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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

The crude protein (N × 6.25) and crude fat contents were 52.7% and 0.32% for raw linseed meal (LM) and 54.7% and 0.15% for water-boiled linseed meal (WB), respectively. Defatted soybean meal (SM) had 48.5% crude protein and less than 1% crude fat. The crude protein and crude fat content of the samples were estimated according to AOAC (1980).

Nitrogen Solubility. Two grams of linseed meal was shaken with 20 mL of solvent [water, 0.5 M NaCl, 1.0 M NaCl, or 2% sodium hexametaphosphate (SHMP)], and the pH of the suspension was adjusted to the desired pH (in the range pH 1–12) by the addition of 5 N HCl or 5 N NaOH. The suspension was shaken mechanically at room temperature (~28 °C) for 1 h and centrifuged at 6000 rpm for 30 min, and the pH of the supernatant was noted. Aliquots of 5 mL of supernatant were taken for nitrogen estimation by the micro Kjeldahl method. The solubilized nitrogen was expressed as the percent of total nitrogen.

Water Absorption Capacity (WAC). This was determined by the method of Sosulski (1962) at room temperature. The values are expressed as grams of water absorbed by 100 g of meal or 100 g of protein.

Fat Absorption Capacity (FAC). This was determined by the method of Sosulski et al. (1976) using a 4-g meal sample and refined groundnut oil. The determinations were carried out at room temperature, and the values are expressed as grams of oil absorbed by 100 g of meal or 100 g of protein.

Bulk Density. This was determined by the method of Wang and Kinsella (1976), and the values are expressed as grams/milliliter of the sample.

Foam Capacity (FC) and Foam Stability (FS). A sample (2 g) of meal was blended with 100 mL of water in a Braun electric blendor. The suspension was whipped at 1600 rpm for 5 min. The mixture was poured into a 250-mL measuring cylinder, and the volume was recorded after 30 s. Foam capacity is expressed as percent increase in volume (Lawhon et al., 1972) by the formula

$$\text{FC} = \frac{\text{vol after whipping} - \text{vol before whipping}}{\text{vol before whipping}} \times 100$$

FC was determined as a function of pH and NaCl concentration. The foam volume was recorded at 5, 10, 20, 30, 45, 60, 90, and 120 min after whipping to determine FS according to Ahmed and Schmidt (1979):

$$\text{FS} = \frac{\text{foam vol after time } t}{\text{init foam vol}} \times 100$$

The effect of temperature on FC was studied by incubating the protein–meal mixture at a given temperature for 20 min. FS was determined as a function of pH and NaCl concentration.

Emulsification Capacity (EC). The method of Beuchat et at. (1975) was used for these measurements at room temperature. A 2-g meal sample and 23 mL of water or NaCl solution were blended for 30 s in a Braun electric blender at 1600 rpm. After complete dispersion, refined groundnut oil was added continuously from a buret and blending continued until there was a phase separation. This was observed visually. Emulsification capacity is expressed as milliliters of oil emulsified by gram of meal.

RESULTS AND DISCUSSION

Nitrogen Solubility. The nitrogen solubility vs. pH profile of LM in water showed a broad solubility minimum between pH 2.0 and 6.0 where solubility was ~20% (Figure 1, parts A and B). It increased above pH 6.0, and at pH 10.0, it was 80%. Below pH 2.0, solubility increased,

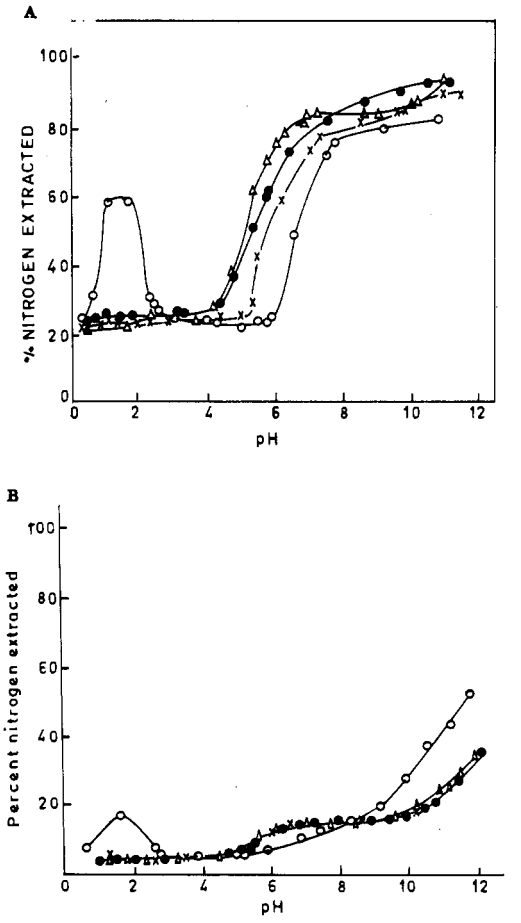

Figure 1. Nitrogen solubility vs. pH profile of (A) raw linseed meal (B) water-boiled linseed meal: ○, water; ●, 0.5 M NaCl; △, 1.0 M NaCl; ×, 2% SHMP.

and at pH 1.0, it was 50%. The solubility of WB was considerably lower at all pH values, and further, the pH range of solubility minimum was wider, i.e., pH 3.0–8.0. At pH 10.0, solubility was only 25% compared to 80% with LM. Heat treatment given to the meal in the preparation of WB reduced the solubility of the proteins. In the presence of NaCl, the solubility minimum of both LM and WB shifted to lower pH values. In the range pH 6.0–11.0, nitrogen solubility of LM was nearly 80% in both 0.5 and 1.0 M NaCl solutions. However, the nitrogen solubility of WB proteins in 0.5–1.0 M NaCl or 2% SHMP was considerably lower than that of LM proteins. The nitrogen solubility profile of LM proteins shows that 22–24% of the total nitrogen was soluble at the point of minimum solubility irrespective of the solvents used. In contrast, WB showed a value of only ~5%. The nonprotein nitrogen content of LM and WB was 12.5% and 2.1%, respectively. Hence, the low solubility of WB proteins may be attributed not only to heat denaturation of proteins but also possibly to the leaching of some amount of nonprotein nitrogen during the detoxification treatment. A study of the physicochemical characteristics of the proteins of WB showed a certain degree of dissociation of the high molecular weight proteins (Madhusudhan and Singh, 1985b); denatured proteins have a low solubility. A reduction in the nitrogen solubility by moist heat treatment has been reported earlier for oilseed and vegetable proteins (Wu and Inglett, 1974), groundnut (Cherry et al., 1975), guar meal (Nath and Narasinga Rao, 1981; Tasneem et al., 1982) and Winged bean (Narayana and Narasinga Rao, 1982).

Water Absorption Capacity (WAC). The WAC of LM, WB, and SM was 345, 443, and 305 g/100 g of flour,

and the values expressed on the protein basis were 717, 897, and 630 g/100 g of protein, respectively. WB showed the highest WAC, and LM had higher WAC than SM. Several factors affect water binding by food proteins, viz. amino acid composition, protein conformation, surface hydrophobicity, etc. (Kinsella, 1982). The extent of hydration strongly correlates with the polar residues, and amides generally inhibit water binding (Kuntz, 1971). Polar amino acids (Anglemier and Montgomery, 1976; Kinsella, 1982) of soybean and linseed meal account for 66.9 and 52.6 g/16 g of N, respectively (FAO, 1972; Mandokhot, 1974). From these data, one could expect a higher WAC for SM than for LM; this is contrary to the results obtained. However, the nature of these polar sites is important as the cationic, anionic, and nonionic sites bind different amounts of water (Kuntz, 1971). Also, it is important to consider whether the protein conformation permits these polar sites sterically available for the water binding. The enhanced water absorption of WB may be due to the denaturation of protein that facilitates the additional binding sites available for water binding (Kinsella, 1982). The higher WAC of WB may also be due to gelation of carbohydrates and swelling of crude fiber due to heat treatment as reported in winged bean (Narayana and Narasinga Rao, 1982).

Fat Absorption Capacity (FAC). The FAC values of LM, WB, and SM were 236, 167, and 167 g/100 g of flour, and the values expressed on the protein basis were 490, 337, and 344 g/100 g of protein respectively. FAC of LM was greater than that of SM, suggesting that linseed proteins are possibly more lipophilic than soy proteins. From the amino acid composition data, the apolar amino acid (Anglemier and Montgomery, 1976; Kinsella, 1982) contents for linseed and soybean were 34.5 and 30.7 g/16 g of N (Mandokhot, 1974; FAO, 1972), respectively. This suggests a direct correlation between FAC and apolar amino acid content. However, FAC of WB was lower than that of LM and comparable to that of SM. FAC of proteins varies depending on the protein source, extent of processing, particle size, temperature, etc. (Lin et al., 1974; Hutton and Campbell, 1977). The reason for lowered FAC of WB compared to LM may be due to heat denaturation of the proteins and masking of the apolar amino acids. A correlation between fat binding capacity of heat-denatured proteins with the surface hydrophobicity has been reported (Nakai, 1983) and lowered FAC of heat-processed proteins has been reported by other workers also (Hutton and Campbell, 1977; Tasneem et al., 1982).

Bulk Density. The values of bulk density of LM, SM, and WB were 0.322, 0.555, and 0.526 g/mL, respectively. Wang and Kinsella (1976) and Dench et al. (1981) reported a negative correlation between both WAC and FAC of alfalfa leaf protein and sesame protein samples. However, a positive correlation of WAC and bulk density and a negative correlation between FAC and bulk density of LM and WB have been observed. Similar observations were made with acid-treated and wet autoclaved guar meal (Tasneem et al., 1982).

Effect of pH on Foam Capacity (FC). The FC of the meal samples as a function of pH (2–12) is shown in Figure 2. In the broad range of solubility minimum of LM proteins (pH 2.0–6.0), more or less constant FC was observed. However, WB showed a steep increase in FC from pH 3.6 to 4.6 and then a constant value until pH 10.0. There was a slight increase in FC beyond pH 10.0. LM proteins showed a significant increase in FC above pH 8.0, and this may be due to the fact that the protein solubility is higher at this pH. The lowered FC of WB proteins

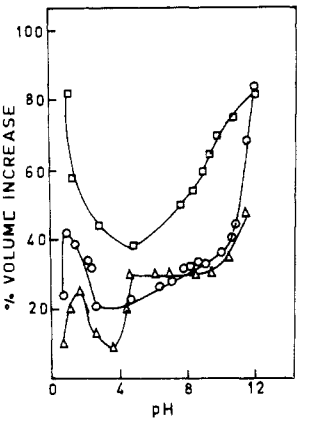

Figure 2. Effect of pH on the foam capacity: □, soybean meal; ○, raw linseed meal; △, water-boiled linseed meal.

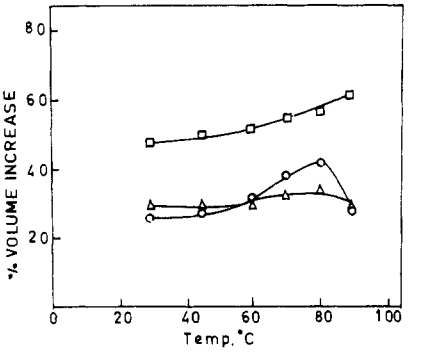

Figure 3. Effect of heating on the foam capacity: □, soybean meal; ○, raw linseed meal; △, water-boiled linseed meal.

relative to that of LM may be due to an overall decrease in the extractability of nitrogen. The minimum FC observed around the solubility minimum of LM proteins was perhaps due to low nitrogen solubility. However, it is not clear as to why there was a steep increase in FC of WB samples after pH 3.6, though these proteins exhibited a broad solubility minimum. SM exhibited a better FC than LM at all pH values. This may be due to the differences in protein as well as nonprotein components such as carbohydrates and minerals (Cherry and McWatters, 1981).

Effect of Heating on the FC. It was observed that FC of LM and WB proteins remained almost constant up to 45 °C and then increased until 80 °C, after which a decrease was noticed (Figure 3). FC of SM increased continuously with the increasing temperature. The increase in FC of WB proteins was not marked, since they were already subjected to heat treatment during detoxification treatment. In general, mild heat treatment results in surface denaturation of the protein to expose the hydrophobic regions of the protein and yet keep it in solution to result in better foaming properties (Tamsma et al., 1969; Richert et al., 1974) as reported in soybean (Beckel et al., 1949; Eldridge, et al., 1963). A lowered FC of LM at higher heating temperatures may be due to the precipitation of proteins.

Effect of NaCl on the FC. Foam capacity as a function of NaCl concentration in the range of 0–1.0 M NaCl is shown in Figure 4. There was a gradual increase in FC from 0 to 0.2 M NaCl and then a gradual decrease afterward. However, FC of SM increased up to even 0.4 M NaCl concentration, and then decreased. At any given NaCl concentration, WB exhibited a higher FC than LM whereas SM showed always higher FC values than either of these. WB in water exhibited a higher FC than LM in the pH range of 4.4–8.0, which is also reflected in the presence of NaCl. The increase in FC of LM proteins upto

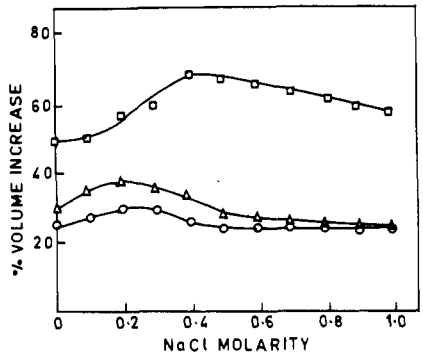

Figure 4. Effect of NaCl molarity on the foam capacity: □, soybean meal; ○, raw linseed meal; △, water-boiled linseed meal.

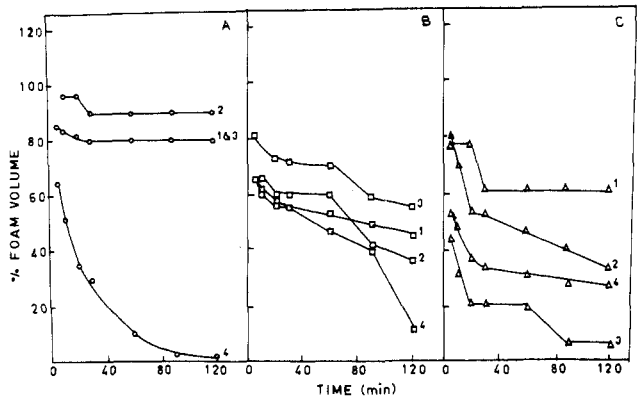

Figure 5. Foam stability as a function of pH of (A) raw linseed meal, (B) soybean meal, and (C) water-boiled linseed meal at the following pH: 1, 2.4; 2, 4.7; 3, 6.5; 4, 9.0.

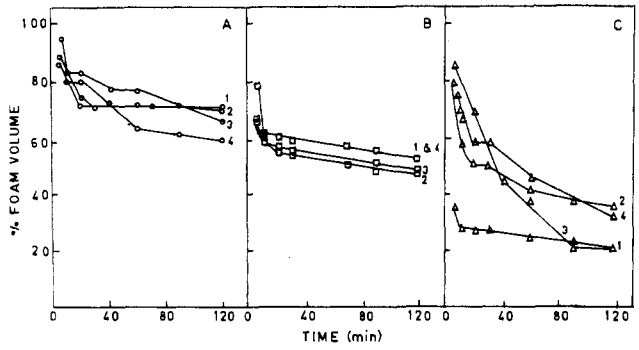

Figure 6. Effect of NaCl molarity on the foam stability of (A) raw linseed meal, (B) soybean meal, and (C) water-boiled linseed meal at the following concentrations (M): 1, 0; 2, 0.4; 3, 0.8; 4, 1.0.

0.2 M NaCl may be due to salting-in of proteins, and at higher salt concentrations, foamability was reduced probably due to salting-out proteins (Kinsella, 1976).

Foam Stability (FS). The FS of the samples (Figures 5 and 6) showed that at pH 2.4, 4.7, and 6.5 FS of LM at 120 min was 80–90% but at pH 9.0 it was only 3%. In contrast, FS of WB was 60% at pH 2.4, and at other pH values, it was 5–30%. In the case of SM, FS was better at pH 2.4 (52%) and 4.7 (60%) than at other pH values. Hence, LM proteins showed better FS at acidic and neutral pH values compared to SM or WB.

There was a decrease in FS of the three samples in the presence of NaCl. FS of LM at 0 and 0.4 M NaCl concentrations had a constant value of 70% between 20 and 120 min and decreased at 0.8 and 1.0 M NaCl concentrations. The stability of foams of SM in the presence of NaCl was improved considerably; however, WB exhibited poor FS even in NaCl solutions. Native proteins give higher

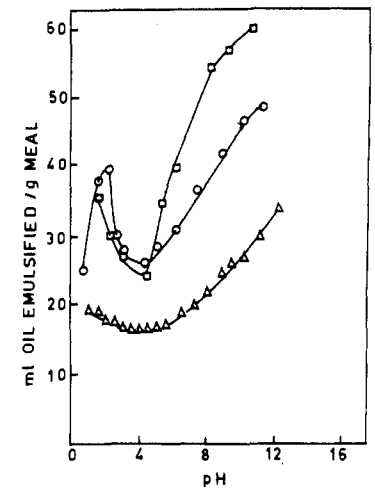

Figure 7. Effect of pH on the emulsification capacity: □, soybean meal; ○, raw linseed meal; △, water-boiled linseed meal.

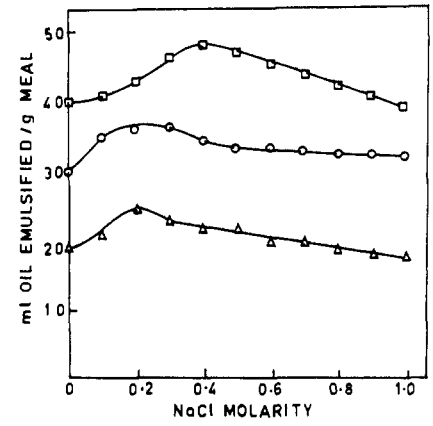

Figure 8. Effect of NaCl molarity on the emulsification capacity: □, soybean meal; ○, raw linseed meal; △, water-boiled linseed meal.

FS than denatured proteins (Yasumatsu et al., 1972). Apart from this fact, the low amounts of nitrogen extracted near neutral pH or in the presence of NaCl may be in the reason for the poor FS of WB. It has been reported that extensively heat-denatured proteins show poor FS values: soybean (Yasumatsu et al., 1972), winged bean (Narayana and Narasinga Rao, 1982), guar metal (Tasneem et al., 1982). The reason for the high and constant FS values at pH 2.4, 4.7, and 6.7 for LM may be due to the broad solubility minimum of LM proteins (pH 3–6). Near the isoelectric point of proteins, they carry no net charge and are more stable than at other pH values. The reductions in FS in the presence of NaCl may be due to the charge repulsions (Bickerman, 1953).

Effect of pH on the Emulsification Capacity (EC). The EC of the samples as a function of pH in the range pH 1–12 is shown in Figure 7. LM and SM gave a U-shaped curved with the minimum at pH 4.5. A broad minimum from pH 3 to 6 was observed in case of WB. In all the cases, the EC vs. pH profile curve resembled the nitrogen solubility vs. pH curve, suggesting that perhaps EC was mainly due to the solubilized proteins. The lowest EC values recorded for LM, WB, and SM were 26, 17, and 24 mL/g of meal, respectively. LM showed better EC values at acidic pH compared to SM; however, SM exhibited better EC values at neutral and alkaline pH range than LM. At any given pH, EC of WB was lower than that of LM, which may be due to poor nitrogen solubility of proteins of the former. The differences in EC values of LM and SM may be due to differences in protein and nonprotein components (McWatters and Cherry, 1977).

Effect of NaCl on EC. The EC profile of the samples as a function of NaCl concentration is shown in Figure 8. The EC values of LM and WB increased gradually from 0 to 0.2 M NaCl and those of SM from 0 to 0.4 M NaCl, after which a gradual decrease was observed. This is similar to the effect of NaCl on FC. EC values of SM were higher than those of LM at a given NaCl concentration. LM exhibited higher EC values than WB, probably owing to increased nitrogen solubility of the former.

The results of the above study show a reduction in nitrogen solubility and FAC and an increased WAC on detoxification of linseed meal. LM showed higher EC at acidic and neutral pH and higher FS than SM, in the presence or absence of electrolytes.

ACKNOWLEDGMENT

The authors thank Dr. M. S. Narasinga Rao for his valuable suggestions. K.T.M. thanks the Council of Scientific and Industrial Research, New Delhi, India, for the award of a Senior Research Fellowship.

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Received for review March 4, 1985. Accepted August 12, 1985.