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Emulsifying and foaming properties of *Phaseolus vulgaris* and *coccineus* proteins

Eleousa A. Makri, Georgios I. Doxastakis *

Laboratory of Food Chemistry and Technology, School of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

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Abstract

Protein isolates from two *Phaseolus* cultivars, common bean (*Phaseolus vulgaris* L.) and scarlet runner bean (*Phaseolus coccineus* L.), were prepared by wet extraction methods (isoelectric precipitation – 4000 rpm, ultrafiltration, extraction with NaCl 2%, and isoelectric precipitation – 9900 rpm). The protein isolates were characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then evaluated for their solubility. The emulsion stability of emulsions produced at pH 7.0 and 5.5 with 1% or 2% or 3% w/v protein isolate was evaluated by average droplet size diameter, viscosity and creaming measurements. Emulsions with 1% protein content were unstable through storage. Emulsions with 3% w/v protein isolate concentration, extracted by ultrafiltration at pH 5.5 from both cultivars, were flocculated; this was more pronounced for *coccineus* isolates. The foaming properties, for the respective foams, were investigated. Foams with 1% w/v protein showed little foaming ability Ultrafiltration isolates produced more foam, which was especially stable at pH 5.5.

Keywords: *Phaseolus vulgaris*; *Phaseolus coccineus*; Protein isolate; SDS–PAGE; Emulsion stability; Foaming stability

1. Introduction

Legume seeds are one of the most important sources of protein, carbohydrates and dietary fibre for human nutrition. *Phaseolus* beans (*Phaseolus* L.) have been cultivated for several thousand years all over the world, especially in America but also in Europe, Asia and Africa (Sathe, 2002; Smart, 1990). Common bean (*Phaseolus vulgaris* L.) is perhaps the most widely cultivated of all *Phaseolus* beans species. It is a highly polymorphic crop and more than 14,000 cultivars exist. Scarlet runner bean (*Phaseolus coccineus* L.) is also a very important sub species, also known for thousands of years, and mostly cultivated in America and Europe.

The exact protein content varies among species and *Phaseolus* beans contain 20–30% protein on a dry weight basis. They have a balanced amino acid composition while they are low in sulfur-containing amino acids, notably methionine and tryptophan, which is common among legumes (Augustin & Klein, 1992; Gueguen & Cerletti, 1994; Sathe, 2002; Sathe, Iyer, & Salunkhe, 1981). They contain vitamins, as well as antinutritional factors, such as proteolytic enzyme inhibitors, phytic acid and lectins (Satterlee & Chang, 1979) and their consumption is negatively affected by the reduced protein digestibility and the hard-to-cook effect (Augustin & Klein, 1992; Gueguen & Cerletti, 1994; Sathe, 2002).

The storage proteins of *Phaseolus* beans are vicilin and legumin and also phytohemaglutin. Vicilin is a 7S globulin and is often referred to as phaseolin. It is comprised of 3–5 subunits and represents 50% of the total protein content. Legumin is an 11–12S, globulin, comprised of acid and basic subunits, and usually sediments

* Corresponding author. Tel.: +30 310 99 7774; fax: +30 310 99 7779.
E-mail address: doxasta@chem.auth.gr (G.I. Doxastakis).

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with vicilin as a single component (Gepts & Bliss, 1986; Kohnhorst, Smith, Uebersax, & Bennink, 1991; Kohnhorst, Uebersax, & Zabik, 1990; Sathe, 2002).

Interest in the production of new food products from protein-rich seeds is constantly increasing. In order to decide upon the most effective ways and conditions for incorporating the protein extracted from these seed into food products, functional properties must be investigated. Functional properties, such as solubility, emulsifying and foaming properties, will be crucial for the production of these new ingredients.

Emulsions are unstable systems. At low protein concentrations or low protein/oil ratios there is insufficient protein present to saturate the interface, created during emulsification, and the resulting emulsion is highly unstable and flocculation occurs. The degree to which an emulsion is flocculated depends on the structure of the adsorbed layer and the thermodynamic quality of the intervening solvent. If the stabilizing film at the oil–water interface ruptures, coalescence occurs and the oil droplets merge into larger spherical globules. Factors that influence this phenomenon are, the viscosities of the dispersed and continuous phases, the droplet deformability, the droplet size and the interdroplet forces, the interfacial tension and the mobility of the adsorbed film. Creaming occurs when the density of the droplets is less than that of the continuous phase. The rate of creaming is greatly enhanced by flocculation of droplets at low-to-medium volume fractions (Dickinson & Stansby, 1988).

Rhelology of emulsions is affected by the rheological properties of the continuous phase, the nature of the droplets, the size distribution, deformability, the internal viscosity and concentration and finally the nature of particle–particle interactions (Barnes, 1994). Emulsions containing insufficient for (near-) saturation protein surface coverage develop a time-dependent increase in low-stress apparent viscosity and associated shear-thinning behaviour. This can be attributed to bridging flocculation (Dickinson & Golding, 1997).

Foams are also important for the food industry. Foams are stabilized by an adsorbed layer of protein at the air–water interface. In particular, when air is injected into a protein solution, entrapment, in the form of bubbles, occurs as a result of adsorption of the protein molecules at the bubble surface. So, an understanding of the kinetics, of the properties of the adsorbed film and the physicochemical changes involved is needed. The basic requirements for a protein to be a good foaming agent are the ability to: (a) adsorb rapidly at the air–water interface during bubbling, (b) undergo rapid conformational change and rearrangement at the interface, and (c) form a cohesive viscoelastic film via intermolecular interactions. The first two criteria are essential for better foam ability, whereas the third is important for the stability of the foam (Damodaran, 1994).

The objective of this study was prepare protein isolates by two different species of *Phaseolus* beans (*Phaseolus vulgaris* and *Phaseolus coccineus*), to characterize the protein isolates and to investigate the influence of the extraction method (isoelectric precipitation and ultrafiltration), protein concentration and, pH in relation to emulsifying and foaming properties (see Fig. 1).

2. Materials and methods

2.1. Materials

Common bean (*Phaseolus vulgaris* L.) and scarlet runner bean (*Phaseolus coccineus* L.) seeds are of Protected Geographical Indication and were provided by "AGROKA" (Kastoria, Greece). Commercially available refined corn oil was obtained from the local market and used without further treatment. Commercial xanthan gum was provided by the Sigma Chemical Co. All the pH adjustments were made with 1 N NaOH and 1 N HCl solutions. NaCl was bought from Riedel-de-Haen and was of analytical grade.

2.2. Protein solubility

Protein solubility was determined in the pH range 2–9 in a 0.1% w/v protein isolate aqueous solution. The suspensions were adjusted to the desired pH value, solubilized thoroughly and then centrifuged (9000 rpm for 30 min). Protein solubility was estimated in the supernatant according to Lowry, Rosebrough, Farr, and Randall (1951).

2.3. Electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed using the system of Laemmli (1970). Electrophoresis was performed with a APELEX Electrophoresis power supply ST 1006T,

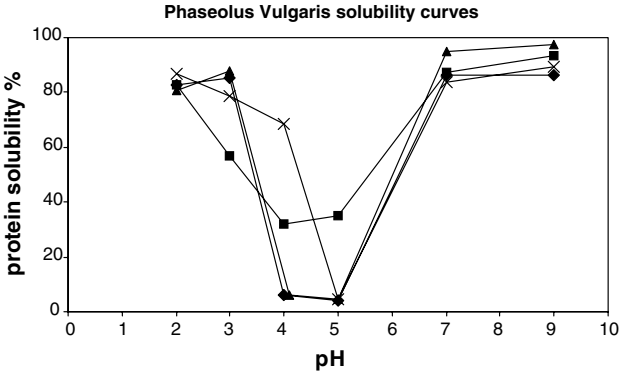

Fig. 1. Solubility curves of V protein isolates at pH values 2–9: (▲) Vhcs; (◆) VpI; (×) Vsalt; (■) VUF.

France, using a constant voltage power supply of 60 mA. The molecular weight (MW) and % amount of total protein were estimated using, as a standard, a protein marker with broad range, Premixed Format P7702S Biolabs, New England.

2.4. Preparation of seed protein isolates

Protein isolates were prepared from *Phaseolus vulgaris* and *Phaseolus coccineus* by an isoelectric precipitation method described elsewhere (Alamanou & Doxastakis, 1995, 1997a, 1997b). The pH of the alkaline extraction was 8.5 and the precipitation of the protein was done at pH 4.5 and all centrifugations described were done at 4000 rpm for 20 min. These isolates will be referred to as VpI and CocpI, respectively. Protein isolates were also prepared by an ultrafiltration method. According to this method, seeds were ground in a kitchen type mill (Braun, Germany) and the resulting flour (~100 mesh) was dispersed in distilled water (1/10); the pH was increased to 8.5 and the dispersion was stirred for 40 min. It was the centrifuged at 4000 rpm for 20 min. The residue was collected and treated as above with (1/5) distilled water. The two supernants were collected, combined and their pH value adjusted to 7.0. The new solution was then forced through an ultrafiltration device, Millipore Pellicon XL, which was operating under 5 bar pressure, with a PLCGC 10K ultrafiltration membrane, 50 cm^2, with a molecular cut-off at 5 kDa These isolates will be referred to as VUF and CocUF, respectively. Protein isolates were also prepared by a salting-out method, so the extraction was made with 2% NaCl, the procedure is described elsewhere and the product is characterized by researchers as globulin fraction (Sathe & Salunkhe, 1981a; Satterlee, Bembers, & Kendrick, 1975). These isolates will be referred to as Vsalt and Cocsalt, respectively. Finally, isolates from the two cultivars were also prepared by the isoelectric method but with all centrifugations were performed at the same time but at a higher speed, 9900 rpm. These isolates will be referred to as Vhcs and Cochcs respectively. The protein content of all isolates was determined by the Kjeldahl method ($N \times 5.7$) (Pearson, 1976).

2.5. Emulsion preparation

The oil-in water emulsions (o/w 50/50 v/v) were prepared by adding corn oil to protein solution which contained legume protein at concentrations 1%, 2% or 3% w/v and had a pH value of 5.5 or 7.0, while mixing with the aid of a mechanical stirrer. The crude emulsion, following mixing for 15 min, was then homogenized with an Ultra-Turrax T-25 homogenizer, IKA Instruments, Germany, equipped with a S25 KG-25F dispersing tool at a speed of 9500 rpm for 1.5 min. A small amount of sodium azide (0.1% w/v) was added to the water phase as a preservative. Emulsification conditions were chosen to result in oil droplets >1 μm. Emulsions were stored at 4 °C. The stability of the emulsions was studied for a period of 70 days.

2.6. Average droplet diameter

Particle size distribution was determined by integrated light scattering, using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK) after 1, 10, 40, 70 days of preparation, in duplicate. Measurements were performed at room temperature after complete dispersion of the oil droplet flocs with the help of 2% SDS. The action of the SDS solution on the breakage of the flocs was confirmed by optical microscopy. The measurements, were made at a dilution of approximately 1:1000 of the emulsion dispersions in distilled water. The refractive indices of the corn oil (1.473) and of the water (1.33) were used for the calculations.

2.7. Creaming

The stabilities of the emulsions against creaming were assessed, by visual observation of the serum appearing at the bottom of samples, stored in flat-bottomed cylindrical glass containers of 2.5 cm diameter and 4.5 cm height, at 25 °C. The creaming was expressed as the percentage of the height of the serum layer over the total height of the emulsion in the cylinder

$$\text{Creaming} = \text{Height of serum layer} \times 100/\text{Total height of the emulsion.}$$

2.8. Rheology measurements

A steady stress rheometer (Brookfield DV-II, LV Viscometer, Brookfield Engineering Laboratories, USA), equipped with the SC4-18/13R small adapter, was used to determine the viscosity-rate of shear of the emulsions after 1 and 70 days of storage.

2.9. Preparation and evaluation of foams

Samples (100 ml) of protein solutions, with 1% or 2% or 3% w/v protein concentration, were adjusted to the desired pH and whipped at maximum speed (1000 rev min^{-1}) for 5 min in a Braun machine, Germany. Then, foams were carefully transferred to a 1-l glass measuring cylinder. The initial foam volume, along with the foam volume and liquid drainage after 30 min were measured.

Foaming capacity was evaluated by relative overrun (Hammershoj & Qvist, 2001)

$$\text{Relative overrun} = V_0/V\text{i},$$

where V_0, foam volume at 0 min and V_i, initial liquid volume before foaming.

Foam stability was studied by comparing the foam after 30 min with the initial foam (0 min) (Hammershoj & Qvist, 2001)

$$\text{Foam stability} = V_{30}/V_0,$$

where V_{30}, foam volume at 30 min and V_0, foam volume at 0 min.

Liquid drainage was calculated from the drainage from the foam in a 30 min period (Hammershoj & Qvist, 2001)

$$\text{Liquid drainage} = (V_i - V_{L30})/(V_i - V_{L0}),$$

where V_i, initial liquid volume before foaming, V_{L30}, volume of liquid at 30 min and V_{L0}, volume of liquid at 0 min after foaming.

2.10. Statistical analysis

Each experiment was repeated at least three times and the data were analyzed using the one-way ANOVA programme. The level of confidence was 95%. Significant differences between means were identified by the LSD procedure.

3. Results

3.1. SDS–PAGE electrophoresis

SDS–PAGE electrophoretic analysis was done for the four V isolates (VpI, VUF, Vhcs, Vsalt) and for the four Coc isolates (CocpI, CocUF, Cochcs, Cocsalt) and is shown in Fig. 2. The VpI isolate showed a band of 48 kDa and a triple band of subunits with molecular weights 44, 42, 41 kDa, which were identified as vicilin. It also showed bands of 31, 26, 25, 22 kDa, which correspond to phytohemagluttin (PHA). There were also two minor bands of 61 and 57 kDa, which were probably breakdown products of an 11S protein. The VUF isolate showed the same bands but the amount of total protein of the 31 kDa band was doubled. There was little difference between VpI and Vhcs, while the Vsalt did not have the 61 kDa band.

The CocpI did not have a very different electriophoretic profile, it exhibited a 47 kDa band, a double of 44 and 41 kDa and another one of 39 kDa which all corresponded to vicilin. It had bands of 31, 24, 21 kDa and these were the PHA fractions. It also had two minor bands of 61 and 57 kDa which, as before, are thought to be the breakdown products of an 11S protein. CocUF had a similar profile but the amount of total protein of the band of 31 kDa of the PHA fraction was doubled as already observed with VUF. There was little difference between CocpI, Cochcs and Cocsalt, apart from the fact that CocpI showed the 57 kDa band in a higher amount compared to the other three. The storage protein of *Phaseolus* is vicilin (7–8S), with MWs 186 kDa, and is typically composed of preproteins of MWs in the range 50–75 kDa. The legumin group (11–12S) has MWs 331 kDa and is comprised of a basic 20 kDa and an acid 40 kDa subunit bound together by disulfide bridge(s), while PHA is comprised of polypeptides of 27–37 kDa (Sathe, 2002; Sathe & Salunkhe, 1981b). Our data are in agreement with the results obtained by Carbonaro, Vecchini, and Carnovale (1993), Kohnhorst et al. (1990), Kohnhorst, Smith, Uebersax, and Bennik (1990), Bernardi, Lupi, and Durante (1990) and Sathe and Salunkhe (1981a).

3.2. Protein solubility

The protein solubility of vulgaris protein isolates in the pH range 2–9, is shown in Fig. 1. The solubility curves for VpI and Vhcs isolates are almost the same. This was expected, taking into consideration that their preparation procedures only differ in the centrifugation speed. Their protein solubility is substantially reduced at pH 4.5 The pH value of minimum solubility of vulgaris proteins is reported to be around 4.0 by most researchers (Carbonaro et al., 1993; Deshpande & Cheryan, 1983; Dzudie & Hardy, 1996; Romo & Bartholomai, 1978; Sathe & Salunkhe, 1981b). Vsalt isolate also has its lowest protein solubility at pH 5.0, since its main protein fractions are very similar to those of pI isolates, a fact that is in agreement with the electophoretic results. This is a little higher than that reported by Sathe and Salunkhe (1981b). On the other hand, VUF isolate shows minimum protein solubility at pH 4–5, but with a different solubility profile. This is probably due to the different extraction procedure followed, since ultrafiltration results in rather different protein fractions, and, more specifically, leads to the extraction of both albumin and globulin protein fractions (Alamanou & Doxastakis, 1997a, 1997b). The solubility curves for the isolates prepared from Coc are almost the same and thus exhibit minimum solubility at pH 4.5.

3.3. Protein content of isolates

While the V and Coc flour have 22.5% and 19.0% protein contents, the protein contents of protein isolates for, VpI, VUF, Vhcs and Vsalt are 72.3%, 66.0%, 75.0% and 82.5%, respectively, and, for the Coc isolates, CocpI, CocUF, Cochcs and Cocsalt, 69.2%, 68.7%, 71.1%, and 71.5%, respectively. The protein content of the isoelectric precipitation isolate is similar to the values given by several authors (DilLollo, Inteaz, Billiaderis, & Barthakur, 1993; Kohnhorst et al., 1990). Also, the Vhcs and Cochcs isolates have rather higher protein contents than those of VpI and CocpI which is in agreement with previous findings by other authors (Kohnhorst et al., 1991).

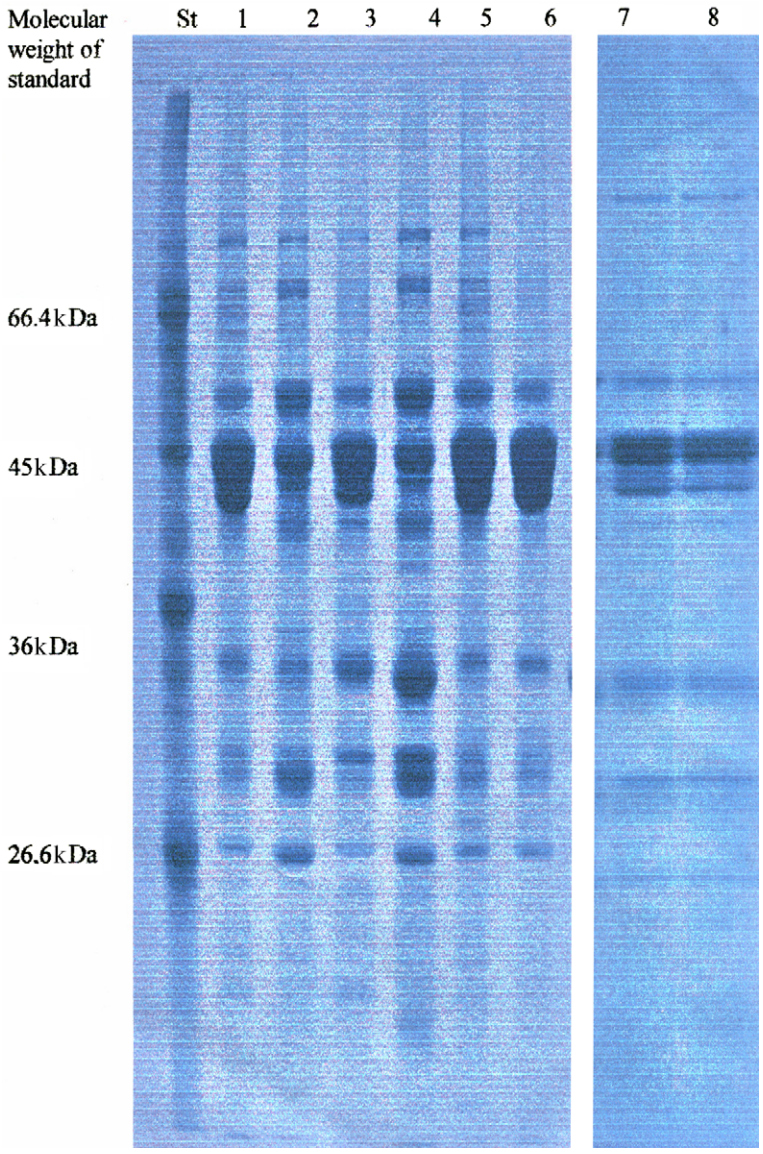

Fig. 2. SDS–PAGE electrophoretogram of protein isolates from *Phaseolus vulgaris* and *Phaseolus coccineus*, (St) Molecular weight standard: (1) VpI; (2) CocpI; (3)VUF; (4) CocUF; (5) Vhcs; (6) Vsalt; (7) Cochcs; (8) Cocsalt.

For the flours, Vsalt and Cocsalt, the values now found are lower than those reported by other researchers (Sathe & Salunkhe, 1981b, 1981c; Satterlee et al., 1975).

3.4. Emulsion stability

Emulsion stability was evaluated with respect to coalescence (mean oil droplet size D[4.3], changes through time). All emulsions studied, showed flocculation even after a few (10 days) of storage. Fig. 3 shows the mean oil droplet size distribution, without the presence of SDS solution during the measurement procedure, for the emulsion prepared with 1% w/v CocUF at pH 5.5, and the trimodal profile indicates that the emulsion is flocculated. All other D[4.3] data presented are after the addition of SDS solution.

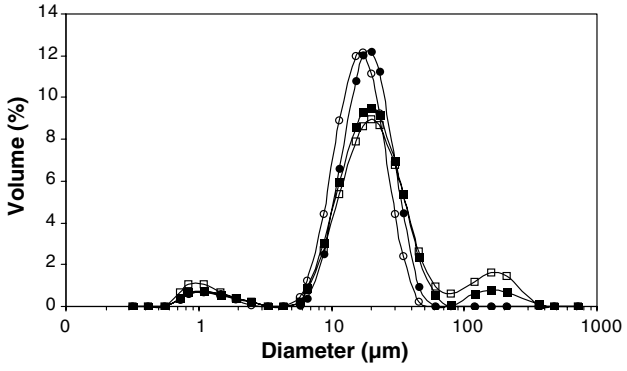

Fig. 3. Oil droplet distribution, of o/w emulsion prepared with 1% w/v CocUF protein isolate at pH 5.5 after 1, 10, 40 and 70 days of ageing, without SDS solution added during the measurement: (○) CocUF 1 day; (●) CocUF 10 day; (■) CocUF 40 day; (□) CocUF 70 day.

Fig. 4 shows the influence of protein concentration, protein extraction method, and pH of emulsion on the mean particle size distribution of emulsions prepared with V isolates. As far as the concentration is concerned, it appears that 1% w/v VpI and VUF are less able to stabilize the emulsion for 70 days, while the emulsions prepared with 2% w/v and 3% w/v protein are more stable and smaller changes in oil droplet size were observed. Furthermore, the initial D[4.3] is smaller for the higher concentrations studied. Method of extraction and pH do not seem to influence the mean droplet size for all the V emulsions studied. At pH 7, the initial size of oil droplets was smaller than at pH 5.5. This is more obvious for the emulsions prepared with 1% protein concentration, which is in agreement with observations made by others researchers (Fidantsi & Doxastakis (2001); Paplamprou, Makri, Kiosseoglou, & Doxastakis, 2005). For 2% w/v VpI emulsion at pH 7.0, the droplet size was almost the same as the 1% w/v VpI emulsions, which did not occur for the rest of the emulsions studied. Emulsions were also prepared with 1% w/v Vhcs and Vsalt, at both pH values studied, for a period of 70 days, and they showed almost the same behaviour was VpI isolates (data not shown).

Fig. 5 shows the D[4.3] values for emulsions prepared with 1%, 2%, 3% w/v CocpI and UF isolates. It appeared that 1% w/v CocpI, CocUF were less able to stabilize the emulsion for 70 days. while the emulsions prepared with higher protein concentration (2% w/v and 3% w/v) showed smaller changes in oil droplet size and were more stable. As far at the method of extraction is concerned, 1% w/v CocpI emulsions were more stable through time, irrespective of the pH. The 2% w/v and 3% w/v emulsions prepared with CocpI had smaller oil

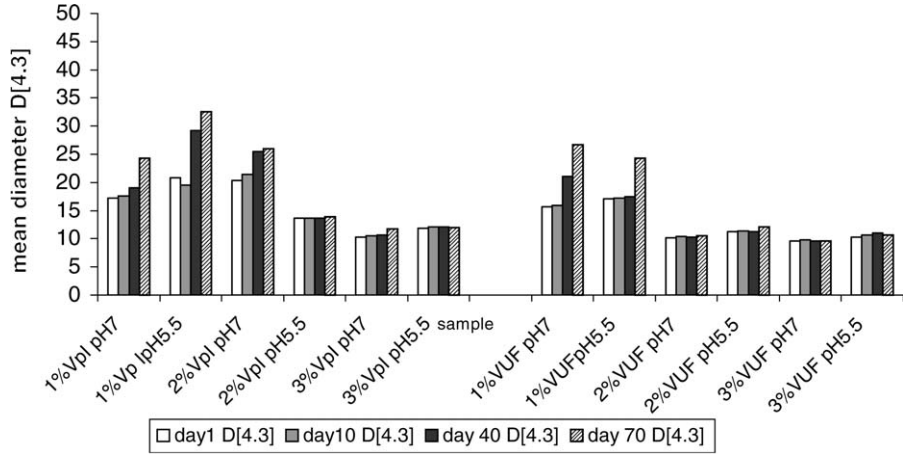

Fig. 4. Mean oil droplet size, D[4.3] of o/w emulsion prepared with 1% or 2% or 3% w/v VpI or VUF protein isolate at pH 7.0 or 5.5 after 1, 10, 40 and 70 days of ageing.

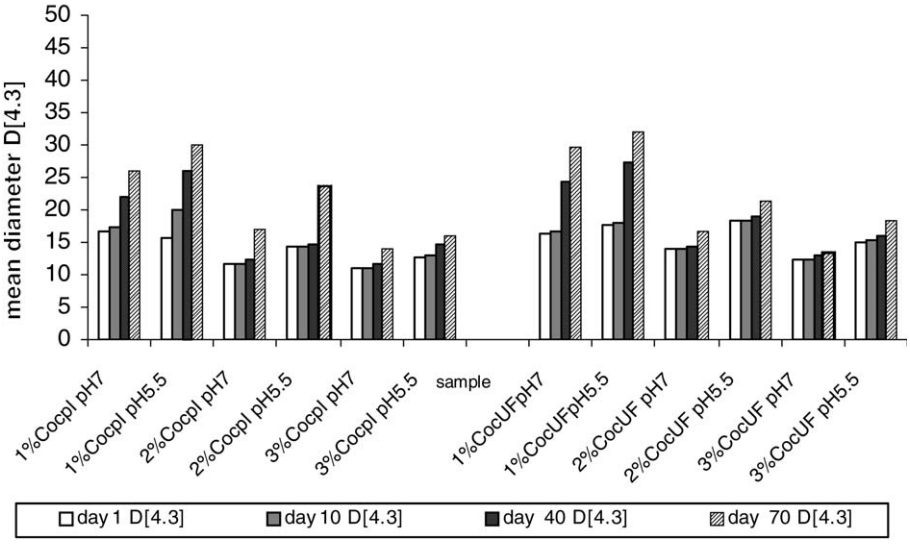

Fig. 5. Mean oil droplet size, D[4.3] of o/w emulsion prepared with 1% or 2% or 3% w/v CocpI or CocUF protein isolate at pH 7.0 or 5.5 after 1, 10, 40 and 70 days of ageing.

droplet sizes than CocUF which enhanced their stability. All Coc emulsions at pH 5.5 had higher D[4.3] values, even from the first day of preparation. In addition to that, for pH 5.5 on the70th day of preparation the droplet size increased rapidly, which could be attributed to the lower protein solubility at this pH value. The D[4.3] values at this pH were much higher, even compared to V emulsions. The role of pH (7.5–7.0–6.5–6.0–5.5–5.0) and method of extraction (pI and UF), with respect to the mean size distribution of emulsions of V and Coc isolates, are presented in Fig. 6. As can be seen, VpI stabilized emulsions better for a period of 70 days at pH 7.0, 5.5 and 5.0. For VUF, CpI and CocUF, the stabilization was better throughout time at pH 7.0 and 5.5. Emulsions were also prepared with 1% w/v Cochcs and Cocsalt at both pH values studied, and their stabilities were measured for a period of 70 days; they exhibited approximately the same behaviour was CocpI isolates (data not shown).

The stability of the dispersed system depends on the formation of a rigid and viscoelastic film around oil droplets. The protein fraction, when adsorbed, unfolds and rearranges to the state of the lowest possible energy at the oil–water interface. Different factors, like the origin of protein, the method of extraction, its concentration, the pH of the aqueous phase, determine the stability of this film. It has been found that the coalescence procedure of legume-stabilized emulsions is greatly influenced by the pH value and the method of protein extraction (Alamanou & Doxastakis, 1997a, 1997b; Fidantsi & Doxastakis, 2001; Tsaliki, Pegiadou, & Doxastakis, 2004; Walstra, 1983).

3.5. Creaming

Creaming is also a type of instability behaviour of emulsions. Fig. 7 presents the creaming profiles of both *Phaseolus* species pI and UF isolates for 1% w/v and 3% w/v protein concentrations. It can be seen that emulsions with 1% w/v protein of both V and Coc create serum quite quickly, within only a few hours of preparation. This is probably due to the fact that the protein is not sufficient to create a rigid film around the oil droplets. The method of protein extraction seems to influence the creaming behaviour since, for both V and Coc, UF isolates form more serum, irrespective of protein concentration. The pH value plays quite an important role, since, at pH 5.5, the emulsions appeared to be less stable towards creaming, in all emulsions studied. This was probably due to the fact, that at pH 5.5, which is near the isoelectric point of the proteins, the emulsions tend to be more unstable. The creaming instability indicates that the emulsions are flocculated, which has already been observed during average droplet diameter measurements. Once droplets become aggregated they cream more rapidly because of the increase in the particle size (Kulmyraev, Silvestre, & McClements, 2000; Silvestre, Decker, & McClements, 1999).

3.6. Viscosity

The rheological behaviour of all emulsions studied was measured for 1, 10, 40, 70 days after preparation. Emulsions at pH 5.5 had higher viscosity than their respective emulsions at pH 7 (data not shown). Fig. 8 shows that emulsions with higher protein content prove to be more viscous but their viscosity is lowered, through-out storage, at the same rate as for the emulsions stabilized with 1% protein isolates. Coc, on the first day of emulsion preparation, have higher viscosity then V. UF isolates compared to pI, for both *Phaseolus* beans on the first day of emulsion storage, proved to be more viscous, confirming that the method of protein preparation can alter the functional properties of the *Phaseolus* isolates due to the extraction of different fractions (Alamanou & Doxastakis, 1997a, 1997b; Sathe &

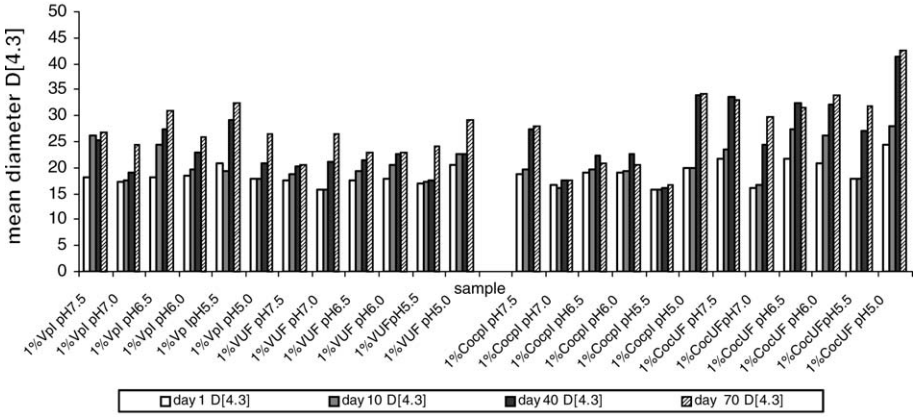

Fig. 6. Mean oil droplet size, D[4.3] of o/w emulsion prepared with 1% w/v VpI or VUF and CocpI or CocUF protein isolate at pH 7.5, 7.0, 6.5, 6.0, 5.5 or 5.0 after 1, 10, 40 and 70 days of ageing.

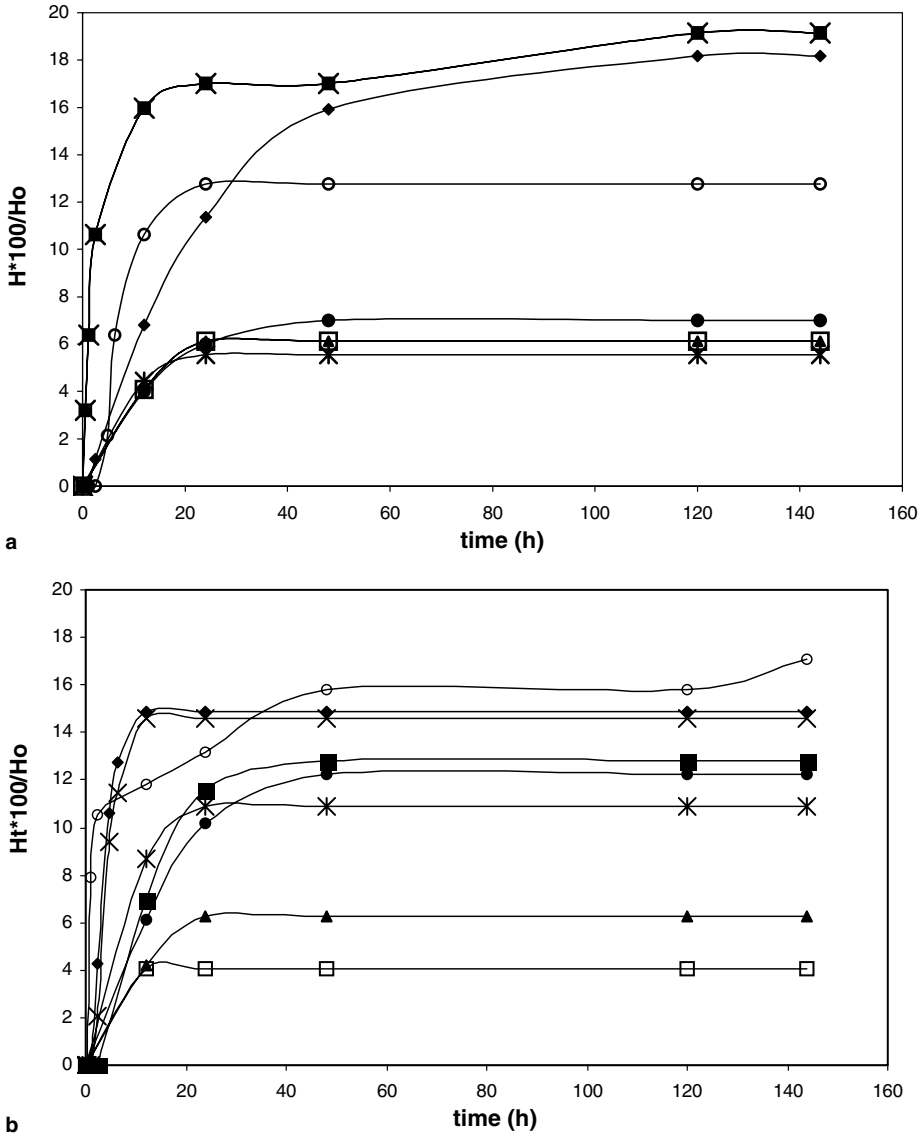

Fig. 7. Creaming profile of o/w emulsion prepared with 1% or 3% w/v VpI and VUF or CocpI and CocUF protein isolate at pH 7.0 or 5.5: (a), (○) 1% w/v VpI pH 7.0; (■) 1% w/v VpI pH 5.5, (◆) 1% w/v VUF pH 7.0; (×) 1% w/v VUF pH 5.5; (□) 3% w/v VpI pH 7.0; (▲) 3% w/v VpI pH 5.5; (✻) 3% w/v VUF pH 7.0; (●) 3% w/v VUF pH 5.5; (b), (○) 1% w/v CocpI pH 7.0; (■) 1% w/v CocpI pH 5.5; (◆) 1% w/v CocUF pH 7.0; (×) 1% w/v CocUF pH 5.5; (□) 3% w/v CocpI pH 7.0; (▲) 3% w/v CocpI pH 5.5; (✻) 3% w/v CocUF pH 7.0; (●) 3% w/v CocUF pH 5.5.

Salunkhe, 1981c). This is because, at pH 5.5, i.e., near to their isoelectric point, the protein molecules are in a more compact form, than at pH 7; adsorbed in this configuration, the loops and tails of the protein molecules around the oil droplets provide more space for the inter-droplet interactions to take place, resulting in an increase in the emulsions rheological properties. Still, after 70 days, no differences could be observed among samples. Emulsions displayed shear-thinning rheological behaviour (i.e., apparent viscosity decreased with increasing shear rate). This indicated that the emulsions were unstable, flocculated and that the protein was insufficient for the long-term storage of the emulsions (Dickinson & Golding, 1997; Kulmyraev et al., 2000).

3.7. Foaming properties

Table 1 presents the influence of protein concentration, protein extraction method, and pH on the foaming properties of V and Coc isolates. As far as the concentration is concerned, 3% w/v protein concentration resulted in more foam than the rest of the concentration studied. UF isolates, from both cultivars, had higher foaming capacity, and, for 3% w/v protein concentration, V proved to be a slightly better foaming agent. For UF isolates, pH 5.5, provided better foaming ability as well as better foaming stability, while, for pI foams, at pH 7.0, more foam was created but better stability was exhibited at pH 5.5. The protein concentration must

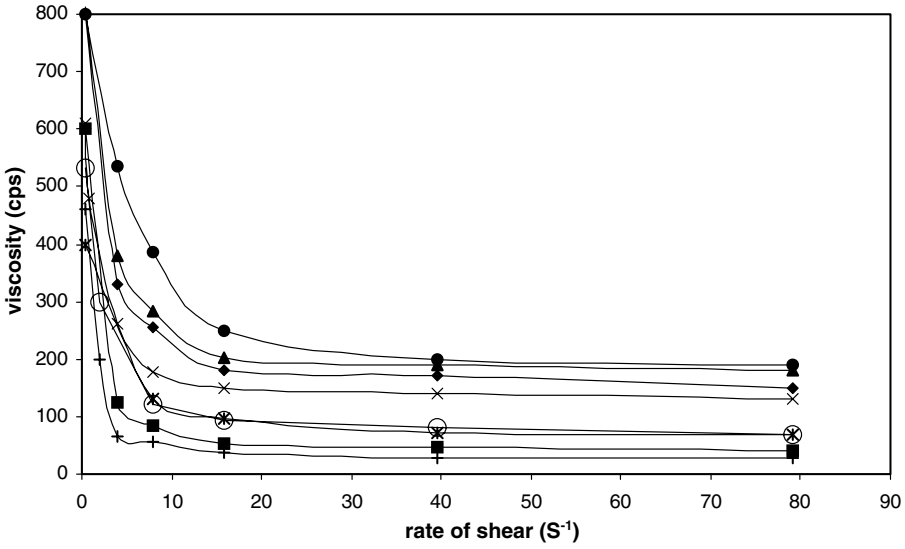

Fig. 8. Viscosity-shear of rate curves for o/w emulsion prepared with 3% w/v VpI and VUF or CocpI and CocUF protein isolate at pH 5.5 after 1 and 70 days of ageing: (×) 3% w/v VpI pH 5.5 day 1; (○) 3% w/v VpI pH 5.5 day 70; (◆) 3% w/v VUF pH 5.5 day 1; (■) 3% w/v VUF pH 5.5 day 70; (▲) 3% w/v CocpI pH 5.5 day 1; (✻) 3% w/v CocpI pH 5.5 day 70; (●) 3% w/v CocUF pH 5.5 day 1; (+) 3% w/v CocUF pH 5.5 day 70.

Table 1
Relative overrun, foam stability and liquid drainage of foams prepared with 1% or 2% or 3% w/v VpI or VUF or CocpI or CocUF at pH 7.0 and 5.5

Sample	Relative overrun	Foam stability	Liquid drainage
1%VpI, pH 7	2.3 (±0.1)	0.63 (±0.03)	0.85 (±0.03)
1%VpI, pH 5.5	2.0 (±0.1)	0.80 (±0.04)	0.80 (±0.03)
2%VpI, pH 7	2.6 (±0.2)	0.65 (±0.05)	0.80 (±0.02)
2%VpI, pH 5.5	2.4 (±0.2)	0.75 (±0.03)	0.70 (±0.02)
3%VpI, pH 7	2.8 (±03)	0.57 (±0.04)	0.75 (±0.03)
3%VpI, pH 5.5	2.7(±0.2)	0.70 (±0.04)	0.65 (±0.05)
1%CocpI, pH 7	2.4 (±0.1)	0.63 (±0.05)	0.80 (±0.03)
1%CocpI, pH 5.5	2.2 (±0.1)	0.61 (±0.02)	0.80 (±0.03)
2%CocpI, pH 7	2.7 (±0.1)	0.73 (±0.03)	0.80 (±0.04)
2%CocpI, pH 5.5	2.8 (±0.2)	0.74 (±0.03)	0.70 (±0.03)
3%CocpI, pH 7	3.0 (±0.2)	0.67 (±0.04)	0.85 (±0.04)
3%CocpI, pH 5.5	2.9 (±0.3)	0.71 (±0.04)	0.75 (±0.03)
1%VUF, pH 7	2.1 (±0.1)	0.24 (±0.05)	0.95 (±0.02)
1%VUF, pH 5.5	2.2 (±0.2)	0.72 (±0.04)	0.80 (±0.03)
2%VUF, pH 7	4.9 (±0.2)	0.86 (±0.05)	0.70 (±0.03)
2%VUF, pH 5.5	5.6 (±0.1)	0.98 (±0.02)	0.25 (±0.05)
3%VUF, pH 7	5.1 (±0.2)	0.86 (±0.03)	0.70 (±0.03)
3%VUF, pH 5.5	5.7 (±0.2)	0.93 (±0.03)	0.25 (±0.05)
1%CocUF, pH 7	2.2 (±0.1)	0.59 (±0.05)	0.90 (±0.05)
1%CoCUF, pH 5.5	1.9 (±0.1)	0.71 (±0.03)	0.85 (±0.03)
2%CocUF, pH 7	3.6 (±0.2)	0.77 (±0.03)	0.80 (±0.03)
2%CoCUF, pH 5.5	4.7 (±0.1)	0.85 (±0.03)	0.60 (±0.05)
3%CocUF, pH 7	4.0 (±0.3)	0.83 (±0.04)	0.70 (±0.05)
3%CoCUF, pH 5.5	4.9 (±0.2)	0.87 (±0.02)	0.65 (±0.05)

be sufficient to cover the air bubble surfaces, create a rigid film around them, and produce enough foam, so a higher protein concentration leads to the formation of more foam (Sathe & Salunkhe, 1981c; Satterlee et al., 1975). The pH alters the configuration of the protein molecules, consequently altering the capacity and stability of the foam created. At pH 5.5, i.e., near to the isoelectric point, the molecules are more compact and less flexible and this seems to contribute to the rigidity and the viscoelasticity of the interfacial film. The extraction method which also results in different molecule fractions (in quality and quantity), influences the foaming properties of the isolates and the isolates have been proved to be better foaming agents (Fidantsi & Doxastakis, 2001; Tsaliki, Kechagia, & Doxastakis, 2002).

Table 2 provides information on the role of the pH of the aqueous phase of the foam, on its the volume and stability. At pH 8.0, the relative overrun was greater

Table 2
Relative overrun, foam stability and liquid drainage of foams prepared with 1% or 2% or 3% w/v VpI or VUF or CocpI or CocUF at pH 8.0, 7.0, 5.5 and 4.5

Sample	Relative overrun	Foam stability	Liquid drainage
1%VpI, pH 8	2.3 (±0.2)	0.66 (±0.03)	0.80 (±0.03)
1%VpI, pH 7	2.3 (±0.2)	0.63 (±0.02)	0.85 (±0.03)
1%VpI, pH 5.5	2.0 (±0.2)	0.80 (±0.06)	0.80 (±0.04)
1%VpI, pH 4.5	1.9 (±0.2)	0.56 (±0.06)	0.85 (±0.02)
1%CocpI, pH 8	2.5 (±0.1)	0.68 (±0.05)	0.75 (±0.03)
1%CocpI, pH 7	2.4 (±0.1)	0.63 (±0.03)	0.80 (±0.03)
1%CocpI, pH 5.5	2.2 (±0.2)	0.61 (±0.03)	0.80 (±0.04)
1%CocpI, pH 4.5	1.9 (±0.1)	0.59 (±0.06)	0.80 (±0.02)
1%VUF, pH 8	2.3 (±0.2)	0.65 (±0.05)	0.80 (±0.03)
1%VUF, pH 7	2.1 (±0.1)	0.24 (±0.05)	0.85 (±0.02)
1%VUF, pH 5.5	2.2 (±0.1)	0.72 (±0.04)	0.80 (±0.03)
1%VUF, pH 4.5	1.6 (±0.3)	0.60 (±0.03)	0.90 (±0.05)
1%CocUF, pH 8	2.5 (±0.2)	0.68 (±0.03)	0.80 (±0.05)
1%CocUF, pH 7	2.2 (±0.1)	0.59 (±0.05)	0.90 (±0.05)
1%CocUF, pH 5.5	1.9 (±0.1)	0.71 (±0.05)	0.85 (±0.03)
1%CocUF, pH 4.5	1.9 (±0.2)	0.68 (±0.04)	0.85 (±0.02)

for all foams prepared. At this pH value, the molecules are more flexible which probably enhances the foam formation. Our results are in agreement with data reported by Satterlee et al. (1975). Again, the stability for the majority of the foams studied was optimized at pH 5.5.

4. Conclusions

Protein isolates, from common bean and scarlet runner bean, were extracted by wet methods and characterized. Only isoelectric-precipitated and ultrafiltration – extracted proteins showed differences, confirmed by SDS–PAGE data, solubility curves and emulsifying and foaming properties. The roles of pH and protein concentration proved to be quiet important for the creation and stability of emulsions and foams. Emulsions with 3% w/v protein isolates at pH 5.5 and extracted by ultrafiltration, from both cultivars, exhibited more pronounced tendency to flocculation, especially the *coccineus* isolates. So, pI isolates, and especially V isolates, could be considered more stable throughout storage. Foams with 1% w/v protein also showed little foaming ability compared to foams prepared with 2% or 3% w/v protein isolates. Foaming capacity was higher for foams prepared at pH 8.0, while ultrafiltration isolates produced more foam, which was especially stable at pH 5.5.

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