Accepted Manuscript

Emulsifying properties of legume proteins at acidic conditions: Effect of protein concentration and ionic strength

Yakoub Ladjal ettoumi, Mohamed Chibane, Alberto Romero

PII: S0023-6438(15)30269-3

DOI: 10.1016/j.lwt.2015.10.051

Reference: YFSTL 5046

To appear in: LWT - Food Science and Technology

Received Date: 7 June 2015

Revised Date: 7 August 2015

Accepted Date: 19 October 2015

Please cite this article as: Ladjal ettoumi, Y., Chibane, M., Romero, A., Emulsifying properties of legume proteins at acidic conditions: Effect of protein concentration and ionic strength, *LWT - Food Science and Technology* (2015), doi: 10.1016/j.lwt.2015.10.051.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Emulsifying properties of legume proteins at acidic conditions: Effect of 1 protein concentration and ionic strength 2 Yakoub LADJAL ETTOUMI^{a*}, Mohamed CHIBANE^b, Alberto ROMERO^c 3 ^aDépartement des Science Alimentaires, FSNV, Université Abderahman Mira, Bejaia, Route 4 5 Targa Ouzemour, Bejaia 06000, Algérie. ^bLaboratoire de Gestion et Valorisation des Ressources Naturelles Assurance Qualité, 6 Université Akli Mohand Oulhadj de Bouira, 10000 Bouira, Algérie. 7 ^cDepartamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla, 41012 8 Sevilla, Spain. 9 *Yakoub LADJAL ETTOUMI 10 Département des Science Alimentaires, FSNV, Université Abderahman Mira de Bejaia, Route 11 Targa Ouzemour, Bejaia 06000, Algérie. 12 E-mail: yakoblajel@hotmail.fr 13 14 Phone:+213 699 44 67 56.

15 Abstract

This work is focused on emulsifying properties of pea, chickpea and lentil protein isolates at acidic conditions (pH 3), as affected by protein concentration and ionic strength. Emulsions characteristics and stability (droplet size distribution, flocculation, coalescence and creaming) were determined. The microstructure of selected emulsions was also studied. Results indicated that emulsifying properties (ability and stability) are dependent to protein concentration and highly sensitive to ionic strength. In our conditions, the best emulsifying properties are found around 1.5% of protein, and at less than 50 mM of NaCl. Clearly, ionic

strength affects emulsions microstructure. Thus, this study indicated that pea, chickpea and
lentil proteins would have great potential as emulsifiers in acidic food formulations.

25 Keywords: Acidic emulsions, legume protein, protein concentration, ionic strength,
26 microstructure.

27 **1. Introduction**

In the time being, the consumer demands originated from health concerns, allergenicity, 28 religious limitations (Halal) and rising trend of vegetarianism have increased the interest of 29 30 food industry in use of functional plant proteins as alternative to animal proteins (Aydemir & Yemenicioglu, 2013; Carbonaro, Maselli, & Nucara, 2014). The production of plant protein 31 isolates is of growing interest to industry because of the increasing applications of plant 32 proteins in food and non-food markets (Zhang, Bo, Wanmeng, & Zhang, 2009). Nevertheless, 33 these applications in the food trade are almost limited to proteins from soybean and wheat, 34 35 whereas other vegetable proteins are available and less used. Thus, searchers, food manufacturers and consumers are looking for alternative protein sources (Boye et al., 2010; 36 Taherian et al., 2011; Toews & Wang, 2013; Liang & Tang, 2014; Shen & Tang, 2014). 37 38 Among these are those from dry legumes that are extensively grown in different parts of the world and, in particular, in the Mediterranean region. 39

There is increased interest in legume proteins as they can be used as good substitute for animal and soybean proteins (Zhang et al., 2009; Joshi et al., 2012; Liang & Tang, 2014; Shen and Tang, 2014). In this regard, the understanding of factors affecting the functional properties of legume proteins enables better control of these properties, which will facilitate the novel application of these proteins. The functional properties such as solubility, water and oil absorption capacity, gelation, foam and emulsion formation of legume protein isolates were studied to some extent previously (Makri, Papalamprou, & Doxastakis, 2005; Boye et

al., 2010; Aydemir & Yemenicioglu, 2013). However, there is lack of fundamental knowledge
and supporting data on the emulsifying properties of legumes proteins. Particularly, studies on
emulsifying properties of legumes protein, taking into account, protein concentration, pH,
ionic strength and relationship between structure and functionality are scarce.

In general, the emulsifying properties of plant proteins are dependent on the physicochemical properties of proteins, including, composition (e.g. vicilin/legume ratio), solubility, conformational stability and Hydrophobicity (Lestari, Mulder, & Sanders, 2011; Kaushal, Kumar, & Sharma, 2012; Liang & Tang, 2014). Also, environmental conditions, including, pH, ionic strength, protein concentration and oil fraction have an effect (Yu, Ahmedna & GoktepeIpek, 2007; Liang & Tang, 2014).

57 Our previous study (Ladjal E., Boudries, Chibane & Romero, 2015) demonstrated that legume 58 protein isolates have good emulsifying ability and stability at pH 3; suggesting their use in 59 acidic formulation, such as, salad dressing and mayonnaise. Thus, the present study is aimed 50 to investigate the effect of protein concentration and ionic strength on emulsifying properties 51 of protein stabilized emulsions at pH 3 using protein isolates derived from three legumes (pea, 52 chickpea and lentil). The microstructure of the selected emulsions is also studied.

63 2. Material and method

64 2.1. Preparation of legume protein isolates

Whole flours of pea (*Pisum sativum*), chickpea (*Cicer arietinum*) and lentil (*Lens culinaris*) were prepared as previously (Ladjal & Chibane, 2015). Legume protein isolates was prepared according to the method of Papalamprou, Doxastakis & Kiosseoglou (2010) with slight modification. In brief, flour (100 g) was mixed with distilled water at a 1:10 ratio (w/v), adjusted to pH 8.0 using 1 M NaOH and stirred at 500 rpm for 45 min at room temperature (20–22 °C). The suspension was then centrifuged at 4,500×g 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),

72 adjusted to pH 8.0, stirred for an additional 45 min, followed by centrifugation (4500×g, 20 min, 4 °C). Both supernatants were pooled and adjusted to pH=4.0 (pea) or pH=4.5 (chickpea 73 and lentil) using 0.1 M HCl to precipitate the protein. The protein was recovered by 74 centrifugation and collected (Karaca, Low, and Nickerson, 2011). The pH adjustment values 75 are deduced from our previous study on the protein solubility (Ladjal & Chibane, 2015). The 76 obtained precipitate was washed twice with distilled water (4° C) and re-dispersed in distilled 77 water with pH adjusted to pH 7 with 1M NaOH, and freeze-dried (Boye et al., 2010). The 78 protein contents were determined in quadruplicate as %N x 6.25 using a Leco CHNS-932 79 nitrogen micro analyser (Leco Corporation, St. Joseph, MI, USA) (Etheridge, Pesti, & Foster, 80 1998). Their protein contents were 85.7±0.6%, 85.9±0.2% and 84.8±0.1% (fresh matter) for 81 pea protein (PP), chickpea protein (CP) and lentil protein (LP), respectively. 82

83 2.3. Emulsions preparation

Three protein solutions with a different protein concentration (0.5 - 2%, w/v) were prepared at 84 85 pH adjusted to 3, 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 86 with sunflower oil at oil fraction $(\phi) = 0.1$, and pre-homogenized using the high-speed 87 dispersing and emulsifying unit (model IKA-ULTRA-TURRAX- T25 basic, IKA Works, 88 Inc., Germany) at 17,000 rpm for 1 min. Then, the pre-homogenized dispersions were further 89 homogenized by a high pressing emulsificator (Emulsiflex-C5, Canada) for one pass at a 90 pressure level of 40 MPa. To investigate the effect of ionic strength, we used the same 91 parameters with protein concentration of 1.5% and the salt concentration (salt dissolved in 92 93 deionised water) varied from 0 mM to 200 mM of NaCl. The fresh emulsions were stored for various periods of time (e.g., 24 h) prior to further analysis. All other materials and chemicals 94 used were purchased from regular suppliers and were of analytical grade. 95

96 2.4. Emulsions characterization

99 2.5. Emulsion microstructure

The microstructure of the selected emulsions was determined by means of confocal scanning laser microscopy a ZEISS LSM 7 DUO (Germany). A small aliquot of freshly prepared emulsion was placed on a microscope slide and covered with a cover slip prior to analysis. Emulsions were colored by Nile bleu. The emission of Nile bleu was detected in the 633 nm). This technique provides images of dark fat droplets and a bright water phase where proteins were found. a 63x objective was used. The microstructure images were analyzed using image analysis software (ZEN_2012SP1_black_SP2_blue).

107 2.6. Statistical analysis

108 Statistical analysis was carried out using analysis of variance (ANOVA) and the significant 109 difference between the samples was determined using LSD test at p < 0.05.

110 **3. Result and discussion**

- 111 3.1. Effect of protein concentration on emulsifying properties
- 112 *3.1.1. Droplet size distribution*

The effects of protein concentration on emulsifying properties were measured at pH 3.0 and an oil fraction of 0.1, at various protein concentrations (0.5, 1, 1.5 and 2%, (w/v)). The $d_{4,3}$ of the droplets, diluted in 1% SDS or deionized water, was calculated and summarized in Table 1. In general, the smaller the droplet sizes of protein-stabilized emulsions, the better the emulsifying ability of the protein is (Shen & Tang, 2014).

Generally, with the exception of PP-stabilized emulsions, CP and LP-stabilized emulsionsexhibited the best emulsifying ability (corresponding to the lowest diameter) at 2%

concentration. But the other concentrations did not display significant difference in their 120 emulsifying ability. This trend was noted both in SDS and water dilutions. For PP emulsion, 121 the smallest droplet size was found at 1.5% in water dilution and at 1% and 1.5% in SDS 122 dilution. From this concentration, there was a slight increase in droplets size indicating an 123 excess of non adsorbing protein which promote droplets flocculation by depletion 124 phenomenon. The high $d_{4,3}$ values of the emulsions containing 0.5% protein suggested that 125 protein content may not be enough to cover the oil droplets and form sufficiently dense 126 adsorption layer (Sanchez and Patino, 2005). As a result, protein acted as bridges among the 127 oil droplets and led to droplets aggregation (Sun and Gunasekaran, 2009; Guo & Mu, 2011). 128 Our results are in agreement with those reported by Sun and Gunasekaran (2009), and 129 corroborate well with those of Joshi et al. (2012), which suggest that the higher the protein 130 concentration (lentil protein), the greater was the reduction in interfacial tension, displaying 131 132 stabilized emulsion with high turbidity and small oil droplet.

133 *3.1.2. Flocculation in fresh emulsions*

The flocculated state of oil droplets was evaluated in terms of flocculation index (FI), as 134 shown in Table 1. The FI (0h) varied with type and concentration of protein. Basically, the FI 135 (0h) varied considerably from 0.88 in 1.5% LP emulsion to 3.72 in 1% PP emulsion. PP-136 stabilized emulsion exhibited the lowest FI (1.19) at 2% concentration. Whereas, 1.5% 137 concentrations displayed the lowest FI in CP and LP emulsions (1.82 and 0.88 respectively). 138 At any concentration (except to 2% PP emulsion), LP-stabilized emulsions exhibited lower 139 flocculation phenomenon than PP and CP emulsions, which might be due to its lower content 140 of SH and SS bounds (Ladjal E. et al., 2015). Thus, SH groups can form bridge and enhance 141 droplets flocculation. In general, FI% progressively decreased to a minimal value, as the 142 concentration increased from 0.5 to 1.5%, while a further increase in concentration (2%) 143 contrarily led to a gradual increase in FI%. An increase in protein concentration enhanced 144

protein adsorption and surface coverage of oil droplets, which effectively inhibited droplet 145 146 aggregation (Sun and Gunasekaran, 2009). However, further increase in protein concentration (up to 1.5% in our case), could promote depletion flocculation. Interestingly, protein 147 stabilized emulsions showed low FI at 1.5% concentration, suggesting that the oil-water 148 interface is saturated by protein molecules. Joshi and collaborators (2012) suggest a 149 concentration of 1% as interface saturation point in lentil protein stabilized emulsion with oil 150 fraction of 10%. According to Liang and Tang (2014), the flocculated state of droplets 151 displayed inverse trend in pea proteins stabilized emulsions, where FI% progressively 152 increased up to a maximal value, as the concentration increased from 0.25 to 1.0 g/100ml, 153 while a further increase in concentration contrarily led to a gradual decrease in FI%. 154

155 *3.1.3. Flocculation and coalescence stability*

Emulsions are inclined to break down over time through a variety of physicochemical mechanisms, including gravitational separation, flocculation, coalescence and Ostwald ripening. The stability of various emulsions formed at various concentrations upon storage of 24 h was evaluated in terms of flocculation (FI) and coalescence indexes (CI).

The FI (24h) varied with concentration and type of proteins. PP-stabilized emulsion exhibited high FI at 0.5 and 1.0% concentration, and low FI at 1.5 and 2% concentrations. In the contrary, CP emulsion showed high flocculation index at 2% concentration, but the other concentration displayed low values. In the case of LP emulsion, the FI decreased when concentration increased to reach the minimal value at 2.0% concentration.

Regarding coalescence, there was also great variation in the results, depending to the type and concentration of protein. CI% values diverged from 2.98% in 1.5% LP emulsion to 46.51% in 2% LP emulsion. PP-stabilized emulsions showed low CI at 0.5% and 2% concentrations, while CI was more or less high at 1% and 1.5% concentrations respectively. In the case of CP emulsions, the lowest CI value was obtained at 1.5% concentration, and the highest value was

obtained at 0.5% concentration. Differently, LP-stabilized emulsions displayed very low CI at
0.5% and 1.5% concentrations, and high values at 1% and 2% concentrations. Basically,
protein-stabilized emulsions showed good emulsifying stability at 1.5% and 2%
concentrations.

This divergence between protein emulsification behaviors might be due to the difference in 174 physicochemical properties, such as, molecular weight, hydrophobicity, free SH and SS 175 bounds as determined previously (Ladjal E. et al., 2015). Clearly, LP-stabilized emulsions are 176 the most stable comparing to the others. According to our results, the underlying mechanism 177 for emulsion instability was by means of droplet flocculation and coalescence. Stable 178 emulsions can be prepared when the interface is completely covered with particles. However, 179 upon insufficient loading of the interface, droplet coalescence cannot be fully prevented, and 180 partial coalescence may take place (Scholten, Moschakis, & Biliaderis, 2014). The thickness 181 of the interfacial layer determines the magnitude and range of the steric repulsion between 182 droplets. Droplet flocculation can be inhibited if the range of the steric repulsion is longer 183 than the range of any attractive interactions, such as van der Waals or hydrophobic (Chung & 184 McClements, 2014). Hence, emulsion stability at high protein concentration can be attributed 185 to the formation of multilayered protein shell around oil droplet which successfully prevents 186 the coalescence of oil droplets Joshi et al. (2012). 187

188 *3.1.4. Creaming stability*

The creaming index of the three protein emulsions at different protein concentrations, upon quiescent storage up to 7 days was investigated. As expected, various emulsions showed different creaming behaviors, depending on the type of proteins and the applied concentration. Clearly, for any emulsion at 0.5% concentration, the creaming index was the highest. It increased with storage to reach the maximum values on the 7th day. It should be noted that

there was no creaming in 2% CP emulsion and 1.5% and 2% LP emulsion even after 7 days of
storage. In the contrary, low concentration (0.5%) displayed clear creaming even after one
day of storage (in the case of PP and LP emulsions).

These observations indicated that increasing the concentration progressively improved the 197 creaming stability of these emulsions. The improvement of the creaming stability could be in 198 part due to the progressive decreases in $d_{4,3}$ (water dilution; Table 1). A similar improvement 199 of creaming stability upon increasing protein concentration has been observed for emulsions 200 stabilized by pea proteins (Liang and Tang, 2014). Higher protein concentration lowered 201 creaming rate possibly due to the unadsorbed protein in the aqueous phase which increase the 202 viscosity (Sun and Gunasekaran, 2009). Hence, higher protein concentration may facilitate the 203 adsorption of protein to the interface of oil droplets, slightly increase their density, and 204 consequently prevent gravitational separation (Piorkowski and McClements, 2013). In 205 addition, increasing protein concentration enhanced the surface coverage of oil droplets 206 207 against flocculation and reduced the scope for protein bridging, leading to decreased creaming (Sun and Gunasekaran, 2009). 208

For each system, there is a critical concentration of proteins, above which the droplet size and emulsion stability, may be independent of concentration (at constant oil fraction). Under the investigated conditions of the present work, and taking into account emulsifying ability and stability, we can approximately see that this critical concentration was about 1.5%.

213 3.2. Effects of ionic strength on emulsifying properties

Another factor that plays a role in protein-emulsifying properties is salt presence. NaCl affects the protein-emulsifying properties mainly by two mechanisms: (1) salts reduce the electrostatic repulsion between droplets through electrostatic screening and (2) high concentrations of electrolytes alter the structural organization of water molecules, which alters the strength of the hydrophobic interactions between non-polar groups (Zhang et al., 2009).

3.2.1. Droplet size distribution 222

Emulsifying properties of various legume proteins (PP, CP and LP) at different ionic strength 223 (0, 50, 100, 150 and 200 Mm NaCl) were evaluated at pH 3, protein concentration of 1.5% 224 and an oil fraction of 0.1 using droplet-size analysis. The $d_{4,3}$ of the droplets, diluted in 1% 225 SDS or deionized water, was calculated and summarized in Table 2. 226

Emulsions were highly sensitive to changes in ionic strength. Although the particle size in 227 SDS dilution was approximately in the same range (ranging from ≈ 1.5 and 4 µm), the mean 228 229 particle diameter in water dilution significantly increased with NaCl addition, indicating that salt addition promoted droplet aggregation without affecting the initial droplet size. At ionic 230 strength of 0 and 50 mM, the particle diameter of emulsions remained relatively small and 231 emulsions were more stable against aggregation. At relatively high ionic strength ($\geq 100 \text{ mM}$), 232 emulsions were instable manifesting droplet aggregation, maintaining relatively small droplet 233 sizes in SDS dilution. The large diameter in water dilutions, at high ionic strength, is due to 234 the screening of the surface charges that encouraged protein-protein interaction, but, 235 236 however, reduced protein-oil interaction (Zhang et al., 2009). The most likely stabilization mechanism preventing droplet aggregation of protein stabilized emulsion is, hence, 237 electrostatic repulsion (Joye and McClements, 2014). For example, at pH 3, the droplets had a 238 239 high net charge, which would generate a strong electrostatic repulsion that prevents droplet aggregation. By increasing the ionic strength, the net charge on the droplets is decreased and 240 the proteins tend to aggregate (solubility decrease), as a result, emulsion is destabilized by 241 flocculation (Joye and McClements, 2014). 242

High flocculation was observed even though droplet size distribution (SDS dilution at 0h) was 244 not clearly affected by ionic strength. All emulsions were destabilized by salt addition and 245 displayed extensive aggregation behavior expressed as FI (0h). FI (0h) values varied from 246 0.88 to 7.09 in 0 mM LP and 200 mM CP-stabilized emulsions, respectively. Basically, the 247 higher the ionic strength, the higher the FI is. Where, low ionic strength exhibited the lowest 248 FI and vice versa. Interestingly, there was a positive correlation between ionic strength and FI 249 (0h). Flocculation is due to "electrostatic screening" phenomenon, which occurs when the 250 ionic strength of the aqueous phase is increased (Piorkowski and McClements, 2013) (i.e. the 251 accumulation of counter-ions around the surfaces, Salminen and Weiss, 2014). Inducing the 252 neutralization of the protein external charge, as a result, the electrostatic repulsions will be 253 reduced, encouraging the protein aggregation. 254

255 *3.2.3. Flocculation and coalescence stability*

Regarding results in Table 2, it can be seen that, after 24h of storage, the flocculation increased with ionic strength increase. An extensive flocculation (FI, 24h) was observed at high ionic strengths. Markedly, for the three protein emulsions, the FI increased with ionic strength increase, to reach the maximal values at 150 mM of ionic strength. This finding corroborate with Joye and McClements (2014)'s suggestions, indicating that by increasing the ionic strength, the net charge on the droplets is shielded or decreased and the emulsion is destabilized by flocculation.

The coalescence phenomenon also was determined as coalescence index (CI %) (Table 2). For example, CI% was ranging from 2.88% to 156 % in 0 mM LP and 200 mM CP emulsions, respectively. For any emulsion, the highest CI% was found at the highest ionic strength (200 mM). It is well known that, coalescence rate can be reduced if the protein, in addition to decreasing the interfacial tension, can form a film with good viscoelastic properties in the oil–water interface, to resist droplet-droplet collision (Lestari et al., 2011).

Based on our results, we can deduce that, at high ionic strength, legume proteins form films with insufficient steric repulsion and weak viscoelastic properties at the interface, which accelerate emulsion destabilization.

272 *3.2.4. Creaming stability*

Table 3 shows the creaming index of the three protein emulsions at different ionic strengths 273 (from 0 to 200 mM), upon quiescent storage up to 7 days. As expected, emulsions showed 274 275 different creaming behaviors, depending to the type of proteins and the applied ionic strength. Clearly, for any emulsion, the creaming index increase when the ionic strength increase. It 276 increased with storage to reach the maximum values on the 7th day. Interestingly, creaming 277 was the lowest at the lowest ionic strength (0 and 50 mM). LP emulsions exhibited the most 278 stable state comparing to PP and CP stabilized emulsion. The ability of an emulsion to resist 279 creaming is highly dependent on the droplet size, density difference between the dispersed 280 and continuous phases, and the viscosity of the continuous phase. Emulsions with smaller 281 droplets, a lower density contrast between phases, and higher viscosity are more stable to 282 creaming (Karaca et al., 2011). 283

284 As reported in Joshi et al. (2012), low salt concentrations enhance solubility because hydrated salt ions especially the anions weakly bind to the charged group of proteins. This phenomenon 285 is known by "the salting-in effect" of proteins resulting into high solubility in the presence of 286 low concentration of salt (Yuliana, Truong, Huynh, Ho, & Ju, 2014). At low concentrations, 287 salts can stabilize proteins through non specific electrostatic interactions, dependent only on 288 the ionic strength of the medium (Hamada, Arakawa, & Shiraki, 2009), and the increased 289 solubility of proteins comes from the water bound with the ions (Joshi et al., 2012). However, 290 at high concentrations, salts exert specific effects on proteins depending on the type and 291 concentration of the salts (Molina-Bolivar, Galisteo-Gonzalez, & Hidalgo-Alvarez, 2001; 292 Hamada et al., 2009). When NaCl concentration increase (above 0.15 mM), it can rather 293

reduce the protein solubility (Joshi et al., 2012). Since, the monovalent Na⁺ ions are 294 counterions for the negatively charged protein molecules, whereas the monovalent Cl⁻ ions are 295 counterions for the positively charged (Rao, Chen, & Chen, 2009), leading to a decrease in 296 electrostatic repulsion, thus enhancing hydrophobic interactions (Yuliana et al., 2014). When 297 net charge of protein molecules is screened sufficiently, molecules will be able to approach 298 closely enough together to aggregate (Chantrapornchai and McClements, 2002). This can be 299 also attributed to the increase of interfacial tension between the protein surface and bulk 300 solvent (Hamada et al., 2009). In the other hand, the decrease in emulsion stability at high 301 ionic strengths can be attributed also to the hydration of adsorbed counterions (Fisicaro, 302 Compari, & Braibanti, 2011). This phenomenon is known by "the salting-out effect". Proteins 303 can interact with water through hydrogen bonding with greater or comparable strength to 304 water-water interactions. This is because the water molecules prefer to form strong hydrogen 305 306 bonds with the ions instead of weaker bonds with the hydrated biopolymers (proteins) (Salminen and Weiss, 2014). This promotes protein-protein interaction and protein aggregate 307 308 formation, which ultimately results into slow diffusion of protein molecules into oil-water 309 interface at high salt concentration (Joshi et al., 2012). Furthermore, it has also been suggested that salt at its high concentrations can compete with protein for water to ionize 310 itself. This competition effectively reduces the availability of water and increases the protein 311 dehydration (Joshi et al., 2012). When the hydration repulsion becomes negligible compared 312 with the van der Waals attraction, the particles will aggregate (Salminen and Weiss, 2014), 313 encouraging creaming. 314

Likewise, the ionic strength has an effect on protein hydrophobicity. Zhang and co-workers (2009) reported that the emulsifying activity and hydrophobicity of chickpea proteins take the same trend as function of ionic strength; they decrease first and then increase with the increase of ionic strength, the lowest values (both parameters) occurred at ionic strength

0.1M. The ionic strength seems also to have an effect on the conformational structure oflegume proteins (Zhang et al., 2009).

321 Under the investigated conditions of the present work, and taking into account emulsifying
322 ability and stability, we can approximately see that the critical ionic strength was in the range
323 of 0-50 mM NaCl.

324 *3.2.5. Emulsion microstructure*

According to the results of emulsifying properties of the investigated emulsions, we selected two emulsions for each protein; 0 mM and 50 mM NaCl (at 1.5% protein concentration). Fig. 1 shows the CLSM microstructural observations of various fresh emulsions formed at 0 mM and 50 mM of NaCl. Proteins are stained in green and oil droplets appear as dark bubbles.

In emulsions at 0 mM, it can be observed that most of the droplets were present in the 329 separated and unflocculated form. However, in emulsions with 50 mM NaCl, droplets were in 330 flocculated state. The results confirmed that emulsions at 0 mM were stable to aggregation, 331 however, emulsions at 50 mM showed even bigger aggregates. This can be explained by the 332 electrostatic screening and dehydration effects of ionic strength on proteins, thus increasing 333 the attractive interactions of protein-protein as detailed above. Herein, these results evidence 334 335 that emulsions instability in presence of salt was related to protein aggregation, leading to physical separation (coalescence and creaming). It should be noted that LP-stabilized 336 337 emulsion was more sensitive to ionic strength, showing intensive aggregation at 50 mM of NaCl. Our results are in agreement with those reported on whey protein stabilized emulsions 338 containing various concentrations of CaCl₂ (Ye and Singh, 2000). Equally, Shao & Tang 339 (2014) reported the same remark about the effect of NaCl on the microstructure of soy 340 protein-stabilized emulsions. 341

This study confirms that, at pH 3, protein concentration and ionic strength (sodium chloride) 343 have a major influence on the characteristics of legume protein-stabilized emulsions, 344 including, droplet size distribution, flocculation, coalescence creaming and microstructure. 345 Basically, it was found that 1.5 % (w/v) of protein and 0 mM to < 50 mM of NaCl are the best 346 conditions to produce stable emulsions with legume proteins. It should be noted that high 347 ionic strength (>50 mM) promotes flocculation and accelerates destabilization of legume 348 protein emulsions, whilst, high protein concentration improves their stability. Our results 349 suggest the use of these proteins as emulsifiers in acid food formulation, such as, mayonnaise 350 and salad dressing. 351

352 Acknowledgments

The authors wish to thank Béjaia University and the Universidad de Seville for the valorous technical support. The authors acknowledge also to the Microscopy Service (CITIUS-Universidad de Sevilla) for providing full access and assistance.

356 **References**

- Aydemir, L. Y., & Yemenicioglu, A. (2013). Potential of Turkish Kabuli type chickpea and
 green and red lentil cultivars as source of soy and animal origin functional protein
 alternatives. *LWT Food Science and Technology*, 50, 686-694.
- Boye, J.I., Aksay, S., Roufik, S., Ribéreau, S., Mondor, M., Farnworth, E., & Rajamohamed
 S.H. (2010). Comparison of the functional properties of pea, chickpea and lentil protein
 concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Research International*, 43(2), 537-546.
- Carbonaro, M., Maselli, P., & Nucara, A. (2014). Structural aspects of legume proteins and
 nutraceutical properties. *Food Research International*.
 <u>http://dx.doi.org/10.1016/j.foodres.2014.11.007</u>.

- Chantrapornchai, W., and McClements, D. J. (2002). Influence of NaCl on optical properties,
 large strain rheology and water holding capacity of heat induced whey protein isolates
 gels. *Food Hydrocolloids*, 16, 467-476.
- 370 Chung, C. & McClements, D.J. (2014). Structure-function relationships in food emulsions:
- 371 Improving food quality and sensory perception. *Food Structure*, 1, 106-126.
- Etheridge, R. D, Pesti, G. M., & Foster, E. H. (1998). A comparison of nitrogen values
 obtained utilizing the Kjeldahl and Dumas combustion methodologies (Leco CNS) on
 samples typical of an animal nutrition analytical laboratory. *Animal Feed Science and Technology*, 73, 21-23.
- 376 Fisicaro, E., Compari, C., & Braibanti, A. (2011). Hydrophobic hydration processes: thermal
- and chemical denaturation of proteins. *Biophysical Chemistry*, 156,51-67.
- Guo, Q., & Mu, T.H. (2011). Emulsifying properties of sweet potato protein: Effect of protein
 concentration and oil volume fraction. *Food Hydrocolloids*, 25, 98-106.
- Hamada, H., Arakawa, T., & Shiraki, K. (2009). Effect of Additives on Protein Aggregation.
 Current Pharmaceutical Biotechnology, 10, 400-407.
- Joshi, M., Adhikari, B., Aldred, P., Panozzo, J.F., Kasapis, S., & Barrow, C.J. (2012).
 Interfacial and emulsifying properties of lentil protein isolate. *Food Chemistry*, 134, 1343–1353.
- Joye, I. J., & McClements, D. J. (2014). Emulsifying and Emulsion-Stabilizing Properties of
 Gluten Hydrolysates. *Journal of Agricultural and Food Chemistry*, 62, 2623-2630

- Karaca, A. C., Low, N., & Nickerson, M. (2011). Emulsifying properties of chickpea, faba
 bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International*, 44, 2742–2750.
- 390 Kaushal, P., Kumar, V., & Sharma, H.K. (2012). Comparative study of physicochemical,
- functional, antinutritional and pasting properties of taro (*Colocasiaesculenta*), rice
 (*Oryza sativa*) flour, pigeonpea (*Cajanuscajan*) flour and their blends. *LWT Food Science and Technology*, 48 (1), 59-68.
- Ladjal, E. Y., & Chibane, M. (2015). Some physicochemical and functional properties of pea,
 chickpea and lentil whole flours. *International food research journal*, 22(3), 987-996.
- Ladjal, E. Y., Boudries, H., Chibane, M. & Romero, A. (2015). Pea, chickpea and lentil
 protein isolates: Physicochemical characterization and emulsifying properties. *Food Biophysics*. Accepted manuscript. http://dx.doi.org/10.1007/s11483-015-9411-6.
- Lestari, D., Mulderb, W. J., & Sanders, J. P.M. (2011). Jatropha seed protein functional
 properties for technical applications. *Biochemical Engineering Journal*, 53, 297-304.
- 401 Liang, H.-N., & Tang, C.-H. (2013). pH-dependent emulsifying properties of pea
 402 [*Pisumsativum* (*L*.)] proteins. *Food Hydrocolloids*, 33, 309-319.
- Liang, H.-N., & Tang, C.-H. (2014). Pea protein exhibits a novel Pickering stabilization for
 oil-in-water emulsions at pH 3.0. *LWT Food Science and Technology*, 58, 463-469.
- Makri, E., Papalamprou, E., & Doxastakis, G. (2005). Study of functional properties of seed
 storage proteins from indigenous European legume crops (lupin, pea, broad bean) in
 admixture with polysaccharides. *Food Hydrocolloids*, 19, 583–594.

408	Molina-Bolivar, J.A., Galisteo-Gonzalez, F., & Hidalgo-Alvarez R. (2001). Specific cation
409	adsorption on protein-covered particles and its influence on colloidal stability. Colloids
410	and Surfaces B: Biointerfaces, 21, 125–135.

- 411 Papalamprou, E.M., Doxastakis, G. I., & Kiosseoglou, V. (2010). Chickpea protein isolates
- 412 obtained by wet extraction as emulsifying agents. *Journal of the Science of Food and*
- 413 *Agriculture*, 90(2), 304–313.
- 414 Piorkowski, D. T., & McClements, D. J. (2013). Beverage emulsions: Recent developments in
 415 formulation, production, and applications: Review. *Food Hydrocolloids*, 1-37.
 416 http://dx.doi.org/10.1016/j.foodhyd.2013.07.009.
- 417 Rao, J.-J., Chen, Z.-M., & Chen, B.-C. (2009). Modulation and stabilization of silk fibroin-
- 418 coated oil-in-water emulsions. *Food Technology and Biotechnology*, 47 (4), 413–420.
- Salminen, H., & Weiss, J. (2014). Electrostatic adsorption and stability of whey protein-pectin
 complexes on emulsion interfaces. *Food Hydrocolloids*, 35, 410-419.
- 421 Sanchez, C. C., & Patino, J. M. R. (2005). Interfacial, foaming and emulsifying characteristics
- 422 of sodium caseinate as influenced by protein concentration in solution. *Food*423 *Hydrocolloids*, 19, 407-416.
- 424 Scholten, E., Moschakis, T., & Biliaderis, C. G. (2014). Biopolymer composites for
 425 engineering food structures to control product functionality. *Food Structure*, 1, 39-54.
- Shao, Y., & Tang, C.-H. (2014). Characteristics and oxidative stability of soy proteinstabilized oil-in-water emulsions: Influence of ionic strength and heat pretreatment. *Food Hydrocolloids*, 37, 149-158.

- Shen, L., & Tang, C.-H. (2014). Emulsifying properties of vicilins: Dependence on the
 protein type and concentration. *Food Hydrocolloids*, 36, 278-286.
- 431 Sun, C., & Gunasekaran, S. (2009). Effects of protein concentration and oil-phase volume
 432 fraction on the stability and rheology of menhaden oil-in-water emulsions stabilized by
- 433 whey protein isolate with xanthan gum. *Food Hydrocolloids*, 23, 165–174.
- Taherian, A. R., Mondor, M., Labranche, J., Drolet, H., Ippersiel, D., & Lamarche, F. (2011).
 Comparative study of functional properties of commercial and membrane processed
 yellow pea protein isolates. *Food Research International*, 44, 2505–2514.
- Toews, R., & Wang, N. (2013). Physicochemical and functional properties of protein
 concentrates from pulses. *Food Research International*, 52, 445-451.
- Ye, A., & Singh, H. (2000). Influence of calcium chloride addition on the properties of
 emulsions stabilized by whey protein concentrate. *Food Hydrocolloids*, 14, 337–346.
- Yu, J., Ahmedna, M., & Goktepe, I. (2007). Peanut protein concentrate: Production and
 functional properties as affected by processing. *Food Chemistry*, 103, 121-129.
- Yuliana, M., Truong, C. T., Huynh, L. H., Ho, Q. P., & Ju Y.-H. (2014). Isolation and
 characterization of protein isolated from defatted cashew nut shell: Influence of pH and
 NaCl on solubility and functional properties. *LWT Food Science and Technology*, 55,
 621-626.
- Zhang, T., Bo, J., Wanmeng, M., & Zhang, W. (2009). Emulsifying properties of chickpea
 protein isolates: Influence of pH and NaCl. *Food Hydrocolloids*, 23, 146-152.
- 449

Table 1

n	itein % v /v)	d _{4,3} (μm)				Indices			
Emulsid		0 h		24 h		FI		e : (e))	
	Em	Pro (Water	SDS	Water	SDS	0 h	24h	CI (%)
PP emulsions	0.5	9.10±0.56 ^a	2.69±0.07 ^b	9.94±0.69 ^a	3.00±0.13 ^b	2.38	2.31	11.52	
	1.0	8.12±0.24 ^b	1.72±0.01 ^d	9.02±0.81 ^{ab}	1.83±0.01 ^d	3.72	3.40	19.18	
	1.5	6.14±0.70 ^c	2.06±0.14 ^c	6.22±0.67 ^c	2.61±0.15 ^c	1.98	1.37	27.02	
	2.0	8.15±0.27 ^b	3.72±0.18 ^a	8.48±0.04 ^b	4.23±0.03 ^a	1.19	1.00	13.70	
Ilsions	0.5	8.48±0.08 ^a	2.51±0.14 ^ª	8.98±0.7 ^ª	3.64±0.00 ^a	2.37	1.46	45.01	
	1.0	8.00±1.85 ^{ab}	2.75±0.03 ^a	7.50±2.07 ^a	3.56±0.07 ^ª	1.91	1.10	29.45	
emi	1.5	7.41±0.14 ^{ab}	2.62±0.29 ^a	8.15±0.25 ^ª	2.69±0.17 ^b	1.82	1.92	6.48	
С	2.0	6.83±0.31 ^b	1.97±0.02 ^b	6.96±0.31 ^ª	2.24±0.13 ^c	2.46	2.11	13.51	
LP emulsions	0.5	6.69±0.25 ^ª	2.85±0.08 ^ª	8.94±0.97 ^a	2.98±0.12 ^b	1.34	2.00	4.31	
	1.0	6.38±0.83 ^a	2.66±0.12 ^b	8.11±0.34 ^a	3.65±0.11ª	1.39	1.21	37.46	
	1.5	5.26±0.20 ^b	2.79±0.13 ^{ab}	5.59±0.19 ^b	2.87±0.17 ^b	0.88	0.94	2.98	
	2.0	5.19±0.12 ^b	2.05±0.03 ^c	5.62±0.62 ^b	3.01±0.2 ^b	1.52	0.86	46.51	

Emulsion characteristics, including volume-mean droplet size $(d_{4,3})$, flocculation and coalescence indices (FI and CI) of legume protein-stabilized emulsions at various protein concentration, freshly prepared or after a storage of 24 h. FI and CI are calculated using mean values of droplet size $(d_{4,3})$.

Values expressed are mean \pm standard deviation. Means in the column (in the same protein emulsion) with different superscript are significantly different at p< 0.05. PP: Pea protein; CP: Chickpea protein; LP: Lentil protein.

Table 2

Emulsion characteristics, including volume-mean droplet size $(d_{4,3})$, flocculation and coalescence indices (FI and CI) of legume protein-stabilized emulsions at various ionic strength, freshly prepared or after a storage of 24 h. FI and CI are calculated using mean values of droplet size $(d_{4,3})$.

nc	Ionic strength (mM NaCl)	d _{4,3} (μm)					Indices		
Emulsic		0 h		24 h		FI		CI (%)	
		Water	SDS	Water	SDS	0 h	24 h		
	0	6.14±0.70 ^c	2.06±0.14 ^c	6.22±0.67 ^c	2.61±0.15 ^b	1.98	1.37	27.02	
ions	50	6.21±0.18 ^c	2.33±0.10 ^c	7.06±0.36 ^c	2.53±0.09 ^b	1.65	1.78	8.55	
muls	100	10.48±0.23 ^b	3.58±0.52 ^ª	14.86±1.06 ^b	4.20±0.72 ^a	1.82	2.33	20.16	
PP el	150	13.91±0.62 ^ª	2.70±0.04 ^{bc}	16.44±1.21 ^{ab}	3.44 ± 0.10^{ab}	4.14	3.77	27.17	
	200	14.05±0.73 ^ª	3.32±0.67 ^{ab}	17.19±0.98 ^ª	4.72±1.50 ^ª	3.06	2.63	36.56	
	0	7.41±0.14 ^d	2.46±0.12 ^a	8.49±0.33 ^c	2.71±0.15 ^b	2.01	2.13	10.16	
ions	50	7.67±0.15 ^{cd}	1.56±0.11 ^c	8.47±1.23 ^c	2.04±0.09 ^c	3.91	3.14	30.70	
muls	100	8.11±0.09 ^c	1.68±0.01 ^c	10.27±2.31 ^{bc}	2.01±0.07 ^c	3.81	4.10	19.36	
CP ei	150	10.64±0.56 ^b	1.90±0.00 ^b	13.03±3.34 ^b	2.26±0.26 ^c	4.60	4.76	18.94	
	200	12.70±0.37 ^ª	1.57±0.01 ^c	17.83±1.61 ^ª	4.03±0.43 ^a	7.09	3.42	156.68	
٩.	0	5.26±0.20 ^d	2.79±0.13 ^b	5.59±0.19 ^d	2.87±0.17 ^{cd}	0.88	0.94	2.86	
	50	5.61±0.32 ^d	2.95±0.22 ^b	6.78±0.29 ^d	3.41±0.21 ^b	0.89	0.98	15.55	

100	9.35±0.62 ^c	4.28±0.10 ^a	11.24±0.71 ^c	5.07±0.20 ^a	1.18	1.21	18.35
150	12.49±0.59 ^b	2.05±0.0 ^c	20.43±1.78 ^b	2.55±0.18 ^d	5.09	7.00	24.55
200	17.13±2.34 ^a	2.31±0.08 ^c	25.09±1.95 ^a	3.23±0.24 ^{bc}	6.41	6.76	39.82

Values expressed are mean \pm standard deviation. Means in the column (in the same protein emulsion) with different superscript are significantly different at p< 0.05. PP: Pea protein; CP: Chickpea protein; LP: Lentil protein.

Table 3

Creaming index of various legume protein emulsions formed at various ionic strengths, upon storage up to 7 days. Each data is means of at least duplicate measurements.

Emulsion	Ionic strength	Creaming index			
Emuision	(mMNaCl)	1 day	4 days	7 days	
	0	0.00	24.50±1.50	29 .00±1.00	
	50	13.33±5.77	25.22±1.34	27.27±2.36	
PP	100	26.69±7.84	34.67±2.27	35.47±2.82	
	150	26.21±3.35	32.20±5.78	33.00±6.45	
	200	31.92±2.98	37.01±5.92	37.54±5.48	
	0	±	±	+	
	50	+	29.00±0.00	34.00±0.00	
СР	100	29.00±0.00	29.00±0.00	29.00±0.00	
	150	30.00±0.00	31.00±0.00	32.00±0.00	
	200	22.72±3.85	23.63±5.14	23.63±5.14	
	0	0.0±0.0	0.0±0.0	ND	
	50	0.0±0.0	0.0±0.0	ND	
LP	100	11.87±0.88	16.87±2.65	ND	
	150	16.31±0.85	20.76±1.08	ND	
	200	29.86±10.80	36.38±7.45	ND	

Values expressed are mean ± standard deviation. PP: pea protein, CP: chickpea protein and LP: lentil protein. ±: Slight creaming not objectively evaluated; +: clear creaming not objectively evaluated; ND: not determined.

Figure list



PP-50 mM-emulsionCP-50 mM-emulsionLP-50 mM-emulsionFig. 1. CLSM images of legume protein-stabilized emulsions at 0 and 50 mM NaCl. PP: Pea protein, CP:Chickpea protein and LP: Lentil protein.

Highlights

- Emulsifying properties of pea, chickpea and lentil protein isolates at pH 3;
- Effect of protein concentration and ionic strength on emulsifying properties of legume proteins at acidic conditions (pH 3).
- Effect of salt on the microstructure of acidic emulsions stabilized by legume proteins.

freepaper.me 💀 فرى پـيـپر