Partially Hydrolyzed Rapeseed Protein Isolates with Improved Functional Properties

Javier Vioque, Raul Sánchez-Vioque, Alfonso Clemente, Justo Pedroche, and Francisco Millán*

Instituto de la Grasa, 41012-Sevilla, Spain

ABSTRACT: Limited rapeseed protein hydrolysates ranging from 3.1 to 7.7% hydrolysis were produced from isoelectric-precipitated protein isolate. Water absorption, oil absorption, whippability, foam capacity and stability, emulsifying activity, and emulsion stability of the hydrolysates were determined. All protein hydrolysates showed better functional properties than the original protein isolate. Foam and emulsion stability decreased as the degree of hydrolysis increased. The hydrolysate with the lowest degree of hydrolysis showed the best functional properties. These improved functional properties make rapeseed protein hydrolysates a useful product to be used in foods such as breads, cakes, ice creams, meat products, desserts, and salad dressings.

Paper no. J9393 in *JAOCS 77,* 447–450 (April 2000).

KEY WORDS: Alcalase, enzymatic protein hydrolysis, functional properties, rapeseed.

Rapeseed is one of the most important oilseed crops, ranking fifth with respect to oil production after soybean, cottonseed, peanut, and sunflower. Oilseeds are of interest as sources of edible proteins. Rapeseed protein isolates, obtained from defatted rapeseed meal and free of antinutritional components such as glucosinolates, phenolics, phytic acid or fiber, can be used as food ingredients (1). But the use of these proteins is often limited by their low solubility and poor functional properties. This is a particular problem in oilseeds, such as rapeseed, because the proteins suffer denaturation during industrial oil extraction that further reduces their solubility.

To improve water solubility as well as other properties, proteins can be hydrolyzed. The production of enzymatic protein hydrolysates has undergone considerable development in recent years. Protein hydrolysates can be classified depending on the degree of hydrolysis (DH). Protein hydrolysates with low DH (<10%), with better functional properties than the original proteins and with enhanced functional properties such as water and oil absorption, foaming capacity or emulsifying activity are used as food ingredients (2). Protein hydrolysates with variable DH are used as flavorings in soups, sauces, and meat products (3). Extensive protein hydrolysates (DH > 10%) are used as protein supplements or in special medical diets, such as in the production of hypoallergenic foods (4). Extensive rapeseed protein hydrolysates are obtained by the sequential use of Alcalase and Flavourzyme and their application as food supplements has been proposed (5). In the present paper we describe the production of limited rapeseed protein hydrolysates and characterization of their functional properties in order to determine potential application of these hydrolysates in foods.

*To whom correspondence should be addressed at Instituto de la Grasa, Avda. Padre García Tejero 4, 41012-Sevilla, Spain.
E-mail: frmillian@cica.es

MATERIALS AND METHODS

Raw materials. Undehulled rapeseed meal (*Brassica campestris* L.), industrially produced by Koipesol (Sevilla, Spain) by means of solvent extraction and flash desolventization, was used as the protein source. Trinitrobenzenesulfonic acid (TNBS) was purchased from the Sigma Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade.

Proteolytic enzyme. The enzymatic complex used was Alcalase 2.4 L (Novo Nordisk, Bagsvaerd, Denmark), a protease of *Bacillus licheniformis* with endopeptidase activity. A main component of the commercial preparation is serine protease subtilisin A. The specific activity of Alcalase 2.4 L is 2.4 Anson Units (AU) per gram. One AU is the amount of enzyme that, under standard conditions, digests hemoglobin at an initial rate that produces an amount of trichloroacetic acid-soluble product that gives the same color with the Folin reagent as 1 meq of tyrosine released per minute.

Total nitrogen determination. Total nitrogen was determined in 0.1-g samples by the micro-Kjeldahl method (6). Crude protein content was estimated using a conversion factor of 6.25.

Preparation of protein isolate. Rapeseed defatted flour (100 g) was suspended in 1 L of 0.25% Na_2SO_3 at pH 10.5. Na_2SO_3 was used to prevent oxidation of polyphenols and to avoid darkening of the final product. The suspension was extracted by stirring for 1 h at room temperature. After centrifuging at 8,000 × *g*, two additional extractions were carried out with half of the volume of alkaline solution. The supernatants were pooled, and the pH of the soluble proteins was adjusted to the isoelectric point (pH 5.0). The precipitate formed was recovered by centrifuging as above. The precipitate was washed with distilled water that had been adjusted to pH 5.0 and freeze-dried.

Hydrolysis. The protein isolate (50 g resuspended in 1 L of water) was hydrolyzed batchwise in a reaction vessel equipped with a stirrer, thermometer, and pH electrode. Hy-

drolysis was carried out for 60 min at room temperature, pH 8, with a substrate concentration (*S*), of 5% (wt/vol) and different enzyme to substrate (*E/S*) ratios. Hydrolysis was stopped by heating at 85°C for 10 min. Hydrolysates were clarified by filtering to remove insoluble substrate fragments, and the filtrate was freeze-dried for further use.

DH. The DH, defined as the percentage of peptide bonds cleaved, was calculated by determining free amino groups by reaction with TNBS according to Adler-Nissen (7). The total number of amino groups was determined in a sample that had been 100% hydrolyzed at 110°C for 24 h in 6 N HCl (10 mg sample in 4 mL HCl).

Gel filtration chromatography. Samples were passed through a PD-10 column (Amersham Pharmacia, Uppsala, Sweden) to remove nonprotein components. Gel filtration was carried out in a Fast Protein Liquid Chromatography system equipped with a Superose 12 HR 10/30 column from Amersham Pharmacia. Injection volume was 200 μL. The eluent was 20 mM phosphate buffer, 0.5 M NaCl buffer pH 8.3 at 0.4 mL/min flow rate. Elution was monitored at 214 nm to detect small peptides lacking aromatic residues. The molecular masses were determined using a calibration curve made with blue dextran 2000 (2,000 kDa), catalase (240 kDa), α-amylase (200 kDa), bovine serum albumin (67 kDa), ovoalbumin (43 kDa), carbonic anhydrase (29 kDa), and ribonuclease A (13.7 kDa) as molecular weight standards.

Water- and oil-holding capacities. The methods of Carcea-Bencini (8) were used with modifications. One gram of protein was stirred with 10 mL of distilled water or corn oil and centrifuged at 2,200 × *g* for 30 min. The volume of the supernatant was measured. The water- or oil-holding capacities were expressed as the number of grams of water or oil retained by 100 g of protein.

Whippability, foam capacity, and foam stability. The methods of Nath and Narasinga-Rao (9) were used with modifications. A Waring Blendor was used to whip 100 mL of 1.5% (wt/vol) protein suspension at low speed in for 5 min. Whippability was expressed as percentage of volume increase. Foam capacity (%) was expressed as foam (volume/total volume) × 100. Foam stability was expressed as the percentages of foam remaining after 15, 30, 60, and 120 min.

Emulsifying activity and emulsion stability. Emulsifying activity was determined by the methods of Naczk *et al.* (10) with modifications. A 3.5-g sample of meal was homogenized with 50 mL of water for 30 s using a polytron homogenizer at 10,000 rpm. Corn oil (25 mL) was added, and the mixture was homogenized for another 30 s. Another 25 mL of corn oil was added, and the mixture was rehomogenized for 90 s. The emulsion was centrifuged at 1,100 × *g* for 5 min. Emulsifying activity was calculated as (volume of the emulsified layer/volume of emulsion before centrifugation) × 100. Emulsion stability was determined using the sample prepared for measuring of emulsifying activity. It was heated for 15 min at 85°C, cooled, and then centrifuged at 1,100 × *g*. Emulsion stability was expressed as the percentage of emulsifying activity remaining after heating.

RESULTS AND DISCUSSION

Production of rapeseed protein hydrolysates. The protein isolate used as starting material for the hydrolysis had a protein content of more than 95% (Table I). This rapeseed protein isolate was hydrolyzed with Alcalase, a well-known nonspecific endoprotease widely used in food research.

Limited protein hydrolysis may improve functional properties of the original material. But, above a certain DH these properties disappear as a consequence of the smaller peptide size. Thus, depending on the substrate and enzyme used, hydrolysates between 1 and 10% DH on average possess better functional properties than the original proteins (2). To obtain rapeseed protein hydrolysates in this range using Alcalase, a very low proportion *E*/*S* ratio is needed because of the high activity and stability of Alcalase (11). Thus, after several trials, an *E*/*S* ratio of 0.042 mg/g was chosen, with an [*S*] of 0.05 g/mL. Under these conditions, a hydrolysate with 7.7% DH was obtained after 60 min at room temperature (Fig. 1). With this DH some bitter peptides can be formed. This bitterness could be masked with the addition of components such as polyphosphates, gelatin, or cyclodextrin.

The gel filtration chromatography profile of the protein isolate shows a main peak that corresponds to the 12S globulin "cruciferin," the major protein component of protein isolates from rapeseed (Fig. 2). In the final hydrolysate the rela-

TABLE 1
Chemical Composition of Rapeseed Defatted Meal and Protein Isolate Calculated on a Dry Weight Basis

Component	Defatted meal (%)	Protein isolate (%)
Ash	9.0	0.52
Fiber	37.9	0.05
Protein	40.4	97.82
Lipids	3.9	1.14
Soluble sugars	6.3	0.35
Polyphenols	2.6	0.12

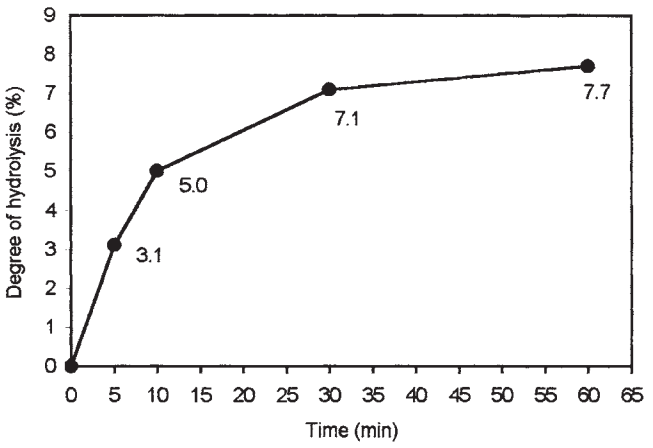

FIG. 1. Limited enzymatic hydrolysis of rapeseed protein isolate with Alcalase. Hydrolysis proceeded for 1 h, at room temperature and pH 8 with an enzyme/substrate ratio of 0.042 mg/g and a substrate concentration of 0.05 g/mL. Degree of hydrolysis (DH) is defined as percentage of peptide bonds cleaved.

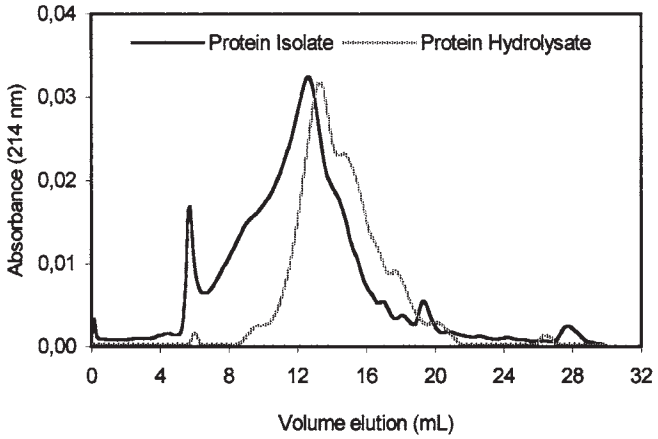

FIG. 2. Gel filtration chromatography of rapeseed protein isolate and protein hydrolysate after Alcalase treatment for 60 min. For conditions see Figure 1.

tive amount of this peak decreased, increasing the amounts of others with lower molecular weights. Thus, after 60 min of hydrolysis, the maximal protein absorbance changed.

Water absorption. Improved water absorption as a result of enzyme hydrolysis has been reported (12). Enzymatic treatment exposes ionizable polar amino acids, such as aspartic and glutamic acids, which are abundant in rapeseed protein isolates (5). These amino acids are capable of binding nearly three times more water than nonionized polar groups. All rapeseed protein hydrolysates obtained had higher water-holding capacities than the unhydrolyzed protein isolate (Table 2). Similar water absorption properties were reported for protein concentrates from *Phaseolus* (13). These values were higher than those observed in commercial preparations of sodium caseinate, nonfat dry milk, soy protein isolate, or dried egg white (14). Thus, because of their high water absorption capacities, these limited rapeseed protein hydrolysates could be used in the food industry to prevent water loss in breads and cakes and to increase yields of cured sausages, canned fish, and frozen products.

Oil-holding capacity. Fat absorption capacity represents the binding of fat by nonpolar side chains of proteins. The digestion of proteins exposes nonpolar side chains that bind hydrocarbon moieties of oil, contributing increased oil absorption. Rapeseed protein hydrolysates had higher oil-holding capacity than the original protein isolate (Table 2). Fat absorption was similar in all protein hydrolysates obtained and slightly below that observed in commercial preparations, such as sodium caseinate or dry egg white, which range between 150 and 200 g/100 g (14).

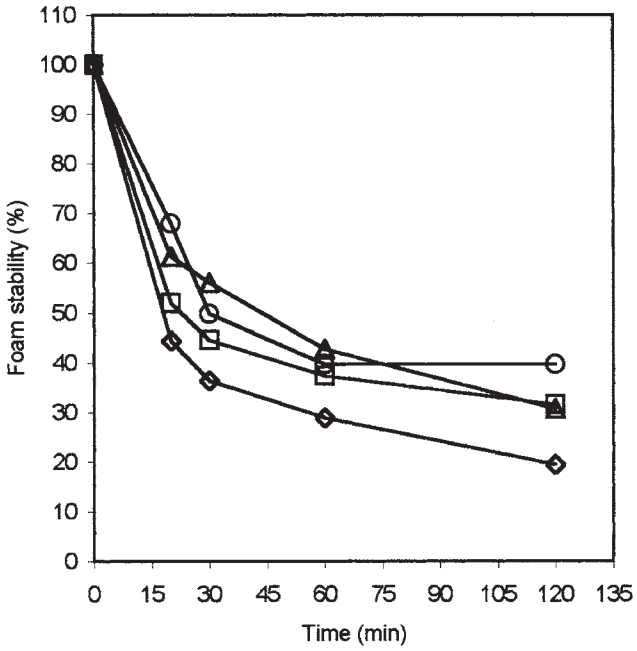

FIG. 3. Foam stability of rapeseed limited protein hydrolysates. ○, 3.1% DH; △, 5.0% DH; □, 7.1% DH; ◇, 7.7% DH. For abbreviation see Figure 1.

Whippability, foaming capacity, and stability. Foams are biphasic colloidal systems, with a continuous liquid phase and a dispersed gas bubble phase. Some food proteins are capable of forming good foams, and their capacity to form and stabilize foams depends on the type of protein, degree of denaturation, pH, temperature, and whipping methods.

It has been reported that limited proteolysis may improve foaming capacity but decrease foam stability (15). Rapeseed protein hydrolysates showed higher foaming capacity and whippability than the original protein isolates. The sample with 3.1% DH possessed the highest value (Table 2).

All protein hydrolysates had lower foam stability than those reported for other rapeseed protein products (4), and this stability decreased with DH (Fig. 3). Apparently, hydrolysates with increased DH are capable of foaming but lack strength to maintain the foam as result of the reduction in peptide size. Foam stability of the protein isolate droped to 0% after 15 min of hydrolysis (data not shown).

Emulsion activity and stability. In many food systems proteins and lipids commonly interact, and thus the ability of pro-

TABLE 2
Functional Properties of Limited Rapeseed Protein Hydrolysates[a]

Degree of hydrolysis (%)	Whippability (% vol increase)	Foam capacity [(foam vol/total vol) × 100]	Water absorption [(g water/g material) × 100]	Oil absorption [g oil/g material) × 100]
0	130 ± 8	18 ± 1	131 ± 11	63 ± 5
3.1	301 ± 19	69 ± 1	585 ± 43	155 ± 8
5.0	253 ± 14	58 ± 3	514 ± 53	133 ± 10
7.1	234 ± 9	59 ± 0	540 ± 10	130 ± 13
7.7	251 ± 24	59 ± 6	551 ± 55	137 ± 9

[a]Data represent the mean ± SD of three independent determinations.

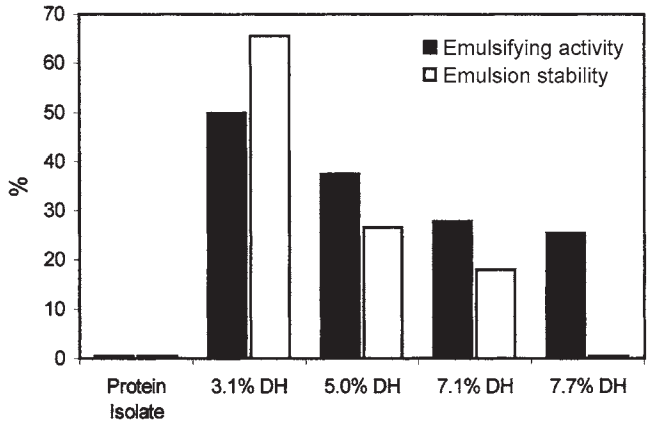

FIG. 4. Emulsifying activity (closed bars) and emulsion stability (open bars) of rapeseed protein isolate and limited protein hydrolysates. For abbreviation see Figure 1.

teins to form stable emulsions is important. The most frequently encountered emulsions are oil-in-water emulsions. It is generally recognized that the emulsifying properties of proteins are improved by limited hydrolysis, owing to the exposure of hydrophobic amino acid residues that may interact with the oil while the hydrophilic residues interact with water.

Rapeseed protein isolate possesses a very low emulsifying activity (Fig. 4) as has been previously reported (16). But when these proteins were hydrolyzed, emulsion activity greatly increased. It has been reported that as DH increases, emulsifying activity and stability decrease (17). Similar results were obtained with rapeseed protein hydrolysates (Fig. 4). Thus, the highest values of emulsifying activity and stability were obtained with the lowest DH. It seems that, as hydrolysis progresses, smaller peptides are generated. These reduce emulsion stability and are less efficient in reducing interfacial tension.

The emulsifying properties of rapeseed protein hydrolysates make them a potential ingredient in food formulations, such as salad dressing, ice creams or mayonnaise. Thus, limited protein hydrolysis is a good procedure to improve functional properties of rapeseed proteins. But the extent of hydrolysis is critical for maximizing the functionality of the product. So, a compromise must be reached between DH and functionality.

The functional characteristics of rapeseed protein hydrolysates make them good substrates to be used in food industry as a natural additive for improving desired functional properties of the final product. Thus, the by-product defatted rapeseed meal can be processed into a value-added product with numerous food applications.

ACKNOWLEDGMENT

This work was supported by research grant from CICYT ALI98-0766.

REFERENCES

1. Zhou, B., Z.-Q. He, H.-M. Yu, and K.D. Mukherjee, Proteins from Double-Zero Rapeseed, *J. Agric. Food Chem. 38*:690–694 (1990).
2. Panyam, D., and A. Kilara, Enhancing the Functionality of Food Proteins by Enzymatic Modification, *Trends Food Sci. Tech. 7*:120–125 (1996).
3. Weir, G.S.D., Protein Hydrolysates as Flavourings, in *Developments in Food Protein*, edited by B.J.F. Hudson, Elsevier, London, 1986, Vol. 4, pp. 175–217.
4. Frokjaer, S., Use of Hydrolysates for Protein Supplementation, *Food Technol. 48*:86–88 (1994).
5. Vioque, J., R. Sánchez-Vioque, A. Clemente, J. Pedroche, J. Bautista, and F. Millán, Production and Characterization of an Extensive Rapeseed Protein Hydrolysate, *J. Am. Oil Chem. Soc. 76*:819–823 (1999).
6. Association of Official Analytical Chemists, *Official Methods of Analysis*, 15th edn., Washington, DC, 1990, pp. 1–11.
7. Adler-Nissen, J., Determination of the Degree of Hydrolysis of Food Protein Hydrolysates by Trinitrobenzensulfonic Acid, *J. Agric. Food Chem. 27*:1256–1262 (1979).
8. Carcea-Bencini, M., Functional Properties of Drum-Dried Chickpea (*Cicer arietinum* L.) flours, *J. Food Sci. 51*:1518–1521, 1526 (1986).
9. Nath, J.P., and M.S. Narasinga-Rao, Functional Properties of Guar Proteins, *Ibid. 46*:1255–1259 (1981).
10. Naczk, M., L.L. Diosady, and L.J. Rubin, Functional Properties of Canola Meals Produced by a Two-Phase Solvent Extraction System, *Ibid. 50*:1685–1688 (1985).
11. Mannheim, A., and M. Cheryan, Enzyme-Modified Proteins from Corn Gluten Meal: Preparation and Functional Properties, *J. Am. Oil Chem. Soc. 69*:1163–1169 (1992).
12. Beuchat, L.R., J.P. Cherry, and M.R. Quinn, Physicochemical Properties of Peanut Flour as Affected by Proteolysis, *Ibid. 23*:616–620 (1975).
13. Chau, C.-F., P.C.K. Cheung, and Y.-S. Wong, Functional Properties of Protein Concentrates from Three Chinese Indigenous Legume Seeds, *J. Agric. Food Chem. 45*:2500–2503 (1997).
14. Ahmedna, M., W. Prinyawiwatkul, and R.M. Rao, Solubilized Wheat Protein Isolate: Functional Properties and Potential Food Applications, *Ibid. 47*:1340–1345 (1999).
15. Chobert, J.M., M.Z. Sitohy, and J.R. Whitaker, Solubility and Emulsifying Properties of Caseins Modified Enzymatically by *Staphylococcus aureus* V8 Protease, *Ibid. 36:*220–224 (1988).
16. Xu, L., and L.L. Diosady, Functional Properties of Chinese Rapeseed Protein Isolates, *J. Food Sci. 59*:1127–1130 (1994).
17. Mahmoud, M.I., W.T. Malone, and C.T. Cordle, Enzymatic Hydrolysis of Casein: Effect of Degree of Hydrolysis on Antigenicity and Physical Properties, *Ibid. 57*:1223–1229 (1992).

[Received September 15, 1999; accepted January 7, 2000]