Food Chemistry **49** (1994) 45–51

Functional properties and amino-acid composition of solvent-extracted flaxseed meals

Janitha P. D. Wanasundara & Fereidoon Shahidi*

Department of Biochemistry, Memorial University of Newfoundland, St. John's, NF, Canada, A1B 3X9

(Received 6 November 1992; revised version received and accepted 15 March 1993)

The functional properties of solvent-extracted flaxseed meals were studied. The solvent systems employed consisted of hexane and absolute or 95% (v/v) alkanol with or without 10% (w/w) ammonia. Approximately 55% of the total nitrogen-containing compounds present in the meals were dissolved in water. Meals extracted with methanol–ammonia–water/hexane had the highest nitrogen-solubility index. The minimum solubility of nitrogenous compounds in hexane-extracted and methanol–ammonia–water/hexane-extracted meals occurred between pH 3·0 and 3·5. Flaxseed meals had a high water-adsorption value, and the two phase solvent extraction did not influence it. Fat adsorption of the meals was influenced by the presence of ammonia in the extraction system, and the pH of these meals was increased by nearly one unit. Solvent-extracted flaxseed meals had improved emulsifying and foaming properties. Extraction with methanol–ammonia–water/hexane did not change the amino-acid composition of flaxseed meals.

INTRODUCTION

Linseed or flax (*Linum usitatissimum* L.) is an important oilseed crop in Canada. The seed (for oil), meal (as a feed), and other parts of the plant (as fibre) provide raw materials for different agri-based industries. However, the utilization of linseed products for edible purposes is limited. The presence of cyanogenic glycosides (Smith *et al.*, 1980; Conn *et al.*, 1981), an antivitamin (Klosterman *et al.*, 1967), and other antinutrients has limited the use of flaxseed in food formulations. Madhusudhan and Singh (1985) and Dev and Quensel (1986, 1988, 1989) have reported the functional properties of linseed protein products and linseed meal. The presence of mucilage in the flaxseed coat may improve the functional properties of the meal intended for use in food products.

We have previously reported the use of a two-phase solvent-extraction system for the processing of flaxseed (Wanasundara *et al.*, 1992). Methanol–ammonia–water/hexane-extracted meals reduced levels of cyanogenic glycosides, phenolic acids, tannins, and soluble sugars and were considered to be of improved nutritional and sensory qualities. However, food utilization of plant proteins requires a thorough knowledge of their functional properties. The present study was undertaken to investigate the functional properties of meals treated with alkanol–ammonia–water. The effect of the above processing methodology on the composition of the amino acids of the meal was also investigated.

MATERIALS AND METHODS

Seeds and meals of flax were obtained from either Omega Nutrition (Vancouver, British Columbia, Canada) or through the Flax Council of Canada (Winnipeg, Manitoba, Canada). The seeds were first ground by using a Moulinex coffee grinder. Ground seeds or meals (75 g) were blended in a Waring blender for 2 min with 500 ml of 95% (v/v) methanol, ethanol, or isopropanol, with or without 10% (w/w) ammonia at a low speed (approximately 10 000 r/min). After a 15-min quiescent period, hexane (500 ml) was added to the mixture, which was then blended again for 2 min at 10 000 r/min. The meal was then separated by vacuum filtration on Whatman No. 41 filter paper, rinsed three times with a total volume of 125 ml of alkanol, and dried overnight at 40°C in a vacuum oven. The meal was further defatted with hexane by using a Soxhlet apparatus and dried as before. Dried meals were stored in airtight plastic bags at room temperature until use.

Food Chemistry 0308-8146/93/$06.00

Functional properties of linseed meals

The pH of a 10% (w/v) dispersion of each meal in CO_2-free distilled water was determined by using a Fisher Accumet 810 pH meter. The nitrogen-solubility index (NSI) of each sample of meal was determined according to the method of AACC (1976). A 5-g portion of meal sample was weighed into a 250-ml Erlenmeyer flask, and distilled water (200 ml) was added to it in small portions while it was stirred thoroughly to obtain a uniform dispersion. The content of each flask was mixed at room temperature for 2 h by using a Burrel wrist-action shaker (Pittsburgh, PA, USA). The mixture was then transferred carefully into a 250-ml volumetric flask. Two drops of antifoam A were added, and the mixture was then diluted to the mark with water while being mixed thoroughly. A 40-ml aliquot was centrifuged for 10 min at 1500 *g*. The supernatant was passed through a funnel equipped with a glasswool plug. A 25-ml volume of the clear liquid was transferred to Kjeldahl tubes for subsequent nitrogen determination. The amount of water-soluble nitrogen in the sample was determined, and the NSI was obtained by expressing the content of water-soluble nitrogen as a percentage of that of total nitrogen in the meal. The effect of pH on the NSI of hexane- and methanol–ammonia–water/hexane-extracted meals was monitored. The pH was adjusted from 2·0 to 11·8 by the addition of a 1% (v/v) solution of HCl or NaOH.

The water adsorption of the meals was determined by a combination of the AACC (1976) method and that of Sosulski (1962). A 2-g sample was dispersed in 20 ml of distilled water. The content was mixed further for 30 s at every 10 min over a 60-min period by using a glass rod. The content was centrifuged at 2000*g* for 15 min, and the supernatant was carefully decanted. Tubes were then inverted and drained for 15 min before being weighed. The adsorbed water was expressed as a percentage increase in the sample weight.

The water-hydration capacity (WHC) of meals was measured by the method of AACC (1976) and that of Quinn and Paton (1979). A 5-g portion of meal was weighed into a preweighed 50-ml centrifuge tube, and distilled water was added in small unmeasured-volume increments while stirring proceeded with a glass rod after each addition until the mixture was thoroughly wet. The stirring rod was wiped on the sides of the tube, which was then centrifuged at 2000*g* for 10 min. The resulting supernatant was discarded and the content weighed. If no supernatant appeared, more distilled water was added, and the procedure was repeated. The approximate water-hydration capacity (WHC_{approx}) was calculated as:

$$WHC_{approx}(gH_2O/g\ meal) = \frac{(\text{Weight of tube + Sediments}) - (\text{Weight of tube + 5·0})}{5{\cdot}0\ g\ meal}$$

To determine the water-hydration capacity accurately, enough meal was added to four pre-weighed tubes (amount of meal = $15/WHC_{approx} + 1$, where 15 is the desired total mass of sample and water). The volume of water to be added was calculated as 15 minus the mass of material (g). To each of the four tubes were added volumes of water equal to 1·5 and 0·5 ml more and 1·5 and 0·5 ml less than that of the calculated value. The content of each tube was vigorously mixed with a stirring rod for 2 min and then centrifuged as before. The two tubes, one with and one without supernatant, represented the limits within which the WHC values occurred. The WHC values were presented as the mid-point between these two volumes divided by the mass of the material in grams.

Fat adsorption was determined by using the methods described by Lin *et al.* (1974) and Sosulski *et al.* (1976). A 2-g sample in 12 ml of pure soybean oil was placed in a 50-ml centrifuge tube, and the contents were stirred for 30 s every 5 min. After 30 min, the tubes were centrifuged at 1600*g* for 25 min. The free oil was decanted, and the absorbed oil was determined as the mass percentage difference.

The emulsifying activity or capacity of each sample was determined by the method of Yasumatsu *et al.* (1972). Meal (1·25 g) was homogenized with 50 ml of water for 30 s, a Polytron homogenizer was used at 10 000 r/min. Pure soybean oil (25 ml) was added to each sample, and the mixture was homogenized for 90 s. The emulsion so obtained was divided evenly into four tubes, which were then centrifuged at 1100*g* for 5 min. Emulsifying activity was calculated by dividing the volume of the emulsified layer by the volume of emulsion before centrifugation and expressing the result as a percentage.

Emulsion stability was determined by using the material prepared for the measurement of emulsifying activity. The mixture was heated at 85°C for 15 min and cooled as described by Naczk *et al.* (1985). After cooling to room temperature, the mixture was evenly divided into four portions, transferred into 50-ml centrifuge tubes, and centrifuged at 1100*g* for 5 min. Emulsion stability was expressed as the percentage emulsifying activity remaining after heating.

Whippability and foam stability were determined by the method of Lin *et al.* (1974). A 100-ml 3% (w/v) dispersion of meal in distilled water was homogenized for 60 s by using a Polytron homogenizer at 10 000 r/min. The mixture was then transferred immediately into a 250-ml measuring cylinder, and the foam volume was noted. The percentage volume increase was calculated as given by Lawhon and Cater (1971) and expressed as whippability. Foam stability was expressed (on the basis of 100 ml of a 3% w/v dispersion) as the volume of the foam remaining after a 0·5, 10, 20, 40, 60, 120 min quiescent period.

Analysis of amino acids

Samples of hexane-extracted and methanol–ammonia–water/hexane-extracted meals (10–15 mg) were digested in 6 N HCl at 110°C under a stream of nitrogen (Blackburn, 1978). The amino-acid composition of the

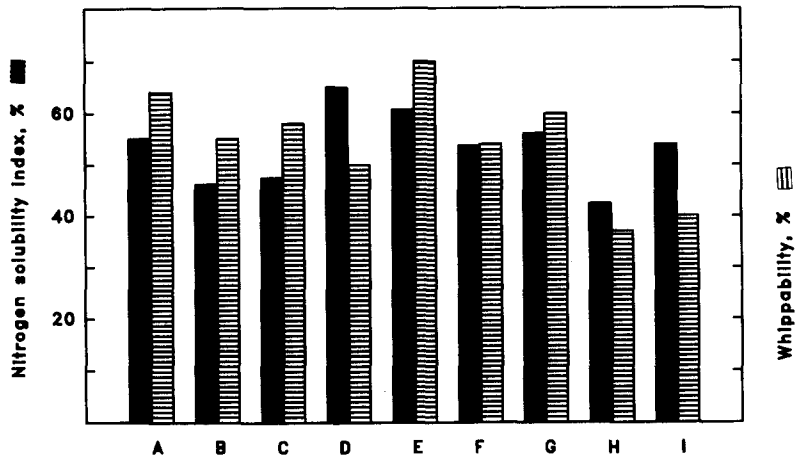

Fig. 1. Effect of two-phase solvent extraction on nitrogen-solubility index and whippability of flaxseed meal. A, hexane only; B, methanol/hexane; C, methanol–water/hexane; D, methanol–ammonia/hexane; E, methanol–ammonia–water/hexane; F, ethanol–ammonia–water/hexane; G, isopropanol–ammonia–water/hexane; H, commercial meal hexane-extracted; I, commercial meal methanol–ammonia–water/hexane-extracted.

hydrolysates was determined by using a Beckman 121 MB amino-acid analyzer (Beckman Instruments, Palo Alto, CA, USA). Cysteine and methionine were determined after performic acid oxidation prior to their digestion in 6 N HCl. Cysteine and methionine were measured as cystic acid and methionine sulphone, respectively (Blackburn, 1978). Analysis of tryptophan was performed after hydrolysis of the samples in 3 N mercaptoethane sulphonic acid at 110°C under vacuum as described by Penke *et al.* (1974).

RESULTS AND DISCUSSION

Extraction of flax meal by a two-phase solvent system consisting of absolute or 95% (v/v) alkanol with or without 10% (w/w) added ammonia and hexane increased the crude protein (N% × 6·25) content of the meals by up to 13% (Table 1). Originally, flaxseed meal contained 42·9% crude protein, on a dry-weight basis. However, the content of non-protein nitrogen of samples remained unchanged. The removal of polar compounds from seeds was responsible for the enhancement of the protein content of treated meals. The pH of meals ranged from 5·98 to 6·82 (Table 1). The presence of minute amounts of ammonia in the meals was responsible for this observation. Shahidi *et al.* (1988) have reported a similar pH effect in canola as a result of its solvent extraction with a methanol–ammonia–water/hexane system.

The nitrogen solubility of flax meals at their natural pH after solvent extraction is given in Fig. 1. Nitrogen solubility is considered a good index of the functional potential of protein-rich products. Hexane-extracted linseed meal at pH 5·98 had 55% of its total nitrogen content in solution. Extraction with methanol or methanol–water lowered the NSI of the meals. Extraction with ethanol and isopropanol–ammonia–water slightly lowered NSI values, but methanol in combination with ammonia was able to improve the NSI of the meals. The dependence of the NSI on the pH of the medium was studied for both hexane-extracted and methanol–ammonia–water/hexane-extracted linseed meals as shown in Fig. 2. The solubility curves for both meals showed one minimum only. The minimum NSI occurred at a pH between 3·0 and 3·5 for both meals. However, the numerical values were slightly lower for hexane-extracted meals than those for methanol–ammonia–water/hexane-extracted meals. Both meals exhibited higher NSI values in the alkali as compared with the acidic range. Thus, at pH ≥ 11·0, more than 69–70% of both meals' nitrogen was in solution.

Table 1. Effect of two-phase solvent extraction on protein enhancement, pH, and water-hydration capacity of linseed meal

Solvent	Protein enhancement (%)	pH	Water-hydration capacity (g H_2O/g)
Laboratory-prepared meal			
Hexane only	0·0	5·98 ± 0·02	5·2 ± 0·2
Methanol/hexane	1·4	5·97 ± 0·01	5·9 ± 0·3
Methanol–water/hexane	8·3	6·02 ± 0·02	5·8 ± 0·2
Methanol–ammonia/hexane	7·2	6·67 ± 0·03	6·3 ± 0·3
Methanol–ammonia–water/hexane	13·3	6·82 ± 0·02	5·8 ± 0·1
Ethanol–ammonia–water/hexane	7·9	6·71 ± 0·04	6·0 ± 0·2
Isopropanol–ammonia–water/hexane	2·5	6·04 ± 0·05	6·5 ± 0·1
Commercial meal			
Hexane only	0·0	6·09 ± 0·01	6·1 ± 0·2
Methanol–ammonia–water/hexane	15·5	6·84 ± 0·04	6·3 ± 0·1

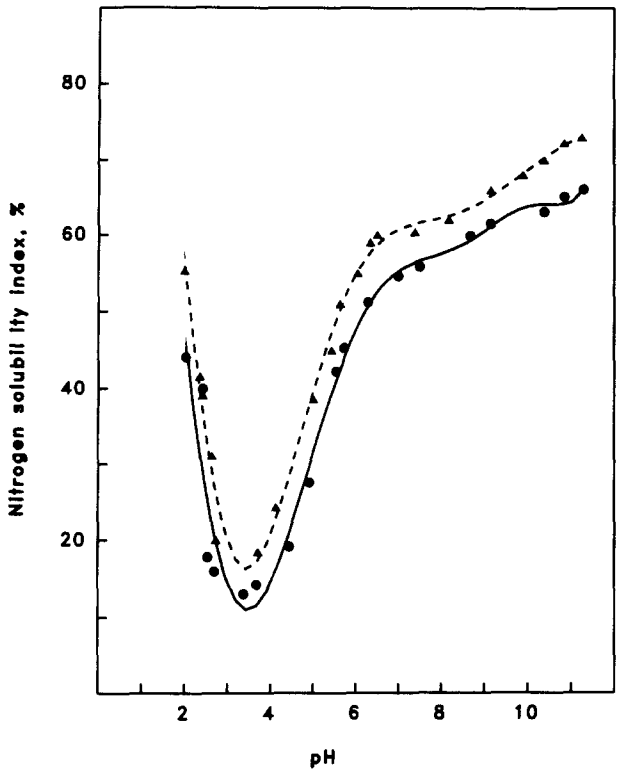

Fig. 2. The nitrogen-solubility index of linseed meal as affected by change of pH in the medium. ●—●, hexane-extracted; ▲—▲, methanol–ammonia–water/hexane-extracted.

Hexane-extracted linseed meal adsorbed 9·7 times as much water as its original weight (Fig. 3). Other solvent-extraction systems did not alter the water adsorption, except for the isopropanol–ammonia–water/hexane system. The water adsorption of linseed meals was considerably higher than that of canola, which adsorbed approximately 3·7 times as much water as its original weight (Shahidi *et al.*, 1988). Bhatty and Cherdkiatgumachai (1990) found that linseed meal can adsorb eight times and its hulls can adsorb thirteen times as much water as their original weights. It has been reported that linseed hulls contain 2·7% mucilage from the outer endosperm (Peterson, 1958), which adsorbs water and swells (Dev & Quensel, 1986). Gelation of carbohydrates and swelling of crude fibre are two important factors that influence the water adsorption of oilseed meals (Narayana & Narasingha Rao, 1982). The higher water adsorption of solvent-extracted meals may be due to the presence of hull polysaccharides. The larger difference in water adsorption of linseed than that of other oilseed meals (canola or soybean) is presumably due to the presence of mucilage in flaxseed meal and may also be due to the existing differences in the conformational characteristics of its proteins. The water-hydration-capacity values (Table 1) suggest that hexane-extracted meals require 5·2 times their weight of water to become hydrated. The ability to hold water physically against gravity is related to the viscosity of the food system and is influenced by the pH, ionic strength, and temperature (Kinsella, 1979). The swelling and expansion of particles by adsorbing water are an important functional property in foods such as processed meats, doughnuts, and custards, where proteins mix with water without dissolution and impart thickening power and viscosity to the food (Hermensson & Åkesson, 1975; Kinsella, 1979).

The fat adsorption of the meals was influenced by the presence of ammonia in the alkanol phase (Fig. 3). Hexane-extracted meals adsorbed 2·6 times as much soybean oil as their original weight. Methanol–ammonia–water/hexane extraction enhanced the fat adsorption of meals by up to 3·2 times. A similar trend was observed when commercially processed linseed meals were subjected to methanol–ammonia–water/hexane-extraction. Kinsella (1982) and Dench *et al.* (1981) have reported that the fat adsorption of a protein meal relies mainly on physical entrapment of the oil by a capillary-attraction process. It is important to note that the degree of water and fat adsorption and their retention during cooking and processing can greatly influence the juiciness, dryness, and mouthfeel and even

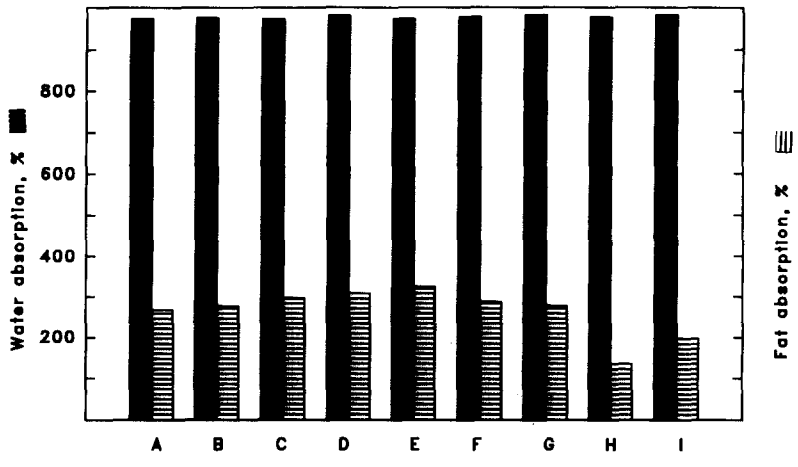

Fig. 3. Effect of two-phase solvent extraction on water and fat adsorption of flaxseed meal. A, hexane only; B, methanol/hexane; C, methanol–water/hexane; D, methanol–ammonia/hexane; E, methanol–ammonia–water/hexane; F, ethanol–ammonia–water/hexane; G, isopropanol–ammonia–water/hexane; H, commercial meal hexane-extracted; I, commercial meal methanol–ammonia–water/hexane-extracted.

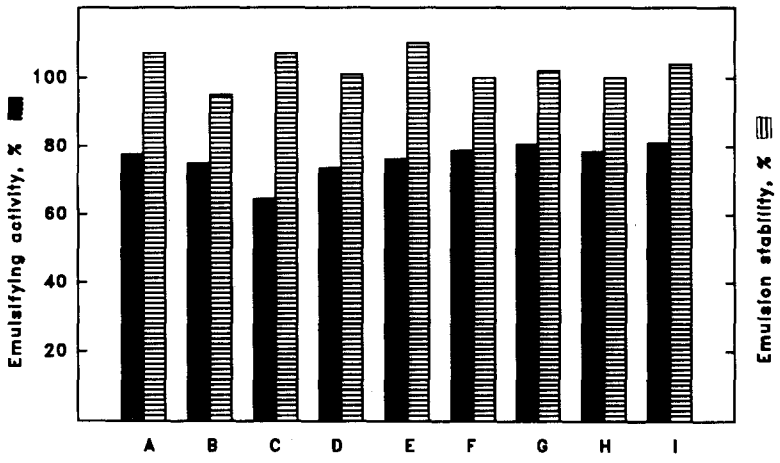

Fig. 4. Effect of two-phase solvent extraction on emulsifying activity and emulsion stability of flaxseed meal. A, hexane only; B, methanol/hexane; C, methanol–water/hexane; D, methanol–ammonia/hexane; E, methanol–ammonia–water/hexane; F, ethanol–ammonia–water/hexane; G, isopropanol–ammonia–water/hexane; H, commercial meal hexane-extracted; I, commercial meal methanol–ammonia–water/hexane-extracted.

the storage stability of products in which oilseed meals are incorporated (Sosulski *et al.*, 1977).

The emulsifying capacity of solvent-extracted flaxseed meals ranged from 64·5 to 80·6% (Fig. 4) of the added soybean oil. Methanol–water/hexane-extracted meals showed a slight decrease in their emulsifying capacity, whereas other solvent-extracted samples did not show much of a change as compared with hexane-extracted samples. Commercial linseed meal had a similar emulsifying capacity to the hexane-extracted meal. All the emulsions formed were fairly stable, since 95–100% emulsifying activity was retained after heating of the emulsions at 80°C for 30 min. Thus heat treatment did not collapse the emulsions. It is established that high protein solubilities (Inklarr & Fourtin, 1969; Lin *et al.*, 1974; Kinsella, 1979) and high fat-adsorption values (Wolf & Cowan, 1975) are closely related to the formation and stabilization of emulsions. According to Dev and Quensel (1986), the mucilage polysaccharides of linseed flour also contribute to the emulsifying activity and emulsion stability of products.

The whippability of the meals was between 55·0 and 70·0% (Fig. 1). Methanol/hexane extraction lowered the foaming ability of meals. The foam produced by all solvent-extracted samples had a fine bubble structure, which may be uniquely characteristic of linseed meal. The change in foam volume with time for hexane and methanol–ammonia–water/hexane-extracted meals is presented in Fig. 5. A similar trend was observed for other meals. The good foam stability of products may be partly attributed to the presence of polysaccharides in the meals. Both non-protein-nitrogen compounds and carbohydrates can stabilize foams (Cherry & McWaters, 1981). According to Sosulski *et al.* (1977) a good nitrogen solubility also ensures good foam stability in a protein meal.

The amino-acid composition of hexane-extracted, methanol–ammonia–water/hexane-extracted, and commercial meals is given in Table 2. The content of essential amino acids of the meals was slightly less than the FAO/WHO (1973) reference values. Methionine, lysine, and tryptophan levels were considerably lower than the reference values. Linseed protein showed an abundance of glutamic acid and had an amino-acid composition similar to that of canola, as reported by Shahidi *et al.* (1992). Commercial linseed meal showed slightly lower values for all amino acids than the laboratory-prepared meals. Methanol–ammonia–water/hexane extraction gave slightly lower amino-acid values but not lower than those of its commercial counterpart.

In conclusion, the observed changes in some functional properties of solvent-extracted linseed meals, particularly the methanol–ammonia–water/hexane-ex-

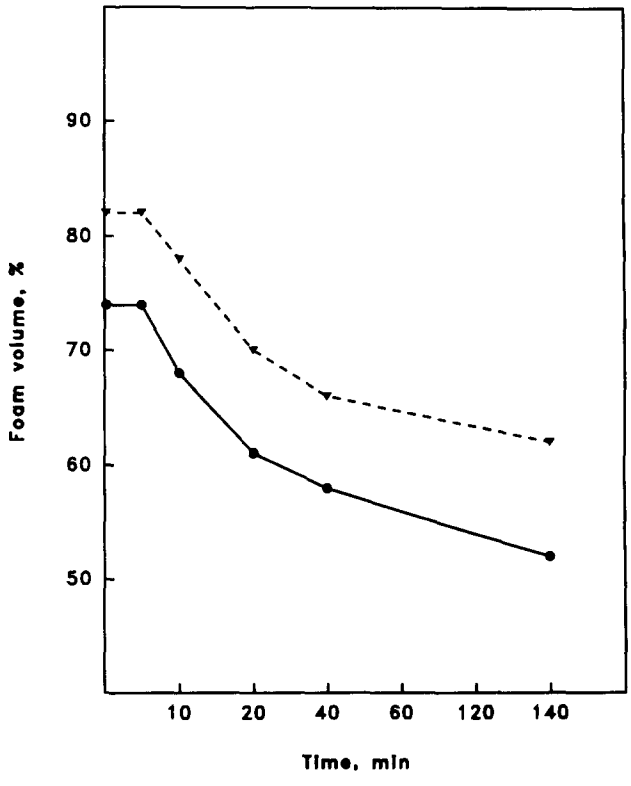

Fig. 5. Time-dependence of foam volume. ●—●, hexane-extracted; ▲—▲, methanol–ammonia–water/hexane-extracted.

Table 2. Amino-acid composition of hexane- and methanol–ammonia–water/hexane-extracted and commercial linseed meals (g/16 g N)

Amino acid	Hexane-extracted	Methanol–ammonia–water/hexane-extracted	Commercial meal
Histidine	2·69 ± 0·24	2·46 ± 0·05	2·36 ± 0·33
Isoleucine	4·78 ± 0·54	4·54 ± 0·15	4·19 ± 0·03
Leucine	6·70 ± 0·62	6·39 ± 0·15	5·96 ± 0·04
Lysine	4·38 ± 0·37	4·14 ± 0·08	3·92 ± 0·02
Methionine	1·45 ± 0·09	1·41 ± 0·05	1·24 ± 0·05
Cysteine	3·29 ± 0·56	3·39 ± 0·21	3·16 ± 0·23
Pheylalanine	5·13 ± 0·05	4·91 ± 0·08	4·63 ± 0·00
Tyrosine	2·21 ± 0·19	2·12 ± 0·03	1·98 ± 0·02
Threonine	3·40 ± 0·30	3·33 ± 0·04	3·00 ± 0·00
Tryptophan	0·46 ± 0·10	0·46 ± 0·05	0·25 ± 0·09
Valine	5·75 ± 0·07	5·64 ± 0·17	5·02 ± 0·06
Alanine	4·81 ± 0·50	4·64 ± 0·10	4·61 ± 0·01
Aspartic acid	9·18 ± 0·60	9·16 ± 0·60	8·03 ± 0·19
Arginine	11·50 ± 0·33	11·20 ± 0·10	9·78 ± 0·06
Glycine	6·44 ± 0·35	6·26 ± 0·25	5·64 ± 0·17
Glutamic acid	16·70 ± 0·43	16·36 ± 0·33	14·45 ± 0·63
Proline	3·64 ± 0·24	3·65 ± 0·10	3·32 ± 0·03
Serine	4·94 ± 0·03	4·99 ± 0·24	4·48 ± 0·03

tracted meals, were favourable. Observed changes in the functional properties of meals may be due to the removal of polar compounds by the solvent-extraction systems employed. The removal of polar compounds, namely, phenolic acids, cyanogenic glycosides, sugars, and phospholipids, from linseed meal by the alkanol phase has previously been reported. Flaxseed meal has some desirable functional characteristics that are important in food products, and some were favourably influenced by the use of the two-phase solvent-extraction processing.

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