Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 88 (2004) 337–346
Food Chemistry
www.elsevier.com/locate/foodchem

Brassica carinata protein isolates: chemical composition, protein characterization and improvement of functional properties by protein hydrolysis

J. Pedroche, M.M. Yust, H. Lqari, J. Girón-Calle, M. Alaiz, J. Vioque, F. Millán *

Consejo Superior de Investigaciones Cientificas, Instituto de la Grasa, Avda. Padre García Tejero 4, 41012 Sevilla, Spain

Received 31 October 2003; received in revised form 16 January 2004; accepted 22 January 2004

Abstract

Brassica carinata defatted flour has been used to prepare protein isolates by alkaline extraction and precipitation at low pH. Different extraction parameters have been tested, and the chemical composition and functional properties of the resulting isolates have been analyzed. All the isolates had a protein content above 90% and a well balanced amino acid composition according to FAO standards except for a low content of lysine. The extraction process reduced antinutritional components by more than 90%. Solubility, water and fat adsorption, emulsifying and foaming properties of the original flour and the isolates have been compared. In addition, hydrolysis of *B. carinata* protein during extraction using the endoprotease preparation Alcalase was performed, and functional properties of hydrolysates with low and high degrees of hydrolysis were studied and compared with those of the original isolates.

Keywords: *Brassica carinata*; Protein isolates; Chemical composition; Functional properties; Antinutritional components

1. Introduction

Plants belonging to the genus *Brassica* are of great economic and nutritional importance and include crops widely used for oil extraction. *Brassica* oil production occupies the fourth position in volume in the world after soybean oil, palm seed oil, and cottonseed oil, and it is the most important oilseed crop in Europe (FAO, 2002). The defatted meal that is left after oil extraction is rich in protein, although its use for human nutrition presents some problems because it also contains antinutritional components such as glucosinolates and phytates, and a high cellulose content. For this reason, the *Brassica* defatted meal is only used for animal feeding and as an organic fertilizer (Duncan, 1991; Liener, 1989).

Brassica carinata (Ethiopian mustard) is related to rapeseed (*B. napus*) and originated from a cross between *B. nigra* and *B. oleracea*. *B. carinata* seeds are consumed in Ethiopia but they have not developed as an oil seed crop elsewhere despite the continued search for cheaper sources of edible and industrial oils. Recently, countries like Spain, Greece, and Italy, with regions characterized by semi-arid climates, are showing interest in the exploitation of this crop for production of biodiesel and solid biomass. In addition, the defatted meal that is left after oil extraction may constitute an important protein source for human nutrition thereby increasing the value of *B. carinata* crops.

Protein isolates (PI) are frequently prepared from defatted meals by solubilization of proteins in alkaline media and precipitation at the isoelectric point. This protocol allows for separation of proteins from non-protein components such as sugars, fiber, and antinutritional chemicals (Fernández-Quintela, Larralde, Macarulla, Marcos, & Martinez, 1993). However, the high temperatures and organic solvents used during the process of oil extraction cause denaturation of proteins, which reduces protein solubility and extrac-

* Corresponding author. Tel.: +34-5-4611550; fax: +34-5-4616790.
E-mail address: frmillan@cica.es (F. Millán).

0308-8146/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2004.01.045

tion yields. In addition, a reduced solubility impairs other functional properties since solubility determines many other functional properties. The functional properties of proteins can be improved by modification in order to facilitate their use in nutritional, medical, and cosmetic applications. This includes proteolytic treatments to produce protein hydrolysates with a degree of hydrolysis of less than 10%, which may result in improved functional properties (Krause & Schwenke, 1995; Mannheim & Cheryan, 1992; Süle, Tömösközi, & Hajós, 1998). The goal of this work was to obtain and characterize PI and hydrolysates using *B. carinata* defatted meal, and to determine their potential as food ingredients by studying their chemical composition and functional properties.

2. Materials and methods

2.1. Materials

B. carinata brown-seeds were a gift from Koipesol Semillas, S.A. (Sevilla, Spain). The seeds were ground and extracted with hexane in a soxhlet extractor for 9 h. The resulting defatted flour was used as the starting material for preparing PI. Protease, amiloglucosidase and α-amylase were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Analytical methods

Moisture, ash and nitrogen contents were determined using AOAC, 1990 approved methods. Total fiber was determined according to the procedure described by Lee, Prosky, and Vries (1992). Soluble sugars and polyphenols were measured using standard curves of glucose (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and chlorogenic acid (Moores, McDermott, & Wood, 1948), respectively. Glucosinolates were determined according to the methods described by Minchinton, Sang, Burke, and Truscott (1982). Phityc acid was determined according to Frühbeck, Alonso, Marzo, and Santidrian (1995).

2.3. Determination of the isoelectric point (IEP)

For determination of the IEP, 15 g of *B. carinata* defatted flour were extracted twice with 300 ml 0.2% NaOH and centrifuged at 8000 rpm for 20 min. Aliquots (40 ml) of the supernatant were titrated with 0.5 N HCl to various pH values, ranging from 2.5 to 6.5, and precipitates were separated by centrifugation as above. Percentages of soluble nitrogen in the supernatants in relation to the total nitrogen extracted were plotted vs. pH to determine the IEP.

2.4. Preparation of protein isolates

B. carinata defatted flour (20 g) was extracted by stirring for 1 h in 200 ml water at pH 10, 11, or 12. After centrifugation at 8000 rpm for 15 min, an additional extraction was carried out for another hour with half the volume of alkaline solutions. Supernatants were pooled and analyzed for nitrogen content. Pellets were dried in an oven at 50 °C, weighed and analyzed for nitrogen content. The pH of the protein extracts was adjusted to 5 and after centrifugation, the pH of the supernatant was adjusted to 3.5 followed by centrifugation. Centrifugation was performed as described above. The precipitates were mixed and freeze-dried.

2.5. Gel filtration chromatography

Lyophilized samples (1 g) were dissolved in 10 ml 0.1 M sodium borate, 0.2 M sodium chloride buffer pH 8.3. Gel filtration was carried out in an FPLC system equipped with a Superose 12 HR 10/30 column from Pharmacia LKB Biotechnology. Volume injection and concentration of the samples were 200 μl and 1.6 mg protein/ml, respectively. Elution was carried out using the same buffer at a flow of 0.4 ml min^{-1}. Elution was monitored at 280 nm. The approximate molecular masses were determined using blue dextran 2000 (2000 kDa), β-amylase (200 kDa), bovine serum albumin (67 kDa) and ribonuclease A (13.7 kDa) as molecular weight standards.

2.6. Amino acid analysis

Samples containing 2 mg of protein were hydrolyzed using 6 N HCl at 110 °C for 24 h under inert nitrogen atmosphere and derivatized with diethyl ethoxymethylenemalonate. Amino acids were analyzed by reversed-phase high-performance liquid chromatography (HPLC) using D,L-α-aminobutyric acid as internal standard as described (Alaiz, Navarro, Giron, & Vioque, 1992). Tryptophan was analyzed by HPLC after basic hydrolysis according to Yust, Pedroche, Girón-Calle, Vioque, Millán, and Alaiz (2004).

2.7. Determination of functional properties

2.7.1. Water adsorption

This was determined using the method of Sosulski (1962). Samples (3 g) were mixed with 25 ml of water and stirred six times for 1 min at 10 min intervals. The mixtures were centrifuged at 3000 rpm for 25 min, and supernatants were removed. Pellets were dried at 50 °C for 25 min and weighed. Water adsorption capacity was expressed as the number of grams of water retained by 100 g of material.

2.7.2. Fat adsorption

For determination of fat adsorption the method of Lin, Humbert, and Sosulski (1974) was used. Samples (0.5 g) were mixed with 6 ml of soy oil. After 30 min the mixtures were centrifuged at 3700*g* for 25 min and the volume of the supernatants was weighed. Oil adsorption capacity was expressed as the number of grams of oil retained by 100 g of material.

2.8. Emulsifying activity and stability

Emulsifying activity was determined according to Naczk, Diosady, and Rubin (1985) with modifications. Samples (3.5 g) were homogenized for 30 s in 50 ml water using a model A Polytron homogenizer (Brinkmann, Wesbury, NY) at setting 6 (approximately 10,000 rpm). Canola oil (25 ml) was added, and the mixture was homogenized again for 30 s. Another 25 ml of canola oil were added, and the mixture was homogenized for 90 s. The emulsion was divided into two 50 ml aliquots and centrifuged at 1100*g* for 5 min. Emulsifying activity was calculated by dividing the volume of the emulsified layer by the volume of emulsion before centrifugation at 1100*g*. The emulsion stability was determined using similar samples as for measurement of emulsifying activity. They were heated for 15 min at 85 °C according to the procedure of Naczk et al. (1985), and after temperature came back to room temperature they were divided into two 50 ml aliquots and centrifuged at 1100*g* for 5 min. The emulsion stability was expressed as the percentage of emulsifying activity remaining after heating.

2.9. Foaming capacity and stability

Foaming capacity and foam stability were determined by the method of Lin et al. (1974). The samples (50 ml of a 3% dispersion in distilled water) were homogenized using a model A Polytron homogenizer at a setting of 6 (approximately 10,000 rpm). The mixture was immediately transferred into a 250 ml graduated cylinder and the foam volume measured. The foaming capacity was expressed as the percentage of volume increase. Foam stability was expressed as foam volume remaining after 20, 40, 60, and 120 min.

2.10. Protein solubility

Samples were dissolved in H_2O (5% p/v) and pH was kept at different values between 2 and 10 using 1 N NaOH or 1 N HCl while stirring at room temperature for 1 h. The samples were then centrifuged at 7500 rpm for 15 min and nitrogen content was determined in the supernatants. Solubility was expressed as the percentage of total nitrogen of the original sample present in the soluble fraction.

2.11. Protein hydrolysis

The procedure for protein hydrolysis was similar to the procedure for preparation of PI, except that the commercial protease Alcalase® 2.4 l (Novo Nordisk, Bagsvaerd, Denmark) was added during extraction. After each extraction and before centrifugation at 7500 rpm for 15 min the protein hydrolysates were heated at 85 °C for 10 min to inactivate the enzyme. After that, the supernatants were collected and stored at −20 °C. With the goal of optimizing the hydrolytic process different enzyme to substrate ratios and pH settings were used. The degree of hydrolysis was determined according to the method of Adler-Nissen (Adler-Nissen, 1979).

3. Results and discussion

3.1. Preparation of protein isolates

The isoelectric point of the proteins in *B. carinata* defatted meal was determined in order to optimize the production of PI. The lowest protein solubility was achieved at pH 3.5 and 5 (Fig. 1). These values are in accordance with the isoelectric points previously described for other members of the same family (El-Nockrashi, Mukherjee, & Mangold, 1977).

Three different pH values (10, 11 and 12) were tested for protein extraction of the defatted meal. The nitrogen balance for both alkaline extraction and isoelectric precipitation is shown in Table 1. Extraction at pH 12 resulted in a value of extracted nitrogen (close to 80%), a value higher than those obtained after extraction at pH 11 or 10 (78% and 55%, respectively). Irrespective of pH used for extraction a similar amount of protein remained soluble during isoelectric precipitation. This may be due to albumin proteins which are easily extractable and fairly soluble at acidic pH (Berot, 1996).

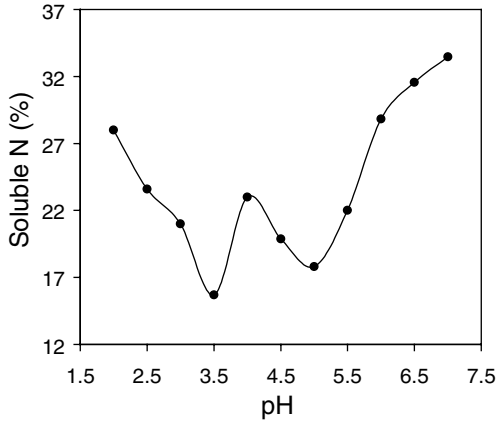

Fig. 1. N-solubility of *B. carinata* defatted meal as a function of pH.

Table 1
Nitrogen balance in the production of PI

		pH used in alkaline protein extraction		
		pH 10	pH 11	pH 12
Extractions	First	43.2 ± 2.8	65.9 ± 2.3	63.1 ± 1.5
	Second	12.2 ± 0.5	11.7 ± 0.7	16.1 ± 1.1
	Total	55.4 ± 3.2	77.6 ± 2.8	79.2 ± 2.3
Isoelectric precipitation	pH 5.0	16.3 ± 0.6	37.2 ± 1.1	40.1 ± 0.8
	pH 3.5	2.7 ± 0.2	7.4 ± 0.2	10.0 ± 0.3
	Total	19.0	44.6	50.1

Data are expressed as percentage and are the mean ± SD of three analyses.

3.2. Chemical characterization of *B. carinata* defatted meal and protein isolates

Proteins represent the main component of defatted meal (Table 2). A high protein content is essential in order to use this by-product for the production of protein concentrates, isolates and hydrolysates. The amino acid composition of the proteins of *B. carinata* meal is balanced in all essential amino acids including lysine (Table 3), although other authors have described varie-

Table 2
Chemical characterization of *B. carinata* defatted meal and PI

	Defatted meal	PI pH 10	PI pH 11	PI pH 12
Proteins	38.9 ± 2.3	92.6 ± 1.7	94.3 ± 2.2	91.1 ± 2.5
Ash	5.2 ± 1.2	1.8 ± 0.3	1.9 ± 0.7	2.3 ± 0.1
Moisture	8.9 ± 0.5	1.3 ± 0.1	1.6 ± 0.1	2.1 ± 0.2
Glucosinolates	5.1 ± 1.1	0.0	0.0	$0.02 \pm 2 \times 10^{-3}$
Phytic acid	3.1 ± 0.6	$0.04 \pm 1 \times 10^{-3}$	$0.03 \pm 1 \times 10^{-3}$	$0.21 \pm 3 \times 10^{-2}$
Fiber	31.8 ± 4.2	$0.03 \pm 1 \times 10^{-3}$	$0.11 \pm 3 \times 10^{-2}$	$0.23 \pm 3 \times 10^{-2}$
Soluble sugars	5.7 ± 0.7	1.3 ± 0.3	1.3 ± 0.4	1.4 ± 0.4
Polyphenols	0.3 ± 0.1	0.6 ± 0.3	0.7 ± 0.1	1.3 ± 0.5

Data are the mean ± SD of three analyses.

Table 3
Amino acid composition of *B. carinata* defatted meal and PI

	Defatted meal	PI pH 10	PI pH 11	PI pH 12	FAO[a]
Aspartic acid[b]	8.8 ± 1.1	11.6 ± 1.3	11.2 ± 2.0	11.3 ± 0.9	
Glutamic acid[c]	20.7 ± 0.6	18.1 ± 0.8	18.7 ± 1.8	17.5 ± 2.3	
Serine	4.9 ± 0.5	5.0 ± 0.6	4.9 ± 0.5	4.9 ± 0.8	
Histidine	2.7 ± 0.3	2.4 ± 0.5	2.4 ± 0.2	2.5 ± 0.5	1.9
Glycine	5.5 ± 1.3	5.7 ± 0.9	5.6 ± 0.2	5.6 ± 1.3	
Threonine	4.6 ± 0.7	4.7 ± 0.4	4.6 ± 0.3	4.7 ± 0.7	3.4
Arginine	8.4 ± 0.8	8.3 ± 1.6	9.1 ± 0.8	8.9 ± 0.7	
Alanine	4.6 ± 0.6	5.0 ± 0.3	5.0 ± 0.5	4.8 ± 0.6	
Proline	6.9 ± 2.5	6.1 ± 2.2	6.6 ± 1.8	6.7 ± 2.1	
Tyrosine	2.8 ± 0.3	2.8 ± 0.4	2.6 ± 0.6	2.9 ± 0.4	6.3[d]
Valine	4.6 ± 0.4	5.5 ± 0.8	6.0 ± 1.3	4.9 ± 0.5	3.5
Methionine	1.8 ± 0.6	1.0 ± 0.4	1.5 ± 0.3	1.0 ± 0.3	2.5[e]
Cysteine	2.0 ± 1.1	1.8 ± 0.7	2.4 ± 0.2	1.9 ± 0.6	
Isoleucine	3.7 ± 0.7	4.1 ± 0.6	4.1 ± 0.4	4.1 ± 0.7	2.8
Leucine	7.2 ± 0.5	8.9 ± 0.5	7.7 ± 0.4	8.5 ± 1.1	6.6
Phenylalanine	4.4 ± 1.1	5.2 ± 0.7	4.3 ± 0.7	5.3 ± 0.7	
Lysine	5.9 ± 1.3	3.8 ± 0.8	3.3 ± 1.1	4.5 ± 0.6	5.8
Tryptophan	0.6 ± 0.3	0.7 ± 0.2	0.7 ± 0.1	0.8 ± 0.1	

Data expressed as g/100 g protein are the mean ± SD of three analyses.
[a] FAO/WHO/ONU. Energy and protein requirement, 1985.
[b] Aspartic acid + asparagine.
[c] Glutamic acid + glutamine.
[d] Phenylalanine + tyrosine.
[e] Methionine + cysteine.

ties of *B. carinata* with a deficit in this amino acid (Mnzava & Olsson, 1990). The nutritional quality of the proteins from the *Brassica* genus has been compared to casein and is better than other plant proteins such as soy, sunflower, pea and wheat proteins (Sarwar et al., 1984).

We found fiber to be the second component in abundance in *B. carinata* defatted meal, with a value of 31.8%. This value is higher than those reported by other authors for the same species (Simbaya, Slominski, Rakow, Campbell, Downey, & Bell, 1995; Sosulski, 1983). This difference may be related to the color of the seeds, since brown seeds (as in our case) have a higher concentration of condensed polyphenols and lignin than yellow seeds (Theander, Aman, Miksche, & Yasuda, 1977). High amounts of fiber may influence negatively protein digestibility (Bell, 1993) and bioavailability of minerals such as manganese (Kies, Aldrich, Johnson, Creps, Kowalski, & Wang, 1987) and zinc (Bales, Freeland-Graves, Lin, Stone, & Dougherty, 1987).

Soluble sugars represent 5.7% of minor components, similar to the value reported by Simbaya et al. (1995) for the same species, and also to the values reported by other authors for other species of the same genus (Mansour, Dworschák, Lugasi, Gaál, Barna, & Gergely, 1993; Slominski, Campbell, & Guenter, 1994). Reduction of sugar content is one of the main goals during the production of protein products because they can have adverse effects on the functional properties of proteins as well as on the bioavailability of some amino acids (Cheftel, 1989). Furthermore, some sugars are responsible of the flatulence observed after the ingestion of certain foods (Reddy, Salunkhe, & Sathe, 1982).

The presence of glucosinolates is one of the main characteristics of Brassicaceae. In *B. carinata* defatted meal glucosinolates represent 5.1% (127 μmol/g meal). This value is higher than those observed by Sosulski (1983) and Mnzava and Olsson (1990). However, Getinet, Rakow, and Raney (1996) reported a range between 69.8 and 158.0 μmol/g meal in a study performed in 300 different cultivars in Ethiopia. Glucosinolates and the products that are released after their hydrolysis are considered antinutritional factors since they reduce the availability of iodine (Schöne et al., 1990). However, a protector effect of some of these hydrolytic products against certain tumors has been recently proposed (Nastruzzi et al., 2000). The predominant glucosinolate in *B. carinata* is sinigrin (Fig. 2). Some authors have shown the anticancerigenic action of sinigrin (Manson et al., 1997; Zheng et al., 2002), therefore the residual presence of glucosinolates in *B. carinata* protein products could be of great interest.

B. carinata defatted meal has 3.1% phytic acid, which is consistent with the values reported by Bell (1996) for the same species and by others for other species of the same genus (Lajolo, Marquez, Filisetti-Cozzi, &

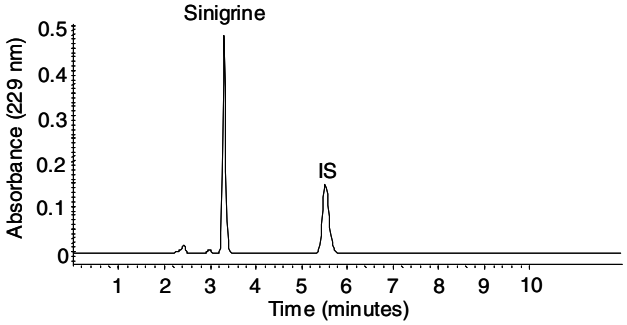

Fig. 2. HPLC analysis of the glucosinolates from *B. carinata* defatted meal. IS: internal standard.

McGregor, 1991; Mansour et al., 1993; Zhou, He, Yu, & Mukherjee, 1990). As glucosinolates, phytic acid has been traditionally considered an antinutritional factor. It can form phytic acid–mineral complexes, decreasing the availability and adsorption of minerals (Gibson, 1994). However, recent investigations have shown that these compounds are also involved in the prevention of decalcifications (Gorecka, Lampart-Szczapa, Janitz, & Sokolowska, 2000; Grases, Prieto, Simonet, & March, 2000) and tumor growth (Vucenik, Tomazic, Fabian, & Shamsuddin, 1992).

Others minor components present in *B. carinata* defatted meal are polyphenols. The content that we have found, 0.3%, is higher than that observed by others, ranging from 0.15% to 0.2% (Bouchereau, Hamelin, Lamour, Renard, & Larher, 1991; Matthäus, 2002). The dark color of the seeds that were used for our experiments indeed suggests a high concentration of polyphenols. Bouchereau et al. (1991) reported that the main phenol in *B. carinata* is sinapine, a chemical that may inhibit digestive proteases (Treviño, 1998). As glucosinolates and phytic acid, polyphenols may also be beneficial because they are strong antioxidants (Matthäus, 2002).

The protein content of the different types of PI that were prepared was higher than 90%, and all of these isolates presented a balanced amino acid composition except for lysine (Table 3). The losses of lysine observed in PI with respect to the defatted meal may be due to the losses of albumins, which are rich in lysine, during the process of preparation of the isolates. It is also possible that the availability of lysine may be decreased by interactions with glucosinolates (Kroll & Rawel, 1996), polyphenols (Gassmann, 1983) and phytic acid (Kroll, 1991).

The concentration of minor components traditionally considered as antinutritional components clearly diminished in the isolates with the exception of polyphenols. Considering the yield of protein extraction and the chemical composition of the PI, a pH value of 11 was chosen for production of PI from *B. carinata* defatted

meal. This pH value provided a higher yield of protein extraction than pH 10, and at the same time a lower yield of non-protein compounds than pH 12.

3.3. Functional properties of *B. carinata* defatted meal and protein isolates

Solubility is one of the most important characteristics of proteins because it is not only important by itself, but it also influences other functional properties. Solubility of *B. carinata* meal is low at acidic or neutral pH, and gradually increases with increasing pH values (Fig. 3). Although protein is the main component in the meal, the heterogeneous nature of the meal may facilitate interactions between proteins and other components that can modify the net charge and hydrophobicity of proteins affecting protein solubility. The process of oil extraction may also decrease protein solubility by denaturing these molecules. Protein solubility at acidic pH is lower in PI than in the original meal (Fig. 3) because certain proteins soluble at low pH are lost during the preparation of PI. However, protein solubility at basic pH is higher in PI because protein solubility in the meal is referred to total protein, a fraction of which is not extracted.

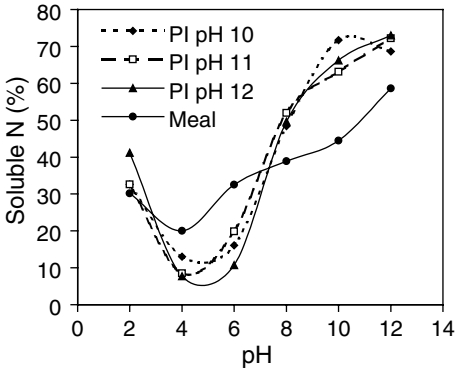

Fig. 3. N-solubility of *B. carinata* meal and PI as a function of pH.

In general, the defatted meal has better functional properties than the PI (Table 4). The different components of the meal are responsible for these functional properties. Gorecka et al. (2000) observed different values of water adsorption between the lupine meal and lupine fiber, the latter showing a higher water adsorption. Also, the presence of polyphenols might be beneficial because they are involved in the stabilization of protein–protein complexes of β-lactoglobuline in the water–air interface, increasing the foaming capacity and stability against the action of surfactants (Sarker, Wilde, & Clark, 1995). In contrast the presence of phytic acid may have a negative effect. Schwenke, Mothes, Marzilger, Borowska, and Kozlowska (1987) and Kroll (1991) observed that phytic acid–protein complexes are formed between pH 3 and 7 in rapeseed, which results in decreased protein solubility especially in the globulin fraction. Functional properties are mostly determined by proteins in protein isolates, since they have a much higher protein content than meals. The functional properties, with the exception of water binding and relative foam stability deteriorate with increasing pH values during the process of obtaining the PI. This could be due to denaturation of proteins at higher pH. The functional properties of the PI obtained by extraction at pH 11 and 12 are similar to those reported in rapeseed PI (Mahajan & Dua, 1995; Mansour, Peredi, & Dworschak, 1992; Thompson, Liu, & Jones, 1982). Only PI obtained at pH 10 have functional properties comparable to those of the defatted meal.

3.4. Protein extraction with Alcalase

Protein solubility and other functional properties may be improved by carrying out a limited protein hydrolysis. For this reason, *B. carinata* defatted meal was partially hydrolyzed during alkaline extraction. Different pH values and enzyme to substrate ratios were tested in order to optimize the yield of protein extraction and the degree of hydrolysis of the protein extracts. In the first series of assays an enzyme to substrate ratio (E/S) of

Table 4
Functional properties of *B. carinata* defatted meal and PI

	Meal	PI pH 10	PI pH 11	PI pH 12
Water adsorption	255.3 ± 10.2	102.7 ± 6.4	99.0 ± 7.2	102.7 ± 6.5
Fat adsorption	202.2 ± 6.4	340.0 ± 10.2	217.0 ± 12.6	209.7 ± 13.4
Emulsifying activity	75.6 ± 4.8	70.0 ± 4.2	54.0 ± 4.3	15.0 ± 4.2
Emulsifying stability	49.4 ± 8.2	33.0 ± 3.6	5.0 ± 2.1	0.0 ± 0.0
Foaming capacity	295.0 ± 9.4	280 ± 6.3	163 ± 7.1	181 ± 4.9
Foaming stability				
At 20 min	91.5 ± 4.3	87.0 ± 2.3	62.5 ± 2.0	66.0 ± 4.2
At 40 min	74.6 ± 3.5	81.0 ± 3.1	52.3 ± 5.3	59.5 ± 3.6
At 60 min	65.5 ± 1.3	73.2 ± 2.5	50.7 ± 4.2	50.6 ± 4.3
At 120 min	56.9 ± 5.4	70.1 ± 1.8	44.6 ± 3.1	48.2 ± 1.9

Data are the mean ± SD of three analyses.

Table 5
Extraction yields (soluble N) and degree of hydrolysis obtained in the protein extracts of *B. carinata* with (0.108 AU/g protein) or without Alcalase at different pHs

	Extraction yields	Degree of hydrolysis
pH 7 – Alcalase	33.5 ± 2.1	0.0
pH 7 + Alcalase	57.5 ± 1.9	15.2 ± 1.1
pH 9 – Alcalase	41.6 ± 3.1	0.0
pH 9 + Alcalase	62.1 ± 3.6	11.6 ± 0.8
pH 10 – Alcalase	55.4 ± 3.2	0.0
pH 10 + Alcalase	51.3 ± 2.4	11.5 ± 1.2

Data are the mean ± SD of three analyses.

Table 6
Extraction yields (soluble N) and degree of hydrolysis obtained in the protein extracts of *B. carinata* with (0.036 AU/g protein) or without Alcalase at different pHs

	Extraction yields	Degree of hydrolysis
pH 7 – Alcalase	33.5 ± 2.1	0.0
pH 7 + Alcalase	48.5 ± 2.6	11.0 ± 1.6
pH 9 – Alcalase	41.6 ± 3.1	0.0
pH 9 + Alcalase	52.4 ± 1.7	8.3 ± 1.3

Data are the mean ± SD of three analyses.

0.108 AU of Alcalase/g protein and pH 7, 9, and 10 were tested (Table 5). pH 7 is optimum for the protease preparation that was used, while pH 10 provides much better protein solubility. pH 9 was chosen as an intermediate value. The highest degree of hydrolysis (15.2%) was reached at pH 7, the optimum pH for the enzyme, while the highest yield of protein extraction was obtained in the presence of Alcalase at pH 9 (62.1%). At pH 7 and 9 the yield of protein extraction in the presence of Alcalase is higher than without the enzyme because the hydrolytic process favors the solubilization of a higher amount of proteins, specially those denatured during the oil extraction process.

A second series of assays were performed using an E/S ratio three times lower than above in order to reduce the degree of hydrolysis of the protein hydrolysates extracts while maintaining the yield of protein extracted at pH 7 and 9 (Table 6). The yield of protein extraction in the presence of Alcalase was 48.5% at pH 7 and 52.4% at pH 9, while the degrees of hydrolysis were 11.0 and 8.3%, respectively. Lower E/S ratios resulted in lower yields of protein extraction and lower degrees of hydrolysis. The assays performed at pH 9 provided the highest yield of protein extraction and a degree of hydrolysis lower than 10%. Therefore, pH 9 was chosen as the optimum to produce a *B. carinata* protein isolate with limited protein hydrolysis. Fig. 4 shows the yields of extraction and the degree of hydrolysis of protein hydrolysates obtained at pH 9 with E/S ratios ranging from 0.036 AU to 0.72 AU Alcalase/g protein. Both parameters increased with the E/S ratio. However this increase is not proportional since an increase of E/S from 0.072 to 0.36 UA Alcalase/g protein approximately doubled the degree hydrolysis, from 8.7% to 17.5%, but the extraction yields remained very similar (from 61.8% to 68.5%).

Hydrolysates obtained using 0.072 and 0.72 AU Alcalase/g protein (denoted PH-LDH and PH-HDH respectively) were selected to study functional properties as compared to the PI (Table 7). 0.72 AU Alcalase/g protein was chosen because this provided the highest

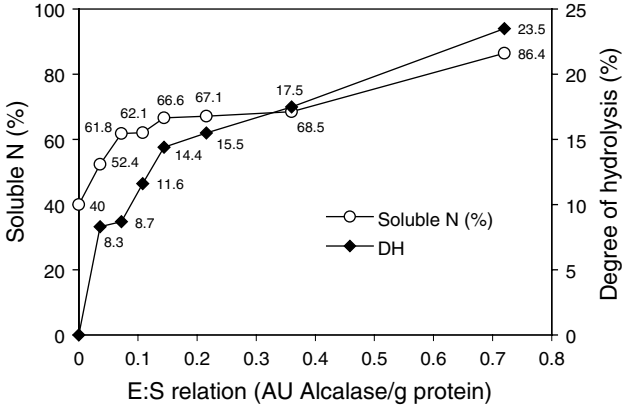

Fig. 4. Extraction yields and degree of hydrolysis at pH 9 using different enzyme to substrate ratio.

Table 7
Functional properties of *B. carinata* deffated meal, protein isolate at pH 11, and protein hydrolysates with low (PH-LDH) and high (PH-HDH) degree of hydrolysis

	Deffated meal	Protein isolate pH 11	PH-LDH	PH-HDH
Water adsorption	255.3 ± 10.2	99.0 ± 7.2		
Fat adsorption	202.2 ± 6.4	217.0 ± 12.6	418.2 ± 7.2	494.4 ± 6.9
Emulsifying activity	75.6 ± 4.8	54.0 ± 4.3	60.5 ± 4.2	9.9 ± 2.6
Emulsifying stability	49.4 ± 8.2	5.0 ± 2.1	84.6 ± 3.1	93.8 ± 4.3
Foaming capacity	295.0 ± 9.4	163.0 ± 4.9	310.0 ± 9.4	141 ± 6.3
Foaming stability				
At 20 min	91.5 ± 4.3	62.5 ± 2.0	58.1 ± 5.2	37.8 ± 3.8
At 40 min	74.6 ± 3.5	52.3 ± 5.3	45.2 ± 4.3	31.5 ± 4.6
At 60 min	65.5 ± 1.3	50.7 ± 4.2	41.9 ± 3.1	26.0 ± 5.1
At 120 min	56.9 ± 5.4	44.6 ± 3.1	25.8 ± 4.6	14.6 ± 2.9

Data are the mean ± SD of three analyses.

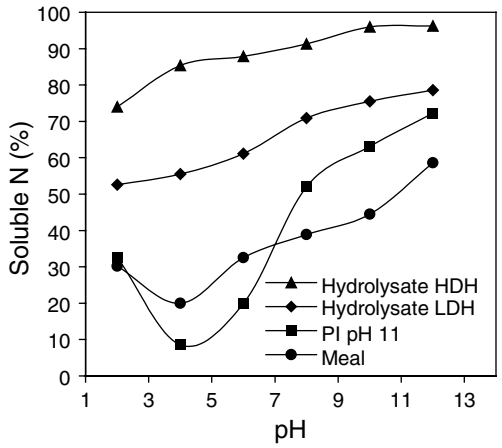

Fig. 5. Solubility of *B. carinata* defatted meal, protein isolate produced by extraction at pH 11 and protein hydrolysates produced in the presence of 0.072 and 0.72 UA Alcalase/g protein.

yield of protein extraction (86.4%), while using 0.072 AU of Alcalase/g protein resulted in a good yield of protein extraction (61.8%) and a degree of hydrolysis lower than 10% (8.7%). As mentioned before, the degree of hydrolysis determines the functional properties of protein hydrolysates (Chobert, Bertrand-Harb, & Nicolas, 1988; van der Ven, Gruppen, Bont, & Voragen, 2001; Vioque, Sánchez-Vioque, Clemente, Pedroche, & Millán, 2000). The protein hydrolysate obtained using 0.72 UA of Alcalase/g protein had a very good solubility in a wide range of pH, from 2 to 12, when compared with the protein isolate obtained at pH 11 and the protein hydrolysate with a low degree of hydrolysis (Fig. 5). However, this improved solubility did not translate into an improvement of interfacial properties, probably because the peptides generated are to small to stabilize the air/water and oil/water interfases. In this sense, the partially hydrolyzed *B. carinata* protein obtained using 0.072 UA Alcalase/g protein showed the best functional properties. Extraction in the presence of 0.72 UA Alcalase/g protein resulted in a protein product with high fat adsorption. This is probably due to a higher exposure of the hydrophobic groups of the protein during the hydrolytic process.

B. carinata has a high potential to be exploited as an oil seed crop. However, this will lead to the production of large amounts of defatted meal as a by-product. This work has focused on developing procedures for the production of protein products using *B. carinata* defatted meal. Although the process of oil extraction is to some extent detrimental to protein quality, alkaline extraction in the presence of Alcalase yields a products rich in protein and with functional properties that could be used by the food industry. Different variables in this procedure have been studied so that the process can be tailored to meet the needs for different types of protein isolates/hydrolysates.

Acknowledgements

This work was supported by research grants AGL2002-02836 (JGC), AGL2001-0526 (FM) and a Ramón y Cajal contract (JGC).

References

Adler-Nissen, J. (1979). Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *Journal of Agricultural and Food Chemistry, 27*, 1256–1262.

Alaiz, M., Navarro, J. L., Giron, J., & Vioque, E. (1992). Amino acid analysis by high-performance liquid chromatography after derivatization with diethyl ethoxymethylenemalonate. *Journal of Chromatography, 591*, 181–186.

AOAC. (1990). *Official Methods of Analysis*, 15th ed. Arlington: Association of Official Analytical Chemists-Washington D.C.

Bales, C. W., Freeland-Graves, J. H., Lin, P. H., Stone, J., & Dougherty, V. (1987). Plasma uptake of manganese response to dose and dietary factors. In C. Kies (Ed.), *Nutrition bioavailability of manganese* (pp. 112–122). Washington DC: American Chemical Society.

Bell, J. M. (1993). Factors affecting the nutritional value of canola meal. *Canadian Journal of Animal Science, 73*, 679–697.

Bell, J. M., & Rakow, G. (1996). Trypsin inhibitor and phytic acid in oil-extracted meals from seeds of several *Brassica* sp. and *Sinaps alba*. *Canadian Journal of Animal Science, 24*, 423–425.

Berot, S., & Davin, A. (1996). Technologie d' extraction et de purification des matières protéiques végétales. In B. Godon (Ed.), *Proteines végétales*. Paris: Technique and Documentation Lavoisier.

Bouchereau, A., Hamelin, J., Lamour, I., Renard, M., & Larher, F. (1991). Distribution of sinapine and related compounds in seeds of *Brassica* and allied genera. *Phytochemistry, 30*, 1873–1881.

Cheftel, J.C. (1989). Proteínas alimentarias. Bioquímica. Propiedades funcionales. Valor nutritivo. Modificaciones químicas; Zaragoza: Acribia S.A.

Chobert, J. M., Bertrand-Harb, C., & Nicolas, M.-G. (1988). Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. *Journal of Agricultural and Food Chemistry, 36*, 883–892.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350–356.

Duncan, A. J. (1991). Glucosinolates. In F. J. P. D'Mello, J. P. Duffus, & C. M. Duffus (Eds.), *Toxic substances in crop plants* (pp. 126–147). Cambridge, Science Park: The Royal Society of Chemistry, Thomas Graham House.

El-Nockrashi, A. S., Mukherjee, K. D., & Mangold, H. K. (1977). Rapeseed protein isolates by countercurrent extraction and isoelectric precipitation. *Journal of Agricultural and Food Chemistry, 25*, 193–197.

FAO/WHO/UNU (2002). *Agricultural Bulletin Board on Data Collection, Disemination and Quality of Statistics*, Geneva: World Health Organization.

Fernández-Quintela, A., Larralde, J., Macarulla, M. T., Marcos, R., & Martinez, J. A. (1993). Leguminosas y concentrados de proteína: Nuevas perspectivas y aplicaciones. *Alimentaria, 239*, 59–63.

Frühbeck, G., Alonso, R., Marzo, F., & Santidrian, S. A. (1995). Modified method for the indirect quantitative analysis of phytate in foodstuffs. *Analytical Biochemistry, 225*, 206–212.

Gassmann, B. (1983). Preparation and application of vegetable proteins, especially proteins from sunflower seed, for human consumption. An approach. *Nahrung, 27*, 351–369.

Getinet, A., Rakow, G., & Raney, J. P. (1996). Glucosinolate content variation in *Brassica carinata* A. Braun germplasm grown at Holetta Ethiopia. *Cruciferae Newsletter, 18*, 84–85.

Gibson, R. S. (1994). Content and bioavailability of trace elements in vegetarian diets. *American Journal of Clinical Nutrition, 59*, 1223S–1232S.

Gorecka, D., Lampart-Szczapa, E., Janitz, W., & Sokolowska, B. (2000). Composition of fractional and functional properties of dietary fiber of lupines (*L. luteus* and *L. albus*). *Nahrung, 44*, 229–232.

Grases, F., Prieto, R. M., Simonet, B. M., & March, J. G. (2000). Phytate prevents tissue calcifications in female rats. *Biofactors, 11*, 171–177.

Kies, C., Aldrich, K. D., Johnson, J. M., Creps, C., Kowalski, C., & Wang, R. H. (1987). Manganese availability for humans. Effects of selected dietary factors. In C. Kies (Ed.), *Nutrition bioavailability of manganese* (pp. 136–145). Washington DC: American Chemical Society.

Krause, J. P., & Schwenke, K. D. (1995). Changes in interfacial properties of legumin from faba beans (*Vicia faba* L.) by tryptic hydrolysis. *Nahrung, 39*, 396–405.

Kroll, J. (1991). Selected functional properties of detoxified rapeseed protein preparations affected by phytic acid. *Nahrung, 35*, 619–624.

Kroll, J., & Rawel, H. (1996). Chemical reactions of benzyl isothiocyanate with myoglobin. *Journal of Science and Food Agriculture, 72*, 376–384.

Lajolo, F. M., Marquez, U. M. L., Filisetti-Cozzi, T. M. C. C., & McGregor, D. I. (1991). Chemical composition and toxic compounds in rapeseed (*Brassica napus*, L.) cultivars in Brazil. *Journal of Agriculture and Food Chemistry, 39*, 1933–1937.

Lee, S. C., Prosky, L., & Vries, J. W. (1992). De Determination of total soluble and insoluble dietary fiber in foods-enzymatic-gravimetric method, MES-TRIS buffer: Collaborative study. *Journal of AOAC International, 75*, 395–416.

Liener, I. E. (1989). Antinutritional factors in legume seeds: state of the art. In J. Huisman, A. F. B. Van der Poel, & I. E. Liener (Eds.), *Recent advances of research in antinutritional factors in legume seeds* (pp. 6–14). The Netherlands: Wageningen-Pudoc.

Lin, M. J. Y., Humbert, E. S., & Sosulski, F. W. (1974). Certain functional properties of sunflower meal products. *Journal of Food Science, 39*, 368–370.

Mahajan, A., & Dua, S. (1995). Functional properties of rapeseed protein isolates. *Journal of Food Science and Technology, 32*, 162–165.

Mannheim, A., & Cheryan, M. (1992). Enzyme-modified proteins from corn gluten meal: preparation and functional properties. *Journal of the American Oil Chemists' Society, 69*, 1163–1169.

Manson, M. M., Ball, H. W., Barrett, M. C., Clark, H. L., Judah, D. J., Williamson, G., & Neal, G. E. (1997). Mechanism of action of dietary chemoprotective agents in rat liver: induction of phase I and II drug metabolizing enzymes and aflatoxin B1 metabolism. *Carcinogenesis, 18*, 1729–1738.

Mansour, E. H., Dworschák, E., Lugasi, A., Gaál, Ö, Barna, É, & Gergely, A. (1993). Effect of processing on the antinutritive factors and nutritive value of rapeseed products. *Food Chemistry, 47*, 247–252.

Mansour, E. H., Peredi, J., & Dworschak, E. (1992). Preparation and functional properties of rapeseed protein products. *Acta Alimentaria, 21*, 293–305.

Matthäus, B. (2002). Antioxidant activity of extracts obtained from residues of different oilseeds. *Journal of Agriculture and Food Chemistry, 50*, 3444–3452.

Minchinton, I., Sang, J., Burke, D., & Truscott, R. J. W. (1982). Separation of desulphoglucosinolates by reversed-phase high-performance liquid chromatography. *Journal of Chromatography, 247*, 141–148.

Moores, R. G., McDermott, D. L., & Wood, T. R. (1948). Determination of chlorogenic acid in coffee. *Analytical Chemistry, 20*, 620–624.

Mnzava, N. A., & Olsson, K. (1990). Studies on tropical vegetables. Part 1: seed amino acids and glucosinolate profile of ethiopian mustards (*Brassica carinata* Braun). *Food Chemistry, 35*, 229–235.

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*, 1685–1689.

Nastruzzi, C., Cortesi, R., Esposito, E., Menegatti, E., Leoni, O., Iori, R., & Palmieri, S. (2000). In vitro antiproliferative activity of isothiocyanates and nitriles generated by myrosinase-mediated hydrolysis of glucosinolates from seeds of cruciferous vegetables. *Journal of Agriculture and Food Chemistry, 48*, 3572–3575.

Reddy, N. R., Salunkhe, D. K., & Sathe, S. K. (1982). Biochemistry of black gram (*Phaseolus mungo* L.): a review. *Critical Reviews in Food Science and Nutrition, 16*, 49–114.

Sarker, D. K., Wilde, P. J., & Clark, D. C. (1995). Control of surfactant-induced destabilization of foams through polyphenol-mediated protein–protein interaction. *Journal of Agriculture and Food Chemistry, 43*, 295–300.

Sarwar, G., Blair, R., Friedman, M., Gumbmann, M. R., Hackler, L. R., Pellett, P. L., & Smith, T. K. (1984). Inter- and intra-laboratory variability in rat growth assays for estimating protein quality of foods. *Journal of the Association of Official Analytical Chemists, 67*, 976–981.

Schöne, F., Jahreis, G., Lange, R., Seffner, W., Groppel, B., Hennig, A., & Ludke, H. (1990). Effect of varying glucosinolate and iodine intake via rapeseed meal diets on serum thyroid hormone level and total iodine in the thyroid in growing pigs. *Endocrinologia Experimentalis, 24*, 415–427.

Schwenke, J. D., Mothes, R., Marzilger, K., Borowska, J., & Kozlowska, H. (1987). Rapeseed protein polyanion interactions-turbimetric studies in systems with phosphate-containing polyanions: phytic acid and octametaphosphate. *Nahrung, 31*, 1001–1013.

Simbaya, J., Slominski, B. A., Rakow, G., Campbell, L. D., Downey, R. K., & Bell, J. M. (1995). Quality characteristics of yellow-seeded Brassica seed meals: protein, carbohydrates and dietary fiber components. *Journal of Agriculture and Food Chemistry, 43*, 2062–2066.

Slominski, B. A., Campbell, L. D., & Guenter, W. (1994). Carbohydrates and dietary fiber components of yellow- and brown-seeded canola. *Journal of Agriculture and Food Chemistry, 42*, 704–707.

Sosulski, F. W. (1962). The centrifuge method for determining flour absorption in hard red spring wheats. *Cereal Chemistry, 39*, 344–350.

Sosulski, F. W. (1983). Rapeseed protein for food use. In B. J. F. Hudson (Ed.), *Developments in food proteins. II* (pp. 109–132). London and New York: Applied Science Publisher.

Süle, E., Tömösközi, S., & Hajós, G. (1998). Functional properties of enzymatically modified plant proteins. *Nahrung, 42*, 242–244.

Theander, O., Aman, P., Miksche, G. E., & Yasuda, S. (1977). Carbohydrates, polyphenols and lignin in seed hulls of different colors from turnip rapeseed. *Journal of Agriculture and Food Chemistry, 25*, 270–273.

Thompson, L. U., Liu, R. F. K., & Jones, J. D. (1982). Functional properties and food applications of rapeseed protein concentrate. *Journal of Food Science, 47*, 1175–1180.

Treviño, J., Rebolé, A., Rodriguez, M. L., Ortiz, L. T., Centeno, C., & Alzueta, C. (1998). Nutritional effect of chlorogenic acid fed o growing broiler chicks. *Journal of Science and Food Agriculture, 76*, 156–160.

van der Ven, C., Gruppen, H., Bont, D. B., & Voragen, A. G. (2001). van der de Emulsion properties of casein and whey protein

hydrolysates and the relation with other hydrolysate characteristics. *Journal of Agriculture and Food Chemistry, 49*, 5005–5012.

Vioque, J., Sánchez-Vioque, R., Clemente, A., Pedroche, J., & Millán, F. (2000). Partially hydrolyzed rapeseed protein isolates with improved functional properties. *Journal of the American Oil Chemists' Society, 77*, 1–4.

Vucenik, I., Tomazic, V. J., Fabian, D., & Shamsuddin, A. M. (1992). Antitumor activity of phytic acid (inositol hexaphosphate) in murine transplanted and metastatic fibrosarcoma, a pilot study. *Cancer Letters, 65*, 9–13.

Yust, M. M., Pedroche, J., Girón-Calle, J., Vioque, J., Millán, F., & Alaiz, M. (2004). Determination of tryptophan by high-performance liquid chromatography of alkaline hydrolysates with spectrophotometric detection. *Food Chemistry, 85*, 317–320.

Zheng, Q., Hirose, Y., Yoshimi, N., Murakami, A., Koshimizu, K., Ohigashi, H., Sakata, K., Matsumoto, Y., Sayama, Y., & Mori, H. (2002). Further investigation of the modifying effect of various chemopreventive agents on apoptosis and cell proliferation in human colon cancer cells. *Journal of Cancer Research and Clinical Oncology, 128*, 539–546.

Zhou, B., He, Z., Yu, H., & Mukherjee, K. M. (1990). Proteins from double-zero rapeseed. *Journal of Agriculture and Food Chemistry, 38*, 690–694.