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Extraction and functionality of membrane-concentrated protein from defatted *Rosa rubiginosa* seeds

Andrés Moure[a], Jorge Sineiro[b], Herminia Domínguez[a,*]

[a]*Departamento de Enxeñería Química, Facultade de Ciencias de Ourense, Universidade de Vigo (Campus Ourense), Edificio Politécnico, As Lagoas 32004, Ourense, Spain*

[b]*Departamento de Enxeñería Química, Universidade de Santiago de Compostela, Avda. das Ciencias sn 15706, Santiago de Compostela, Spain*

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Abstract

Rosa rubiginosa protein was extracted either with distilled water or in the presence of 0.5 M NaCl and further concentrated by ultrafiltration membranes. Temperature and pH influenced the extractability of both protein and polyphenolic compounds. Up to 55–60% of the initial protein was solubilized during alkaline extraction. More than 90% of the solubilized protein was recovered in the unwashed concentrate, which contained more than 80% pure protein (d.b.). Grinding and the presence of NaCl during extraction influenced both the nutritional and functional properties of the protein products. These latter properties could be related to the molecular weight distribution of the extracted protein. The protein concentrated by ultrafiltration was highly soluble in the range of neutral and alkaline pH and the concentrates showed water- and oil-holding capacities up to 26.75 and 16.33%, respectively. The solid byproduct after protein extraction has potential for food uses due to both the high dietary fibre content, and the water and oil absorption capacities.

Keywords: *Rosa rubiginosa*; Protein extraction; Ultrafiltration membranes; Functional properties

1. Introduction

Aqueous solutions are suitable solvents for the extraction of seed proteins, since the major protein fractions in seeds are those soluble in water and in salt solutions. Aqueous technology is comparatively simple and cheap and was successfully used for the extraction of proteins from rapeseed (El Nockrashy, Mukherjee, & Mangold, 1977), canola (Tzeng, Diosady, & Rubin, 1990), grapeseed (Fantozzi & Betschart, 1979), peanut (Rustom, López-Leiva, & Nair, 1991), tomato seed (Liadakis, Tzia, Oreopoulou, & Thomopoulos, 1995, 1998) and almonds (Sze-Tsao & Sahe, 2000), among others. The development of processes for the isolation of vegetable protein is gaining increasing commercial interest, due to the functional properties, prolonged shelf life and UV-radiation absorbing properties of these proteins. Formulation of existing and new protein food products, based on ingredients with desirable properties, is the main application. A growing demand for vegetable non-alcoholic-based protein products, as substitutes for animal types in cosmetics, has also arisen.

Operational conditions, during extraction and isolation, affect the yield, recovery and properties of the protein. The presence of salts influences the protein extraction (Bello & Okezie, 1989; Liadakis et al., 1998; Sathe, 1994) and the functional properties of emulsions, gels and foams (Mwasaru, Muhammad, Bakar, & Che Man, 2000). Ultrafiltration (UF) processes can be used to recover low molecular weight protein, thus avoiding the generation of effluents with high organic load and the consumption of chemicals required for isoelectric precipitation (IE). Membrane technology was used for preparing isolates from jojoba meal (Abbott et al., 1991; Nabetani, Abbott, & Kleiman, 1995), *Crambe abyssinica* (Massoura, Vereijken, Kolster, & Derksen, 1998a) and recovered more protein than isoelectric precipitation, as reported for soy (Lawhon, Rhee, & Lusas, 1981) and coconut (Chakraborty, 1985). The nutritional and functional properties of the UF isolates are superior to those of IE isolates, as observed for peanut (Lawhon et al., 1981), cottonseed (Choi, Lawhon, & Lusas, 1984),

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

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coconut (Chakraborty, 1985) and *Crambe abyssinica* (Massoura, Vereijken, Kolster, & Derksen, 1998b).

Dog rose (*Rosa moschata*, *Rosa rubiginosa*) is native to Europe and shows important expansion in the Andean areas; in Chile 15,000 ha are destined for this crop. The fruit contains carotenoids, vitamins, minerals, 20–40 times more vitamin C than citrus fruits (Gómez, Malec, & Vigo, 1993) and antioxidants (Cao, Björk, Trajkovski, & Uggla, 2000). The seeds, initially considered a byproduct and burned, contain highly valuable oil with dermatological properties for treating queloids, scars and early ageing lines (Valladares, Palma, Sadoval, & Carvajal, 1986). According to Rodríguez et al., (1987), in the Bio-Bio Region (Chile), 22,000 tons of seeds are produced annually. After oil extraction, the defatted seeds are used either as fuel or as feed supplement. Alternatively, the defatted meal can be fractionated into the major nutritional components for use as a potential source of both dietary fibre and protein. In addition, the aqueous extracts of *R. rubiginosa* defatted seeds also possess antioxidant activity (Moure, Franco, Sineiro, Domínguez, Núñez, & Lema, 2001b). The protein content of the meal is low (Malec, Civeira, & Vigo, 1993; Moure et al., 2000a), but extraction and production of concentrates with more than 60% pure protein would allow the evaluation of (1) the nutritional and functional properties and (2) the feasibility of an in situ fractionation to increase the added value of this underexploited crop.

Studies dealing with characterization of nutritional and functional properties of the protein from *R. rubiginosa* are scant. The aim of this work was to use aqueous processing for the extraction and further recovery, with membrane technology, of the protein from defatted *R. rubiginosa* seeds. In addition, the functional properties of the protein extracted with water and with NaCl were evaluated.

2. Materials and methods

2.1. Materials

R. rubiginosa seeds, kindly supplied by Professor R. Chamy (Univ. Católica de Valparaíso, Chile), were used. The seeds were ground in a coffee grinder without refrigeration. The external peeling action led to different compositions among the different particle size ranges. Two particle sizes were used during this work: 0.5–1 mm and <0.5 mm. The ground seeds were defatted overnight at room temperature, using 15 g of hexane per gram of seed. After separation by filtration, the solid phase was subjected to another extraction step under the same conditions. The defatted seeds contained, on a dry basis, 24.6 protein, 64.0 neutral detergent fibre and 1.8% ash for the particles <0.5 mm in size, and 8.2 protein, 76.2 neutral detergent fibre and 1.9% ash for the particles in the range 0.5–1 mm.

2.2. Protein extraction and isolation

The defatted meals (5–15 g) were extracted at 30–60 °C for 90 min with aqueous solutions in either one or two stages. The liquid/solid ratio was 20 g/g, selected to avoid mass transfer limitations; the environmental implications derived from the use of such a high value were minimized, due to the possible whey reutilization after membrane separation. Temperature and pH of the extracting solution were selected to maximize protein extraction yield and the effect of the addition of 0.5 M NaCl during extraction was also studied. Solid and liquid phases were separated by vacuum filtration and the pooled liquid phases from the first and second extraction stages were further subjected to UF in a Filtron unit to concentrate the solubilized protein (Fig. 1). Previous studies of the native protein by Gel Filtration Chromatography (GFC) revealed that the range of sizes for the water- and salt-soluble proteins from *R. rubiginosa* was 15–116 kDa (Moure, Franco, Rúa, Sineiro, Domínguez, & Núñez, 1998). Based on the mentioned study, a sequence of 10 and 5 kDa molecular weight cut-off membranes was used. The retentate was dehydrated by freeze-drying and analyzed for protein content, nutritional and functional properties. The permeate was also characterized, in order to evaluate the feasibility of further reutilization in the following extraction steps.

2.3. Gel electrophoresis

Molecular weight distribution in the concentrates obtained by UF was analyzed with sodium dodecylsulfate-polyacrylamide gel (10%) electrophoresis (SDS-PAGE), according to the method of Laemmli (1970). Freeze-dried samples were solubilized to a final concentration of 10 mg/ml in electrophoresis buffer and heated at 90°C for 3 min. Forty micrograms of protein were loaded in each well and the electrophoresis was run at 40 mA for 1 h. The gels were stained for protein detection with Coomassie Blue R-250. Phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa) and trypsin inhibitor (21.5 kDa) were obtained from Bio-Rad and used as molecular weight markers.

2.4. Analytical methods

Moisture and ash were determined according to standard methods (AOAC, 1997). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined with the method of Goering and van Soest (1970). Protein in the liquid was measured by the Lowry method, and the nitrogen

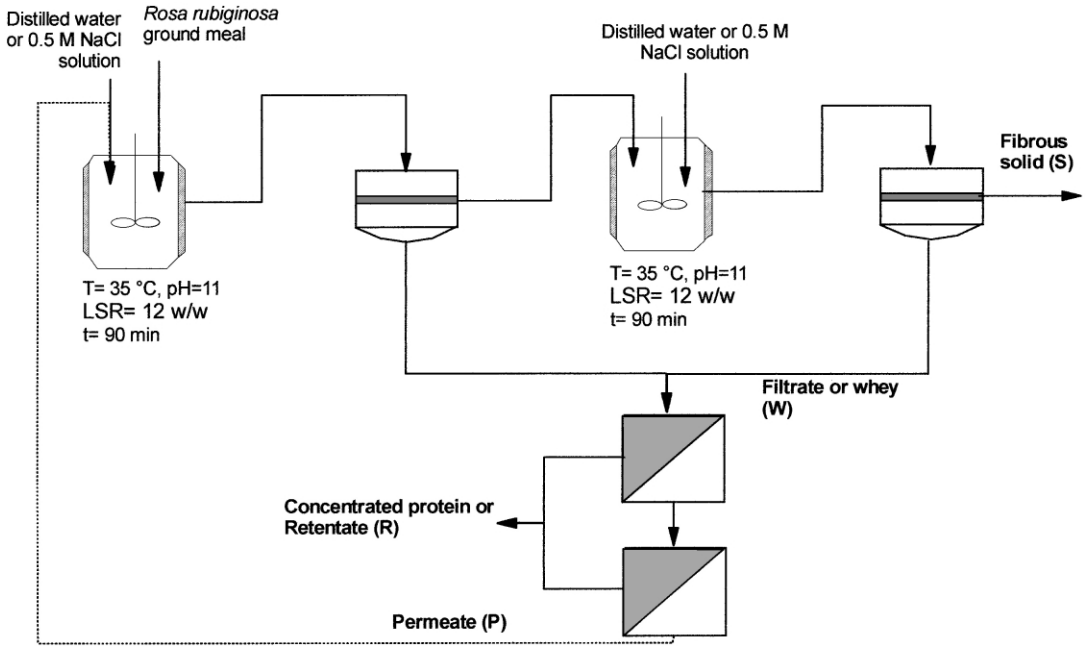

Fig. 1. Flow diagram of the extraction of protein from *Rosa rubiginosa* and further isolation with ultrafiltration membranes.

content in the solids was determined by Kjeldhal; the factor 6.25 was used to convert nitrogen into protein. Colour values L^*, a^*, b^* were measured with a Macbeth Colour Eye 2180, standardized against a white tile (10 degree, illuminant D65, $L^* = 95.773$, $a^* = -0.145$, $b^* = 1.549$). Total polyphenolic compounds were spectrophotometrically determined according to the Folin-Denis method (AOAC, 1997), using chlorogenic acid (Sigma Chem. Co) as standard. In vitro protein digestibility, measured as ADC (Apparent Digestibity Coefficient), was measured by a multienzyme assay with trypsin, chymotrypsin and peptidase (Hsu, Vavak, Satterlee & Miller, 1977). Available lysine was determined by the TNBS method with D,L-lysine (Merck) as standard (Hall, Trinder, & Givens, 1973). Reducing sugars were determined with the Nelson–Somogyi method with glucose (Sigma Chem. Co) as standard.

2.5. Functional properties

2.5.1. Water and oil absorption

The samples of isolates and fibre (1 g) were mixed with distilled water or with oil (100 ml) in an Ultra-Turrax T50. After 30 min at room temperature, the mixtures were centrifuged at 5000 ***g*** for 30 min. The liquid retained by the solids was measured and the water and oil absorption capacity was expressed as gram bound per gram sample on a dry basis (Okezie & Bello, 1988).

2.5.2. Whippability and foam stability

A sample (0.5 g) was mixed with 40 ml distilled water in an Ultra-Turrax T50 for 2 min. The blend and the liquid from rinsing the blender jar were transferred into a 100 ml 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.

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

These were determined as reported by Pearce and Kinsella (1978). A 0.1% (w/v) protein solution (pH 7.0, 20 ml) and 6.6 ml of soy oil were homogenized for 1 min in an Ultraturrax T-50 to produce an emulsion. Inmediately after homogenization, aliquots of 50 μl 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. Emulsifying activity index was measured as initial absorbance, and emulsion stability index (ESI) was calculated by the equation:

$$\mathrm{ESI(min)} = T_0 \times t/(T_0 - T_{10})$$

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

2.5.4. Gelification

This was determined according to the method of Coffmann and García (1977), and measured as the Least Gelation Concentration (LGC). Sample suspensions of 2, 4, 6, 8, 10, 12, 14, 16% (w/v) were prepared in 50 ml distilled water and pH was adjusted to 7.0 with 0.1 N NaOH or 0.1 N HCl. The dispersions were

poured into test tubes in 5 ml aliquots, heated to 100°C in a water bath for 1 h and cooled to 4°C for 2 h.

2.5.5. Protein solubility

This was determined in a 10% (w/v) suspension over a pH range from 2 to 12 according to the method of Beuchat, Cherry, and Quinn (1975). The suspensions were stirred at room temperature for 90 min and then centrifuged at 5000 ***g*** for 30 min. The protein content in the supernatant was analysed by the Lowry method and expressed as percent of the total protein in the concentrate, measured by Kjeldahl.

3. Results and discussion

3.1. Effect of pH and temperature

The effect of temperature on the extraction yield of both protein and polyphenolic compounds from *R. rubiginosa* was studied at acid (4.5) and at alkaline (11) pH. Extraction experiments were performed during 360 min in a single extraction stage using a particle size less than 0.5 mm with LSR = 20 g/g. Phase separation was accomplished by filtration. Fig. 2 shows protein extractability, determined in the extract by the Lowry method, confirmed by the unextracted protein in the solid samples measured by Kjeldahl, and calculated as percent of the total extractable protein in the initial sample. The extraction temperature, in the range 30–50°C, did not affect the protein yield. However, at 60°C a higher extraction yield was obtained, both at acid and at alkaline pH. Parallel to the increase in the extracted protein at the higher temperature assayed, a reduction in the residual protein was observed. The sum of the protein extracted in the liquid and the protein remaining in the solid was around 100%; any difference was probably due to interferences in the values calculated by the Lowry method.

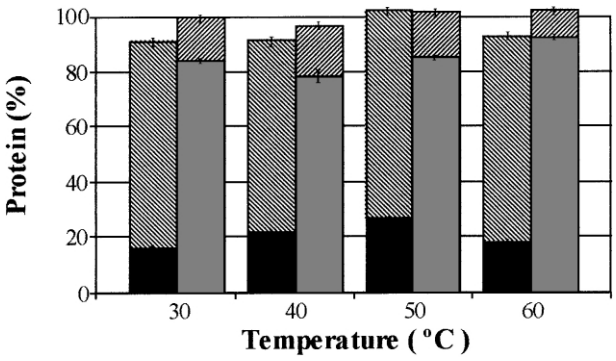

Fig. 2. Protein distribution in the extract, determined by the Lowry method, after acid (▬) and after alkaline (▬) extraction, and in the solid residue, determined by Kjeldahl, after acid (▨) and after alkaline (▨) extraction. *Rosa rubiginosa* defatted seeds were extracted with distilled water during 360 min with LSR = 20 g/g.

The highest yield of solubilized protein was obtained under alkaline conditions. The protein yields after alkaline extraction are usually higher than extraction yields at acidic pH and have been correlated with the presence of low molecular weight subunits for alkaline extraction of coconut (Chakraborty, 1985). The protein yield achieved after alkaline extraction was comparable with the values reported for the isolation of other vegetable proteins. Up to 40% total protein was extracted from tomato seed (Liadakis et al., 1995), 45% from grapeseed (Corrao, Gattuso, Fazio, Cilluffo, & Pirrone, 1979), 60% from soy flakes and flours (Boatright & Hettiarachchy, 1995), 70% from winged bean (Bello & Okezie, 1989), 64% from jojoba meal (Nabetani, Abbott, & Kleiman, 1995), 66% from *Crambe abyssinica* oilseed (Massoura et al., 1998a) and 80–87% from chickpea (Sánchez-Vioque, Clemente, Vioque, Bautista, & Millán, 1999).

The influence of the extraction temperature on protein extractability differs greatly from seed to seed, and for the same seed it can be affected by the operational conditions. The effect of temperature on the protein extraction was non-significant for the neutral and alkaline extraction of faba bean (McCurdy & Knipfel, 1990) and pigeon pea (Mizubuti, Biondo, Souza, da Silva, & Ida, 2000) and for the extraction, at neutral pH, of winged beans (Bello & Okezie, 1989). These latter authors found higher increases in protein extractability with increasing temperature at acidic than at alkaline pH. Protein extraction yield and content in the isolates from tomato seed were enhanced by raising temperature in the range 30–50°C (Liadakis et al., 1995) and similar behaviour was reported during the extraction of peanut protein (Rustom et al., 1991). For the following extraction experiments, pH was adjusted and maintained at 11 and temperature was fixed at 35°C. Although higher yields could be attained at 60°C, prolonged exposure under these conditions could denature protein (Kwon, Bae, Park, & Rhee, 1996; Massoura et al., 1998a).

The fibre content in the solid residue increased with the protein extraction yields, and reached up to 88% of the solid dry weight (Table 1). The increase in NDF content with rising temperature was apparent both at acid and at alkaline extraction pH, and could more likely be ascribed to the reduction in protein content of the solid, than the solubilization of hemicelluloses at the moderate temperatures employed. The cellulose content, calculated from the difference between ADF and ADL, increased slightly with temperature. Lignin content, as observed from the non-significantly different ADL values, remained fairly constant with temperature. Colour values for lightness (L^*) and for red (a^*) and yellow colour (b^*) are also indicated in Table 1. Darkening of the meal increased as the residual protein decreased.

Fig. 3 shows the extraction kinetics of water-extractable polyphenolic compounds measured as chlorogenic acid equivalents in the liquid phase. At acidic pH, increases in temperature increased the extraction yield. During extraction at 50 and 60°C a slight degradation of the polyphenolic compounds was noticeable. A similar and more marked trend was observed at alkaline pH at temperatures over 50 °C, the degradation effect being more pronounced than the enhanced extractability. After 1 h almost all the water-extractable polyphenolic compounds were extracted. The extraction yield at acidic conditions was comparable with that obtained after three consecutive extraction stages (2.8×10^{-2} g/100 g freeze-dried extract; Moure et al., 2001b).

Table 1
Effect of temperature on the fibre composition (% d.b.) and colour (L^*, a^*, b^*) of the meal from *Rosa rubiginosa* after protein extraction with distilled water at LSR = 20 during 360 min

(°C)	Acidic pH[a]			Alkaline pH[a]		
	NDF	ADF	ADL	NDF	ADF	ADL
30	73.02±0.64	52.57±0.42	23.83±3.59	80.17±0.75	64.31±0.22	27.81±1.04
40	79.56±4.15	61.55±1.19	26.35±1.27	77.13±1.80	61.79±0.49	23.51±0.05
50	80.84±2.33	59.26±0.35	23.94±0.13	85.12±1.40	65.57±0.13	24.14±0.15
60	68.22±0.46	51.24±1.55	22.48±0.47	88.43±0.19	66.63±0.22	25.90±0.48
	L^*	a^*	b^*	L^*	a^*	b^*
30	67.67±0.39	6.10±0.01	16.36±0.07	61.62±0.42	7.92±0.04	16.26±0.26
40	66.09±0.33	6.96±0.15	17.40±0.30	60.75±0.48	8.09±0.04	16.46±0.16
50	59.46±0.22	6.52±0.11	15.05±0.20	59.85±0.67	6.78±0.03	15.50±0.36
60	63.60±0.94	6.81±0.04	16.38±0.15	60.52±0.30	7.28±0.04	17.06±0.33

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

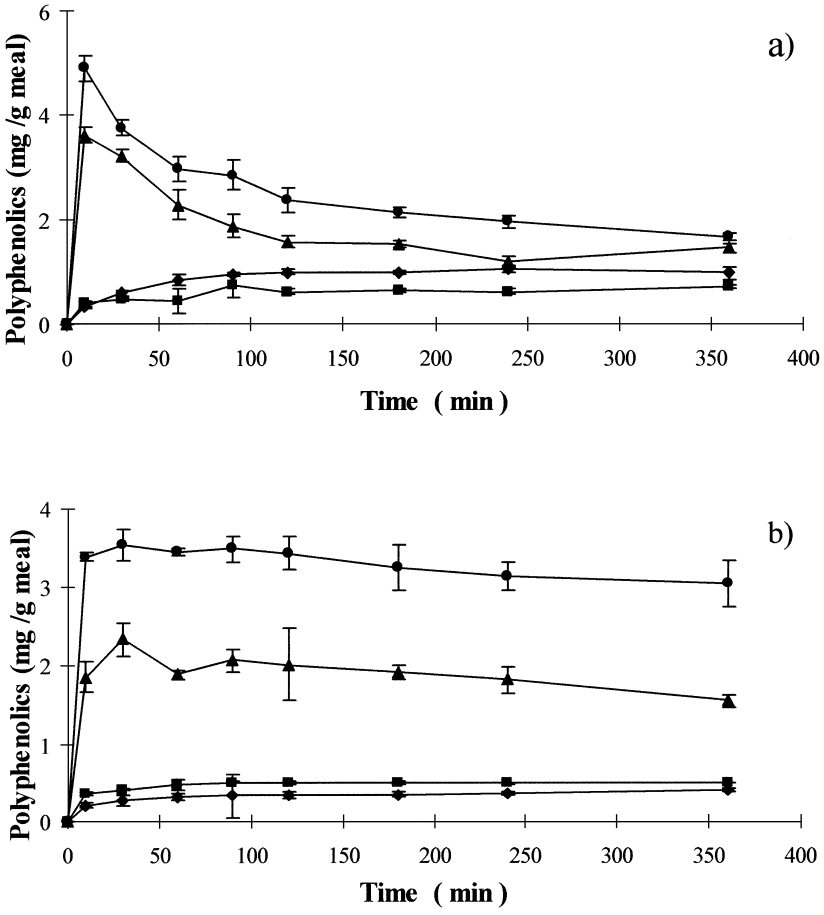

Fig. 3. Extraction kinetics of polyphenolic compounds from *Rosa rubiginosa* at (a) acidic and (b) alkaline pH. ◆, 30°C; ■, 40°C; ▲, 50°C; ●, 60°C.

3.2. Effect of particle size and presence of salts on the nutritive and functional properties

The effect of particle size on the protein extraction from *R. rubiginosa* defatted seeds was studied, but particle size reduction involved additional features in the meal, related to composition and probably to protein quality. The mechanical action, applied for grinding, removed the external layers of the seeds; with higher fibre content and, consequently, the protein content of smaller size was higher than the protein content of the higher size ones. The quality-related effect could be caused by a slight heat exposure during mechanical grinding. The salts and the pH affect the yield and physicochemical properties of the extracted proteins. Once the protein has been extracted, the presence of salts can also alter the solubility of proteins by affecting protein–protein and protein–water interactions (Mwasaru et al., 2000).

The extraction of proteins soluble in water and in salt solutions is desirable since both groups (albumins and globulins) have the highest lysine content (Obatolu & Cole, 2000). Absence of salts was reported to be optimal for protein extraction from pigeon pea (Mizubuti, Biondo, Souza, da Silva, & Ida, 2000) and faba bean (McCurdy & Knipfel, 1990). These conditions were also selected for wheat (Wu, 1993), peanut (Rustom et al., 1991) and tomato seed meal (Liadakis et al., 1998). Based on literature data, the addition of salts to the extraction system allows the solubilization of more protein and their presence influences physico-chemical properties of the proteins, such as electrostatic and hydrogen bonds and hydrophobic interactions (Phillips, Yang, & Kinsella, 1991). NaCl was used in the next experiments since, among salts, it presents several advantages derived from the ionic strength and selective solubilization (Liadakis et al., 1998). The NaCl concentration selected was 0.5 M, also chosen for the extraction of protein from coconut (Kwon et al., 1996) and from *Gevuina avellana* (Moure, Rúa, Sineiro, & Domínguez, 2000). Higher and lower values have been reported: up to 0.3 M NaCl was optimal for winged bean (Bello & Okezie, 1989) and faba bean (McCurdy & Knipfel, 1990), 0.8 M for tomato seed (Liadakis et al., 1998) and 1 M for coconut (Chakraborty, 1985) and for sunflower (Venktesh & Prakash, 1993).

Extraction experiments were carried out at pH 11 and LSR 12 during 90 min and protein was recovered with UF membranes. Table 2 summarizes the distribution of protein and sugars (where available) in the pooled filtrate (W) from a sequence of two extraction steps, in both the retentate (R) and permeate (P) obtained after membrane processing. The proteins extracted with NaCl were more efficiently recovered than those extracted with distilled water. The performance of the combined extraction and UF steps was superior for the smaller particles, due to the lower protein loss in permeate. Up to 84 and 92% of the protein solubilized with distilled water and with 0.5 M NaCl, respectively, were recovered from the smaller *R. rubiginosa* particles. These values were comparable with or higher than those obtained during UF of protein extracted from coconut (Chakraborty, 1985; Kwon et al., 1996), cottonseed (Choi et al., 1984), *Crambe abyssinica* (Massoura et al., 1998a) and jojoba (Nabetani et al., 1995) with recovery yields between 23 and 97% of the solubilized protein. A fraction of 40–70% of the solubilized reducing sugars was concentrated in the retentate. Since washing steps were not considered in the proposed extraction process (Fig. 1), the sugars remained in the protein concentrate. The sugar and protein content in the permeate was low, particularly during processing of the smaller particle size. The reuse of the permeate stream for further extraction stages could be feasible and would reduce both the water consumption and the generation of effluents. This possibility is indicated by a dotted line in the flow diagram of Fig. 1.

Table 3 summarizes the composition of isolates extracted from *R. rubiginosa* defatted seeds in the presence and in the absence of 0.5 M NaCl. The purer protein concentrates were produced with water extraction from the more finely ground particles. Grinding had no effect on the protein content of concentrates extracted with 0.5 M NaCl. The protein purity ranged from 65 to 83.5% and, by successive washings with water, these values could be increased by 3–13% (McCurdy & Knipfel, 1990; Sánchez-Vioque, Clemente, Vioque, Bautista, & Millán, 1999).

Nutritional and functional properties of protein concentrates are also shown in Table 3. In vitro protein digestibility was slightly lower for the concentrates produced from the finely ground seeds, probably due to heat-denaturation. For this particle size, extraction in the presence of 0.5 M NaCl yielded more digestible protein. Although the globular structure of the proteins was observed to influence their digestibility by hindering the action of digestive enzymes (Sánchez-Vioque et al., 1999), NaCl extracted proteins were more digestible than water extracted ones. The in vitro digestibility of the membrane-concentrated protein from *R. rubiginosa* was comparable with that of isolates from sesame seeds (El-Adawy, 1997), lower than the value for chickpea (Sánchez-Vioque et al., 1999) and higher than that for pea globulins and albumins (Clemente, Sánchez-Vioque, Vioque, Bautista, & Millán, 1998). Available lysine showed a similar and significant trend.

The functional properties of proteins are affected by both the isolation method and interactions with other constituents (lipids, carbohydrates, salts). The physical, chemical and conformational properties, especially size, shape, amino acid composition and sequences, charge and charge distribution, determine the functional properties

(Damodaran, 1997). Since the different protein contents of the samples could also influence the protein behaviour (Akintayio, Oshodi, & Esuoso, 1999; Vani & Zayas, 1995), the assays for testing the functional properties were done on the basis of the same protein concentration. However, properties such as emulsification are strongly affected by quality and not by quantity (Prinyawiwatkul, Beuchat, McWatters, & Phillips, 1997).

The foaming properties are influenced by the protein type, method of preparation, composition, solubility, concentration, pH, presence of salts and interactions between exposed hydrophobicity and solubility (Massoura et al., 1998b; Mwasaru, Muhammad, Bakar, & Che Man, 1999b; Obatolu & Cole, 2000; Venktesh & Prakash, 1993). Interactions of mixtures of proteins and molecular changes resulting from processing treatments also affect foaming behaviour (Vani & Zayas, 1995). Molecular flexibility, surface and molecular hydrophobicity, net charge and charge distribution and hydrodynamic properties, affect foam formation and stability, which is related to the ability to form a cohesive, viscoelastic film through intermolecular interactions (Akintayo et al., 1999; Damodaran & Paraf, 1997; Wagner & Gueguen, 1999). The foam stability after 30 min was higher for the *R. rubiginosa* proteins extracted in the presence of distilled water from particles smaller than 0.5 mm (Fig. 4). The foam stability after 2 h was slightly higher for the NaCl-extracted protein of the same particle size. The protein products from the less-ground particles, extracted with distilled water, showed higher foam capacity or whippability than concentrates extracted with 0.5 M NaCl, but foams were unstable. The better whippability of protein extracted from the less-ground particles might suggest a certain denaturation degree of the protein extracted from the more finely-ground particles, probably due to heat exposure during grinding. Moderate NaCl concentrations were reported to improve the foaming capacity of pigeon pea protein concentrates (Akintayo et al., 1999). Venktesh and Prakash (1993) observed differences in foam stability in the presence of NaCl, depending on the heat-treatment of the sample. Similar behaviour was observed in samples subjected to dry heating, whereas,

Table 2
Distribution of *Rosa rubiginosa* solids, protein and sugars from extraction in either one or two stages at 35°C, pH = 12, LSR = 20 g/g during 90 min[a]

Extraction conditions	Before UF (W)		Permeate (P)		Retentate (R)	
	Protein	Sugars	Protein	Sugars	Protein	Sugars
Distilled water < 0.5 mm[b]	61.0±2.40	1.52±0.04	1.68±0.37	0.53±0.08	51.1±1.98	1.29±0.08
Distilled water 0.5–1 mm[c]	55.4±4.19	0.93±0.02	7.24±1.81	0.50±0.02	42.6±3.11	0.72±0.03
0.5 M NaCl < 0.5 mm[b]	55.6±1.72	2.12±0.05	1.86±0.28	0.69±0.06	51.2±2.80	1.59±0.12
0.5 M NaCl 0.5–1 mm[c]	56.3±0.83	–	4.44±0.35	–	45.0±1.9	–

[a] Values are indicated as percentage of total content in defatted seeds.
[b] One stage.
[c] Two stage.

Table 3
Functional properties of from *Rosa rubiginosa* produced by ultrafiltration in either one or two-extraction stages[a]

Composition (%, d.b.)	Distilled water		0.5 M NaCl	
	< 0.5 mm[b]	0.5–1 mm[c]	< 0.5 mm[b]	0.5–1 mm[c]
Protein	83.5±2.62	77.4±3.93	65.6±0.99	66.2±1.54
Soluble sugars (%)	7.85±0.12	14.20±0.07	5.78±0.13	8.93±0.13
Nutritional property				
Digestibility, A.D.C. (%)	78.51±0.26	81.41±0.26	80.22±0.26	82.31±3.56
Available lysine (g/16 g N)	1.42±0.07	1.70±0.05	1.93±0.08	n.d.
Functional property				
Water absorption (g/g)	2.37±0.11	8.79±1.06	7.05±0.34	26.75±0.47
Oil absorption (%)	11.06±0.95	13.04±0.10	11.06±1.14	16.33±0.28
LGC (%)	8	6	6	6
Emulsifying activity index (EAI)	53.4±3.3	82.4±3.7	73.9±3.1	73.3±1.8
Emulsion stability index (ESI)	58.4±2.0	47.5±1.4	80.4±0.2	68.6±4.9
Whippability (%)	56	84	20	64

[a] Extraction conditions were 35 C, pH = 12, LSR = 20 g/g, 90 min.
[b] One stage.
[c] Two stage.

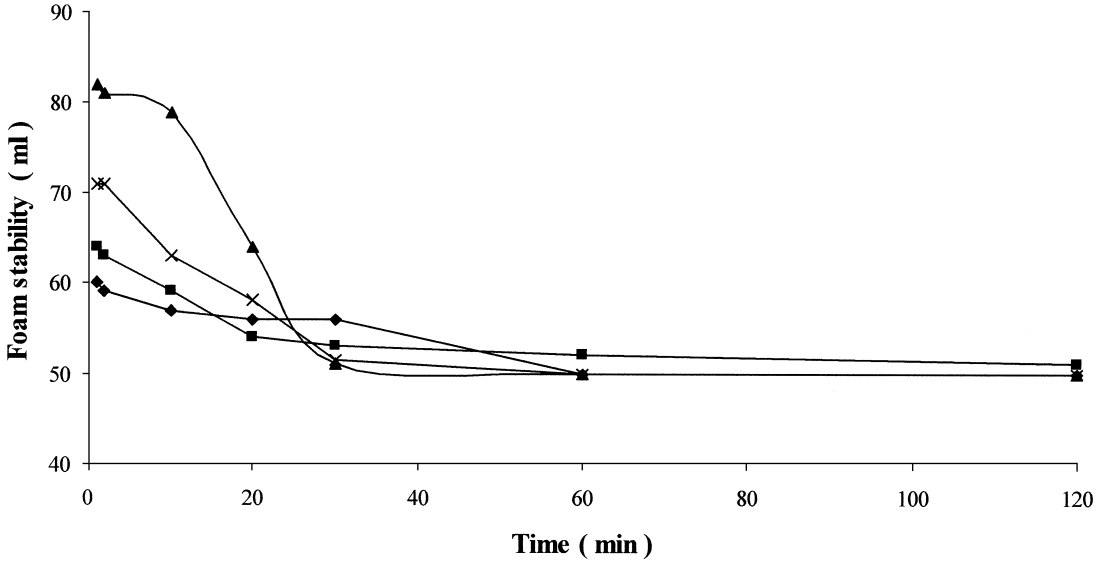

Fig. 4. Foam stability of protein extracted from *R. rubiginosa* defatted seeds ground to 0.5–1 mm extracted with distilled water (-x-) or with 0.5 M NaCl (-▲-) and from particles smaller than 0.5 mm extracted with distilled water (-■-) or with 0.5 M NaCl (-◆-), and isolated with ultrafiltration membranes.

for samples severely heat-treated, the higher foam volume and stability corresponded to water-extracted samples. However, Mwasaru et al., (1999b) observed that the denaturation degree did not significantly affect pigeon pea and cowpea protein foam stability. Mwasaru et al., (2000) proposed that the better solubilization and foam expansion of pigeon pea and cowpea protein in the presence of NaCl (not during their extraction) could be due to the fact that NaCl reduced the denaturation surface. The high foam stability observed for *R. rubiginosa* protein concentrates was comparable with that reported for soybean isolate (Sze-Tao & Sathe, 2000; Xie & Hettiarachchy, 1998), and almond isolate (Sze-Tao & Sathe, 2000), and slightly lower than for isolates from rapeseed (Mahajan, Bahrdwaj, & Dua, 1999), winged bean (Okezie & Bello, 1988) and tobacco leaves (Sheen, 1991).

Surface hydrophobicity and protein concentration are the major characteristics defining the emulsifying properties of a protein (Wagner & Gueguen, 1999). Proteins are surface-active to different degrees, although all are amphiphilic and most of them contain similar proportions of polar and non-polar aminoacid residues. Differences in their conformation and the susceptibility to unfold at interfaces, related to flexibility and adaptability of the conformation to changes in the environment, explains the surface activity (Damodaran & Paraf, 1997). The emulsifying capacity of the concentrates, from the less-ground particles extracted with distilled water, was considerably higher than for the concentrates from the smaller particles, but this emulsion was the least stable. For NaCl-extracted protein, the particle size of the initial meal did not affect the emulsifying activity.

Due to the lack of washing steps after membrane processing, the protein concentrates of *R. rubiginosa* contained 5–14% reducing sugars. Increased sugar content led to increased foam and emulsifying capacity and to decreased foam and emulsifying stability. The presence of sugars, even when they are not chemically bound to proteins, could affect the functional properties of protein. The interaction between proteins and carbohydrates could be responsible for the functional properties observed, as determined for different sugars (trehalose, raffinose, stachyose, sucrose; Murray & Liang, 1999). Glycosylation, succinylation and acylation improve the functional properties of vegetable proteins (Cayot, Roullier, & Tainturier, 1999; Gruener & Ismond, 1997; Sheen, 1991).

Water-absorption is a function of size, shape, hydrophilic and hydrophobic interactions and is affected by the presence of lipids, carbohydrates and amino acid residues on the surface, since most non-polar amino acid residues and polar groups are not hydrated in the interior (Damodaran & Paraf, 1997). The water-absorption capacity was higher for concentrates from particles higher than 0.5 mm, especially for those extracted in the presence of NaCl. Since the lower values corresponded to the protein extracted from the more-ground samples, probably grinding caused a certain degree of heat-denaturation that reduced the water-absorption capacity. Severely heat-treated flours showed lower water-absorption capacities, as observed for proteins from oilseeds (Venktesh & Prakash, 1993) and legumes (Prinyawiwatkul et al., 1997). On the other hand, the water-absorption capacity can increase with heat treatment, due to the increased proportion of low molecular weight proteins with a high percentage of

charged amino acids and to partial denaturation, unfolding and insolubilization. The water-absorption of *R. rubiginosa* proteins was comparable with that of soy isolate (King, Aguirre, & de Pablo; 1985, Okezie & Bello, 1988), winged bean isolate (Okezie & Bello, 1988), and wheat meal (Li & Lee, 1996) and higher than those of pigeon pea and cowpea isolates (Mwasaru et al., 1999b). Values were inferior to those reported for meals from sunflower (Lin, Humbert, & Sosulski, 1974) and barinas nut (Padilla, Alvarez, & Alfaro, 1996) and to those obtained for isolates from linseed (Madhusudhan & Singh, 1985), peas (Sumner, Nielsen, & Youngs, 1980) and tomato seed (Liadakis et al., 1998).

A slight increase in the oil absorption, which depends on the number of non-polar side chains on the proteins, was observed for the isolates obtained from the less-ground seeds and, for this size range, the value was higher for the extraction in the presence of NaCl. Oil-holding capacity was higher than that of wheat meal (Li & Lee, 1996), coconut concentrate (Kwon et al., 1996) and of isolates from winged beans and soybean (Okezie & Bello, 1988), tomato seed (Liadakis et al., 1998), peanut (Monteiro & Prakash, 1994), faba bean, field peas (Sosulski & McCurdy, 1987), pigeonpea, cowpea (Mwasaru et al., 1999b) and tobacco leaves (Sheen, 1991). Oil-absorption capacities of protein from *R. rubiginosa* were lower than values obtained from pea isolates (Sumner et al., 1980) and for meal of barinas nut (Padilla et al., 1996), sunflower (Lin et al., 1974) and linseed (Madhusudhan & Singh, 1985).

The protein solubility of the isolates was determined, since it is an indicative of the protein denaturation and of the interactions that relate to other functional properties, although a high solubility is not a prerequisite for good emulsifying properties (Prinyawiwatkul et al., 1997). The nitrogen solubility profiles of the concentrates are presented in Fig. 5. Maximum solubility occurs at neutral and alkaline pH, except for the concentrate produced from the particle size <0.5 mm, particularly the one extracted with 0.5 N NaCl, which presents maximum solubility at the acidic and alkaline pH values. At neutral and alkaline pH the protein solubility of the concentrates produced from the higher particle size was slightly higher than that of concentrates from smaller particles. These products were less soluble at very acidic pH and NaCl decreased protein solubility at low pH. Similar effects were reported for protein isolates from pigeon and cowpea (Mwasaru et al., 2000), Chilean hazelnut (Moure et al., 2000c), peanut (Monteiro & Prakash, 1994) and coconut (Kwon et al., 1996). The reason could be that the number of charges is reduced, NaCl could compete with protein for water and the increased hydrophobic interaction and protein aggregation reduce solubility.

The gelation capacity is related to the interaction between proteins, carbohydrates and lipids and with the exposed hydrophobicity and especially the sulphydryl groups of proteins (Chau & Cheung, 1998; Mwasaru, Muhammad, Bakar, & Che Man, 1999a; Obatolu & Cole, 2000). Hydrogen and ionic bonds could be responsible for the stabilization of gel. The least gelation concentration (LGC) was lower for the concentrates extracted with NaCl and for those extracted with distilled water from the higher particle size assayed. LGC are comparable with the values reported for isolates from pigeon pea (Akintayo et al., 1999) and cowpea (Mwasaru et al., 1999b) and lower than for isolates from mung bean (Coffmann & García, 1977), winged bean, soy (Okezie & Bello, 1988) and yellow peas (Soetrisno & Holmes, 1992) and for meals of barinas nut (Padilla et al., 1996), chickpea (Carcea Bercini, 1986) and lupin (King et al., 1985). According to

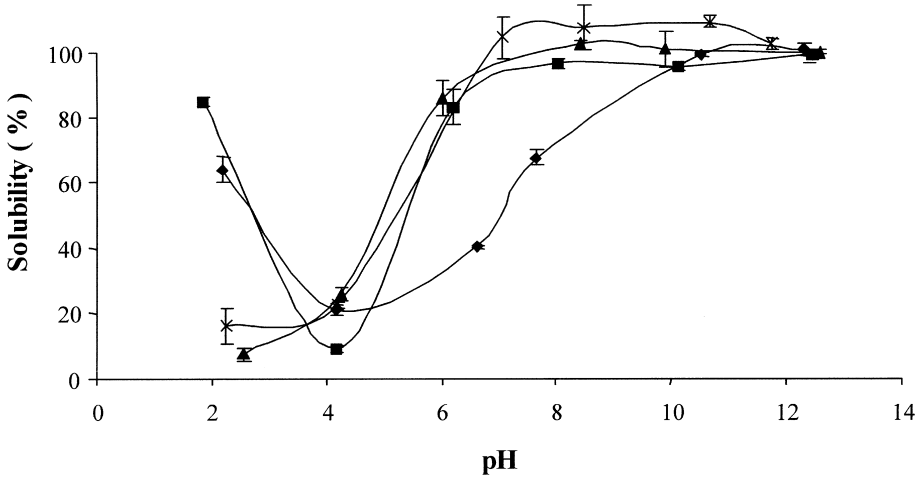

Fig. 5. Solubility profiles of the protein from *Rosa rubiginosa* defatted seeds ground to 0.5–1 mm extracted with distilled water (-x-) or with 0.5 M NaCl. (-▲-) and from particles smaller than 0.5 mm extracted with distilled water (-■-) or with 0.5 M NaCl (-◆-), and concentrated by ultrafiltration. Soluble protein was determined by the Lowry method, and expressed as percent of the protein content in concentrate, measured by the Kjeldahl method.

Mwasaru et al. (1999b), the LGC decreases with increasing denaturation; therefore the protein from the more finely-ground particles should not have been denatured to a higher degree than protein from the less-ground ones.

The differences in the functional properties, caused by the different compositions of the proteins extracted with distilled water and with NaCl, were confirmed by gel electrophoresis. Fig. 6 shows the SDS-PAGE pattern of proteins from distilled water and from NaCl extracted *R. rubiginosa* protein. In the protein concentrates from smaller particles extracted with distilled water, the bands at 33 and 56 kDa are not visible, whereas the band at 60 kDa was more intense than in concentrates extracted with NaCl. The protein concentrates extracted from the higher particle size assayed, presented three light bands at 40, 26 and 19 kDa and a more marked one at 60 kDa was observed for the water extracted protein. The concentrates produced from the more finely ground particles showed the presence of more bands than those from particles higher than 0.5 mm; the bands were more intense and differences between the two solvents could be noticed. Therefore, both the particle size and the solvent influence the protein extractability. The bands corresponding to proteins of lower molecular weight are more marked for the concentrates with higher foam stability; analogous behaviour has been reported for pea albumins (Lu, Quillien, & Popineau, 2000).

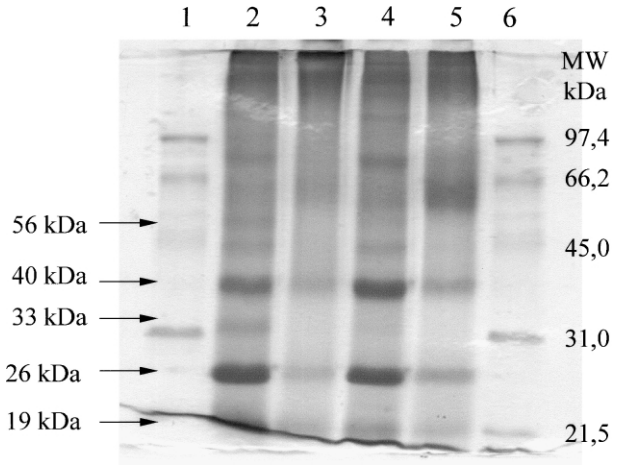

Fig.6. SDS-PAGE patterns of protein concentrates from *Rosa rubiginosa* defatted seeds of size less than 0.5 mm extracted with 0.5 M NaCl (lane 2) and with water (lane 4), and from defatted seeds of size between 0.5 and 1 mm extracted with 0.5 M NaCl (lane 3) and with distilled water (lane 5). Lane 1 and 6: Molecular weight of the standards.

Contrary to the trends in ADC, whippability, solubility and absorption capacities for the protein from the smaller particles, the gelation ability of the concentrates seems to suggest that the type of protein and not the thermal denaturation is responsible for the observed behaviour properties.

Table 4 shows the water- and oil-absorption capacities of the fibre fraction obtained as solid byproduct from the protein extraction of *R. rubiginosa*. The solid residue from the particles higher than 0.5 mm contained more than 90% total detergent fibre; the higher content was observed for this particle size, regardless of the extracting solvent. The in vitro digestibility of the fibre products was around 70% and no clear trend with regard to extracting solvent or particle size was observed. The water-absorption capacity of the fibre was in the range of those reported for other seeds or for commercial fibre formulations (Abdul-Hamid & Luan, 2000). The oil-absorption capacity of the fibre was similar to that of fibre from defatted rice bran and higher than a commercial fibre product reported by Abdul-Hamid and Luan (2000).

In conclusion, the protein products obtained after aqueous extraction of *R. rubiginosa* defatted meals and

Table 4
Functional properties of the fibre unextracted solid from the extraction of *Rosa rubiginosa* in one stage and two-stages[a]

	Distilled water		0.5 M NaCl	
Composition (%, d.b.)	< 0.5 mm[b]	0.5–1 mm[c]	< 0.5 mm[b]	0.5–1 mm[c]
Protein	8.7	2.1	10.0	1.4
N.D.F.	70.58±1.71	92.61±0.79	66.05±0.89	90.46±0.76
A.D.F.	51.86±0.73	67.36±1.38	47.19±6.41	66.65±0.54
A.D.L.	20.71±1.58	26.28±1.43	19.55±1.71	26.25±2.12
Ash	0.83±0.07	0.81±0.02	6.64±0.46	2.14±0.19
Nutritional property				
in vitro digestibility (%)	69.73±0.13	70.00±0.26	72.99±0.89	66.11±0.38
Available lysine (g/16 g N)	0.012±0.001	0.020±0.001	0.032±0.005	0.033±0.001
Functional property				
Water absorption (g/g)	4.66±0.03	3.43±0.17	3.30±0.12	1.98±0.05
Oil absorption (g/g)	6.29±0.05	7.13±0.36	2.61±0.11	1.79±0.06

[a] Extraction conditions were 35°C, pH = 11, LSR = 20 g/g, time 90 min
[b] One stage.
[c] Two stage.

membrane separation show good nutritional and functional properties. A fibre rich product with acceptable water- and oil-absorption capacities can be obtained. Additional operational and environmental advantages could be derived from recycling the permeate from UF to the first extraction stage, since the water consumption and the generation of liquid effluents would be reduced or avoided.

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