

## Nutritional potential and functional properties of sweet and bitter lupin seed protein isolates

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### Abstract

Protein isolates were prepared from both sweet and bitter lupin seed flours by two different methods, i.e. by alkaline water extraction/isoelectric precipitation (PI) and by micellisation (MI), and studied with regard to nutritional quality and functional properties. Protein solubility of both lupin seed flours was increased as sodium chloride concentration increased up to 1.0 M, then decreased. The minimum protein solubility of bitter lupin seed flour was quite sharp at pH 4.5, while it exhibited a broad pH range of 4.3–4.9 for sweet lupin seed flour. No significant ( $P < 0.05$ ) differences were found between any isolates in their dry matter, fiber, lipids and moisture contents. Isolates-PI from both sources had significantly ( $P < 0.05$ ) higher crude protein and ash contents than their isolates-MI. Bitter and sweet isolates-PI had lower values of total essential amino acids and higher values of tyrosine, phenylalanine, threonine, tryptophan and valine than isolates-MI. There was no significant ( $P > 0.05$ ) difference between bitter isolates in their alkaloid contents, while both sweet lupin isolates were free of total alkaloids. Bitter lupin isolate-PI had significantly ( $P < 0.05$ ) lower tannins, but sweet lupin isolate-MI had a significantly ( $P < 0.05$ ) higher phytic acid content than other isolates. Isolates-MI from both sources had higher chemical scores than their isolates-PI. The first and second limiting amino acids were total sulfur amino acids and valine, respectively, for all types of isolates. Sweet lupin isolate-MI had a higher essential amino acid index and protein efficiency ratio than other isolates. The protein solubility index, fat absorption and emulsification capacities of both isolates-MI were significantly ( $P < 0.05$ ) higher than their isolates-PI. Both sweet lupin isolates had significantly ( $P < 0.05$ ) higher water absorption capacity than bitter lupin isolates. Sweet lupin isolate-MI had significantly ( $P < 0.05$ ) higher foam capacity and foam stability than other lupin isolates. © 2001 Elsevier Science Ltd. All rights reserved.

**Keywords:** Lupin seed flour; Protein isolate; Amino acid; Antinutritional factors; Biological value; Functional properties

### 1. Introduction

Lupin is a valuable ancient leguminous plant, which grows well in different soils and climates. It has been used as food by people around the Mediterranean area and by those living in the Andean highlands (Morrow, 1991; Dervas, Doxastakis, Hadjisavva-Zinoviadi, & Triantaafillakos, 1999). The main interest in lupin for foods is related to its high content of protein which is considered as a good source of lysine and generally poor in the sulfur-containing amino acids (Lampart-Szczapa, 1996).

The main antinutritional substances are various alkaloids of the quinolizidine group (Mohamed & Rayas-Duarte, 1995). Many lupin varieties have high levels of alkaloids (bitter tasting compounds) that make the seed

unpalatable and sometimes toxic. Lupin alkaloids have been removed from the seed by boiling for 30 min, then steeping in running water for 3 days (Rahma & Narasinga Rao, 1984). Also, the production of protein isolates can overcome this problem because alkaloids are water-soluble and would be removed during preparation of the isolates, which can be used as functional ingredients in human food (Sousa, Morgan, Mitchell, Harding, & Hill, 1996). However, German plant breeders produced alkaloid-free lupin (sweet lupin), which can be directly consumed by humans (Hudson, 1979). Sweet lupin could be used as a source of protein, which is especially lower in antinutritional factors and would not need to be heat-treated since trypsin inhibitors and haemagglutinins are practically absent (Chango, Vilaume, Bau, Schwertz, Nicolas, & Mejean, 1998).

Functional properties of any protein material are very important in food applications. Functional properties of several different species of *Lupinus* have been reported.

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Sosulski, Chakraborty, and Humbert (1978) and Sathe, Deshpande, and Salunkhe (1982) studied the emulsifying and solubility properties of protein isolates prepared from lupin seed flour. Sosulski and Youngs (1979) mentioned that the protein concentrates and isolates from lupins have potential as an additional source of protein for human nutrition. Lupin protein has a high nutritive value if supplemented with methionine and could replace soy concentrate in countries where soy-bean must be imported (Ruiz & Hove, 1976).

The interest in utilisation of lupin has increased worldwide, with Australia emerging as a major advocate. New markets in Australia for lupins are now being considered for human foods as well as animal feed (Hough & Jacobs, 1994). Also, products containing full-fat toasted lupin flour are now on sale in Belgium, Holland and Germany (Feidheim, 1998).

This paper studies the chemical composition, nutritional quality and functional properties of sweet and bitter lupin seed protein isolates prepared by alkaline water extraction/isoelectric precipitation (PI) and by micellisation (MI).

## 2. Materials and methods

### 2.1. Materials

Lupin seeds of both varieties, bitter (*Lupinus termis*) and sweet (*Lupinus albus*), were obtained from the local market at Shibin El-Kom City, Egypt. The seeds were cleaned and rendered free of dust, then stored in polyethylene bags in the refrigerator until used.

### 2.2. Methods

#### 2.2.1. Preparation of lupin seed flours

Lupin seeds were crushed, using a household mill (Braun, Germany), then defatted by soaking in n-hexane for 48 h with several changes of the solvent. The defatted flour was air-dried at room temperature ( $\sim 25^{\circ}\text{C}$ ) and ground again to pass through a 60-mesh (British Standard Screen) sieve. The fine flour of each seed variety was then used for preparing protein isolates.

#### 2.2.2. Preparation of protein isolates by alkaline water extraction/isoelectric precipitation (isolate-PI)

One kg of flour was suspended in 10 l distilled water containing 0.25%  $\text{Na}_2\text{SO}_3$ , then adjust to pH 9.0 using 1 M NaOH. The suspension was stirred for 1 h at room temperature, then centrifuged at  $3000 \times g$  for 30 min. In order to obtain higher yields, the extraction and centrifugation were repeated on the residue. The extracts were combined and acidified to pH 4.5 for both bitter and sweet lupin proteins. The precipitate was recovered

by centrifugation at  $3000 \times g$  for 30 min, then neutralised by 1.0 M NaOH to pH 7 and washed by distilled water, several times. The neutralised precipitate was freeze-dried (Lab Conco Freeze Dry 64312, Kansas, Missouri), then milled using a household mill (Braun, Germany) and finally sieved through 60-mesh.

#### 2.2.3. Preparation of protein isolates by micellisation (isolate-MI)

One kg of lupin seed flour was suspended in 5 l of 1.0 M NaCl solution, then stirred for 2 h at room temperature. The suspension was centrifuged at  $3000 \times g$  for 30 min; then the residue was extracted again as described above. The combined supernatant was diluted ten-fold with distilled water and left to stand at  $4^{\circ}\text{C}$  for 18 h. The supernatant was discarded and the precipitate was centrifuged at  $3000 \times g$  for 30 min. The precipitated isolate was freeze-dried and treated as mentioned for isolate-PI.

#### 2.2.4. Chemical composition

Moisture (14.004), fat (14.018), ash (14.006), crude fiber (14.020) and protein  $\text{N} \times 6.25$  (14.026) were determined as described by AOAC (1990).

#### 2.2.5. Antinutritional factors

Total tannins were determined colorimetrically as described in AOAC (1990). Phytic acid was determined by the method of Wheeler and Ferrel (1971). The alkaloids were extracted and determined by the method of Jenkins, Knevel, and Di-Gangi (1967) with slight modification as described by Ruiz (1978).

#### 2.2.6. Amino acids

Amino acids were determined using a Mikrotechna AAA 881 automatic amino acid analyser according to the method of Moore and Stein (1963). Hydrolysis of the samples was performed in the presence of 6 M HCl at  $110^{\circ}\text{C}$  for 24 h under a nitrogen atmosphere. Sulfur-containing amino acids were determined after performic acid oxidation. Tryptophan was chemically determined by the method of Miller (1967).

#### 2.2.7. In-vitro protein digestibility and available lysine

This was determined as described by Salgó, Granzler, and Jecsei (1984) by measuring the change in the sample solution pH after incubation at  $37^{\circ}\text{C}$  with trypsin-pancreatin enzyme mixture for 10 min. Available lysine was determined by the procedure of Fields (1972) using 2,4,6-trinitrobenzenesulfonic acid.

#### 2.2.8. Biological values

Biological values of lupin protein isolates were determined on the basis of their amino acid profiles. Chemical scoring of amino acids was calculated using the FAO/WHO (1973) reference pattern. Essential Amino

Acid Index was calculated according to Oser (1959) using the amino acid composition of the whole egg protein published by Hidvégi and Békés (1984) as standard. Protein efficiency ratio (PER) was estimated according to the following regression equation proposed by Alsmeyer, Cunningham, and Happich (1974):  $PER = -0.468 + 0.454 (\text{leucine}) - 0.105 (\text{tyrosine})$ .

### 2.2.9. Functional properties

Protein solubility of lupin seed flours, as a function of pH and ionic strength (using sodium chloride at the concentration range of 0.2 to 2.0 M), was determined according to the method of King, Aguirre, and Pablo (1985) and El-Adawy, Rahma, El-Bedawy, and Gafar (1999), respectively. Protein solubility index of lupin isolates in distilled water and 1.0 M sodium chloride solution was determined by the method described by Rahma and Narasinga Rao (1979). Water and fat absorption capacities were estimated according to Sosulski (1962) and Sosulski, Humbert, Bui, and Jones (1976), respectively, and expressed as grams of water or sunflower oil bound per gram isolate. Foam capacity and foam stability were assessed according to the method of Lawhon, Rooney, Carter, and Matti (1972) using 1% protein solution in a Braun blender at 1600 rpm for 5 min. The percentage increases in foam volume were recorded as foam capacity. The change in volume of foam, after 15, 30, 45, 60, 90 and 120 min of standing at room temperature ( $\sim 30^\circ\text{C}$ ), was recorded as foam stability. Emulsification capacity (milliliter oil/g isolate) was determined as described by Beuchat, Cherry, and Quinin (1975).

### 2.2.10. Statistical analysis

Results are expressed as the mean value  $\pm$  standard deviation (S.D.) of three separate determinations, except amino acid contents. The data were statistically analysed using analysis of variance and least significant difference according to SAS (1985). Significant differences were determined at the  $P < 0.05$  level.

## 3. Results and discussion

### 3.1. Protein solubility of lupin seed flours

Fig. 1 shows the relationship between sodium chloride concentration and lupin seed flour protein solubility. There was an increase in protein solubility as sodium chloride concentration increased up to 1 M, for both lupin seed flours. However, beyond these concentrations the protein solubility of both flours was decreased. The proteins of sweet lupin seed showed the highest solubility before and after the inflection concentration. Generally, protein solubility is known to increase with moderately increasing salt concentrations due to the

salting-in effect and, at a higher salt concentrations, the protein solubility does not increase, as it is then likely to undergo salting-out. Therefore, the concentration of 1 M sodium chloride was used as suitable extraction solution during preparation of sweet and bitter lupin seed protein isolates by micellisation.

Bitter and sweet lupin seed flours gave a U-shaped curve in the pH range of 1–12, with a solubility minimum which is similar to many oil seed and legume proteins (Fig. 2). The minimum solubility of bitter seed flour proteins was quite sharp at pH 4.5 with 17.6% protein in the solution. Meanwhile, sweet lupin seed proteins exhibited a broad range of minimum solubility at a pH range of 4.3–4.9 with 15.1% protein in the solution. On both sides of the isoelectric pH, the solubility was higher at alkaline pHs than that at acidic pHs and comparable for both flours. The solubility increased slowly between pH 9 and 12. Therefore, the pHs 9 and 4.5 were chosen as suitable for future protein extraction and precipitation, respectively, during preparation of bitter and sweet lupin protein isolates by alkaline water extraction/isoelectric precipitation.

### 3.2. Proximate composition of lupin protein isolates

Table 1 shows the proximate composition of bitter and sweet lupin protein isolates prepared by alkaline water extraction/isoelectric precipitation (PI) and by micellisation (MI). Significant ( $P < 0.05$ ) differences were observed between protein isolates-MI of bitter and sweet lupins and their isolates-PI in crude protein, ash

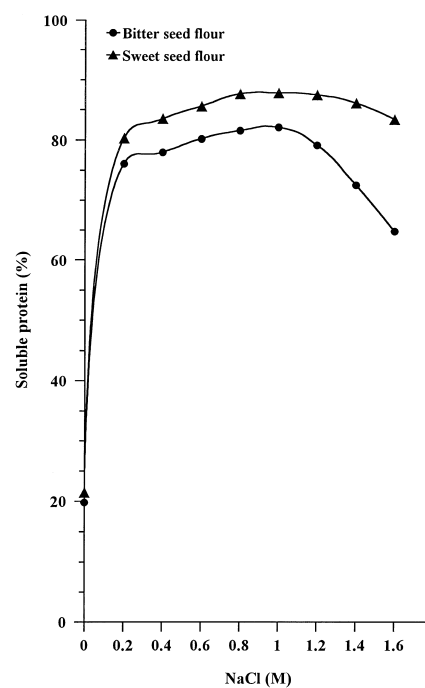


Fig. 1. Effect of sodium chloride concentration on the extracted protein of bitter and sweet lupin seed flours.

content and nitrogen free extract. The isolates-PI, of bitter and sweet lupin, had higher protein contents than their isolates-MI. Ruiz and Hove (1976) reported that the protein isolate of *L. angustifolius* (prepared by alkaline water extraction/isoelectric precipitation) had 92.5% protein. However, the results obtained were higher than that reported by Lampart-Szczapa (1996) for lupin isolate prepared by micellisation. The isolates-PI of bitter and sweet lupin had significantly ( $P < 0.05$ ) higher ash contents than their isolates-MI. This could be due to sodium chloride formation through the

neutralisation process during preparation of protein isolates by alkaline water extraction/isoelectric precipitation (PI). Non-significant ( $P > 0.05$ ) differences were observed among lupin protein isolates in their dry matter, lipids, fiber and moisture contents. The results of crude fiber were higher than reported by Millan, Alaiz, Hernandez, Sanchez, and Bautista (1995) for lupin isolate prepared by alkaline water extraction/isoelectric precipitation.

### 3.3. Amino acids of lupin protein isolates

Amino acid contents of bitter and sweet lupin protein isolates are presented in Table 2. Total essential amino acids of bitter and sweet isolates-PI were lower than their isolate-MI. However, isolates-PI of bitter and sweet lupin had higher values of tyrosine, phenylalanine, threonine, tryptophan and valine than their isolates-MI. The remaining essential amino acids in isolates-MI (except leucine in sweet isolate-MI) were higher than in isolate-PI. Compared to the FAO/WHO (1973) reference pattern, all isolates are rich in leucine, isoleucine and total aromatic amino acids. In addition, all isolates had lower total essential amino acids than FAO/WHO (1973). All isolates are deficient in sulfur-containing amino acids as are most vegetable proteins. Meanwhile, isolates-MI of bitter and sweet lupin had higher sulfur-containing amino acids than isolates-PI by 25%. Lampart-Szczapa (1996) reported that the production of protein isolate by micellisation resulted in high levels of the sulfur amino acids. Generally, this study gave data higher than that reported by Millan et al. (1995) for total amino acids of lupin protein isolate prepared by alkaline water extraction/isoelectric precipitation. Glutamic acid, aspartic acid and arginine were the major non-essential amino acids in all protein isolate samples. Their percentages ranged from 25.0 to 26.74%, 9.70–10.73 and 8.29–8.95% of the total amino acids, respectively.

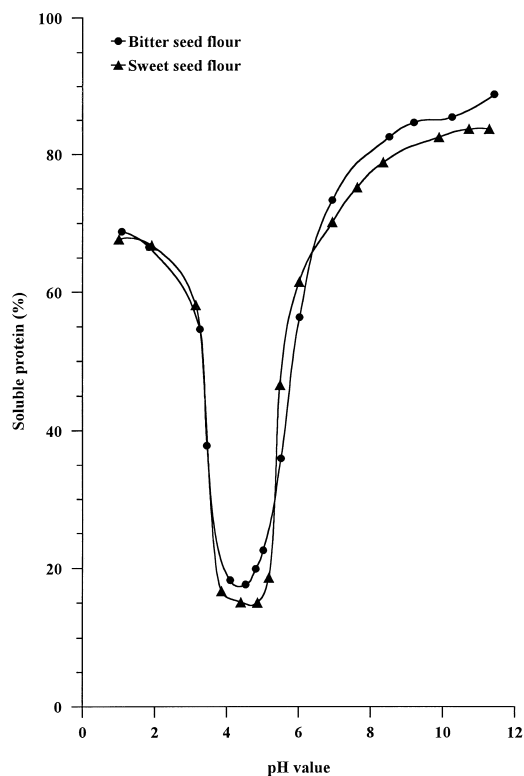


Fig. 2. Effect of different pH on the protein solubility of bitter and sweet lupin seed flours.

Table 1  
Chemical composition of bitter and sweet lupin protein isolates (g/100 g sample)<sup>a</sup>

Chemical constituents	Bitter lupin protein		Sweet lupin protein		LSD
	Isolate-PI	Isolate-MI	Isolate-PI	Isolate-MI	
Dry matter	97.07a ± 0.655	96.84a ± 0.418	97.55a ± 0.355	97.42a ± 0.39	0.736
Crude protein (N × 6.25)	91.25b ± 2.347	87.78a ± 1.314	91.03b ± 1.272	88.75a ± 1.543	1.653
Total ash	1.26b ± 0.056	1.06a ± 0.058	1.35b ± 0.086	1.02a ± 0.098	0.163
Crude fiber	0.81a ± 0.110	1.06a ± 0.168	0.74a ± 0.104	0.89a ± 0.047	0.217
Total lipids	0.15a ± 0.053	0.19a ± 0.050	0.21a ± 0.067	0.19a ± 0.062	0.109
Nitrogen free extract <sup>b</sup>	3.60a ± 0.346	6.77c ± 0.303	4.22b ± 0.204	6.57c ± 0.26	0.521
Moisture	2.93a ± 0.566	3.16a ± 0.418	2.45a ± 0.355	2.58a ± 0.391	0.991

<sup>a</sup> Means ± standard deviation of means of three determinations. Means in the same row with different letters are significantly different ( $P < 0.05$ ). LSD, Least significant differences; Isolate-PI, Protein isolate prepared by alkaline water extraction/isoelectric precipitation; Isolate-MI, Protein isolate prepared by micellisation.

<sup>b</sup> Calculated by difference.

### 3.4. Antinutritional factors and in-vitro digestibility of lupin protein isolates

Antinutritional factors and in-vitro protein digestibility of lupin isolates are shown in Table 3. Non-significant ( $P > 0.05$ ) differences were seen between both bitter lupin isolates in their contents of total alkaloid. However, both sweet lupin isolates were free of total alkaloids. Our results agree well with those reported by Millan et al. (1995); they found that the total alkaloid contents of bitter lupin isolates prepared by alkaline water extraction/isoelectric precipitation, were very low and constituted 0.05%. Generally, the low alkaloids in both bitter isolates were advantageous as supplements in some food products, to avoid a bitter taste.

Bitter lupin isolate-PI had significantly ( $P < 0.05$ ) lower tannin contents than the other isolates, while sweet lupin isolate-MI had significantly ( $P < 0.05$ ) higher phytic acid content than the other isolates. Lampart-Szczapa (1996) found that phytic acid contents were 0.63 and 0.89% for protein isolates of sweet lupin prepared by alkaline water extraction/isoelectric precipitation and by micellisation, respectively.

A non-significant ( $P > 0.05$ ) difference was found in in-vitro protein digestibility between sweet and bitter protein isolates-MI. However, bitter lupin isolate-PI had significantly ( $P < 0.05$ ) higher in-vitro protein digestibility than the other isolates. This could be due to destruction of their tannin contents. Barroga, Laurena, and Mendoza (1985) reported that the tannins play an

Table 2  
Amino acid compositions of bitter and sweet lupin protein isolates (g/16gN)

Amino acid	Bitter lupin protein		Sweet lupin protein		FAO/WHO (1973)
	Isolate-PI <sup>a</sup>	Isolate-MI <sup>b</sup>	Isolate-PI	Isolate-MI	
Isoleucine	4.34	4.58	4.45	4.75	4.00
Leucine	7.14	7.22	7.41	7.34	7.00
Lysine	4.44	4.68	4.30	4.65	5.50
Cystine	1.04	1.50	1.00	1.59	–
Methionine	0.61	0.61	0.68	0.59	–
Total sulfur amino acids	1.65	2.11	1.68	2.18	3.5
Tyrosine	5.26	5.02	5.47	5.24	–
Phenylalanine	3.68	3.59	3.52	3.47	–
Total aromatic amino acids	8.94	8.61	8.99	8.71	6.00
Threonine	3.53	3.47	3.45	3.42	4.00
Tryptophan	0.79	0.76	0.88	0.74	1.00
Valine	3.82	3.45	3.89	3.50	5.00
Total essential amino acids	34.56	34.88	35.05	35.29	36.00
Histidine	3.18	3.12	3.21	3.14	–
Arginine	8.31	8.87	8.29	8.95	–
Aspartic acid	10.62	10.15	10.73	9.70	–
Glutamic acid	26.7	25.0	26.63	25.07	–
Serine	4.77	4.81	5.24	5.18	–
Proline	4.71	5.02	3.66	4.99	–
Glycine	3.36	4.14	3.64	4.00	–
Alanine	3.66	4.02	3.55	3.68	–
Total non-essential amino acids	65.35	65.12	64.95	64.71	–

<sup>a</sup> Isolate-PI, protein isolate prepared by alkaline water extraction/isoelectric precipitation.

<sup>b</sup> Isolate-MI, protein isolate prepared by micellisation.

Table 3  
Antinutritional factors and in-vitro protein digestibility of bitter and sweet lupin protein isolate<sup>a</sup>

Materials	Antinutritional compounds			In-vitro protein digestibility (%)
	Total alkaloids (%)	Tannins (%)	Phytic acid (%)	
Bitter lupin protein isolate-PI	0.09a ± 0.03	0.32a ± 0.04	0.18a ± 0.05	90.8c ± 0.95
Bitter lupin protein isolate-MI	0.15a ± 0.05	0.44b ± 0.05	0.21a ± 0.06	87.6a ± 0.98
Sweet lupin protein isolate-PI	0.00	0.42b ± 0.05	0.21a ± 0.05	88.8b ± 0.84
Sweet lupin protein isolate-MI	0.00	0.49b ± 0.07	0.33b ± 0.06	86.9a ± 1.31
LSD	0.06	0.09	0.14	1.18

<sup>a</sup> Means ± standard deviation of means of three determinations. Means in the same column with different letters are significantly different ( $P < 0.05$ ). LSD, Least significant differences; Isolate-PI, Protein isolate prepared by alkaline water extraction/isoelectric precipitation; Isolate-MI, Protein isolate prepared by micellisation.

important role in reduction of protein digestibility of mung bean flour. The value of in-vitro protein digestibility of sweet lupin isolate-MT confirmed those reported by Lampart-Szczapa (1996).

### 3.5. Biological values and available lysine of lupin protein isolates

The computed biological values and available lysine of lupin protein isolates are shown in Table 4. On the basis of chemical score, both lupin isolates-MI had higher value than isolates-PI. In addition, sweet lupin isolate-MI had a higher chemical score than the other isolates. The first and second limiting amino acids were total sulfur amino acids and valine for all types of isolates, respectively. Generally, these data agree well with those reported by Millan et al. (1995) for lupin protein isolate prepared by alkaline water extraction/isoelectric precipitation. However, the third limiting amino acid was threonine for sweet lupin isolate-PI; it was tryptophan in other isolates. Sweet lupin isolate-MI had a higher essential amino acid index (MEAAI) than other isolates, while bitter lupin isolate-PI had the lowest PER. Generally, chemical score, EAAI and PER values of sweet lupin isolate-MI were higher than other lupin protein isolates. This could be due to the intrinsic content of sweet lupin isolate-MI essential amino acids. Therefore, it would be interesting to utilise sweet lupin isolate by blending with legumes and oil seed proteins to improve its biological values.

Bitter and sweet isolate-MI had significantly ( $P < 0.05$ ) higher available lysine than their isolates-PI. Available lysine ranged from 3.9 to 4.15 g/16 g N. The relatively large amounts of lysine in bitter and sweet isolates make it a good supplement, especially in food doughs.

### 3.6. Functional properties of lupin protein isolates

The functional properties of bitter and sweet lupin isolates are shown in Table 5 and Fig. 3. The protein solubility index of both lupin isolates-MI were significantly ( $P < 0.05$ ) higher than their isolates PI in 0.1

M NaOH and NaCl. The highest protein solubility was observed in 0.1 M sodium hydroxide, while the lowest protein solubility index was in 0.1 M sodium chloride. This may be due to the method used in preparation of the isolate. In fact, sodium chloride solubilises albumins and globulins, while sodium hydroxide extracts albumins, globulins and other protein fractions.

Sweet lupin protein isolates had significantly ( $P < 0.05$ ) higher water absorption capacities than bitter lupin isolates. This could be due to exposing a greater number of water-binding sites during preparation of sweet lupin protein isolates than bitter lupin protein isolates. Our results were higher than those reported by Sathe et al. (1982) for water absorption capacity of lupin protein concentrate (155 g H<sub>2</sub>O/100 g sample). The results may be advantageous for lupin isolates in some foods, especially comminuted meat and baked doughs, which require protein with good water absorption capacity.

Both bitter and sweet lupin isolates-MI had significantly ( $P < 0.05$ ) higher fat absorption capacities than their isolates-PI. The highest fat absorption capacity of isolates-MI may be due to alteration of the protein during isolation, resulting in a structure with more oil-binding sites. Our results were higher than those reported by Abdeen (1987), who found that the fat absorption of lupin isolate prepared by alkaline water extraction/isoelectric precipitation ranged from 200 to 221 g oil/100 g isolate. Kinsella (1976) reported that the ability of proteins to bind fat is an important phenomenon since fats act as flavor-retainers and increase the mouth feel of food.

Bitter and sweet isolates-PI had significantly ( $P < 0.05$ ) lower emulsification capacities than their isolates-MI. Also, the emulsification capacity of sweet isolate-MI was higher ( $P < 0.05$ ) than the other isolates. To evaluate the emulsification properties of a protein, its solubility index is usually determined (Kinsella, 1976). A positive correlation between solubility and the ability of a protein to emulsify oil has been reported by many studies (El-Adawy & Khalil, 1994; El-Adawy et al., 1999). The lupin protein isolate has good emulsification properties, comparable to other known vegetable

Table 4  
Biological values and available lysine of bitter and sweet lupin protein isolates<sup>a</sup>

Materials	Chemical score (%)	Limiting amino acids			EAAI (%)	PER	Available lysine (g/16g N)
		First	Second	Third			
Bitter lupin protein isolate-PI	47.14	Met + Cys	Valine	Tryptophan	56.7	2.22	3.94b
Bitter lupin protein isolate-MI	60.3	Met + Cys	Valine	Tryptophan	58.2	2.28	4.15a
Sweet lupin protein isolate-PI	48.0	Met + Cys	Valine	Threonine	58.0	2.32	3.90b
Sweet lupin protein isolate-MI	62.3	Met + Cys	Valine	Tryptophan	58.7	2.34	4.10a

<sup>a</sup> Means in the same column with different letters are significantly different ( $P < 0.05$ ). Isolate-PI, protein isolate prepared by alkaline water extraction/isoelectric precipitation; Isolate-MI, protein isolate prepared by micellisation; EAAI, Essential amino acids index; PER, Protein efficiency ratio.

Table 5  
Functional properties of bitter and sweet lupin protein isolates<sup>a</sup>

Materials	Protein solubility index in		Water absorption capacity (g/100g isolate)	Fat absorption capacity	Emulsification capacity (ml oil/g protein)	Foam capacity (% volume increase)
	NaOH (0.1 M)	NaCl (0.1 M)				
Bitter lupin protein isolate-PI	95.12a±1.76	21.76a±1.51	209.6a±1.76	281.5b±1.63	164.0a±3.42	104a±2.76
Bitter lupin protein isolate-MI	98.01b±1.98	98.43b±2.54	212.5a±1.97	290.3c±1.51	196.2b±2.96	120b±2.96
Sweet lupin protein isolate-PI	95.63a±2.01	22.62a±1.43	225.7b±2.08	271.6a±3.36	169.4a±2.84	106a±3.12
Sweet lupin protein isolate-MI	98.93b±2.14	98.79b±2.65	229.4b±2.99	285.4c±2.41	210.2c±3.64	146c±2.83
LSD	2.47	3.52	3.68	3.82	5.27	4.65

<sup>a</sup> Means±standard deviation of means of three determinations. Means in the same column with different letters are significantly different ( $P < 0.05$ ). LSD, Least significant differences; Isolate-PI, Protein isolate prepared by alkaline water extraction/isoelectric precipitation; Isolate-MI, Protein isolate prepared by micellisation.

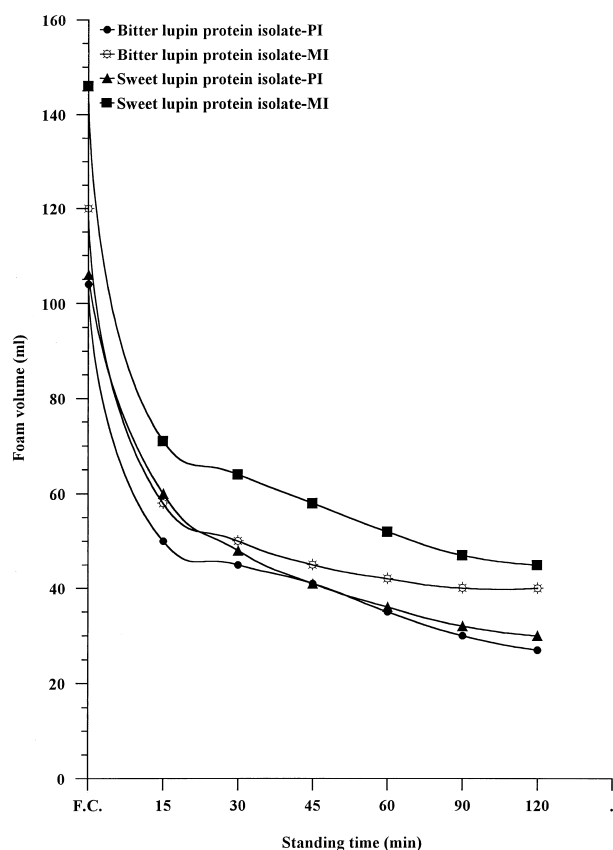


Fig. 3. Foam stability of bitter and sweet lupin protein isolates.

proteins such as soybean. Therefore, its incorporation into meat products, e.g. minced meat analog, will be of great interest. This adds another possible use of lupin protein isolates in food products.

Sweet lupin isolate-MI had significantly ( $P < 0.05$ ) higher foam capacity than other lupin isolates. This could be due to the higher protein solubility index. Deshpande, Rangnekar, Sathe, and Salunkhe (1983) reported that improved foaming of flours may largely be due to a higher globulin fraction of their total protein. Generally, our results agree well with those reported by Abdeen (1987) for lupin protein isolate prepared by

alkaline water extraction/isoelectric precipitation. This could be an advantage in using such materials in soft drinks as a source of protein and it could fit well in bakery products such as cakes, biscuits and breads.

Fig. 3 shows the foam stability of bitter and sweet lupin isolates. Foam stability at room temperature decreased markedly within the first 15 min and then the decrease was gradual up to 90 min and almost stable after that. Sweet lupin isolate-MI had the highest foam stability, followed by bitter isolate-MI then sweet isolate-PI and bitter isolate-PI. Our results agree well with those reported by Abdeen (1987), who found that the foaming stability of lupin protein isolates decreased with increasing time, also after 1.5 h of standing, the foams were less stable. Generally, this decrease may be due to the collapsing and bursting of the formed air bubbles.

#### 4. Conclusions

This study reveals that the sweet and bitter lupin protein isolates prepared by PI and/or by MI were relatively comparable in their nutritional quality and functional properties. Therefore, these results suggest possible use of both sweet and bitter lupin protein isolates as nutrient supplements and as functional agents in many food systems.

#### References

- Abdeen, E. M. I. (1987). *Studies on chemistry and technology of some plant foods*. MSc thesis, Faculty of Agriculture, Kafr El-Sheikh, Tanta University, Egypt.
- Alsmeyer, R. H., Cunningham, A. E., & Happich, M. L. (1974). Equations predict PER from amino acid analysis. *Food Technology*, 28, 34–40.
- AOAC. (1990). *Official methods of analysis* (15th ed.). Washington, DC: Association of Official Analytical Chemists.
- Barroga., C. F., Laurena, A. C., & Mendoza, E. M. (1985). Polyphenol in mung bean (*Vigna radiata* L. Wilczek) determination and removal. *Journal of Agricultural and Food Chemistry*, 23, 1006–1009.

- Beuchat, L. R., Cherry, I. P., & Quinin, M. R. (1975). Physicochemical properties of peanut flour as affected by proteolysis. *Journal of Agricultural Food and Chemistry*, 23, 616–620.
- Chango, A., Villaume, C., Bau, H., Schwertz, A., Nicolas, J., & Mejean, L. (1998). Effects of casein, sweet white lupin and sweet yellow lupin diet on cholesterol metabolism in rats. *Journal of Science and Agriculture*, 76, 303–309.
- Dervas, G., Doxastakis, G., Hadjisavva-Zinoviadi, S., & Triantafyllakos, N. (1999). Lupin flour addition to wheat flour doughs and effect on rheological properties. *Food Chemistry*, 66, 67–73.
- Deshpande, S. S., Rangnekar, P. D., Sathe, S. K., & Salunkhe, D. K. (1983). Functional properties of wheat-bean composite flours. *Journal of Food Science*, 48, 1659–1662.
- El-Adawy, T. A., & Khalil, A. H. (1994). Factors affecting the emulsification capacity of some legumes flours. *Egypt Journal of Food Science*, 22, 249–259.
- El-Adawy, T. A., Rahma, E. H., El-Bedawy, A. A., & Gafar, A. M. (1999). Properties of some citrus seeds. Part 2. Functional properties of seed flours. *Nahrung*, 43, 379–384.
- FAO/WHO. (1973). *Energy and protein requirements* (Report of FAO Nutritional Meeting Series No. 52). Rome: FAO.
- Feldheim, W. (1998). Sweet lupin flour — a very healthy asset. Available at: <http://www.dotfood.com/feature/flour.htm>
- Fields, R. (1972). The rapid determination of amino groups with TNBS. *Methods Enzymol.*, 25B, 464–468.
- Hidvégi, M., & Békés, F. (1984). Mathematical modeling of protein quality from amino acid composition. In R. Lásztity, & M. Hidvégi (Eds.), *Proc. Int. Assoc. Cereal Chem. Symp.* (pp. 205–286). Budapest: Akadémiai Kiadó.
- Hough, G. H., & Jacobs, J. L. (1994). The use of lupin as a feed for dairy and beef cattle. In *Proc. First Australian Lupin Technical Symp.* (pp. 58–66). Perth, Western Australia: Department of Agriculture.
- Hudson, B. J. F. (1979). The nutritional quality of lupin seed. *Qual-Plant Foods Human Nutrition*, 24, 245–257.
- Jenkins, G. L., Knevel, A. M., & Di-Gangi, F. E. (1967). *Quantitative pharmaceutical chemistry* (6th ed.). New York, Toronto, Sydney, London: McGraw-Hill Book Company.
- King, J., Aguirre, C., & Pablo, S. (1985). Functional properties of lupin protein isolates (*Lupinus albus* cv. Multolupa). *Journal of Food Science*, 50, 82–87.
- Kinsella, J. E. (1976). Functional properties of proteins in foods: a survey. *CRC. Critical Review of Food Science and Nutrition*, 7, 219–280.
- Lampart-Szczapa, E. (1996). Preparation of protein from lupin seeds. *Nahrung*, 40, 71–74.
- Lawhon, J. T., Rooney, L., Carter, C. M., & Math, K. F. (1972). A comparative study of the whipping potential of an extract from several oilseed flours. *Cereal Science Today*, 17, 240–243.
- Millan, F., Alaiz, M., Hernandez, P. I., Sanchez, V. R., & Bautista, J. (1995). Study of neutral lipids of *Lupinus mutabilis* meal and isolates. *Journal of American Oil Chemists' Society*, 72, 467–471.
- Miller, E. L. (1967). Determination of the tryptophan content in feeding stuffs with particular reference to cereals. *Journal of Science and Food Agriculture*, 18, 381–386.
- Mohamed, A. A., & Rayas-Duarte, P. R. (1995). Composition of *Lupinus albus*. *Cereal Chemistry*, 72, 634–647.
- Moore, S., & Stein, W. H. (1963). Chromatographic determination of amino acids by the use of automatic recording equipment. In S. P. Colowick, & N. O. Kaplan (Eds.), *Methods in enzymology*, Vol. 6 (pp. 815–860). New York: Academic Press.
- Morrow, B. (1991). The rebirth of legumes. *Food Technology*, 45, 96–121.
- Oser, B. L. (1959). An integrated essential amino acid index for predicting the biological value of proteins. In A. A. Albanese (Ed.), *Protein and amino acid nutrition* (pp. 295–311). New York: Academic Press.
- Rahma, E. H., & Narasinga Rao, M. S. (1979). Characterization of sunflower proteins. *Journal of Food Science*, 44, 579–582.
- Rahma, E. H., & Narasinga Rao, M. S. (1984). Effect of debittering treatment on the composition and protein components of lupin seed (*Lupinus termis*) flour. *Journal of Agricultural and Food Chemistry*, 32, 1026–1030.
- Ruiz Jr., L. P. (1978). Alkaloid analysis of sweet seed by GLC. *N. Z. Journal of Agricultural Research*, 21, 241–247.
- Ruiz Jr., L. P., & Hove, E. L. (1976). Conditions affecting production of protein isolate from lupin seed kernel. *Journal of Science and Food Agriculture*, 27, 667–674.
- Salgó, A., Granzler, K., & Jecsei, J. (1984). Simple enzymatic methods for predication of plant protein digestibility. In R. Lásztity, & M. Hidvégi (Eds.), *Proc. Int. Assoc. Cereal Chem. Symp.* (pp. 311–321). Budapest: Akadémiai Kiadó.
- SAS. (1985). *SAS users guide: statistics version* (5th ed.). Cary, NC, USA: SAS Institute.
- Sathe, S. K., Deshpande, S. S., & Salunkhe, D. K. (1982). Functional properties of lupin seed (*Lupinus mutabilis*) proteins and protein concentrates. *Journal of Food Science*, 47, 491–497.
- Sosulski, F. W. (1962). The centrifugal method for determining flour absorption in hard red spring wheats. *Cereal Chemistry*, 39, 344–347.
- Sosulski, F. W., Humbert, E. S., Bui, K., & Jones, J. D. (1976). Functional properties of rapeseed flours, concentrate and isolate. *Journal of Food Science*, 41, 1349–1352.
- Sosulski, F. W., & Youngs, C. G. (1979). Yield and functional properties of classified protein and starch fractions from eight flours. *Journal of American Oil Chemists' Society*, 56, 292–295.
- Sosulski, F. W., Chakraborty, P., & Humbert, E. (1978). Legumes based limitation and blended milk products. *Canadian Institute of Food Science and Technology*, 11, 117–121.
- Sousa, I. M. N., Morgan, P. J., Mitchell, J. R., Harding, S. E., & Hill, S. E. (1996). Hydrodynamic characterization of lupin proteins solubility, intrinsic viscosity and molar mass. *Journal of Agricultural and Food Chemistry*, 44, 3018–3021.
- Wheeler, E. I., & Ferrel, R. E. (1971). A method for phytic acid determination in wheat and wheat fractions. *Cereal Chemistry*, 48, 312–316.