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

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**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.

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

## 1. Introduction

In the time being, the consumer demands originated from health concerns, allergenicity, religious limitations (Halal) and rising trend of vegetarianism have increased the interest of 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 isolates is of growing interest to industry because of the increasing applications of plant proteins in food and non-food markets (Zhang, Bo, Wanmeng, & Zhang, 2009). Nevertheless, these applications in the food trade are almost limited to proteins from soybean and wheat, 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; Taherian et al., 2011; Toews & Wang, 2013; Liang & Tang, 2014; Shen & Tang, 2014). Among these are those from dry legumes that are extensively grown in different parts of the world and, in particular, in the Mediterranean region.

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).

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

## 2. Material and method

### *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),

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 and lentil) using 0.1 M HCl to precipitate the protein. The protein was recovered by centrifugation and collected (Karaca, Low, and Nickerson, 2011). The pH adjustment values are deduced from our previous study on the protein solubility (Ladjal & Chibane, 2015).The obtained precipitate was washed twice with distilled water (4°C) and re-dispersed in distilled water with pH adjusted to pH 7 with 1M NaOH, and freeze-dried (Boye et al., 2010). The protein contents were determined in quadruplicate as %N x 6.25 using a Leco CHNS-932 nitrogen micro analyser (Leco Corporation, St. Joseph, MI, USA) (Etheridge, Pesti, & Foster, 1998). Their protein contents were 85.7±0.6%, 85.9±0.2% and 84.8±0.1% (fresh matter) for pea protein (PP), chickpea protein (CP) and lentil protein (LP), respectively.

### *2.3. Emulsions preparation*

Three protein solutions with a different protein concentration (0.5 - 2%, w/v) were prepared at 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 with sunflower oil at oil fraction (ø) = 0.1, and pre-homogenized using the high-speed dispersing and emulsifying unit (model IKA-ULTRA-TURRAX- T25 basic, IKA Works, Inc., 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. To investigate the effect of ionic strength, we used the same parameters with protein concentration of 1.5% and the salt concentration (salt dissolved in 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 used were purchased from regular suppliers and were of analytical grade.

### *2.4. Emulsions characterization*

Droplet-size distribution ($d_{4,3}$), flocculation and coalescence indices (FI and CI) as well as creaming indices of prepared emulsions were evaluated as reported by Liang & Tang (2013).

*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).

*2.6.Statistical analysis*

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

## 3. Result and discussion

*3.1. Effect of protein concentration on emulsifying properties*

*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 emulsions exhibited the best emulsifying ability (corresponding to the lowest diameter) at 2%

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

*3.1.2. Flocculation in fresh emulsions*

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

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

155 *3.1.3. Flocculation and coalescence stability*

156 Emulsions are inclined to break down over time through a variety of physicochemical
157 mechanisms, including gravitational separation, flocculation, coalescence and Ostwald
158 ripening. The stability of various emulsions formed at various concentrations upon storage of
159 24 h was evaluated in terms of flocculation (FI) and coalescence indexes (CI).

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

165 Regarding coalescence, there was also great variation in the results, depending to the type and
166 concentration of protein. CI% values diverged from 2.98% in 1.5% LP emulsion to 46.51% in
167 2% LP emulsion. PP-stabilized emulsions showed low CI at 0.5% and 2% concentrations,
168 while CI was more or less high at 1% and 1.5% concentrations respectively. In the case of CP
169 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 physicochemical properties, such as, molecular weight, hydrophobicity, free SH and SS bounds as determined previously (Ladjal E. et al., 2015). Clearly, LP-stabilized emulsions are the most stable comparing to the others. According to our results, the underlying mechanism for emulsion instability was by means of droplet flocculation and coalescence. Stable emulsions can be prepared when the interface is completely covered with particles. However, upon insufficient loading of the interface, droplet coalescence cannot be fully prevented, and partial coalescence may take place (Scholten, Moschakis, & Biliaderis, 2014). The thickness of the interfacial layer determines the magnitude and range of the steric repulsion between droplets. Droplet flocculation can be inhibited if the range of the steric repulsion is longer than the range of any attractive interactions, such as van der Waals or hydrophobic (Chung & McClements, 2014). Hence, emulsion stability at high protein concentration can be attributed to the formation of multilayered protein shell around oil droplet which successfully prevents the coalescence of oil droplets Joshi et al. (2012).

*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 $7^{th}$ day. It should be noted that

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

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

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

213 *3.2. Effects of ionic strength on emulsifying properties*

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

To investigate the effect of ionic strength on emulsifying properties of pea, chickpea and lentil protein, we fixed the same parameters (pH 3, oil fraction 0.1) using the critical protein concentration (1.5%), and then we tested different ionic strengths (0- 200 mM).

*3.2.1. Droplet size distribution*

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

Emulsions were highly sensitive to changes in ionic strength. Although the particle size in SDS dilution was approximately in the same range (ranging from ≈ 1.5 and 4 µm), the mean 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 strength of 0 and 50 mM, the particle diameter of emulsions remained relatively small and emulsions were more stable against aggregation. At relatively high ionic strength (≥100 mM), emulsions were instable manifesting droplet aggregation, maintaining relatively small droplet sizes in SDS dilution. The large diameter in water dilutions, at high ionic strength, is due to the screening of the surface charges that encouraged protein–protein interaction, but, however, reduced protein–oil interaction (Zhang et al., 2009). The most likely stabilization mechanism preventing droplet aggregation of protein stabilized emulsion is, hence, electrostatic repulsion (Joye and McClements, 2014). For example, at pH 3, the droplets had a 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 the proteins tend to aggregate (solubility decrease), as a result, emulsion is destabilized by flocculation (Joye and McClements, 2014).

*3.2.2. Flocculation in fresh emulsions*

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

*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.

*3.2.4. Creaming stability*

Table 3 shows the creaming index of the three protein emulsions at different ionic strengths (from 0 to 200 mM), upon quiescent storage up to 7 days. As expected, emulsions showed 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 increased with storage to reach the maximum values on the $7^{th}$ day. Interestingly, creaming was the lowest at the lowest ionic strength (0 and 50 mM). LP emulsions exhibited the most stable state comparing to PP and CP stabilized emulsion. The ability of an emulsion to resist creaming is highly dependent on the droplet size, density difference between the dispersed and continuous phases, and the viscosity of the continuous phase. Emulsions with smaller droplets, a lower density contrast between phases, and higher viscosity are more stable to creaming (Karaca et al., 2011).

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 is known by “the salting-in effect” of proteins resulting into high solubility in the presence of low concentration of salt (Yuliana, Truong, Huynh, Ho, & Ju, 2014). At low concentrations, salts can stabilize proteins through non specific electrostatic interactions, dependent only on the ionic strength of the medium (Hamada, Arakawa, & Shiraki, 2009), and the increased solubility of proteins comes from the water bound with the ions (Joshi et al., 2012). However, at high concentrations, salts exert specific effects on proteins depending on the type and concentration of the salts (Molina-Bolivar, Galisteo-Gonzalez, & Hidalgo-Alvarez, 2001; Hamada et al., 2009). When NaCl concentration increase (above 0.15 mM), it can rather

reduce the protein solubility (Joshi et al., 2012). Since, the monovalent $Na^+$ ions are counterions for the negatively charged protein molecules, whereas the monovalent $Cl^-$ ions are counterions for the positively charged (Rao, Chen, & Chen, 2009), leading to a decrease in electrostatic repulsion, thus enhancing hydrophobic interactions (Yuliana et al., 2014). When net charge of protein molecules is screened sufficiently, molecules will be able to approach closely enough together to aggregate (Chantrapornchai and McClements, 2002). This can be also attributed to the increase of interfacial tension between the protein surface and bulk solvent (Hamada et al., 2009). In the other hand, the decrease in emulsion stability at high ionic strengths can be attributed also to the hydration of adsorbed counterions (Fisicaro, Compari, & Braibanti, 2011). This phenomenon is known by "the salting-out effect". Proteins can interact with water through hydrogen bonding with greater or comparable strength to water-water interactions. This is because the water molecules prefer to form strong hydrogen 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 formation, which ultimately results into slow diffusion of protein molecules into oil-water 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 itself. This competition effectively reduces the availability of water and increases the protein dehydration (Joshi et al., 2012). When the hydration repulsion becomes negligible compared with the van der Waals attraction, the particles will aggregate (Salminen and Weiss, 2014), encouraging creaming.

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 of legume proteins (Zhang et al., 2009).

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

*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 separated and unflocculated form. However, in emulsions with 50 mM NaCl, droplets were in flocculated state. The results confirmed that emulsions at 0 mM were stable to aggregation, however, emulsions at 50 mM showed even bigger aggregates. This can be explained by the electrostatic screening and dehydration effects of ionic strength on proteins, thus increasing the attractive interactions of protein-protein as detailed above. Herein, these results evidence 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 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 containing various concentrations of $CaCl_2$ (Ye and Singh, 2000). Equally, Shao &Tang (2014) reported the same remark about the effect of NaCl on the microstructure of soy protein-stabilized emulsions.

## 4.Conclusion

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

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449

**Table 1**

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 ($d_{4,3}$).

| Emulsion | Protein % (w /v) | $d_{4,3}$ (µm) | | | | Indices | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 h | | 24 h | | FI | | CI (%) |
| | | Water | SDS | Water | SDS | 0 h | 24h | |
| 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 |
| CP emulsions | 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 |
| | 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 |
| | 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±0.11$^{a}$ | 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 |

Values expressed are mean ± 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 ($d_{4,3}$).

| Emulsion | Ionic strength (mM NaCl) | $d_{4,3}$(µm) | | | | Indices | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 h | | 24 h | | FI | | CI (%) |
| | | Water | SDS | Water | SDS | 0 h | 24 h | |
| PP emulsions | 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 |
| | 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 |
| | 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 |
| | 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 |
| CP emulsions | 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 |
| | 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 |
| | 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 |
| | 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 |
| LP | 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 ± 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 (mMNaCl) | Creaming index | | |
|---|---|---|---|---|
| | | 1 day | 4 days | 7 days |
| **PP** | 0 | 0.00 | 24.50±1.50 | 29 .00±1.00 |
| | 50 | 13.33±5.77 | 25.22±1.34 | 27.27±2.36 |
| | 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 |
| **CP** | 0 | ± | ± | + |
| | 50 | + | 29.00±0.00 | 34.00±0.00 |
| | 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 |
| **LP** | 0 | 0.0±0.0 | 0.0±0.0 | ND |
| | 50 | 0.0±0.0 | 0.0±0.0 | ND |
| | 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

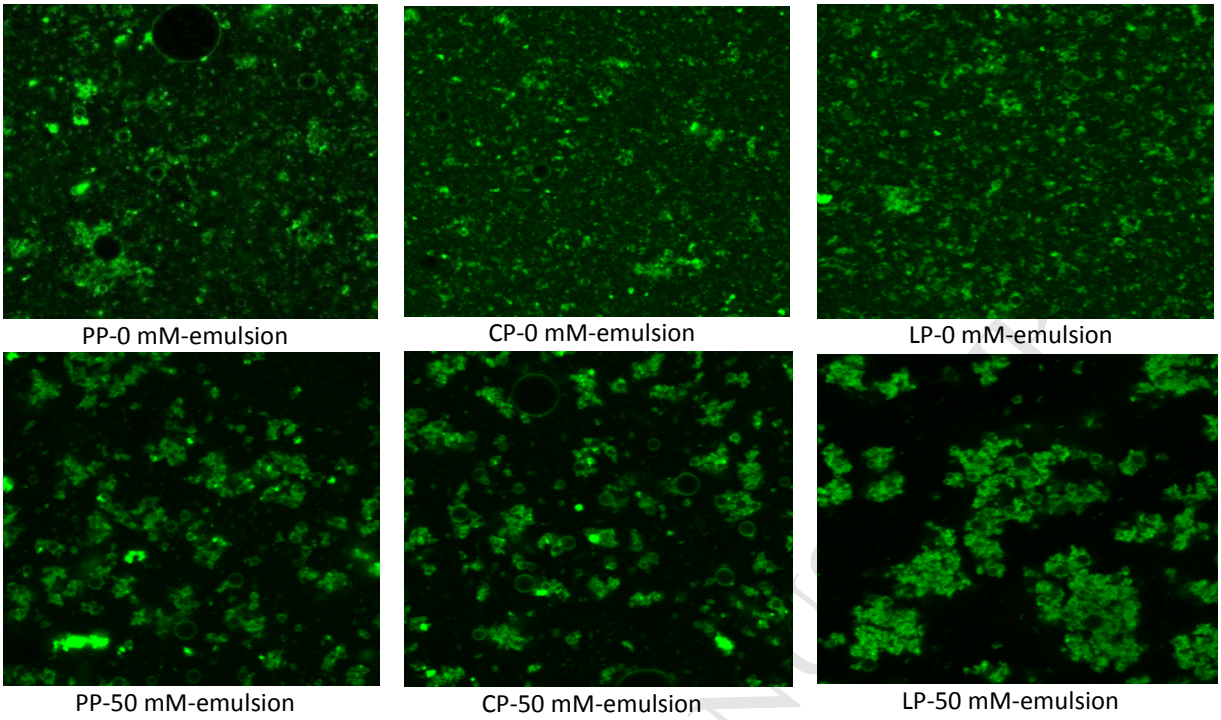


Fig. 1. CLSM images of legume protein-stabilized emulsions at 0 and 50 mM NaCl. PP: Pea protein, CP: Chickpea protein and LP: Lentil protein.

## 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.