component during water boiling.

The electrophoretic pattern of LM and WB proteins showed similarity to each other except for the appearance of two faint and fast-moving bands in WB proteins (Figure 3, parts A and B); this could be due to dissociated proteins.

The sedimentation velocity pattern of WB proteins consisted of four peaks with $S_{20,w}$ values of 1.9, 5.1, 7.7, and 8.4, and that of LM proteins consisted of four peaks with $S_{20,w}$ values of 1.4, 5.0, 9.0, and 14.0 (Figure 4). The $S_{20,w}$ values reported for LM are in agreement with the values reported earlier (Madhusudhan and Singh, 1983). The reduced $S_{20,w}$ value of high molecular weight proteins of WB could be due to dissociation of proteins.

The SDS PAGE experiment was performed to determine whether any significant difference in subunit composition of proteins due to detoxification exists. Both LM and WB patterns showed 11 bands each. However, the area under peak 10 was considerably reduced in WB proteins in comparison to LM proteins.

The $E^{1\%}_{1cm}$ of LM and WB proteins was 10.1 and 9.6, respectively, in 1 M NaCl solutions. The absorption spectrum of LM and WB proteins is shown in Figure 5. The absorption maximum of WB proteins shifted to lower

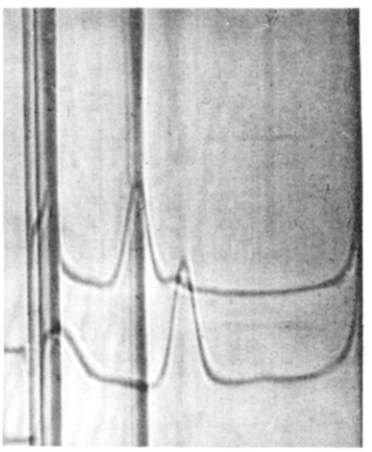

Figure 4. Sedimentation velocity pattern of linseed proteins: top, water-boiled linseed meal; bottom, raw linseed meal. Conditions: photograph taken 30 min at 59 780 rpm; bar angle 70°; sedimentation proceeding from left to right.

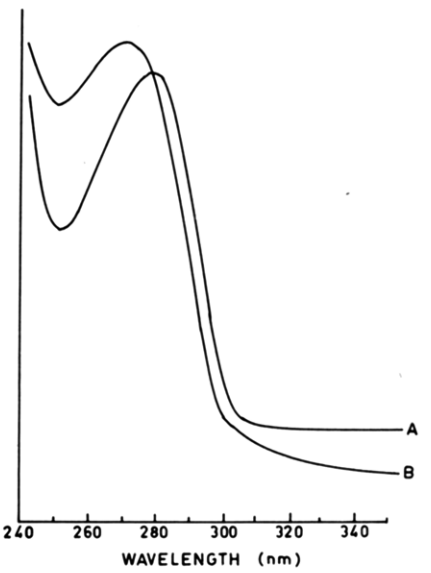

Figure 5. Ultraviolet absorption spectra of linseed proteins in 1 M NaCl: A, raw linseed meal; B, water-boiled linseed meal.

wavelength, i.e. 272 nm in contrast to the absorption maximum of 280 nm for LM proteins. The absorption minimum for both the samples was at 250 nm. There was no difference in the phosphorus content of proteins. But the carbohydrate content of WB proteins was 7% in contrast to the value of 5% obtained with LM proteins. Heat treatment might have led to the interaction of proteins with other constituents of meal, notably carbohydrates, resulting in the formation of complexes altering the spectrum. Such an observation was also reported in guar meal proteins (Nath et al., 1981), groundnut proteins (Srikanta and Narasinga Rao, 1974), and sunflower proteins (Madhusudhan et al., 1985). There was no difference in the fluorescence emission maximum of LM and WB proteins.

The results suggest that no changes occurred in the amino acid composition of linseed meal proteins due to detoxification treatment. However, dissociation of the major protein fraction and an increase in low molecular weight proteins due to heat treatment occurred. This may be responsible for the increased in vitro digestibility of WB.

ACKNOWLEDGMENT

The authors thank Dr. M. S. Narasinga Rao for his valuable suggestions. K.T.M. thanks the Council of Sci-

entific and Industrial Research, New Delhi, India, for the award of a Senior Research Fellowship.

Registry No. L-Lysine, 56-87-1.

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Received for review March 4, 1985. Revised manuscript received August 14, 1985. Accepted September 3, 1985.

Effect of Heat Treatment on the Functional Properties of Linseed Meal

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Nitrogen solubility, fat and water absorption capacity, bulk density, foam capacity and stability, and emulsification capacity of raw linseed meal (LM) and water-boiled linseed meal (WB) were determined and compared with those of defatted soybean meal (SM). Water boiling reduced the nitrogen solubility of linseed meal in water, NaCl, and sodium hexametaphosphate. The water absorption capacity of LM was 345 g compared to 443 g of WB whereas the fat absorption capacity of LM and WB was 236 and 167 g/100 g of flour, respectively. On the other hand, SM exhibited lower water (305 g) and fat absorption (167 g) than LM. SM showed higher foam capacity and emulsification capacity than LM. The foam stability of LM was better than that of SM. Heat processing diminished the foam capacity and stability and emulsification capacity of linseed meal.

The protein flours derived from nonconventional sources must possess appropriate interaction characteristics with other components of food (e.g., water, lipid) to facilitate their incorporation in less expensive food formulations and for extending traditional foods (Kinsella, 1982). In this laboratory, a systematic study on detoxification of linseed meal, isolation, characterization, and physicochemical properties of total proteins, and 12S and 1.6S proteins of linseed meal has been carried out. Water boiling of linseed meal was found to remove the toxic constituents as tested by chick experiments (Madhusudhan and Singh, 1983; Madhusudhan and Singh, 1985; Madhusudhan and Singh, 1985a; Madhusudhan et al., 1984).

Though there are a few reports on the use of linseed in dairy and bakery industries (Strobele, 1970; Steller, 1971; Trinkl, 1971), no information is available on the functional properties of linseed proteins. In this study, the functional properties of raw and water-boiled linseed meal are compared with those of soybean meal.

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MATERIALS AND METHODS

Linseed, Khategaon variety, was purchased from M/s Flour and Foods Ltd., Indore, India.

Defatted linseed meal was prepared as described earlier (Madhusudhan and Singh, 1983). Water-boiled linseed meal was prepared as follows: Defatted linseed meal (30 mesh) was added to boiling water, 5 times the weight of meal, and boiling was continued for 15 min. Water was added to this mixture, 15 times the weight of meal taken, amounting to a total ratio of meal to water of 1:20. The slurry was centrifuged (Westfalia Separator, W. Germany) at 12 000 rpm, and the resultant wet sludge was dried at 40 °C under a vacuum shelf drier (F. J. Stokes Machinery Co.) and passed though 60-mesh (BSS) sieve. Defatted soybean meal, used for comparison, was prepared from Bragg variety, after dehulling, flaking, and defatting with food grade hexane at ambient temperature. The defatted flakes were passed through a 60-mesh (BSS) sieve.

0021-8561/85/1433-1222$01.50/0