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Full Length Research Paper

Production of functional protein hydrolysates from Egyptian breeds of soybean and lupin seeds

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Enzymatic hydrolysis is an agro-processing aid that can be utilized in order to improve nutritional quality of protein extracts from many sources. In this study, protein extracts from ungerminated and/or germinated local Egyptian soybean and lupin flours were hydrolyzed using the enzyme papain. The hydrolysis processes were carried out for 2 h and aliquots were withdrawn at different time intervals. We have analysed the protein hydrolysate after 30 min hydrolysis as an example of a partially hydrolyzed protein, and after 120 min as an example of greatly hydrolyzed protein. The hydrolysate (2 h treatment at 80°C and pH 7.4) from both soybean and lupin flour contained significantly decreased trypsin inhibitor activity and urease activity, and a reduced phytate content, which improved the overall protein quality. Hydrolysis caused almost complete inactivation of urease in all soybean and lupin samples regardless if the seeds were germinated or not. High protein content, nitrogen solubility and *in vitro* protein digestibility was shown after hydrolysis. Total protein content (in g/100 g extract) increased in hydrolyzed samples from 48.1 to 51-60 for soybean (dependent on pre-treatment) and from 36.8 to 39.9-48.6 for lupin. Total essential amino acid content was also increased in papain hydrolyzed samples, compared to that in raw and germinated legumes. More specifically, the amount of lysine, sulphur amino acids, histidine, and to a certain extent isoleucine and threonine increased in samples from both legume species. All soybean samples exhibited antioxidant activity while in lupin samples, only those subjected to hydrolysis showed activity. Generally, it was clearly observed that the longer the duration of enzymatic hydrolysis (within the time frame of the experiment), the higher the improvement of the nutritional quality.

Key words: Lupin, soybean, germination, enzymatic hydrolysis, papain, protein extract, nutritive value.

INTRODUCTION

Legume seeds contain 20-25% protein, which is 2-3 times higher than the content in cereals, and have therefore been considered as leading candidates for protein supply to malnourished areas of the world (Chavan et al., 1989). Utilization of oilseed and legume protein has however had a lower potential, partly due to deficiency of some of the essential amino acids in these proteins, and also due to the presence of antinutritional factors associated with these proteins (Kavas and Nehir, 1992). In order to improve the nutritive value and digestibility, and to reduce antinutritional factors, a number of methods can be applied and include soaking, dehulling, and germination (Sripriya et al., 1997), fermentation (Czarnecka et al., 1998), cooking (Kaankuka et al., 1996), heat treatment (Mulimani and

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Abbreviations: AOA, Antioxidant activity; EAA, essential amino acids; TIA, trypsin inhibitor activity; and UA urease activity.

Paramjyothi, 1994), and irradiation (Joseph and Dikshit, 1993). Of the above methods, focus is in this work directed towards germination, which is here used in combination with enzymatic hydrolysis for improvement of nutrient availability.

Seed germination is a biological process in which the plant restores its activity to full potential and under which enzymes are very active. Appropriate seed germination conditions may reduce some antinutritional factors or decrease the need of additional treatments such as cooking time and temperature required to inactivate these factors (Bau et al., 1997) . In addition, the relative nutrition value, protein efficiency ratio, as well as the content of lysine, methionine and tryptophan (Hamad and Fields, 1979), and several vitamins (Banerjee et al., 1955) can be increased, leading to increased protein digestibility (Schulze et al., 1997), and consequently improvement of nutritional quality. The content of trypsin inhibitors and phytates may also decrease, but considerable amounts of these factors are still present after germination (Donangelo et al., 1995; Silva and Trugo, 1996).

Hydrolysis of food proteins is carried out for many reasons*, e.g*. improving nutritional characteristics, retarding deterioration, imparting texture, increasing or decreasing solubility, adding emulsifying capacity, preventing undesired interactions, removing off-flavours or odours, and removing toxic or inhibitory ingredients (Feeney, 1986; Lahl and Grindstaff, 1989; Lahl and Braun 1994). The hydrolysis process generates smaller peptides with improved nutritional characteristics compared to the original protein. Enzymatic hydrolysis of protein *in vitro* mimics parts of the enzymatic hydrolysis of the *in vivo* ingested protein. In fact, *in vitro* proteolysis can also be considered a pre-digestion of proteins that enhances absorption (Villaneuva et al., 1999), and it has been demonstrated that some short peptides are absorbed in the digestive tract at a higher rate than free amino acids (Mathews et al., 1076). This suggests an advantage of protein hydrolysates with respect to equivalent amino acid mixtures in applications such as diets for surgical patients and for the elderly (Frøkjer, 1994). Protein hydrolysates have also been used in sports nutrition and weight control (Frøkjer, 1994), and in special medical diets for some diseases such as pancreatitis, short bowel syndrome, Crohn's disease and food allergies (Schmidl et al., 1984). Antioxidant activity (AOA) of protein hydrolysates prepared from soybean (Pena-Ramos and Xiong, 2002) rice bran (Paraman et al., 2004) and whey protein (Pena-Ramos et al., 2004) has been reported. Reports have also shown that their essential amino acid content do meet the human nutritional requirements with the exception of lysine, and methionine (Papavergou et al., 1999).

In the present study, the cysteine protease papain (a papaya enzyme, accepted as dietary supplement) was used for hydrolysis of a local Egyptian breed of soybean (with or without germination). In addition, a local breed of lupin seeds was subjected to the same treatment. Very little research has, to the best of our knowledge, been conducted to evaluate the nutritional value of protein hydrolysates prepared from lupin seeds. The aim here is to evaluate the effect of the enzymatic treatment on the antinutritional properties, protein solubility and digestibility, and the antioxidant activity of the hydrolyzed protein products. The essential amino acid composition of the protein hydrolysates was also investigated.

MATERIALS AND METHODS

Seeds and reagents

Local Egyptian breeds of soybean (*Glycine max* L. variety Giza) and lupin (*Lupinus albus* L. variety Giza) were obtained from the Agricultural Research Centre, Giza, Egypt. Papain (EC 3.4.22.2, ref *P*-3375 Sigma) activity while carrying out legume hydrolysis was *ca*. 8.9 units/mg. All other chemical reagents used in the experimental analysis were of analytical grade.

Seed pre-treatments

The seeds were cleaned by rinsing twice with distilled water, sterilized with 1% sodium hypochlorite solution for 1 min, again rinsed twice with fresh distilled water, and finally dried in a ventilated oven at 40°C. The dried seeds were divided into four sets (Table 1): one kept as a raw sample, one used for germination treatment (germinated), and the above two sample sets were further subjected to hydrolysis treatment (raw-hydrolysed, and germinated-hydrolysed). Lupin seeds were crushed to smaller fragments and then milled in a Tecator Cyclotec mill using 1-mm sieve to flours. Break and reduction flours were collected together and blended thoroughly. The blended flour was used throughout this research. Soybeans were milled to flour directly in a Tecator Cyclotec mill.

Germination

Germination was carried out by spreading the soaked grains on blotting papers moistened with distilled water containing 5 gml^{-1} of penicillin and streptomycin to inhibit mould growth. The seeds were kept in the dark to germinate at 25°C for 72 h and kept wet throughout germination by spraying them with distilled water every 12 h. The unimbibed seeds were discarded and the sprouts were rinsed twice in distilled water and dried in a ventilated chamber at 80°C. The dried sprouts were crushed and then milled as described above and the flour was kept at -20°C until used.

Enzymatic hydrolysis

The hydrolysis of soybean and lupin (ungerminated or germinated) powders was carried out using papain enzyme. Optimum hydrolysis conditions determined for papain using soy bean and lupin meals as substrates were enzyme: substrate ratio [E/S] = 0.03 g/g, pH 7.4 at 80°C. The dispersion of the powder was mixed with papain enzyme in a ratio of 1:10 (w/v). The mixture was adjusted to pH 7.4 by adding 0.1 M NaOH and maintained at 80°C for 120 min in a thermostatic reaction vessel under continuous agitation by a magnetic stirrer, in order to ensure the stability of the substrate solution at the selected pH before adding the enzymes.

Table 1. Different treatments of soybean and lupin seeds.

Legume	Treatment		Hydrolysis time	Abbreviation
	Germination	Hydrolysed		
Soybean	No	No	-	S
	Yes	No	-	SG
	Yes	Yes	30 min	SG-h30
	Yes	Yes	120 min	SG-h120
	No	Yes	30 min	SUG-h30
	No	Yes	120 min	SUG-h120
Lupin seed	No	No	-	L
	Yes	No	-	LG
	Yes	Yes	30 min	LG-h30
	Yes	Yes	120 min	LG-h120
	No	Yes	30 min	LUG-h30
	No	Yes	120 min	LUG-h120

The degree of hydrolysis was considered a measure of *in vitro* protein digestibility. Each determination was performed in triplicate. The hydrolysis process was stopped by dropping the pH to 3.0 and then the mixture was kept at 80°C for 15 min with gentle stirring. The mixture was decolorized by adding 2% charcoal with constant stirring at 55°C for 20 min then filtered using Whatman filter paper No 4. Aliquots of the filtrates (hereinafter termed hydrolysate) were taken for determination the antioxidant activity. The rest of the protein hydrolysates were freeze dried and kept -20°C for further analysis.

Legume seed protein extracts

The flours of untreated (raw) and treated (germinated) samples were subjected to defatting in a Soxhlet apparatus using n-hexane (1:3 w/v) at a temperature of about 40°C until the fat content was reduced to about 5%. Defatted samples were spread to dry at room temperature until all traces of solvent were removed, and saved for further analysis. Fine powder from air-dry seeds was used for estimation of nitrogen fractions. Antinutritional factors, antioxidant activity, and amino acid and protein fractions were analyzed in defatted seed powder.

Analyses

Protein content: Nitrogen content was determined by the microkjeldahl technique according to AOCS (1998) methods of analysis. The conventional nitrogen-to-protein conversion factor of 6.25 was used for estimating the protein content. Nitrogen solubility was determined according to Lyman et al. (1953). *In vitro* protein digestibility (IVPD) was determined according to Hsu et al. (1977) using an enzyme mixture. The protein solutions, 6.25 mg ml^{-1} in distilled water, were adjusted to pH 8.0 with 0.1 M NaOH while stirring at 37°C in a water bath. The enzyme mixture (1.6 mg ml^{-1} trypsin, 3.1 mg ml^{-1} chymotrypsin and 1.3 mg ml^{-1} peptidase) was maintained in an ice bath and adjusted to pH 8.0 with 0.1 M NaOH. The multienzyme solution was added to the protein solution at a ratio of 1:10 (v/v) . The pH decrease was recorded over a 10 min period with a pH meter. Percentage protein digestibility (*Y*) was then calculated from the equation $Y = 210.46 – 18.10X$, where *X* is the pH after 10 min.

Amino acid analysis: Amino acid content was determined as described by Moore et al. (1958). The analysis was performed in Central Service Unit, National Research Centre, Egypt using LC3000 amino-acid analyzer (Eppendorf -Biotronik, Germany). The technique was based on the separation of the amino acids using strong cation exchange chromatography followed by the ninhydrin colour reaction and photometric detection at 570 nm. Standard amino acids were used for comparison of resulting profiles, allowing quantitation of amino- acid residues. The defatted powdered seeds were hydrolyzed with 6 N HCl at 110°C in Teflon capped vials for 24 h. After vacuum removal of HCl, the residues were dissolved in a lithium citrate buffer, pH 2.2. Twenty l of the solution were loaded onto the cation exchange column (pre-equilibrated with the same buffer), then four lithium citrate buffers with pH values of 2.2, 2.8, 3.3 and 3.7, respectively, were successively applied to the column at a flow rate of 20 ml/min. The ninhydrin flow rate was 10 ml/h under these conditions and a typical analysis required 160 min.

Cysteine and methionine were determined as cysteic acid and methionine sulfone, respectively, after oxidation with performic acid (Schram et al., 1954). An amino acid standard containing cys was treated parallel with the samples and used to quantify the cys content. The amino acid content of the reference protein was taken from FAO/WHO (1991). The essential amino acid (EAA) score was also calculated:

$$\text{EAA score} = \frac{\text{g of EAA in 100 g of test sample}}{\text{g of EAA in 100 g of FAO/WHO ref. pattern}} \times 100$$

Antinutritional properties: Trypsin inhibitor activity was determined in quadruplicate according to Hammerstrand et al. (1981) with benzoyel-D,L-arginine *p*- nitroanilide. Urease activity was estimated as described in AOCS (1908) methods of analysis. Phytate content was determined as described by Wheeler and Ferrell (1971) and as modified by Alonso et al. (1995). Results are expressed as percentage phytic acid by using phytic acid as standard.

Antioxidant activity (AOA): Defatted dried plant material was extracted by continuous mixing in 80% ethanol, 1-2 h at 50°C. After filtration, ethanol was evaporated, and then the extracts were dried, filtered and concentrated to dryness using a rotary evaporator. AOA was determined according to the -carotene/linoleate coupled oxidation described by Marco (1968) and modified by Velioglu et al. (1998). The rate of bleaching of -carotene solution was

Table 2. Effect of germination and/or hydrolysis on protein, N-solubility and *in vitro* digestibility of soybean and lupin samples[1].

Sample[2]	Protein (%)	Retention (%)[3]	N-Solubility (%)	Retention (%)	Digestibility (%)	Retention (%)
S	48.1 ±		86.2 ±		90.3 ±	
SUG-h30	58.0 ±	20.1	90.4 ±	4.9	92.8 ±	2.8
SUG-h120	60.2 ±	25.2	95.0 ±	10.2	98.5 ±	9.1
SG	49.8 ±	3.5	87.3 ±	1.3	92.7 ±	2.7
SG-h30	51.0 ±	6.0	97.0 ±	12.5	95.8 ±	6.1
SG-h120	55.4 ±	15.2	100 ±	16.0	98.9 ±	9.5
L	36.8 ±		83.3 ±		91.8 ±	
LUG-h30	39.9 ±	8.4	97.4 ±	17.0	93.3 ±	1.6
LUG-h120	48.6 ±	32.1	100 ±	20.0	97.5 ±	6.2
LG	37.9 ±	3.0	85.5 ±	2.6	93.6 ±	2.0
LG-h30	42.1 ±	14.4	87.5 ±	1.3	95.2 ±	3.7
LG-h120	45.5 ±	32.6	96.5 ±	15.8	98.6 ±	7.4

[1]Values are means ± SE of three replicates.
[2]Symbols as in Table 1.
[3]Retention is percentage relative to content in raw beans.

determined by the difference in absorbance at 470 nm between the initial reading (time = 0 min) and the last reading for which the bleaching remained essentially linear (time = 120 min). The absorbance was evaluated every 15 min, and was plotted against time. The difference in absorbance between a blank and the hydrolysate was an indication of the AOA.

RESULTS AND DISCUSSION

Enzymatically hydrolyzed proteins are expected to have several advantages over the original undigested proteins *e.g*. improved solubility and digestibility; improved functional properties; as well as reduced content of antinutritional factors. Hence, enzymatic hydrolysis can be considered as a biotechnological process that leads to the production of functional foods. In this study, proteins from soybean and lupin flours (either ungerminated or germinated) were hydrolyzed using the enzyme papain. This enzyme was selected based on its accepted use as a dietary supplement, being isolated from papaya, a fruit that is consumed as a food source. The hydrolysis processes were carried out for 2 h and aliquots were withdrawn at 10, 20, 30, 60 and 120 min (data not shown) . Based on these data, protein hydrolysates after 30 and 120 min were selected as examples of a partially hydrolyzed and a greatly hydrolyzed protein, respectively. Thus, untreated soybean (S), ungerminated soybean (SUG) and germinated soybean (SG) were analysed after being exposed to the hydrolysing enzyme for 30 min (SG-h30), (SUG-h30) and 120 min (SG-h120), (SUG-h120. Similarly, ungerminated lupin (LUG) and germinated (LG) samples were subjected to the hydrolysis treatment for 30 min (LUG-h120, LG-h30) and 120 min (LUG-h120, LG-h120), respectively.

Protein, nitrogen solubility and *in vitro* protein digestibility

The enzyme hydrolysis treatments resulted in a remarkable increase in the protein content (displayed as %, calculated from the amount in g/100 g extracted seed flour), while germination resulted in only a slight increase (Table 2). For both soybean and lupin seed flours it was found that the longer the duration of hydrolysis the higher the increase in protein content, with the highest value observed in ungerminated hydrolysed samples (SUG-h120 and LUG-h120) (Table 2). A probable explanation is that some insoluble constituents (at pH 7.4) in the flour are removed during the hydrolysis process, hence leading to the higher protein ratio. Perez and Saad (1984) have likewise reported high protein content in thesame protein hydrolysates (prepared using Neutrase and Alcalase, and then spray dried or freeze dried). In their work, the protein content of hydrolysates was 66.3 - 66.9% (compared to a content of maximum 60% in our work (Table 2), while nitrogen solubility was about 85%. Claver and Zhou (2005) hydrolyzed wheat germ protein with several enzymes and has reported that the highest increase in solubilized protein was also observed after using a combination of Alcalase and Fluorozyme (85 and 80%, respectively). However, in their work, papain treatment also showed increased solubilization of the protein during the first 300 min of hydrolysis and reached 73% protein content.

Table 3. Essential amino acid composition and score of papain hydrolysed lupin samples[1].

Amino Acids	FAO/WHO reference value[3]	Amino acid content (gram per 100 grams protein)											
		Raw		Ungerminated lupin[5]				Germinated lupin					
		L	EAA Score[4]	LUG-h30	EAA Score	LUG-h120	EAA Score	LG	EAA Score	LUG-h30	EAA Score	LUG-h120	EAA Score
Lys	5.8	5.54	0.95	14.6	2.52	15.8	2.72	5.54	1.13	17.68	3.05	19.17	3.30
His	1.9	6.56	3.49	6.98	3.67	7.21	3.79	2.52	1.31	3.27	1.72	3.04	1.60
Val	3.5	4.15	1.19	1.69	0.48	2.11	0.60	3.84	1.10	2.08	0.59	2.35	0.67
Thr	3.4	3.07	0.90	3.60	1.06	4.80	1.41	1.89	0.55	2.97	0.87	2.69	0.79
Cys + Met[2]	2.5	2.03	0.81	4.83	1.92	5.64	2.25	2.33	0.93	6.27	2.51	3.46	1.38
Leu	6.6	6.56	0.99	4.20	0.64	3.92	0.59	8.74	1.32	4.62	0.70	4.43	0.67
Ile	2.8	4.70	1.68	5.91	2.11	6,02	2.15	5.09	1.82	5.74	2.05	5.83	2.08
Tyr + Phe	6.3	6.28	1.0	7.30	1.16	7.93	1.26	9.61	1.52	6.60	1.05	5.81	0.92
Total EAA	32.8	38.89	1.18	49.11	1.5	53.43	1.63	39.56	1.20	49.23	1.5	46.78	1.43

[1]Analysis performed in duplicates, (Trp and Arg determination not applicable by the acid hydrolysis method).
[2]Total of aromatic amino acids (Tyr, Phe) as well as sulfur amino acids (Cys, Met) were calculated.
[3]FAO/WHO. Protein quality evaluation; Food and Agriculture Organization of the United Nations. Daily requirements for human adults.1991, Rome, Italy. P. 66.
[4]Essential amino acids **(**EAA) score of FAO/WHO reference pattern.
[5]Symbols as in Table 1.

Table 4. Amino acid composition and essential amino acid score of papain hydrolysed soybean samples[1].

Amino Acids	FAO/WHO reference value[3]	Amino acid content (gram per 100 grams protein)											
		Raw		Ungerminated soybean[5]				Germinated soybean					
		S	EAA Score[4]	SUG-h30	EAA Score	SUG-h120	EAA Score	SG	EAA Score	SG-h 30	EAA Score	SG-h120	EAA Score
Lys	5.8	6.43	1.11	8.30	1.43	9.61	1.65	6.44	1.11	10.8	1.86	11.32	1.95
His	1.9	2.68	1.41	4.21	2.22	5.06	2.66	2.99	1.57	5.78	3.04	6.2	3.26
Val	3.5	4.35	1.24	2.14	0.60	2.43	0.69	4.72	1.35	1.57	0.45	1.55	0.44
Thr	3.4	3.14	0.92	2.44	0.71	3.42	1.01	1.97	0.58	3.04	0.89	2.81	0.85
Cys + Met[2]	2.5	1.01	0.40	2.92	1.16	3.74	1.48	1.91	0.76	3.85	1.54	4.43	1.77
Leu	6.6	7.06	1.07	5.33	0.80	5.81	0.88	7.73	1.17	4.90	0.74	5.09	0.77
Ile	2.8	5.26	1.88	6.01	2.14	4.60	1.64	4.74	1.69	5.96	2.14	5.93	2.12
Tyr + Phe	6.3	8.48	0.71	6.80	1.08	7.32	1.16	9.12	1.45	7.94	1.60	7.73	1.23
Total EAA	32.8	38.41	1.17	38.15	1.16	41.99	1.28	39.62	1.21	43.84	1.34	45.06	1.37

[1]Analysis performed in duplicates (Trp and Arg determination not applicable by the acid hydrolysis method).
[2]Total of aromatic amino acids (Tyr, Phe) as well as sulfur amino acids (Cys, Met) were calculated.
[3]FAO/WHO. Protein quality evaluation; Food and Agriculture Organization of the United Nations. Daily requirements for human adults.1991, Rome, Italy. P. 66.
[4]Essential amino acids **(**EAA) score of FAO/WHO reference pattern.
[5]Symbols as in Table 1.

Table 5. Trypsin inhibitor activity, urease activity and phytate content in germinated and/or hydrolysed of soybean and lupine samples[1].

Sample[2]	Trypsin inhibitor Activity[3]	Difference (%)	Urease activity[4]	Difference (%)	Phytate content[5]	Difference (%)[6]
S	4.50 ± 0.28	-	2.03 ± 0.13	-	6.0 ± 0.16	-
SUG-h30	1.69 ± 0.17	62.6	nil	100	2.3 ± 0.07	61.6
SUG-h120	0.11 ± 0.03	97.5	nil	100	2.0 ± 0.08	66.6
SG	2.11 ± 0.19	53.1	1.78 ± 0.09	12.3	5.3 ± 0.15	11.7
SG-h30	2.00 ± 0.16	55.6	0.06 ± 0.02	97	3.1 ± 0.10	48.3
SG-h120	1.37 ± 0.14	69.5	nil	100	2.2 ± 0.07	63.3
L	1.55 ± 0.16	-	0.52 ± 0.07	-	4.2 ± 0.11	-
LUG-h30	1.13 ± 0.20	29.5	0.04 ± 0.00	92.3	1.3 ± 0.05	69.1
LUG-h120	0.16 ± 0.05	89.5	0.02 ± 0.00	96.1	1.0 ± 0.04	76.1
LG	1.55 ± 0.15	-	0.37 ± 0.06	28.2	3.1 ± 0.11	26.2
LG-h30	1.55 ± 0.14	-	nil	100	2.8 ± 0.06	33.3
LG-h120	1.47 ± 0.22	51.4	nil	100	1.9 ± 0.04	54.8

[1]Values are means ± SE of three replicates.
[2]Symbols as in Table 1
[3]Trypsin inhibitor activity expressed in trypsin inhibitor units (TIU)/mg protein.
[4]Urease activity expressed in enzyme units/ mg protein.
[5]Phytate content expressed mg phytate/g flour.
[6]Retention is percentage relative to content in raw beans.

One of the most important physicochemical and functional properties of protein hydrolysates is their solubility over a wide range of pHs, temperatures, nitrogen concentrations and ionic conditions (Mahmoud, 1994). Increased solubility of diverse proteins can be gained by enzymatic hydrolysis (Turgeon et al., 1992), and studies have shown that even limited or partial hydrolysis leads to substantially increased solubility of the resulting hydrolysate, particularly at the isoelectric point of the parent protein. Results from the nitrogen solubility tests proved that also in our case, hydrolysis improved solubility of the proteins in the soy bean and lupin flours tested (Table 2). A longer hydrolysis time solubilized more protein, and thus also led to higher nitrogen solubility. The enhanced solubility of the hydrolysates is reported to be due to their smaller molecular size (Turgeon et al., 1992) and the newly exposed amino and carboxyl groups that increase the hydrophilicity of the hydrolysates (Phillips and Beuchat, 1981). The values higher than 100% observed here result from use of the conventional Kjeldahl factor of 6.25 used for nitrogen-to-protein conversion. That explains why some references used a conversion factor of 5.75 to estimate protein content in soybeans (Lyman et al., 1953).

In vitro protein digestibility was improved in all hydrolysates under examination. In accordance with this, highly hydrolyzed lupin proteins (120 min) showed higher digestibilities than partially hydrolyzed (30 min). In addition, germination resulted in a slight increase in protein digestibility in comparison to raw soybean and lupin samples (Table 2). The increase in the protein digestibility is probably connected to the reduction in trypsin inhibitor activity (TIA) (as indicated in Table 5), as protease inhibitors can reduce digestion by decreasing or inhibiting the action of the pancreatic enzymes trypsin and chymotrypsin (Liu, 1997) . Ritter et al. (1987) have also reported that reduction of phenolics and phytate (the latter also shown to decrease as a result of hydrolysis (Table 5) in soy protein were connected to slightly enhanced digestibility compared to the controls. The unfolding of native protein structure during the cause of hydrolysis is yet another factor that likely facilitates digestibility.

Amino acid contents

The essential amino acid (EAA) composition of germinated and /or hydrolyzed soybean and lupin protein are presented in Tables 3 and 4. The amino acid profiles of raw legumes revealed that the level of the majority of EAA were comparable to those of the FAO/HO requirements (1991). The content can, however, be as reported by Oshodi et al. (1995) to dependent on environmental factors, and the conditions under which

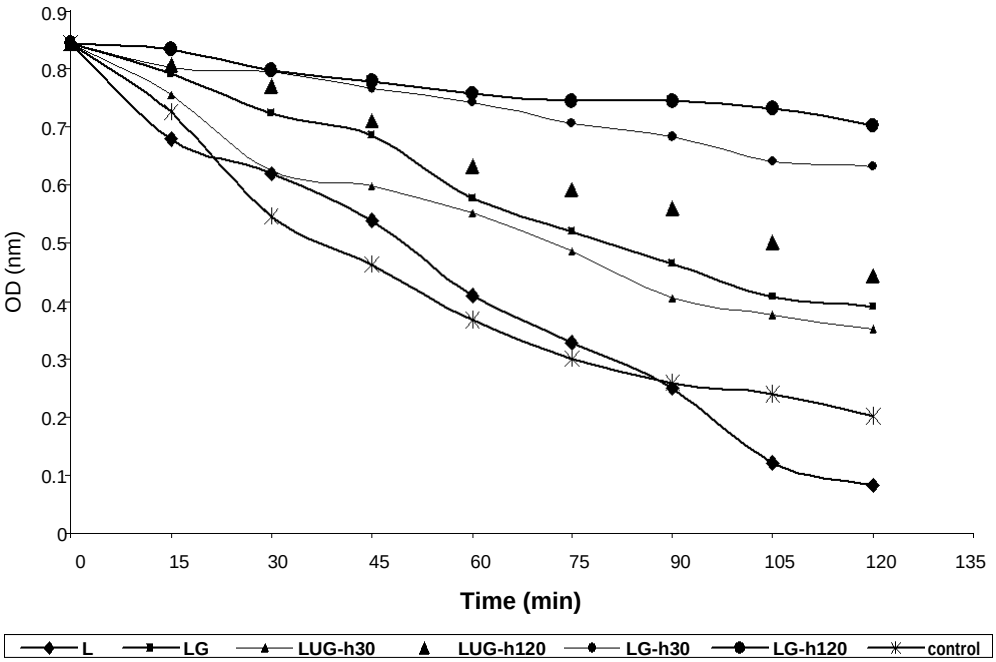

Figure 1. Antioxidant activity of ungerminated, germinated, and hydrolysed lupin in a -carotene linoleate system. L, raw lupin; LG, germinated lupin; LUG-h30, ungerminated lupin hydrolysed for 30 min; LUG-h120, ungerminated lupin hydrolysed for 120 min; LG-h30, germinated lupin hydrolysed for 30 min; LG- h120, germinated lupin hydrolysed for 120 min. Values are means of three replicates. OD on Y-axis is at 470 nm.

food legumes were grown could hence influence their amino acid composition.

In the current study, the total EAA did not differ much as a consequence of germination, while hydrolysis in all cases led to increases. This trend was especially obvious for the lupin samples (Table 3), while germination pre-treatment of soy samples resulted in a slightly higher EAA content at the corresponding hydrolyzing time (Table 4). The amount of lysine, sulphur amino acids, and histidine were generally increased after hydrolysis, while the content of isoleucine, threonine was either increased or unaffected, and leucine, valine and aromatic amino acids decreased.

Germination of soybean and lupin did not result in increased lysine content, but resulted in higher Lys-content in the following hydrolysis step. In addition, there was a large increase in sulphur amino acids after hydrolysis of germinated soybean and lupin samples. This is in agreement with the results on soy bean hydrolysis by Misharina et al. (1987), who also proposed a plant scale production technology for high protein enzymatic hydrolysates from soybean that resulted in 5-7 times higher amino acid content than in conventionally produced samples. Our results however contradict the observations of Arias and Felacio (1988), who found that hydrolysis of an insoluble soybean isolate with *Penecillium duponti* K 1104 (and of defatted sesame seed by a bacterial proteinase) did not result in significant differences in amino acid composition between the hydrolysates and the original material. In this work, papain hydrolysis improved the content of both Lys and Met-residues, both of which were previously identified to be present in low content in legume seeds.

Antinutritional factors

The effect of germination and/or enzymatic hydrolysis on the levels of three antinutritional factors, trypsin inhibitor activity (TIA), urease activity (UA) and phytate content, was analysed in the extracts of soybean and lupin flour, and the reduction of each factor was calculated based on the original values obtained for the untreated ones (Table 5).

The highest reduction in TIA was achieved after hydrolysis of ungerminated samples, using both soybean and lupin flour extracts. Germination of soybean (without any following hydrolysis) still resulted in 53.1% reduction in TIA, while germinated lupin did not show any reduction in TIA compared to the untreated sample. Papain hydrolysis considerably decreased TIA also in the germinated sample of lupin origin (120 min hydrolysis resulted in 51.4% reduction), while for soybean, the reduction obtained between the germination and hydrolysis step was significantly smaller.

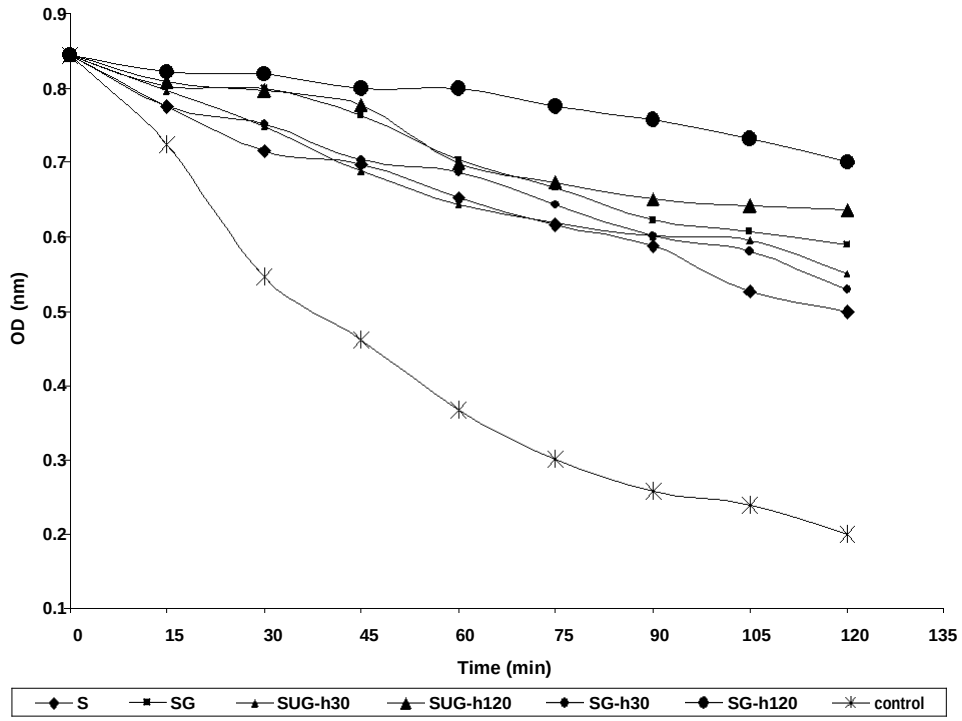

Figure 2. Antioxidant activity for ungerminated, germinated, and hydrolysed soybeans in a -carotene linoleate system. S, raw soybean; SG, germinated soybean; SUG-h30, ungerminated soybean hydrolysed for 30 min; SUG-h120, ungerminated soybean hydrolysed for 120 min; SG-h30, germinated soybean hydrolysed for 30 min; SG-h120, germinated soybean hydrolysed for 120 min. Values are means of three replicates. OD on Y-axis is at 470 nm.

Urease activity (UA) in germinated soy and lupin samples (without hydrolysis) was reduced by 12 and 28%, respectively. Papain hydrolysis then caused almost complete inactivation of urease in all soybean and lupin samples regardless if they were germinated or not (Table 5).

The reduction of phytate follows the same trend as those of TIA and UA. Germination only resulted in a small phytate reduction in soy and lupin samples compared to germinated-hydrolysed samples. The longer the hydrolysis time, the higher was the phytate reduction (Table 5).

It is clear that enzymatic hydrolysis of soybean and lupin flour protein with papain enzyme (2 h at 80°C and E/S = 0.003 and pH 7.4) caused a significant reduction of all three antinutritional factors (TIA, UA and phytate content, Table 5), thus improving the digestibility of the hydrolyzed proteins. Huo et al. (1993) have previously reported on the inactivation of trypsin inhibitor, chymotrypsin inhibitor and lectins of raw soybean by incubation with microbial proteases. It is not unusual that enzymatic protein hydrolysis using external enzymes causes a reduction in the antinutritional factors present in seed proteins, since enzymatic hydrolysis imitates the fermentation and germination processes that are known to activate endogenous enzymes.

Antioxidant activity of protein hydrolysates

The most prominent antinutritional factors in lupin seeds are bitter and toxic quinolizidine alkaloids, which occur at concentrations up to 2.5% (Papavergou et al., 1999). These alkaloids can be removed from defatted lupin extracts (extracted by hexane) by aqueous alcohols (*e.g.* methanol, ethanol, and isopropanol) to produce a de-bittered protein concentrate (Chajuss, 1989) . Before evaluating antioxidant activity (AOA), protein extracts were hence pre-treated with ethanol after which AOA was measured by a -carotene/linoleate system, in which the bleaching rate of a -carotene solution was determined by the difference in absorbance readings between a control (blank) and the protein hydrolysate (Figures 1 and 2).

Raw lupin hydrolysates (L) did not exhibit AOA, but after germination (LG) slightly higher levels were observed (Figure 1). Furthermore, papain hydrolysis boosted the AOA in both ungerminated (LUG) and germinated (LG) lupin samples. The levels observed were increased by both germination and with hydrolysis time.

All soybean samples exhibited AOA (Figure 2). Both germination and/or enzymatic hydrolysis increased the AOA. This is contradictory to the papain hydrolysis res-

ults obtained by Pena-Ramos and Xiong (2002), who found that a papain hydrolysed soy isolate did not exhibit AOA (while pepsin, chymotrypsin and Fluorozyme hydrolysates all showed AOA). This indicates that the AOA-levels may be dependent on the soybean breed.

Bishov and Henick (1975) have reported the hydrolyzed vegetable proteins themselves to be the primary antioxidants, and that they act in synergy with phenolic antioxidants. The effect of amino acids on the oxidation of linoleic acid and its methyl ester has been verified by Marcuse (1962), who found that the amino acids tested (except cysteine) had a potential antioxidant effect, i.e. amino acids can act as pro-oxidants as well as antioxidants depending on the circumstances. The AOA of different amino acids may differ, being especially pronounced in the case of histidine and tryptophan which are strongly antioxidative. According to Elias and McClements (2004), the antioxidant capacity and oxidation kinetics of methionine, cysteine and tryptophan (in oil in water emulsions) renders them effective as antioxidants, and these amino acids are oxidized before the lipid component of the emulsions thus inhibiting lipid oxidation by acting as endogenous scavengers. The essential amino acid composition of the soybean and lupin hydrolysates (Tables 3 and 4) showed obvious increases in lysine, sulphur amino acid and histidine (residues reported to have antioxidant activity). Figures 1 and 2 show that the longer hydrolysis (120 min), resulted in higher AOA. Naturally, the longer time of hydrolysis also resulted in shorter peptides and lower molecular weights.

Concluding remarks

The legume seeds selected for this study are important sources of protein, carbohydrate, minerals, and also good energy sources due to their high oil content. The present data focus on the seeds as sources of protein, and demonstrate that enzymatic papain hydrolysis is an efficient tool for preparing high quality protein hydrolysates of these local soybean and lupin breeds. The protein hydrolysates contain low levels of antinutritional trypsin inhibitor activity, urease activity and low phytate content. They also have a high protein content, nitrogen solubility and *in vitro* protein digestibility. Combining germination with hydrolysis in soybean and lupin hence appears nutritionally advantageous. The hydrolysates also have antioxidant properties which can be safely incorporated in medicinal foods, instant foods and nutritious beverages.

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