

Pea, Chickpea and Lentil Protein Isolates: Physicochemical Characterization and Emulsifying Properties

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Received: 17 June 2015 / Accepted: 30 July 2015 / Published online: 7 August 2015
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Abstract This work is focused on physicochemical and emulsifying properties of pea (PP), chickpea (CP) and lentil (LP) proteins. We evaluated the molecular weight distributions, surface net charge, free sulfhydryl group (SH) and disulfide bond (SS) contents, protein solubility and thermal stability of the protein isolates. Their emulsifying properties (droplet size distribution, flocculation, coalescence and creaming) were also determined as function of pH values. The three protein isolates exhibit similar physicochemical properties, including good solubility and high thermal stability despite a high degree of denaturation. In addition, we analysed the influence of pH on stability of oil-in-water (O/W; 10 wt%/90 wt%) emulsions stabilized by the legume protein isolates. Concerning emulsifying ability and stability, the most unfavourable results for all three protein isolates relate to their isoelectric point (pI=4.5). A significant improvement in

emulsion stability takes place as the pH value departs from the pI. Overall, this study indicates that pea, chickpea and lentil proteins have great potential as food emulsifiers.

Keywords Differential scanning calorimetry · Emulsifying properties · Emulsions · Legume protein · Physicochemical characterization

Introduction

Dry legumes or pulses are the edible seeds of leguminous crops. Legumes are considered the second most important source of human food after cereals. They are an inexpensive source of proteins and other nutrients such as starch, dietary fibre, vitamins, minerals and polyphenols. In general, legumes are good sources of proteins especially when consumed in combination with cereals [1]. Although total human food consumption of legumes globally has risen over the last four decades, this has been driven primarily by population growth. Unfortunately, global mean consumption of legumes per capita is on the decline [2]. Finding new uses for these seeds and thereby creating new demand is critical to the success of the legume industry. New demand is likely to come mainly from a change in focus from marketing legumes as commodities to highlighting and promoting their use as higher value food ingredients [3].

Proteins as concentrates or isolates are used as a functional ingredient primarily to increase nutritional quality (i.e., to include essential amino acids, health promoting polypeptides, etc.) and to provide desirable sensory characteristics such as structure, texture, flavour, and colour to formulated food products. The protein concentrates and isolates used by the food industry today are mostly derived from soybean, whey and wheat. However, because of dietary restrictions and

Headings · Physicochemical characterization of pea, chickpea and lentil protein isolates;

- Good solubility, high thermal stability and high degree of denaturation
- Emulsifying properties are pH dependent with good results at pH values away from pI
- Investigated legume proteins could be useful as emulsifiers in food formulations

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preferences (related allergenicity, vegetarianism, Halal requirements, etc.), food manufacturers and consumers are looking for alternative protein sources [4]. Legumes can be considered highly suitable for the preparation of protein isolates because of their high protein content, low cost and wide acceptability [4]. Recently, there has been growing interest in separating pulse crops into their component parts (protein, starch and fibre) [1, 5–8], and using these components as ingredients in food systems [9–12]. In this context, there is a need for up-to-date information on novel and emerging technologies for the processing of legume proteins, techniques for fractionating into ingredients (legumin and vicilin), and their functional and nutritional properties, as well as novel and potential applications. Knowledge of chemical, physical, and functional characteristics of protein concentrates or isolates are critical for identifying possible food applications [13].

Several studies have been conducted on legume proteins [1, 7, 13, 14]; however, a survey of the literature has shown that there is a dearth of information on the relationship between physicochemical characteristics of legume proteins and their functionality with which to predict potential uses and optimize their properties for food applications. Therefore, the present study aimed to investigate and compare the physicochemical and emulsifying properties of protein isolates derived from three legumes, pea, chickpea and lentil, which are among the most cultivated and consumed legumes in Algeria and for which limited biochemical and functional data are available. Further, a focus on these legumes is justified by their relatively low cost and widespread availability. Studying such legumes may provide useful information to consumers and also incentives to food manufacturers to promote the consumption and production of value-added foods for improving human health.

Material and Method

Material

Seed Material (Flour Preparation)

Dry seeds of pea (*Pisum sativum*), chickpea (*Cicer arietinum*) and lentil (*Lens culinaris*), commonly cultivated in Algeria, were harvested from the region of Ain Defla, Algeria, in July and August 2012. Samples were cleaned to remove foreign materials and damaged seeds. Seeds were crushed initially in a traditional stone mill and then in an analytical mill (IKA A11 basic, Germany), sieved through a 500- μ m screen (Tap sieve shaker AS 200 Retsch, Germany), and kept in dark airtight glass containers at room temperature until use. All chemicals used were purchased from regular suppliers and were of analytical grade.

Preparation of Legume Protein Isolates

Legume protein isolates were prepared according to the method of Papalamprou, Doxastakis, and Kiosseoglou [15] with slight modifications. In brief, flour (100 g) was mixed with distilled water at a 1:10 ratio (*w/v*), adjusted to pH 8.00 using 1 M NaOH, and stirred at 500 rpm for 45 min at room temperature (20–22 °C). The suspension was then centrifuged at 4500g for 20 min at 4 °C to collect the supernatant. The resulting pellet was re-suspended in distilled water at a ratio of 1:5 (*w/v*), adjusted to pH 8.00, stirred for an additional 45 min, followed by centrifugation (4500 \times g, 20 min, 4 °C). Both supernatants were pooled and adjusted to pH=4.0 (pea) or pH=4.5 (chickpea and lentil) using 0.1 M HCl to precipitate the protein. The protein was separated by centrifugation and collected [16]. The pH adjustment values are deduced from our previous study on protein solubility [17]. The precipitate obtained was washed twice with distilled water (4 °C) and re-dispersed in distilled water with pH adjusted to pH 7 with 1 M NaOH, and freeze-dried [1]. The protein content of the isolates was determined in quadruplicate as %N \times 6.25 using a Leco CHNS-932 elemental analyzer (Leco Corporation, St. Joseph, MI, USA). It was found to be 85.7 \pm 0.6 %, 85.9 \pm 0.2 % and 84.8 \pm 0.1 % (fresh matter) for pea protein (PP), chickpea protein (CP) and lentil protein (LP), respectively.

Methods

SDS-PAGE Electrophoresis

SDS-PAGE was performed according to the method of Laemmli [18] with slight modifications. Continuous and stacking gels were prepared of 10 and 3.5 % of acrylamide, respectively. A buffer containing 2 M Tris-base, containing 0.15 % SDS pH 8.8 was used for the separating gel. A solution of 0.027 M Tris-base, and 0.38 M glycine pH 8.3 with 0.15 % SDS was used as running buffer. Ten μ l of each sample was loaded onto the gel. Precision Plus Protein standards (Bio-Rad Calibration kit, Richmond, CA, USA) were used as a reference, and Coomassie Brilliant Blue was used for staining.

Free Sulfhydryl Group (SH) and Disulfide Bond (SS) Contents

The free SH and SS contents of protein samples were described by Tang and Sun [19]. Protein samples (75 mg) were dissolved in 10 ml of Tris-Gly buffer (0.086 M Tris, 0.09 M glycine, 0.004 M EDTA, pH 8.0) containing 8 M urea. The solution was gently stirred overnight until a homogeneous dispersion was achieved. For measuring SH content, 4 ml of the Tris-Gly buffer was added to 1 ml of protein solution. Then 0.05 ml of Ellman's reagent (5,5-dithio-bis-2-nitrobenzoic acid in Tris-Gly buffer, 4 mg/ml methanol) was

added, and absorbance was measured at 412 nm after 20 min in a Genesis-20 spectrophotometer (Thermo Scientific, USA). For total SH content [SH and reduced SS] analysis, 0.05 ml of 2-ME and 4 ml of Tris-Gly buffer were added to 1 ml of the protein solution. The mixture was incubated for 1 h at room temperature. After an additional hour of incubation with 10 ml of 12% TCA, the mixtures were centrifuged at 5000g for 10 min. The precipitate was twice resuspended in 5 ml of 12 % TCA and centrifuged to remove the 2-ME. The precipitate was dissolved in 10 ml of Tris-Gly buffer. Then, 0.04 ml of Ellman's reagent was added to 4 ml of this protein solution, and the absorbance was measured at 412 nm after 20 min. The content was calculated as follows:

$$\mu\text{mol SH/g} = 73.53 * A_{412} * D / C$$

where A_{412} is the absorbance at 412 nm, C is the sample concentration (mg/ml), D is the dilution factor (5 and 10 are used for SH and total SH [SH and reduced SS] content analysis, respectively), and the constant 73.53 is calculated as $10^6 / (1.36 \cdot 10^4)$ (10^6 being for the conversion from molar basis to $\mu\text{M/ml}$ basis and from mg solids to g solids and $1.36 \cdot 10^4$ the molar absorptivity). The SS content was calculated by subtracting the SH content from the total SH group content and dividing the result by 2.

Zeta Potential Measurements

The zeta potential measurements of protein isolates as function of pH were measured using a Zetasizer Nano ZS (Malvern Instruments, Malvern, Worcestershire, UK) as described by Tang and Sun [19]. For this, protein samples were prepared at 1 % at different pH values with buffers. Prior to analysis, the samples were stirred at 20 °C, and then, centrifuged at 10,000g for 10 min in a RC5C Sorvall centrifuge (Sorvall Instruments, Wilmington, DE, USA). After that, the samples were measured in triplicate at 20 °C. The zeta potential was calculated from the electrophoretic mobility using the Henry equation and the Smoluchowski approximation.

Protein Solubility

Protein solubility was determined at various pH values ranging from 2 to 8 following the Chau and Cheung [20] method, with slight modifications. In brief, 10 mg of protein was dispersed in 20 ml of the corresponding buffer. The dispersions were continuously stirred for 30 min at room temperature (25 °C) and centrifuged at 15,000g for 10 min at 10 °C. The amount of protein in the supernatant was measured by the method of Bradford [21]. Protein solubility was expressed as BSA equivalents (mg BSAE/g fresh protein).

Differential Scanning Calorimetry (DSC)

Thermal transition of various protein samples was examined by DSC using a Q20 (TA Instruments, USA). For each protein, approximately 2 mg of the sample was weighed into an aluminium liquid pan, and 5 μl of deionised water was added. Pans were hermetically sealed and heated from 20 to 120 °C at a rate of 5 °C/min. A sealed empty pan was used as a reference. Onset temperature (T_o) and peak transition or denaturation temperature (T_d) were computed from the thermograms by Universal Analysis 2000, Version 4.1D (TA Instruments Waters LLC).

Emulsion Preparation

Three protein solutions with a constant protein concentration of 1.0 % (w/v) were prepared with pH values adjusted to 3, 4.5, 7 and 8, covering the pH range of most common food-stuffs, stirred using a magnetic stirrer for 2 h at room temperature and, then, stored overnight at 4 °C to allow complete hydration. Each protein solution or dispersion was mixed with sunflower oil at oil fraction (ϕ)=0.1, and pre-homogenized using the high-speed dispersing and emulsifying unit (T 25 basic ULTRA-TURRAX, IKA-Werke, Staufen, Germany) at 17,000 rpm for 1 min. Then, the pre-homogenized dispersions were further homogenized by a high pressing emulsificator (Emulsiflex-C5, Canada) for one pass at a pressure level of 40 MPa. The fresh emulsions were stored for various periods of time (e.g., 24 h) prior to further analysis.

Characterization of Emulsions

Droplet-size distribution, flocculation and coalescence indices (FI and CI) as well as creaming indices of prepared emulsions were evaluated as reported by Liang and Tang [22].

Droplet-Size Distribution (DSD)

Droplet-size distribution of various freshly prepared or stored (24 h) emulsions were measured using a Malvern MasterSizer 2000 (Malvern Instruments, Malvern, Worcestershire, UK). Deionized water or 1 % (w/v) SDS solution was used as the dispersant. The role of SDS is to produce displacement of protein molecules from the interface applying gentle agitation in order to disrupt droplets flocs (Chen and Dickinson, 1998; Puppo et al., 2005). Volumetric diameter ($d_{4,3}$), which is inversely proportional to the specific surface area droplet, was calculated as follows:

$$d_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}$$

where n_i is the number of droplets with diameter d_i . All measurements were conducted at least in triplicate.

Flocculation and Coalescence Indices (FI and CI)

Freshly prepared (0 h) or stored (after 24 h) emulsions were diluted in deionized water with and without 1 % SDS (w/v), and the $d_{4,3}$ of droplets was determined as the above. The FI (%) was calculated as follows:

$$FI(\%) = \left[\frac{(d_{4,3} \text{ in water})}{(d_{4,3} \text{ in SDS})} - 1 \right] \times 100,$$

and CI (%) of the emulsions after 24 h of quiescent storage as follows:

$$CI(\%) = \left[\frac{d_{4,3}(24 \text{ h})}{d_{4,3}(0 \text{ h})} - 1 \right] \times 100$$

where $d_{4,3}(24 \text{ h})$ and $d_{4,3}(0 \text{ h})$ were the $d_{4,3}$ of droplets in the emulsions, freshly prepared (0 h) or after storage of 24 h, respectively. For this experiment, all the emulsions were assessed with 1 % SDS as the diluent.

Creaming

Glass test tubes were filled with 10-ml of each emulsion and then stored in a vertical position at ambient temperature. Height of serum (Hs) and total height of emulsion (Ht) were recorded at regular intervals up to day 10 of quiescent storage at room temperature. Mean and standard deviations of three replicates are reported. The creaming index, the percentage of creaming, was calculated as $(Hs/Ht) \times 100$. In addition, emulsions were classified as (+) or (\pm) to indicate considerable or slight creaming respectively, although sometimes this could not be assessed because the separation was unclear.

Statistical Analysis

At least three replicates were carried out for each measurement. Statistical analyses were performed with t tests and one-way analysis of variance (ANOVA, $p < 0.05$) using PASW Statistics for Windows (Version 18, SPSS, Chicago, IL). Standard deviations were calculated for some selected parameters.

Results and Discussion

Physicochemical Characterization of Legume Protein Isolates

SDS-PAGE

Figure 1 shows the SDS-PAGE profiles of the three proteins tested. The profiles exhibit a wide variety of

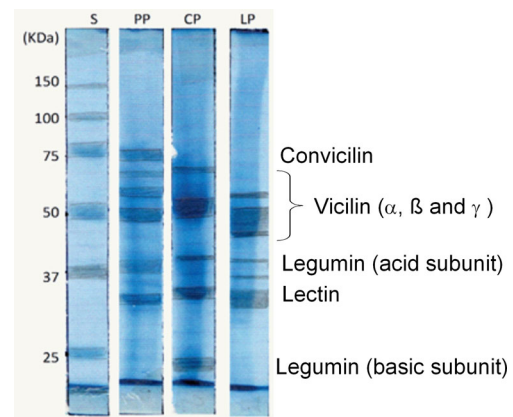


Fig. 1 SDS-PAGE electrophoresis. S standard, PP Pea protein, CP Chickpea protein, LP Lentil protein

polypeptide subunits of molecular weight (Mw) between 25 and 75 kDa. Most bands may be attributed to the polypeptide constituents of legumin and vicilin. As described by Shevkani et al. [23] the subunits of 70 kDa correspond to convicilin and the subunits around 50 kDa might be ascribed to vicilin, which described as an oligomeric protein consisting of three polypeptide subunits, α , β and γ - subunits with Mws between 43 and 53 kDa. The polypeptide subunits around 37 and 25 kDa may be attributable to acidic and basic subunits, respectively, of legumin (a hexameric protein). The bands of ~ 32 kDa might be ascribed to phytohemagglutinin (lectin) [24]. Similar results have also been obtained in SDS-PAGE analysis of other legume proteins [22].

Free SH and SS Contents

Results of free SH (including total and exposed) and SS contents are shown in Table 1. The total SH groups ranged from 31.0 $\mu\text{mol/g}$ in LP to 59.7 $\mu\text{mol/g}$ in CP. These results are in agreement with the sulphur content (results not shown) and are generally consistent with the differences in amino acid composition [25]. The highest content of exposed SH groups was found in CP (19.3 $\mu\text{mol/g}$), followed by LP (16.1 $\mu\text{mol/g}$) and PP (15.8 $\mu\text{mol/g}$). Approximately, half (1/2) of the free SH groups in LP were exposed on the surface of the molecules, while in PP and CP, only a third (1/3) of the total free SH groups were exposed (Table 1). In any case, the relatively low amounts of exposed SH indicates the high degree of protein denaturation found with the legume proteins studied in this work [26]. This fact is due to protein aggregation during processing steps (solubilisation, precipitation and lyophilisation) in order to isolate [27]. In fact, the SS contents are higher than those reported in the literature [22, 28] in various different legume protein fractions.

Table 1 Free SH (exposed and total) and SS contents

Systems	Exposed SH ($\mu\text{mol/g}$)	Total SH ($\mu\text{mol/g}$)	S-S bonds ($\mu\text{mol/g}$)
PP	15.8 \pm 0.7 ^b	45.1 \pm 1.9 ^b	14.7 \pm 0.6 ^b
CP	19.3 \pm 1.8 ^a	59.7 \pm 1.3 ^a	20.2 \pm 0.4 ^a
LP	16.1 \pm 0.7 ^b	31.0 \pm 2.0 ^c	7.5 \pm 0.7 ^c

PP Pea protein, CP Chickpea protein, LP Lentil protein

Values expressed are mean \pm standard deviation. Means in the column with different superscript are significantly different at $p < 0.05$

Surface Charge (Zeta Potential, ζ)

Surface charge is an important characteristic of proteins, determining their functional properties [23]. The surface net charge of extracts at various pH values was evaluated by zeta potential (ζ) analysis, as shown in Fig. 2. All the tested samples exhibited similar ζ profiles. With the pH decreasing from 8 to 2, the ζ values gradually changed from a maximum negative value to a positive maximum. This is consistent with the fact that, the electrostatic repulsion pattern may be gradually changed from negatively charged to positively charged proteins as a result of gradual protonation of carboxyl groups and amino groups of the proteins [29]. Upon further decreases in pH, however, the ζ slightly decreased. This decrease may be due to the hydrolysis of glutamine and/or asparagine into glutamic acid and/or glutamic acid under highly acidic pH conditions [19]. The charges reveal that the pH value of 4.5 (or somewhat lower in the case of LP) corresponds to the isoelectric region. These results are in agreement with those reported by Tang and Sun [19, 29] in various different protein fractions.

Protein Solubility (PS)

The solubility of a protein is the manifestation of the equilibrium between protein-solvent (hydrophilic) and the protein-protein (hydrophobic) interactions [29]. It was investigated at pHs ranging from 2 to 8 (Fig. 3) and expressed in mg of

BSA per g of protein extract. As expected, the solubility profiles for protein extracts were very similar and were found to be pH-dependent. The protein solubility of extracts reached a minimum at pH ca. 4.5 (121, 177 and 210 mg/g for lentil, chickpea and pea, respectively), and increased gradually above and below this pH to reach maxima under highly alkaline (pH 8) and highly acidic (pH 2) conditions. This reflects the pI (pH 4.5). In general, at higher pH values, the increased net negative charge on the protein dissociates the protein aggregates, and the solubility may increase, whilst at lower pH values, the increased net positive charge contributes to the solubility [30]. Generally, the relationship of nitrogen solubility with pH was similar for all three extracts and in agreement with those for other legume proteins reported in the literature [1, 23, 29, 31, 32].

Further, the PS profiles are in good agreement with the zeta potential profiles, indicating that the solubility of legume proteins was closely dependent upon their surface net charge. For food applications, solubility is an important parameter that influences the potential usefulness of a protein in different food matrices [31]. Indeed, it is probably the most critical parameter, because it affects other properties, such as emulsification ability, foaming and gelling [31], and hence to a large extent, it determines the suitability of a protein as an ingredient in various food applications [33]. Their high solubility under acidic conditions could make these proteins very promising candidates for use in acidic food products.

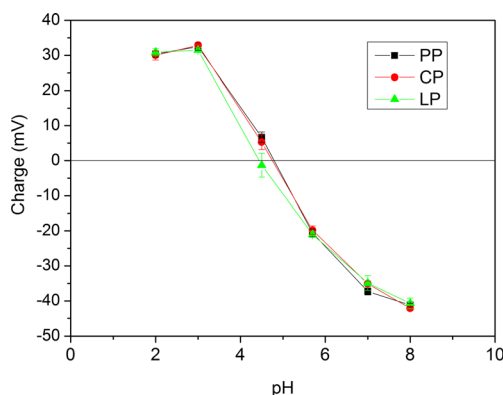


Fig. 2 Zeta potential (ζ) profiles of legume protein isolates as a function of pH values. PP Pea protein, CP Chickpea protein, LP Lentil protein

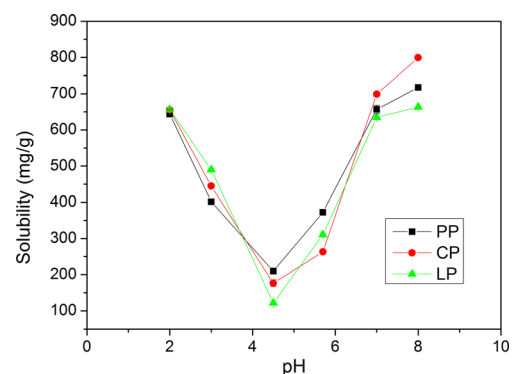


Fig. 3 Solubility profiles of protein extracts as a function of pH values. PP Pea protein, CP Chickpea protein, LP Lentil protein

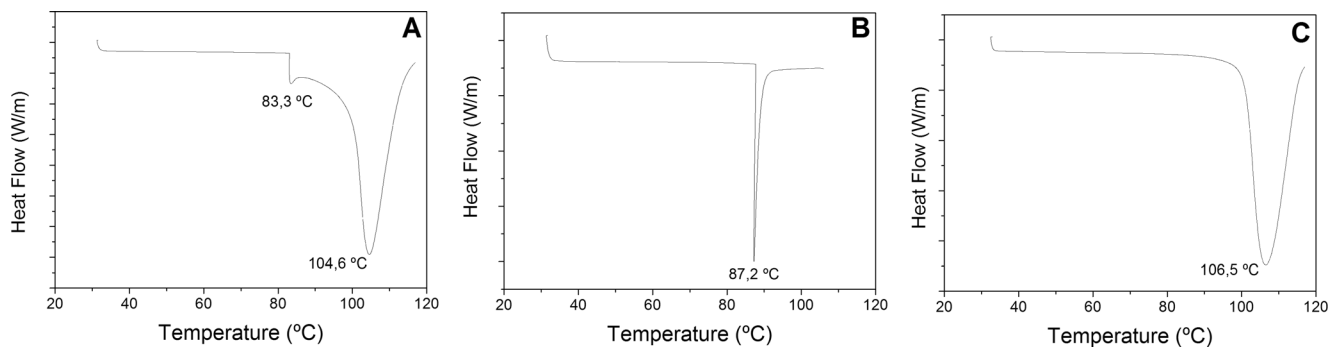


Fig. 4 DSC thermograms of protein extracts: A. Pea protein, PP; Chickpea protein, CP; Lentil protein, LP

Differential Scanning Calorimetry (DSC)

Thermal properties of globular proteins are also important, since they may be related to their heat-induced aggregation and gelation behaviours [19]. A higher denaturation temperature (T_d) is usually associated with higher thermal stability for a globular protein. The T_d can also reflect the disruption of hydrogen bonds maintaining tertiary and quaternary structures of proteins, particularly tertiary ones. Thus, a higher T_d for the proteins suggests that the polypeptides have a more compact tertiary structure [29].

The thermal stability of the proteins, using T_d as the indicator, was evaluated by DSC (Fig. 4). In all the thermograms, there was a prominent endothermic peak, clearly attributable to thermal transition of proteins. PP displayed another weak peak at 83.3 °C. This peak might correspond to the denaturation of vicilin [14]. The highest T_d value was observed for LP (106.5 °C) followed by PP (104.6 °C). CP was less thermally stable, with a T_d of 87.2 °C. The data suggest that the thermal stability decreased in the following order: LP>PP>CP. Although higher thermal stability is usually associated with higher disulfide bond contents for a globular protein. Thermal stability of a protein may be related to other parameters, including its acidic/basic amino acid ratio and polar uncharged amino acid contents and polypeptide heterogeneity [29]. Besides differences in protein structure, the interactions of proteins with residual salts in the isolates might also be responsible for higher thermal stability [23]. Meanwhile in the present

study, higher pea and lentil protein thermostability could be indicative of higher legumin content, since legumin hexamers are more compact and hence harder to thermally-unfold than vicilin trimers [25].

Characterization of Emulsions

Emulsifying Ability

Emulsifying ability is another important physicochemical property that may directly affect the application of proteins in food formulations. The emulsifying properties of the legume proteins (PP, CP and LP) were evaluated at pH 3 to 8 at the same protein concentration (1.0 % (w/v)) and an oil fraction of 0.1 using droplet-size analysis. Figure 5 shows typical droplet-size distribution profiles of each of the fresh emulsions, diluted in 1 % SDS. The $d_{4,3}$ of the droplets, diluted in 1 % SDS or deionized water, is summarized in Table 2. The $d_{4,3}$ in 1 % SDS can reflect ability of a protein to help dispersion of an oil phase into an aqueous phase, since the presence of 1 % SDS may prevent oil droplet aggregation, thus keeping individual droplets separate in an emulsion. In the presence of 1 % SDS, all the emulsions displayed a prominent distribution peak, but the magnitude and location of the peak varied with the type of proteins and the pH values (Fig. 5). In general, the smaller the droplet sizes (in 1 % SDS) of protein-stabilized emulsions, the better the emulsifying ability of the protein [34]. All the samples exhibited

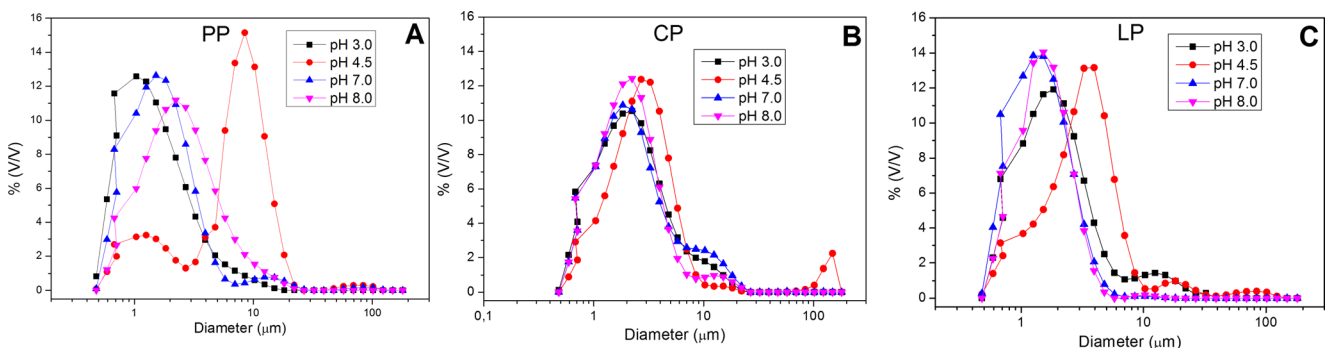


Fig. 5 DSD as function of pH values. A. Pea protein, PP; Chickpea protein, CP; Lentil protein, LP

Table 2 Emulsion characteristics, including volume-mean droplet size ($d_{4,3}$), flocculation and coalescence indices of legume protein-stabilized emulsions at pH 3–8

	pH	d _{4,3} (μm)				Indices	
		0 h		24 h		Flocculation index (%)	Coalescence Index (%)
		Water	SDS	Water	SDS		
PP	3	7.8±0.1	1.7±0.1	8.6±0.1	1.9±0.1	3.6±0.1	12.3±0.2
	4.5	23.9±1.7	7.4±0.3	26.8±6.7	13.0±0.1	2.2±0.1	76.2±0.3
	7	5.6±0.2	2.5±0.1	5.6±0.1	2.9±0.1	1.2±0.1	13.5±0.2
	8	5.0±0.1	2.9±0.1	5.0±0.1	2.9±0.1	1.0±0.1	1.1±0.2
CP	3	6.7±1.0	2.7±0.1	9.0±0.4	2.9±0.4	1.5±0.1	4.8±0.1
	4.5	21.2±0.1	7.8±0.8	27.1±0.1	13.0±0.1	1.7±0.1	66.3±0.4
	7	7.0±0.4	3.1±0.4	7.1±1.2	3.7±0.4	1.3±0.1	19.2±0.3
	8	4.1±0.1	2.5±0.2	4.8±0.1	3.1±0.1	1.0±0.1	24.3±0.4
LP	3	6.7±1.1	2.6±0.1	8.3±1.0	3.5±0.1	1.6±0.1	35.2±0.3
	4.5	23.6±1.4	4.7±0.1	34.7±0.4	6.6±1.3	4.0±0.2	39.8±0.3
	7	8.1±0.7	1.5±0.1	9.8±0.5	1.7±0.1	4.4±0.2	10.7±0.2
	8	4.3±0.2	1.8±0.1	4.5±0.5	1.9±0.1	1.4±0.1	6.2±0.1

Values expressed are mean±standard deviation

PP Pea protein, CP Chickpea protein, LP Lentil protein

similar dependence of emulsifying ability on the pH, with ability at a minimum (corresponding to the maximum value of $d_{4,3}$) at pH 4.5 (around pI), and progressively increasing ($d_{4,3}$ decreasing) as the pH deviated from 4.5 (Table 2). This result is consistent with the pH-dependent surface charge and solubility of these protein isolates (Figs. 2 and 3), confirming the fact that solubility of proteins

plays an important role in their emulsifying properties. Comparing the legume protein isolates, the best results were found for PP under acidic and LP under basic conditions. Notably, however, the emulsifying properties of proteins are complex, usually being affected by their molar mass, hydrophobicity/solubility, conformation stability and charge and physicochemical factors such as

Table 3 Creaming index of various legume protein emulsions formed at various pH values (3–8), upon storage up to 10 days

Emulsions	pH	Creaming index (%)				
		1 day	3 days	5 days	7 days	10 days
PP	3.0	0.0	±	±	±	±
	4.5	44.1±0.1	44.1±0.1	46.1±1.4	46.1±1.4	46.1±1.6
	7.0	0.0	0.0	±	±	±
	8.0	0.0	±	±	±	±
CP	3.0	0.0	±	±	±	±
	4.5	68.3±5.0	71.3±2.0	71.3±2.0	72.3±1.6	73.3±0.5
	7.0	+	+	+	+	+
	8.0	0.0	±	±	±	±
LP	3.0	±	±	±	±	±
	4.5	54.4±1.5	58.3±1.0	58.3±3.1	58.3±3.1	58.3±3.1
	7.0	+	+	+	+	+
	8.0	±	±	±	±	±

Values expressed are mean±standard deviation

PP pea protein, CP chickpea protein, LP lentil protein

±: Slight creaming not objectively evaluated; +: clear creaming not objectively evaluated

pH, ionic strength, temperature, protein concentration and the amino acid profile [35].

Emulsion Flocculation

Table 2 shows the droplet diameter ($d_{4,3}$) of various fresh emulsions. At any pH, droplets diluted in deionized water, globally, shifted toward larger sizes, compared to those observed in 1 % SDS, indicating bridging flocculation of oil droplets. The flocculated state of oil droplets, evaluated in terms of FI, is reported in Table 2. The FI varied with pH and the type of proteins. In the cases of PP and CP, the FI decreased with increasing pH, but this pattern was not observed in LP. In any case, for all three emulsion samples, the FI had the lowest values at pH 8 and high values, but not always the highest, at pH 4.5, indicating that protein emulsifying properties are related to other parameters and not only to their solubility. Generally, emulsions stabilized by LP exhibit more bridging flocculation (FI 0 h) than those stabilized by PP or CP. This might be due to LP exhibit higher Td, maintaining a tertiary structure, and lower SH and SS contents. Therefore, LP is more unable to as readily unfold and form a film at the oil/water interface to stabilize the emulsion when compared to the other legume protein isolates (PP and CP).

Emulsion Stability

Emulsions are thermodynamically unstable due to a variety of physicochemical mechanisms, including gravitational separation, flocculation, coalescence and Ostwald ripening. The stability of each of the emulsions formed at various pH values (3–8) upon storage for 24 h was evaluated in terms of the CI (Table 2) and a creaming index (Table 3). A low CI indicates the formation of strong viscoelastic film at the interface and higher emulsion stability. As expected, the CI was the highest for all samples at pH 4.5, with better results (lower CI values) at extreme pH values. This is consistent with the fact that good solubility is a prerequisite for proteins to exhibit good emulsifying properties [32]. Table 3 shows the creaming index of the three protein emulsions at various pH values (3–8), upon quiescent storage up to 10 days. As expected, the emulsions showed different creaming behaviours, depending on the type of protein and the pH. Notably, the creaming index was high at pH 4.5 for all the emulsions, even after one day of storage. It increased with storage to reach the maximum values, 46.1, 73.3 and 58.3 % for PP, CP and LP emulsions, respectively. At extreme pH values (pH 3 and 8), all the stabilized emulsions were more or less stable, showing slight creaming, which suggests that PP, CP and LP could be used as emulsifiers under such conditions. On the other hand, the creaming instability at pH 4.5 could be largely attributed to large droplet sizes and decreased stability against coalescence [32]. The properties of the continuous phase may also influence a

variety of different instability mechanisms in emulsions. The addition of substances that increase the viscosity or gel strength of the continuous phase, e.g., starches, gums, sugars, and/or proteins, retards droplet movement [36].

Conclusion

Legume protein isolates (pea, chickpea and lentil) investigated were found to have similar SH and SS profiles, a similar degree of denaturation and similar physicochemical properties including, good solubility and high thermal stability. Emulsifying properties (droplet size distribution, flocculation, coalescence and creaming) as function of pH are favourable with relatively stable emulsions as pH values move away the pI (ca. pH 4.5). These physicochemical properties and emulsifying capacities should make legume proteins useful in a variety of formulations, such as bakery products, soups, dairy products, gluten-free foods, mayonnaise, and salad dressing, as well as new food products. Further studies are in progress on the functional and processing characteristics of these proteins and their emerging food applications.

Acknowledgments The authors wish to thank Béjaia University and the University of Seville for technical support. The authors are also grateful to the Microscopy and Microanalysis research services of the University of Seville (CITIUS-Universidad de Sevilla) for providing full access to their facilities and assistance with the experiments.

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