ELSEVIER

Food Chemistry 78 (2002) 179–186

Food Chemistry

www.elsevier.com/locate/foodchem

Characterisation of protein concentrates from pressed cakes of *Guevina avellana* (Chilean hazelnut)

Andrés Moure[a], Herminia Domínguez[a,*], María Elvira Zúñiga[b], Carmen Soto[b], Rolando Chamy[b]

[a]*Departamento de Enxeñería Química, Universidade de Vigo (Campus Ourense), Edificio Politécnico, As Lagoas, 32004 Ourense, Spain*
[b]*Escuela de Ingeniería Bioquímica, Universidad Católica de Valparaíso, Avda, Brasil 2147, Valparaíso, Chile*

Received 10 October 2001; received in revised form 21 November 2001; accepted 21 November 2001

Abstract

Protein concentrates, produced by aqueous extraction and membrane filtration from *Guevina avellana* pressing cakes, were characterized with regard to nutritional and functional properties. The effect of a previous enzymatic treatment, carried out with the aim of enhancing oil extractability during pressing, was also evaluated. Thermal conditioning of the seeds, before pressing, influenced oil and protein extractability, as well as the nutritional quality and functional properties. The protein concentrates contained up to 65% protein with an in vitro apparent digestibility coefficient in the range 75–80%. They presented a reduced ability to bind water, but they retained almost up to ten times their weight of oil.

Keywords: Enzyme aided oil extraction; Pressing; *Guevina avellana*; Membrane filtration; Protein concentrates; Functional properties

1. Introduction

Chilean hazelnut (*Guevina avellana* Mol) is an oilseed with 40–53% (d.b.) oil, which has excellent cosmetic properties. It works by filtering the infrared and UV low spectrum radiation, with application as a solar protection agent and skin regenerator. The oil is composed of more than 85% monounsaturated fatty acids, having isomers unusually found in plants, and 6% of both saturated and polyunsaturated fatty acids (Bertoli, Fay, Stancanelli, Gumy, & Lambelet, 1998; Mella & Masson, 1984).

Industrial oil extraction is achieved by pressing, leaving the cake with 10–12% of oil, which spoils and limits human food application of the meal. Severe pressing conditions (temperature) cause a concomitant reduction of the product (oil and protein) quality, but the use of lower pressing temperatures or cold pressing (avoiding previous thermal conditioning) reduces the extraction efficiency.

During most conventional extraction processes, the pressing cake is further extracted by hexane (Ward, 1976; Young, Poot, Biernoth, Krog, Davison, & Gunstone, 1994). Due to the environmental, toxicological and operational risks of this solvent (Norris, 1982; Witting & Dimick, 1984) several studies have aimed at avoiding the solvent extraction step by either (1) using alternative solvents (water or ethanol) or (2) increasing the efficiency of the pressing process by using enzymes.

The application of an enzymatic digestion to degrade the seed cell walls can enhance the oil extractability from seeds in aqueous (Christensen, 1989; Fullbrook, 1984; Rosenthal, Pyle, & Niranjan, 1996; Rosenthal, Pyle, Niranjan, Gilmour, & Trinca, 2001; Tano-Debrah & Ohta, 1995), pressing (Domínguez, Sineiro, Núñez, & Lema, 1996; Moure, Franco et al., 2001; Sosulski & Sosulski, 1990; Zúñiga, Chamy, & Lema, 2001) and solvent oil extraction processes (Domínguez, Núñez, & Lema, 1995; Shankar, Agrawal, Sarkar, & Singh, 1997; Sosulski, Sosulski, & Coxworth, 1988) and also it improves the protein digestibility of the residual meal (Domínguez, Núñez, & Lema, 1994; Rosenthal et al., 2001; Sosulski & Sosulski, 1990). Previous studies, reporting the optimal conditions for an enzymatic treatment during the extraction process from *G. avellana* dehulled seeds, have been published (Zúñiga, Soto, Mora, Chamy, & Lema, 2001).

* Corresponding author. Tel.: +34-988-387082; fax: +34-988-387001.
E-mail address: herminia@uvigo.es (H. Domínguez).

0308-8146/02/$ - see front matter
PII: S0308-8146(01)00397-1

Production of purified vegetable protein is gaining increasing commercial importance due to the consumer preferences for vegetable sources of food and cosmetic ingredients. The protein can be solubilized in alkaline media and further recovered, either by isoelectric precipitation or by membranes (Kwon, Bae, Park, & Rhee, 1996; Lawhon, Manak, Rhee, & Lusas, 1981; Nabetani, Abbott, & Kleiman, 1995; Tzeng, Diosady, & Rubin, 1990). This latter alternative was successfully applied to hexane-defatted *G. avellana* seeds (Moure, Rúa, Sineiro, & Domínguez, 2001).

The aim of the present study was to assess the effect of the pressing conditions and the effect of the enzyme treatment on the composition and characteristics of the cakes from dehulled *G. avellana* seeds. In addition, the composition, nutritional and functional properties of protein concentrates produced by ultrafiltration membranes were evaluated.

2. Materials and methods

2.1. Materials

G. avellana seeds, purchased in Chilean local markets, were manually dehulled and stored at 4 °C until use.

2.2. Enzyme treatment

Before enzymatic treatment, clean seeds were ground to a particle size lower than 1.4 mm and cooked at 100 °C for 20 min. Enzymatic hydrolysis was carried over 6 h with 1% (w/w) E/S ratio of the commercial enzyme (Olivex: Celluclast, 1:1) mixture at 40 °C and 45% moisture. After treatment, enzymes were inactivated for 20 min at 100 °C (Zúñiga, Soto et al., 2001).

2.3. Pressing

Prior to the pressing operation, samples were dried in a vacuum oven at 60 °C up to 3–4% of moisture. Pressing was done in a manual laboratory hydraulic press (Carver Press, New Jersey, USA) for 20 min in the range of 29.4–49 MPa. For thermal conditioning prior to pressing, dried seeds were cooked at 60 °C for 5 min. Then, residual cakes were hexane-defatted under mild conditions (room temperature) overnight.

2.4. Protein extraction

The defatted cakes were subjected to extraction at 35°C for 90 min with a LSR (Liquid to Solid Ratio) of 12 g water/g meal. The pH of the extracting solution was maintained at 11. Solid and liquid phases were separated by centrifugation (Sigma 3k10, Osterode, Germany) and vacuum-filtration. A second extraction stage was performed after the solid:liquid separation of phases: (1) by vacuum-filtration when the cakes were produced by pressing of thermally conditioned seeds and (2) by vacuum-filtration and ultrafiltration (UF) membranes for separating the liquid phase produced from cold-pressed cakes, due to the operational difficulties encountered when separating them only by vacuum-filtration. Protein concentrates from cold-pressed cakes were produced by pooling the UF retentates from the two extraction stages, and those from thermally conditioned seeds were produced by UF of the pooled vacuum filtrates. Ultrafiltration was carried out through a Filtron unit (Pall Corporation, Madrid, Spain), equipped with 10 and 5 kDa cut-off Omega Screen Channel membranes in series. Fig. 1 shows the flow diagram for the extraction processes from cold-pressed (a) and from thermal-treated seeds (b). the final protein product (R) was freeze-dried, and analyzed for nutritional and functional properties, whereas the permeate could optionally be recycled for further extractions (streams indicated by dotted lines).

2.5. Analytical methods

Nitrogen content in the meals and cakes was determined by the Kjeldahl method, using the factor 6.25 to convert this value to protein. Soluble protein was determined by the Lowry method. Detergent fibre content was determined according to the procedure of Goering and van Soest (1970). In vitro digestibility was measured with the Apparent Digestibility Coefficient (ADC), determined by digestion with trypsin, chymotrypsin and peptidase (Hsu, Vavak, Satterlee, & Miller, 1977). Available lysine was determined by the TNBS method with D,L-lysine (Merck, Darmstadt, Germany) as standard (Hall, Trinder, & Givens, 1973). In vivo digestibility was evaluated on 24 male rats, which were fed during a month with a non-protein diet, a casein diet and a diet with *G. avellana* pressed meal, obtained by enzyme-assisted cold-pressing. PER value was calculated by the increase of weight of the different groups (Young & Pellett, 1991).

2.6. Functional properties

2.6.1. Water and oil absorption

One gram of each sample was mixed with 100 ml distilled water or with 100 ml oil in an Ultra-Turrax T50 (Janke & Kunkel, Staufen, Germany). The samples were then allowed to stand at room temperature for 30 min, centrifuged at 5000 ***g*** for 30 min and the liquid retained by the solids measured. The water and oil absorption capacities was expressed as grams of water or oil bound per gram of the sample on a dry basis (Okezie & Bello, 1988).

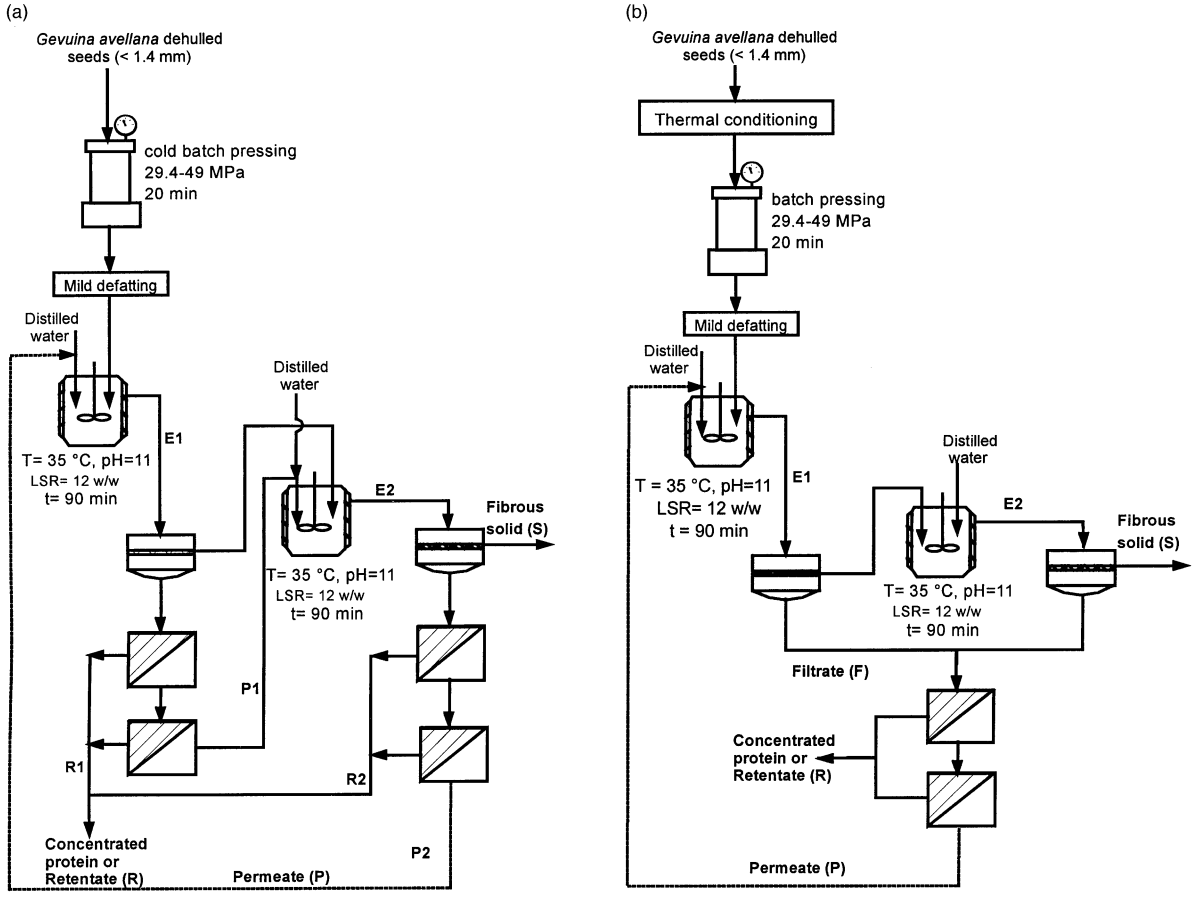

Fig. 1. Flow diagram of the process used to produce concentrates from *Guevina avellana* dehulled seeds subjected to cold-pressing (a) and to thermal conditioning before pressing (b).

2.6.2. Foam stability and foam capacity

One half gram of each sample was mixed with 40 ml distilled water in an Ultra-Turrax T50 for 2 min. The blend was transferred into a graduated cylinder and the blender jar was rinsed with 10 ml distilled water, which was then added to the graduated cylinder. The volume was recorded before and after whipping and measured as the percent of volume increase due to whipping. Foam volume changes in the graduated cylinder were recorded at intervals of 1, 10, 30, 60, 90 and 120 min.

2.6.3. Least gelation concentration

Sample dispersions of 2, 4, 6, 8, 10, 12, 14 and 16% (w/v) were prepared in 50 ml distilled water according to Coffmann and García (1977). Each dispersion was adjusted to pH 7.0 with 0.1 N NaOH or 0.1 N HCl and mixed for 2 min. The dispersions (5 ml aliquots) were poured into test tubes, heated to 100 °C in water bath for 1 h, followed by rapid cooling under cold running tap water. The tubes were further cooled to 4 °C for 2 h. Least Gelation Concentration (LGC) is the concentration above which the sample remained in the bottom of the inverted tube.

2.6.4. Emulsifying activity index (EAI) and emulsion stability index (ESI)

Twenty millilitres of a 0.1% (w/v) protein solution (pH 7.0) were homogenized with 6.6 ml of soy oil during 1 min in an Ultraturrax T-50. Immediately after homogenization, aliquots of 50 μl of the emulsion were diluted to 5 ml with a 0.1% sodium dodecyl sulfate (SDS) solution and its absorbance was determined at 500 nm in a (Perkin Elmer, Lambda 1) spectrophotometer (Pearce & Kinsella, 1978). EAI was measured as initial absorbance, and ESI was calculated by the equation:

$$\text{ESI (min)} = T_0 \cdot t / (T_0 - T_{10})$$

where: T_0 was the turbidity at 0 min, and T_{10} was the turbidity at 10 min after the homogenization, $t = 10$ min.

3. Results and discussion

Table 1 summarizes the composition, nutritional and functional properties of *G. avellana* meals obtained after defatting the pressing cakes with hexane. Thermal

Table 1
Composition, nutritive and functional properties of meals produced from *Guevina avellana* cakes

	Cold-pressing		Thermal conditioning and pressing	
	Control	Enzyme-treated	Control	Enzyme-treated
Composition				
Total protein (%)	19.9±0.51	20.0±0.69	20.7±1.41	22.8±0.73
NDF[a] (%)	27.4±3.47	9.20±0.40	27.3 ±0.86	24.8±1.56
Nutrional properties				
in vitro digestibility (ADC,%)	75.8	80.0	78.0	74.4
Functional properties				
Water absorption capacity (g/g)	6.41±0.05	3.28±0.03	6.92±0.13	4.01±0.41
Oil absorption capacity (g/g)	3.30±0.26	2.53±0.14	3.56±0.48	2.58±0.01
LGC (%)	14	12	–	16
EAI (%)	4.20±0.26	2.20 ±0.50	5.70±0.30	4.0±0.27
ESI (%)	72.2±0.95	24.4±2.21	46.1±1.05	16.7±2.01

[a] NDF, neutral detergent fibre.

conditioning of the seeds before pressing did not significantly influence the protein content of the meals, although those subjected to thermal conditioning had a slightly higher content, especially the enzyme-treated ones. The total fibre content was, as expected, lower for the meals from enzyme-treated seeds, but was not influenced by the thermal treatment of the seeds in controls. The degradative effect of these hydrolytic enzymes on the cell walls of *G. avellana* was also noticed by the increased sugar content of the enzyme-treated seeds (Zúñiga, 1998). In addition, for other oilseeds, degradation has been described in microstructural and compositional studies (Dourado, Vasco, Gama, Coimbra, & Mota, 2000; Sineiro, Domínguez, Núñez, & Lema, 1998; Sosulski & Sosulski, 1990). Whereas the enzyme-treatment enhances the protein digestibility of cold-pressed seeds, it decreases that of cakes subjected to thermal treatment. Probably the browning reactions between proteins and sugars, released after the enzymatic treatment, reduced the protein digestibility.

The water and the oil absorption capacities (WAC and OAC, respectively) of meals from enzyme-treated seeds were lower than for those produced from controls, but the difference for the oil absorption capacities was less pronounced. The thermal treatment before pressing did not significantly affect the ability to bind water and oil, but WAC was slighlty higher for the meals from thermally treated seeds. Similarily, Venktesh and Prakash (1993) reported that thermal treatment increased the water and oil absorption capacities of sunflower flours. The increase is proportional to the severity of the thermal treatment, since steaming has been reported to be more efficient than dry heating for increasing the WAC and OAC (Prinyawiwatkul, Beuchat, & McWatters, 1993) and this effect increases with temperature (King, Aguirre, & de Pablo, 1985). The WAC values of *G. avellana* meals is in the range of those reported for meals from *Sesamum indicum* and *Brassica napus* (Mahajan, Bhardwaj, & Dua, 1999). The OAC values are comparable to those reported for these latter seeds and for mixtures of corn and soya meals (Obatolu & Cole, 2000). The gelation ability of cold-pressed meals was better than that of thermal-treated ones. The direct relationship between the gelification capacity and the digestibility of the meals could be due to the influence of denaturation on the gel formation (Damodaran, 1997).

The emulsification properties of control meals were higher than those of enzyme-treated ones, and the EAI of meals from thermal-treated seeds were slightly higher than those of meals from cold-pressed ones. However, emulsions formed with meals from control and from cold-pressed seeds were more stable than those from thermally conditioned and from the enzyme-treated ones. The reduction in the EAI caused by the enzyme treatment was less noticeable for meals from thermally treated seeds. Dry heating, as opposed to autoclaving was reported to decrease the emulsification stability of sunflower meals (Venktesh & Prakash, 1993). The enzyme treatment, which influences the meal composition, has a more marked effect on the functional properties of the meals than the thermal treatment before pressing. In relation to the values reported for meals from oilseeds, the emulsification activity of *G. avellana* meals was low, but the stability was comparable to those found for canola (Naczk, Diosady, & Rubin, 1985), sesame (Mahajan et al., 1999) and corn (Obatolu & Cole, 2000). Fig. 2a shows volume recordings during the foam stability tests of concentrates from *G. avellana* pressing cakes.

The protein extraction and recovery yield, after two extraction stages, and protein recovery by membrane ultrafiltration are summarized in Table 2 and the

composition and properties of the concentrates are shown in Table 3. Protein values were calculated from Lowry determinations and confirmed by the Kjeldahl method. The protein yield, expressed as g protein/100 g of initial protein in the meal, is summarized in Table 2 for each stream of Fig. 1. The protein was more easily extractable from meals produced from enzyme-treated seeds, as can be inferred from the higher yield in the stream leaving the first extractor (E1). The effect of the thermal treatment was different for control and for enzyme-treated seeds. In the second extraction stage the protein yield was higher from enzyme-treated meals only if they had been thermally conditioned before pressing. The total protein yield from the two extraction stages was significantly higher from enzyme-treated samples. The protein yield from thermal-conditioned seeds was significantly higher if an enzyme treatment was applied. Probably the protein became denatured and less soluble after the thermal treatment, but only after a hydrolytic treatment of the cell walls could it be extracted. The final protein product (R1 + R2 and R for cold-pressed and for thermally conditioned seeds respectively) showed higher protein yield and recovery for the enzyme-treated seeds, regardless of previous thermal conditioning of the seeds. Maximal recovery (85.8% of the solubilized protein) was achieved in concentrates from cold-pressed seeds.

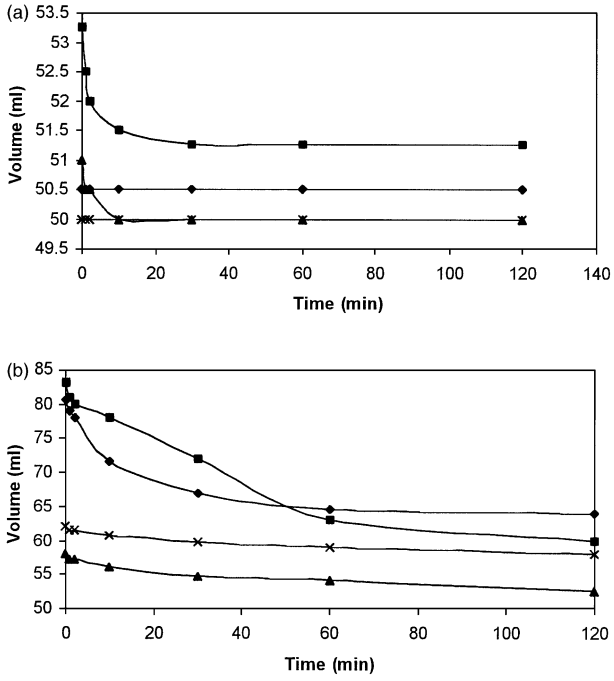

Fig. 2. Foam stability of meals from pressed cakes of *Guevina avellana* (a) and concentrates (b). (–■–) Thermal conditioning before pressing, control; (–□–) Thermal conditioning before pressing, enzyme-treated; (–▲–) Cold pressing, control and (–*–) Cold pressing, enzyme-treated.

The enzyme treatment did not influence the protein content of the concentrates from cold-pressed seeds, whereas it markedly reduced the protein purity of concentrates from thermally treated seeds. The higher protein extraction yields (indicated in Table 2) correspond to the lower protein purity in concentrates (Table 3); probably, together with protein, other compounds were extracted during alkaline solubilization, and such compounds could be further recovered by membranes. The concentrates from enzyme-treated seeds contained more sugars, also the initial meals contained more sugars; due to the hydrolytic action of the enzymes on the cell wall. The trend observed for the digestibility of concentrates was similar to that found for pressing cakes and, like the value for in vivo digestibility of crude meal from hexane-defatted seeds, was 80.0±1.5 (Zúñiga, 1998). The available lysine of concentrates was significantly lower than that of hexane-defatted meal (5.5 g/16 g N), due probably to the severity of the thermal conditioning before and after pressing, since alkaline processing affected this property in lower degree (Moure, Rúa et al., 2001).

The WAC of the protein concentrates was low, irrespective of the thermal conditioning of the seeds, and that of concentrates from cold-pressed seeds was at least three times higher than that of concentrates from thermal-treated seeds. The WAC values are comparable to those reported for coconut concentrates and isolates produced by membrane technology (Kwon et al., 1996) or by

Table 2
Extracted protein (as percent initial protein in the meal) and recovered protein (as percent of solubilized protein) from pressed cakes of *Guevina avellana* (streams indicated in Fig. 1)

Stream	Cold-pressing		Stream	Thermal-conditioned before pressing	
	Control	Enzyme		Control	Enzyme
E1	48.7±3.4	60.9±1.9	E1	43.8±0.8	61.7±3.2
E2	20.8±1.7	12.5±0.8	E2	10.6±0.1	14.5±0.3
R1	47.0±1.0	56.2±0.7	F	52.3±0.7	78.7±0.6
R2	9.6±1.4	6.8±0.3	R	35.4±3.3	55.5±0.1
P1	5.4±0.3	5.7±0.8	P	14.9±0.8	21.1±0.3
P2	4.5±0.05	3.3±0.5			
Extracted protein (%)	69.5	73.5		52.3	77.6
Recovered protein (% solubilized)	84.6	85.8		67.7	70.5

isoelectric precipitation (Chakraborty, 1985), and from peas isolated by isoelectric precipitation (Mwasaru, Muhammad, Bakar, & Che Man, 1999). All the *G. avellana* concentrates showed good capacity for oil retention, not significantly influenced by thermal or enzyme treatment of the seeds. The OAC of concentrates from this seed were comparable to those of sunflower isolates (Ayhllon-Meixueiro, Vaca-García, & Silvestre, 2000).

In the range of sample concentrations usually employed for testing the gelification ability, gelation of *G. avellana* concentrates was not possible. Probably the additional denaturation, caused by the alkaline protein extraction, could lower the gelation ability, therefore increasing the LGC values.

Good emulsification ability of *G. avellana* concentrates was observed. Emulsification properties (EAI and ESI) of protein concentrates from meals subjected to enzyme treatment were higher than those of their respective controls. Similarly, EAI and ESI of protein concentrates from heat-treated seeds before pressing were higher than those of protein concentrates from cold-pressed seeds. Foam stability of *G. avellana* meals and concentrates are presented in Fig. 2, which shows volume recordings with time. The foam capacities of concentrates from thermal-treated seeds were significantly higher than those produced from cold-pressed seeds. The enzymatic treatment did not have a significant effect, on this property, on concentrates from thermally conditioned seeds, whereas it improved the foam capacity of concentrates from cold-pressed seeds. Probably both the thermal and the enzyme treatment can alter the hydrophilic/hydrophobic balance of the protein surface, thus affecting the protein ability to interact with air and the foam capacity (Damodaran, 1997; Were, Hettiarachchy, & Kalapathy, 1997). The effect of thermal conditioning could be more pronounced, since the enzyme treatment only influences the foam capacity of protein from cold-pressed seeds.

Table 4 summarizes the fibre composition of the solid residue after two extraction stages. Higher total fibre content (NDF) was observed for concentrates from enzyme-treated seeds than from controls, either cold-pressed or thermally conditioned seeds before pressing. Probably the higher removal of soluble compounds from enzyme-treated samples during alkaline extraction, which also resulted in lower protein purity of the concentrates (Table 3), increased the relative fibre content in the solid residue.

From data presented in this work, it can be concluded that the application of thermal conditioning and enzyme treatment of *G. avellana,* with the aim of improving oil extraction from seeds, influences the quality of the meal and the nutritional and functional properties of the concentrates obtained from the pressing cakes, by alkaline solubilization and membrane ultrafiltration.

Table 4
Fibre content (NDF) of the fibrous residues (S) after protein extraction, in two stages, from *Guevina avellana* cakes (data are expressed as weight percent of the solid residue)

	Cold-pressing		Heat-treated pressing	
	Control	Enzyme-treated	Control	Enzyme-treated
NDF[a] (%)	27.8±2.86	32.0±1.05	27.3±0.86	45.4 ±0.75
ADF[b] (%)	23.5±0.38	25.0±2.3	23.8±1.71	27.9±1.48
ADL[c] (%)	4.9±0.76	7.0±2.16	7.9±1.13	5.2±0.16

[a] NDF, neutral detergent fibre.
[b] ADF, acid detergent fibre.
[c] ADL, acid detergent lignin.

Table 3
Composition, nutritive and functional properties of concentrates produced from *Guevina avellana* cakes

	Cold-pressing		Heat-treated pressing	
	Control	Enzyme-treated	Control	Enzyme-treated
Composition				
Total protein (%)	53.3±1.2	51.5±2.0	65.6±1.4	46.1±3.2
Reducing sugars (%)	3.81	4.67	3.96	5.06
Nutritional properties				
In vitro digestibility (ADC,%)	75.8	80.0	78.0	74.4
Available lysine (g/16 g N)	1.95	1.99	3.25	1.87
Functional properties				
Foam capacity (%)	37.6±2.21	51.1±4.36	127±2.12	132±11.3
Water absorption capacity (g/g)	0.96±0.09	1.94±0.09	0.39±0.005	0.32±0.03
Oil absorption capacity (g/g)	9.53±0.22	9.94±0.84	9.62±0.16	8.93±1.3
EAI (%)	85.9±7.49	95.7±3.59	252±5.13	317±3.53
ESI (%)	29.2±2.40	42.1±1.10	61.1±4.56	270±31.14

Acknowledgements

This work was funded by the INCO-DC Program of European Commission (ERBIC 18 CT 970206) and Project FONDECYT Nº 1.000.300 (CONICYT, Chile).

References

Ayhllon-Meixueiro, F., Vaca-García, C., & Silvestre, F. (2000). Biodegradable films from isolate of sunflower (*Helianthus annuus*) proteins. *Journal of Agricultural and Food Chemistry*, *48*, 3032–3036.

Bertoli, C., Fay, L. B., Stancanelli, M., Gumy, D., & Lambelet, P. (1998). Characterization of chilean hazelnut (*Guevina avellana* Mol) seed oil. *Journal of the American Oil Chemists' Society*, *75*, 1037–1040.

Chakraborty, P. (1985). Functional properties of coconut protein isolate obtained by ultrafiltration. *Journal of Food Science and Technology*, *22*, 248–254.

Christensen, F. M. (1989). Rewiew: enzyme technology versus engineering technology in the food industry. *Biotechnology and Applied Biochemistry*, *11*, 249–265.

Coffmann, C. W., & Garcia, V. V. (1977). Functional properties and amino acid content of a protein isolate from mung bean flour. *Journal of Food Technology*, *12*, 473–484.

Damodaran, S. (1997). Food proteins: an overwiew. In S. Damodaran, & A. Paraf (Eds.), *Food proteins and their applications* (pp. 1–24). New York: Marcel Dekker.

Domínguez, H., Núñez, M. J., & Lema, J. M. (1994). Enzymatic pretreatment to enhance oil extraction from fruits and oilseeds: a review. *Food Chemistry*, *49*, 271–286.

Domínguez, H., Núñez, M. J., & Lema, J. M. (1995). Enzyme-assisted hexane extraction of soya bean oil. *Food Chemistry*, *54*, 223–231.

Domínguez, H., Sineiro, J, Núñez, M. J., & Lema, J. M. (1996). Enzymatic treatment of sunflower kernels before oil extraction. *Food Research International*, *28*, 537–545.

Dourado, F., Vasco, P., Gama, F. M., Coimbra, M. A., & Mota, M. (2000). Characterisation of Rosa Mosqueta seeds: cell wall polysaccharide composition and light microscopy observations. *Journal of Science and Food Agriculture*, *80*, 1859–1865.

Fullbrook, P. D. (1984). Extraction of vegetable oils. UK Pat. Appl. GB 2 127 425A.

Goering, H. K., & van Soest, P. J. (1970). Forage fiber analyses (Apparatus, Procedures and some Applications). Agriculture Handbook, 379. USDA. Agricultural Research Service, 5-11.

Hall, R. J., Trinder, N., & Givens, D. I. (1973). Observations on the use of 2,4,6-trinitrobenzensulphonic acid for the determination of available lysine in animal protein concentrates. *Analyst*, *98*, 673–686.

Hsu, H. W., Vavak, D., Satterlee, L. D., & Miller, G. A. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science*, *42*, 1269–1273.

King, J., Aguirre, C., & de Pablo, S. (1985). Functional properties of lupin protein isolates (*Lupinus albus* cv *Multolupa*). *Journal of Food Science*, *50*, 82–87.

Kwon, K. S., Bae, D., Park, K. H., & Rhee, K. C. (1996). Aqueous extraction and membrane techniques improve coconut protein concentrate functionality. *Journal of Food Science*, *61*, 753–756.

Lawhon, J. T., Manak, L. J., Rhee, K. C., & Lusas, E. W. (1981). Production of oil and protein food products from raw peanuts by aqueous extraction and ultrafiltration. *Journal of Food Science*, *46*, 391–395.

Mahajan, A., Bhardwaj, S., & Dua, S. (1999). Traditional processing treatments as a promising approach to enhance the functional properties of rapeseed (*Brassica campestris* Var. *toria*) and sesame seed (*Sesamum indicum*) meals. *Journal of Agricultural and Food Chemistry*, *47*, 3093–3098.

Mella, M. A., & Masson, L. (1984). Materias grasas vegetales de consumo habitual y potencial en Chile. *Grasas y Aceites*, *35*, 240–245.

Moure, A., Franco, D., Santamaría, R. I., Soto, C., Sineiro, J., Domínguez, H., Zúñiga, M. E., Núñez, M. J., Chamy, R., López-Munguía, A., & Lema, J. M. (2001). Enzyme-aided alternative processes for the extraction of oil from *Rosa rubiginosa* seeds. *Journal of the American Oil Chemists' Society*, *78*, 437–440.

Moure, A., Rúa, M. L., Sineiro, J., & Domínguez, H. (2001). Aqueous extraction and membrane isolation of protein from defatted *Guevina avellana*. *Journal of Food Science* (in press).

Mwasaru, M. A., Muhammad, K., Bakar, J., & Che Man, Y. B. (1999). Effects of isolation technique and conditions on the extractability, physicochemical and functional properties of pigeonpea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) protein isolates. I. Physicochemical properties. *Food Chemistry*, *67*, 435–443.

Nabetani, H., Abbott, T. P., & Kleiman, R. (1995). Optimal separation of jojoba protein using membrane processes. *Industrial Engineering Chemistry and Research*, *34*, 1779–1788.

Naczk, M., Diosady, L. L., & Rubin, L. J. (1985). Functional properties of canola meals produced by a two-phase solvent extraction system. *Journal of Food Science*, *50*, 1692 .

Norris, F. A. (1982). Extraction of fats and oils. In D. Swern (Ed.), *Bailey's industrial oil and fat products* (pp. 178–253). Canada: John Wiley.

Obatolu, V. A., & Cole, A. H. (2000). Functional property of complementary blends of soybean and cowpea with malted or unmalted maize. *Food Chemistry*, *70*, 147–153.

Okezie, B. O., & Bello, A. B. (1988). Physicochemical and functional properties of winged bean flour and isolate compared with soy isolate. *Journal of Food Science*, *53*, 450–454.

Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry*, *26*, 716–723.

Prinyawiwatkul, W., Beuchat, L. R., & McWatters, X. (1993). Functional property changes in partially deffated peanut flour caused by fungal fermentation and heat treatment. *Journal of Food Science*, *58*, 1318–1323.

Rosenthal, A., Pyle, D. L., & Niranjan, K. (1996). Aqueous and enzymatic processes for edible oil extraction. *Enzyme Microbial Technology*, *19*, 402–420.

Rosenthal, A., Pyle, D. L., Niranjan, K., Gilmour, S., & Trinca, L. (2001). Combined effect of operational variables and enzyme activity on aqueous enzymatic extraction of oil and protein from soybean. *Enzyme Microbial Technology*, *28*, 499–509.

Shankar, D., Agrawal, Y. C., Sarkar, B. C., & Singh, B. P. N. (1997). Enzymatic hydrolysis in conjunction with conventional pretreatments to soybean for enhanced oil availability and recovery. *Journal of the American Oil Chemists' Society*, *74*, 1543–1547.

Sineiro, J., Domínguez, H., Núñez, M. J., & Lema, J. M. (1998). Microstructural features of enzymatically treated oilseeds. *Journal of Science of Food and Agriculture*, *78*, 491–497.

Sosulski, K., & Sosulski, F. W. (1990). Enzyme pretreatment to enhance oil extractability in canola. In F. Shahidi (Ed.), *Rapeseed/canola production, chemistry, nutrition and processing technology* (pp. 277–289). New York: Van Nostrand Reinhold.

Sosulski, K., Sosulski, F. W., & Coxworth, E. (1988). Carbohydrase hydrolysis of canola to enhance oil extraction with hexane. *Journal of the American Oil Chemists' Society*, *65*, 357–361.

Tano-Debra, K., & Ohta, Y. (1995). Enzyme-assisted aqueous extraction of shea fat: a rural approach. *Journal of the American Oil Chemists' Society*, *72*, 251–256.

Tzeng, Y.-M., Diosady, L. L., & Rubin, L. J. (1990). Production of canola protein materials by alkaline extraction, precipitation, and membrane processing. *Journal of Food Science*, *55*, 1147–1151.

Venktesh, A., & Prakash, V. (1993). Functional properties of the total proteins of sunflower (*Helianthus annuus* L.) seed.- Effect of physical

and chemical treatments. *Journal of Agricultural and Food Chemistry*, *41*, 18–23.

Ward, J. A. (1976). Processing high oil content in continuous screw presses. *Journal of the American Oil Chemists' Society*, *53*, 261–264.

Were, L., Hettiarachchy, N. S., & Kalapathy, U. (1997). Modified soy proteins with improved foaming and water hydration properties. *Journal of Food Science*, *62*, 821–823, 850.

Witting, L. A., & Dimick, P. S. (1984). Effects of processing on food lipids. In M. Rechcigl Jr. (Ed.), *Handbook of nutritive value of processed food. Food for human use* (pp. 416). Boca Raton, USA: CRC Press.

Young, F. V. K., Poot, C., Biernoth, E., Krog, N., Davison, N. G. J., & Gunstone, F. G. (1994). Processing of fats and oils. In F. D. Gunstone, J. L. Harwood, & F. B. Padley (Eds.), *The lipid handbook,* (2nd ed.). (pp. 249–254). London: Chapman & Hall.

Young, V. R., & Pellett, P. L. (1991). Protein evaluation, amino acid scoring and the Food and Drug Administration's proposed food labeling regulations. *Journal of Nutrition*, *121*, 145–150.

Zúñiga, M. E. (1998). Aplicación de la tecnología enzimática en la extracción de aceite vegetal por prensado. PhD thesis, Universidad de Santiago de Compostela.

Zúñiga, M. E., Chamy, R., & Lema, J. M. (2001). Canola and chilean hazelnut products obtained by enzyme-assisted cold-pressed oil extraction. In Wilson, R.F. *Proceedings of the World Conference and Exhibition on Oilseed Processing and Utilization*. Champaign, USA: AOCS Press 31, 203–209.

Zúñiga, M. E., Soto, C., Mora, A., Chamy, R., & Lema, J. M. (2001). Enzymatic pre-treatment on the chilean hazelnut *Guevina avellana* mol oil extraction by pressing. *Biotechnology and Bioengineering* (submitted for publication).