This document is a postprint version of an article published in LWT – Food Science and Technology © Elsevier after peer review. To access the final edited and published work see https://doi.org/10.1016/j.lwt.2018.08.033

1 **Characterization of functional properties of proteins from *Ganxet* beans**
2 **(*Phaseolus vulgaris* L. var. *Ganxet*) isolated using an ultrasound-assisted**
3 **methodology**

4 Tomás Lafarga [a], Carlos Álvarez [b], Gloria Bobo [a], and Ingrid Aguiló-Aguayo [a*]

5 [a] IRTA, XaRTA-Postharvest, Parc Científic i Tecnològic Agroalimentari de Lleida, Parc de
6 Gardeny, Edifici Fruitcentre, 25003, Lleida, Catalonia, Spain.
7 [b] Department of Food Quality and Sensory Analysis, Teagasc Food Research Centre, Dublin 15,
8 Ireland.
9
10
11 [*]**Corresponding author**:
12 Dr. Aguiló-Aguayo
13 Phone: +34 973003431, email: Ingrid.Aguilo@irta.cat
14
15
16 **Abbreviations:**
17 a_w: water activity; ISP: isoelectric solubilisation-precipitation; WHC: water holding
18 capacity; OHC: Oil holding capacity; MW: molecular weight; GPC: *Ganxet* protein
19 concentrate; DW: dry weight; FC: foaming capacity; FS: foaming stability; EC:
20 emulsifying capacity; ES: emulsion stability; C^*_{ab}: Chroma; δE: difference from the
21 control; S.D.: standard deviation; ANOVA: Analysis of variance.

Abstract

This study investigated different methods of extraction of protein from *Ganxet* beans (*Phaseolus vulgaris* L. var. Ganxet) and evaluated the functional properties of these valuable proteins. Overall, ultrasound processing (40 kHz, 250 W) resulted in higher yields and increased percentages of material solubilized and proteins recovered. The highest percentage of recovered protein was obtained after extraction using 0.4 M NaOH followed by ultrasound processing for 60 min and was calculated as 78.73±4.88% ($p<0.05$). Extraction using 0.4 M NaOH followed by sonication for 60 min resulted in the highest yield and percentage of solubilized material calculated as 37.98±0.02 and 54.58±0.19%, respectively ($p<0.05$). The water- and oil-holding capacities of the *Ganxet* protein concentrate were calculated as 2.33±0.12 and 2.69±0.32 g of water or oil per g of protein concentrate, respectively. The highest emulsifying capacity was observed at pH 8.0 and was calculated as 69.4±0.8%.

Keywords: functional properties, vegetable proteins, protein extraction, protein solubilization, common beans, Ganxet beans, ultrasound-assisted extraction

37 **1. Introduction**

38 Common beans (*Phaseolus vulgaris* L.) are a group of plants that fix atmospheric nitrogen
39 and are included among pulses. Dry seeds of common beans are excellent protein sources.
40 Indeed their protein content can be up to three fold higher than in cereals (Rivera, Roselló,
41 & Casañas, 2015) and contribute to approximately 6 g of protein per capita and per day
42 in several developing countries (Luna-Vital, Mojica, González de Mejía, Mendoza, &
43 Loarca-Piña, 2015). *Ganxet* beans (*Phaseolus vulgaris* L. var. *Ganxet*) are easily
44 recognized for the squashed and hooked shape of their seeds and are one of the most
45 prestigious bean landraces cultivated in Europe. Indeed, the *Ganxet* bean has Protected
46 Designation of Origin status from the EU (EU, 2011) and is known for its high protein
47 content, which ranges between 24 and 29% (Rivera et al., 2015).
48 Plant-derived proteins are cheaper to produce, when compared to animal-derived
49 proteins, and an increase in their utilization replacing the latter could be beneficial in
50 terms of preventing the effects of climate change (Garcia-Vaquero, Lopez-Alonso, &
51 Hayes, 2017). Isoelectric solubilisation-precipitation (ISP) enabled protein recovery from
52 a variety of sources including seaweed (Kadam, Álvarez, Tiwari, & O'Donnell, 2017) and
53 fish (Álvarez, Lélu, Lynch, & Tiwari, 2018). When applied to vegetable sources, this
54 strategy is generally used in combination with enzymatic hydrolysis, which involves the
55 use of enzymes that degrade the cell wall, facilitating the release of proteins and other
56 strategies such as chemical hydrolysis or subcritical water hydrolysis (Kadam et al.,
57 2017). Non-conventional strategies such as the use of high pressure processing,
58 pressurized liquid extraction, microwaves, or sonication can also be used to enhance the
59 extraction rate and yield of the process. Sonication has shown to be efficient in facilitating
60 the extraction of several compounds from plant sources such as oils (Samaram et al.,
61 2015), phenolic compounds (Rodrigues, Fernandes, de Brito, Sousa, & Narain, 2015),

62 and carbohydrates (Chen, You, Abbasi, Fu, & Liu, 2015). This technology can also be
63 used for enhancing the extraction of proteins from vegetable-derived sources. Indeed,
64 Roselló-Soto et al. (2015) recently reported a significant improvement in the extraction
65 of proteins from olive kernel after processing using either electrical discharges, pulsed
66 electric fields, or ultrasounds. In a different study, the use of ultrasounds in combination
67 with sequential extraction of proteins allowed the recovery of practically 100% of total
68 protein from mackerel (Álvarez et al., 2018).

69 Proteins are used in the food industry not only for their nutritional importance but also
70 for their excellent techno-functional properties. The majority of proteins currently utilized
71 as techno-functional ingredients in the food industry are derived from soy or animal
72 sources. However, the utilization of animal-derived proteins is restricted by cultural,
73 religious, and traditional factors. In addition, the proportion of individuals choosing to
74 follow a vegan diet has increased significantly in recent years especially in more affluent
75 countries (Radnitz, Beezhold, & DiMatteo, 2015) and the utilization of vegetable-derived
76 proteins for the development of vegan foods is one of the top trends in the food industry.
77 Therefore, the aim of the present study was to investigate the efficiency of ultrasound-
78 assisted ISP (ISP-US) processing on the protein extraction yield from *Ganxet* beans and
79 to optimize such extraction using environmentally friendly technologies. The effect of the
80 extraction methodology on the average molecular weight (MW) of the isolated proteins
81 was studied and some key techno-functional properties were determined.

82 **2. Materials and methods**

83 **2.1 Chemicals and reagents**

84 Sodium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate,
85 sodium hydroxide, and hydrochloric acid were purchased from Sigma-Aldrich
86 (Barcelona, Spain). The Quick Start™ Bradford Protein assay kit was purchased from
87 Bio-Rad Laboratories (Barcelona, Spain). Sodium dodecyl sulphate (SDS), tris-tricine 4-
88 20% SDS gels, Coomassie Blue G-250, Coomassie Brilliant Blue R-250 de-staining
89 solution, and the Bio-SafeTM Coomassie Blue G-250 stain were purchased from Fischer
90 Scientific (Dublin, Ireland). Borges Solnatur® sunflower oil (Borges Branded Foods,
91 Lleida, Spain) was purchased locally.

92 **2.2 Protein extraction and determination**

93 Dried *Ganxet* beans were kindly provided by Fundació Miquel Agustí, Barcelona, Spain.
94 Sample processing was performed at the pilot plant of the IRTA Fruitcentre in Lleida,
95 Spain. Dried *Ganxet* seeds were milled to a thin powder using a MINIMOKA GR-020
96 grinder (Taurus Group, Barcelona, Spain) and passed through a sieve of 1 mm. Sieved
97 samples were mixed with alkaline solutions of different concentrations, listed in Table 1,
98 at a sample:solvent ratio of 1:10 (w/v). The mixture was homogenized for 30 s using a T-
99 25 digital ULTRA-TURRAX® homogenizer (IKA, Staufen, Germany) at 14,000 rpm.
100 Homogenized samples were immediately placed in a stirrer allocated in a cold room at 4
101 ºC for 15 min. After this period samples were either left untreated or processed using an
102 ultrasonic bath (JP Selecta S.A., Barcelona, Spain) operating at 4 ºC, 40 kHz, and 250 W
103 for 30 or 60 min. Samples were then centrifuged using a Sigma 3-18 KS centrifuge
104 (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at 10,000 × *g* for 20 min.
105 Proteins in the supernatant were precipitated by shifting the pH value to 5.5 (value

106 selected based on preliminary trials in which we obtained the highest recoveries).
107 Precipitates obtained were separated by centrifugation at 10,000 × *g* for 20 min, frozen,
108 freeze-dried using a Crydos-50 freeze-dryer (Telstar, Barcelona, Spain), and stored at -20
109 ºC until further analysis.

110 The total protein content of the dried *Ganxet* beans was determined in duplicate using a
111 LECO FP628 Protein analyser (LECO Corp., MI) based on the Dumas method. The
112 protein content of the solubilized proteins was determined using the Quick Start™
113 Bradford Protein assay kit (Bio-Rad Laboratories Inc., CA, USA) following the
114 manufacturers' instructions and using BSA as a standard. The yield of the extraction
115 process was calculated as g of *Ganxet* protein concentrate (GPC) obtained per 100 g of
116 *Ganxet* bean on a dry weight (DW) basis. The percentage of protein recovered was
117 calculated based on the yield and the protein content in the GPC using the equation:

118 $$Recovered\ protein\ (\%) = \frac{W_{GPC} \cdot PC_{GPC}}{W_G \cdot PC_G} \cdot 100$$

119 where W_{GPC} is the amount of GPC obtained expressed in g, PC_{GPC} is the protein content
120 in the precipitated GPC expressed as g of protein per 100 g of GPC, W_G is the amount of
121 milled and sieved *Ganxet* beans used for protein precipitation expressed in g, and PC_G is
122 the protein content of *Ganxet* beans expressed as g of protein per 100 g of *Ganxet* bean.
123 The water activity (a_w) of the isolated dried proteins was measured in triplicate using an
124 AquaLab meter (Decagon Devices Inc., WA, USA) at 22.0 ± 0.9 ºC.

125 **2.4 SDS-PAGE profile**

126 MW profile of proteins extracted from *Ganxet* bean was determined by means of SDS-
127 PAGE electrophoresis following Laemmli's methodology (Laemmli, 1970). Freeze-dried
128 samples were solved in 0.25 M NaOH solution to a final concentration of 6 mg/mL
129 extract/buffer, and vortexed for 30 s. Laemmli buffer was added to the samples at a

130 sample:solvent ratio of 1:1 (v/v) and the mixture was boiled for 5 minutes at 95 ºC in the
131 presence of β-mercaeptanol. Pre-cast mini-gels of 4-20% gradient from Bio-Rad were
132 employed and a volume of 15 µL of sample was loaded. A MiniProtean II (Bio-Rad)
133 apparatus was employed to run the gels, using a tris-glycine buffer, 50 V were employed
134 for the first 30 minutes, followed by 190 V until the end of the run, as recommended by
135 the manufacturer. Finally, Coomassie staining was carried out and Precision Plus
136 Protein™ Dual Xtra Prestained Protein Standards, ranging from 2 to 250 kDa, were
137 employed. Molecular weight of samples were calculated based on the distance moved
138 from the separation gel, according the a calibration curve (r^2 = 0.996).

139 **2.5 Colour evaluation**

140 Colour recordings of the precipitated and freeze-dried GPC were taken in triplicate using
141 a Minolta CR-200 colorimeter (Minolta INC, Tokyo, Japan). CIE values were recorded
142 in terms of *L** (lightness), *a** (redness/ greenness), and *b** (yellowness/blueness).
143 Calibration was carried out using a standard white tile provided by the manufacturer and
144 the D65 illuminant, which approximates to daylight. Chroma (C^*_{ab}) and difference from
145 the control (δE) were calculated following the methodology described by Wibowo et al.
146 (2015).

147 **2.6 Assessment of functional properties**

148 The water- (WHC) and oil-holding capacity (OHC) of the *Ganxet* protein concentrates
149 were determined following the method of Raikos, Neacsu, Russell, and Duthie (2014).
150 Determinations were carried out in triplicate for each sample and replicate and results
151 were expressed either as g of water or sunflower oil per g of protein concentrate.
152 The foaming capacity (FC) of the isolated proteins was determined as described by Poole,
153 West, and Walters (1984) with some modifications. Briefly, *Ganxet* proteins were re-

154 suspended in ultrapure water at a concentration of 1.5% (w/v) and the pH was adjusted to
155 either 2, 4, 6, 8, or 10. Protein suspensions were homogenized using a T-25 digital
156 ULTRA-TURRAX® homogenizer (IKA, Staufen, Germany) at 10,000 rpm for 1 min and
157 the volume of foam generated was measured in a graduated cylinder. FC was measured
158 as the volume of foam generated as a percentage of the initial volume and foaming
159 stability (FS) was expressed as the percentage of decrease of foam volume over time as
160 described by Garcia-Vaquero et al. (2017).
161 The emulsifying capacity (EC) of the GPC was determined following the methodology
162 of Naczk, Diosady, and Rubin (1985) with minor modifications. Briefly, the freeze-dried
163 proteins were re-suspended in ultrapure water at a concentration of 1.5% (w/v) and the
164 pH was adjusted to either 2, 4, 6, 8, or 10. The protein solution was homogenized using a
165 T-25 digital ULTRA-TURRAX® homogenizer (IKA, Staufen, Germany) at 14,000 rpm
166 for 30 s. To create emulsions, sunflower oil was added to the aqueous phase containing
167 the suspended protein at an oil to protein solution ratio of 3:2 (v/v) following the
168 methodology published by Garcia-Vaquero et al. (2017). Oil was added in 2 steps.
169 Approximately half of the oil was added and in the first step the mixture was homogenized
170 at 14,000 rpm for 30 s. In the second step, the remaining oil was added and the mixture
171 was further homogenized at 14,000 rpm for 90 s. The emulsion was placed in centrifuge
172 tubes and the volume of the emulsion layer was measured. The EC and the emulsion
173 stability (ES) were determined as previously described by Garcia-Vaquero et al. (2017).
174 ES was determined immediately after the emulsion was created.

175 **2.7 Statistical analysis**

176 Results are expressed as mean ± standard deviation (S.D.). Differences between samples
177 were analysed using analysis of variance (ANOVA) with JMP 13 (SAS Institute Inc.,
178 Cary. USA). Where significant differences were present, a Tukey pairwise comparison

179 of the means was conducted to identify where the sample differences occurred. The
180 criterion for statistical significance was $p<0.05$. To identify relationships between
181 parameters, bivariate Pearson's correlation analysis was carried out.

182 **3. Results and discussion**

183 3.1 Protein extraction

184 The total protein content of *Ganxet* beans was calculated as 24.7 ± 0.4% which is in the
185 range 24 - 29% previously reported by Rivera et al. (2015). Similar protein contents were
186 reported previously for other pulses including faba beans, lentils, and lupins (Boye, Zare,
187 & Pletch, 2010; Lizarazo et al., 2015).
188 The current study evaluated the use of alkaline extraction for obtaining high protein
189 recoveries from *Ganxet* beans. The effect of US processing (40 kHz, 250 W) during 30
190 or 60 min on different parameters of the extraction process such as yield, percentage of
191 solubilized material, and percentage of protein recovered was also evaluated. Results,
192 listed in Table 1 demonstrated that both, pH and US processing, significantly affected the
193 extraction process (p<0.05). When the extraction was carried out using lower NaOH
194 concentrations (T1-T6), US processing did not affect the amount of GPC obtained per
195 100 g of raw material. However, as the resulting GPCs contained a higher protein content,
196 the amount of protein recovered was significantly higher when compared to the untreated
197 samples (p<0.05). US processing resulted in increased yields when the extraction was
198 done using NaOH at 0.3 and 0.4 M (T7-T12; p<0.05). Similar results were obtained after
199 US processing of peanuts (Ochoa-Rivas, Nava-Valdez, Serna-Saldívar, & Chuck-
200 Hernández, 2017), seaweed (Kadam et al., 2017), and fish (Álvarez et al., 2018). Other
201 novel technologies such as pulsed electric fields or microwaves also resulted in increased
202 protein recovery yields previously (Sarkis et al., 2015). Both the percentage of raw sample
203 solubilized and percentage of protein recovered were significantly higher after US
204 processing for 60 min when the extraction was performed using solutions of NaOH of
205 different concentrations but mainly 0.3 and 0.4 M (p<0.05). This could be caused by the
206 collapse of bubbles generated during US processing, which liberates energy that promotes

207 a deeper penetration of the solvent into the cell material and enhances the mass transfer
208 from and to the interface (Ochoa-Rivas et al., 2017). The pH used for protein extraction
209 also affected the percentage of solubilized material, which was found to be significantly
210 higher after extractions performed using higher NaOH concentrations (T7-T12; $p<0.05$).
211 A similar trend was observed for the GPC yield, which was significantly higher when
212 using higher sodium hydroxide concentrations especially 0.4 M (T10-T12; $p<0.05$).
213 Results suggest that the cell wall was disrupted by the strong alkali conditions facilitating
214 the release of proteins. It is important to highlight that in the current study, solubilized
215 proteins were precipitated by adjusting the pH to 5.5. Although we observed higher
216 recoveries at this pH, the precipitation step was not optimized for this raw material and
217 not all the solubilized material is precipitated. The precipitation yield depends on the
218 extraction conditions and the precipitation process seems to be more efficient at high
219 NaOH molarities, and this could be the reason for the observed increase in the protein
220 yield. Similar results were obtained after protein extraction from seaweed, where an
221 increase in the pH from 8.5 to 11.0 resulted in a two-fold protein recovery rate (Parniakov
222 et al., 2015). The percentage of protein recovered was higher after US processing
223 ($p<0.05$). When the extraction was performed using a sodium hydroxide concentration
224 of 0.2 M followed by US processing during 60 min (T6), an increase in the protein
225 recoveries were observed when compared to those obtained after processing for 30 min
226 (T5; $p<0.05$). A similar trend was observed after extraction using 0.3 and 0.4 M NaOH,
227 (T12-T11 and T9-T8, respectively) but the observed differences were not statistically
228 significant. For those samples left unprocessed, the concentration of sodium hydroxide
229 used did not affect the percentage of protein recovered.
230 Previous studies reported higher protein yields after alkaline extraction when compared
231 to acid extraction (Chen & Jaczynski, 2007; Taskaya, Chen, & Jaczynski, 2009). A recent

232 study published by Álvarez et al. (2018) suggested that sequential alkaline/acid extraction
233 assisted by US was efficient in recovering approximately 100% of total protein in
234 mackerel whole fish and this strategy could be useful in increasing the protein yield and
235 optimizing the extraction process.

236 **3.2 SDS-PAGE protein profile**

237 Lyophilized samples were analyzed in order to determine the MW distribution of the
238 extracted proteins and to see if the differences observed in the calculated yields were due
239 to the fact that different proteins were extracted under different conditions. Because of
240 the extraction procedure, which was carried out at high pH values, the solubility of the
241 extracted proteins at pH 4.0-7.5 was very low. Therefore, proteins soluble at neutral pHs
242 were probably not extracted and remained in the insoluble material. In addition, the high
243 pH values needed to solubilize the *Ganxet*-derived proteins, which were higher than those
244 accepted by the column, did not allow a size-exclusion analysis. According to Figure 1,
245 no differences in the protein profile of the samples extracted under different conditions
246 are appreciable, meaning that higher yields were originated by increased extraction levels
247 of the same proteins. In addition, no degradation or protein fragmentation was observed
248 due to the increased sodium hydroxide concentration and neither because of the increased
249 ultrasound processing time. Two high MW bands (92.98 and 74.08 kDa), corresponding
250 to convicilin were observed, followed by the most abundant protein with a MW of 41.29
251 kDa, which could be vicilin. Two minor bands of 30.83 and 26.12 kDa were also
252 observed, which according to literature will probably represent α- and β-legumin. Finally,
253 very weak bands corresponding to molecular sizes of 12.03 and 9.89 kDa were also
254 obtained. The protein profile observed was highly similar to those reported by Mirali, El-
255 Khouri, and Rizq (2007) and Nikolić, Đorđević, Torbica, and Mikić (2012), which were
256 found to be highly dependent on the variety of *Vicia faba* employed. These same authors

257 reported how the polymorphism present in *Vicia* species could alter the profile of the
258 extracted proteins.

259 **3.3 Functional properties**

260 Functional properties of proteins extracted only under the highest yield conditions, that is
261 using 0.4 M NaOH of followed by US processing for 60 min, were assessed and compared
262 to those of previously reported vegetable-derived proteins.

263 **3.3.1 Colour**

264 Table 2 lists the colour parameters of both protein-rich powders. The L^* parameter which
265 denotes lightness and varies from 0 (black) to 100 (white) was measured as 91.40 ± 1.63.
266 This value was high when compared to that obtained for other vegetable-derived proteins
267 such as hempseed meal protein isolates prepared by either micellization (82.80 ± 0.31) or
268 isoelectric precipitation (56.39 ± 0.29) (Hadnađev et al., 2018) or kidney bean, field pea,
269 or amaranth protein isolates which showed L^* values of 79.6 ± 0.1, 88.1 ± 0.2, or 78.0 ±
270 0.8, respectively (Shevkani & Singh, 2015). This denotes a lighter appearance of *Ganxet*
271 proteins when compared to proteins derived from other vegetables. The GPC showed a
272 similar C^*_{ab} value, a quantitative indicator of the intensity of a distinctive hue, when
273 compared to other vegetable-derived proteins (Garcia-Vaquero et al., 2017; Hadnađev et
274 al., 2018), suggesting that the colour intensity of the precipitated *Ganxet* proteins was
275 similar to that of previously isolated vegetable-derived proteins. In addition, the δE
276 combines the change in L^*, a^*, and b^* values to quantify the colour deviation from a
277 standard reference sample, in this case, previously reported protein-rich powders derived
278 from vegetables. Those samples with $\delta E > 3$ display a visible colour deviation (Wibowo
279 et al., 2015). The δE was higher than 3 when comparing GPC with proteins derived from
280 kidney bean, field pea, amaranth (Shevkani & Singh, 2015), hemp seed (Hadnađev et al.,
281 2018), soybean, pigeon, and cowpea (Garcia-Vaquero et al., 2017) and other beans (Wani,

282 Sogi, Shivhare, & Gill, 2015). To the best of the authors' knowledge, there are no studies
283 on the colour of *Ganxet* protein extracts and the colour of the protein concentrate obtained
284 herein was perceptually different to that of other vegetable-derived proteins. However,
285 no visible colour deviation ($\delta E > 3$) was observed between GPC and powders derived
286 from milk such as a whey protein isolate and sodium caseinate (Krupa-Kozak, Bączek, &
287 Rosell, 2013) which are currently commercialized protein-rich powders.

288 **3.3.2 Water activity**

289 The a_w of the GPC isolated in the current study was 0.180 ± 0.002, measured at 22.0 ±
290 0.9 ºC. The low a_w value suggests that the generated powder would be stable during
291 storage as a_w values in the range 0.1 – 0.3 usually do not enable microbial growth. The
292 a_w values obtained herein were low when compared to those reported previously for
293 proteins isolated from varied sources including seaweed (Garcia-Vaquero et al., 2017) or
294 blood (Lafarga, Rai, O'connor, & Hayes, 2016). Results suggest that GPC could be
295 commercialized as a dry powder as its a_w value is comparable to that of soluble powders
296 commercialized such as coffee or chocolate (Schmidt & Fontana, 2007).

297 **3.3.3 Water- and oil-holding capacity**

298 Interactions of water and oil with proteins are important for the food industry because of
299 their effects on the flavour and texture of foods (Kumar, Ganesan, Selvaraj, & Rao, 2014).
300 The WHC of the extracted GPC was 0.98 ± 0.10 g of water per g of GPC. These values
301 were lower when compared to those obtained for different varieties of kidney beans
302 including *French*, *Yellow*, and *Master* beans which varied from 5.34 to 5.85 g of water
303 per g of sample (Wani et al., 2015). However, results obtained herein were in line with
304 those reported previously for cowpea proteins and within the range of the commercial
305 values of protein concentrates (Ragab, Babiker, & Eltinay, 2004). Similar WHC values
306 were also reported recently for hemp seed protein isolates prepared by micellization,

307 calculated as 0.80 ± 0.03 g/g (Hadnađev et al., 2018). High WHC values help to maintain
308 freshness and moist mouth feel of foods and have been associated with reduced moisture
309 loss in packed bakery goods (Garcia-Vaquero et al., 2017). High WHC values are
310 desirable in viscous foods such as sausages, custards, or baked products as this would
311 help to hold water without dissolution of protein, providing thickening and viscosity
312 (Seena & Sridhar, 2005). Differences in WHC values could be attributed to different
313 protein structures and low availability of polar amino acids which have been shown to be
314 primary sites for water interaction of proteins (Li, Shu, Yan, & Shen, 2010).
315 In addition, the OHC of the GPC generated herein was calculated as and 2.33 ± 0.12 g of
316 sunflower oil per g of GPC. This value was low when compared to that obtained
317 previously for kidney bean which ranged from 5.8 to 6.9 g of oil per g of sample (Wani
318 et al., 2015) and *Bambara* groundnut protein, which ranged from 6.7 to 7.2 g of oil per g
319 of sample (Adebowale, Schwarzenbolz, & Henle, 2011). The OHC value of GPC was
320 higher than that of hemp seed proteins, which ranged between 1.62 ± 0.06 and 1.79 ±0.02
321 g of oil per g of protein isolate, depending on the isolation method (Hadnađev et al.,
322 2018). The use of different vegetable oils between the current paper and the above
323 mentioned studies could partially explain the observed differences in OHC values, as
324 functional properties can be affected by using different vegetable oils (Garcia-Vaquero et
325 al., 2017). Similar OHC values were reported for mung beans, which ranged from 1.00
326 to 3.38 mL of oil per g of sample (Li et al., 2010), and chickpea isolates which varied
327 from 2.08 to 2.96 mL/g (Kaur & Singh, 2007), depending on the cultivar.

328 **3.3.4 Foaming capacity and foam stability**

329 Figure 2 shows the FC and FS of *Ganxet* bean proteins. The highest FC, calculated as
330 65.0 ± 3.5%, was observed at pH 2.0 (p<0.05). Results were in line with those obtained
331 for other protein sources such as seaweed (Kumar et al., 2014) or cowpea (Ragab et al.,

332 2004). Results were also comparable to those reported by Li et al. (2010), who calculated
333 the FC of several mung beans which ranged between 33.00 ± 2.20 and 67.50 ± 1.04%,
334 depending on the cultivar. In the current study, an increase was observed in the FC at pH
335 10 when compared to pH 4-8 ($p<0.05$) and no statistically significant differences where
336 observed in the FC when assessed at pHs 4, 6, or 8. Proteins in foams contribute to the
337 uniform distribution of fine air cells in food matrices promoting smoothness and lightness
338 of foods (Garcia-Vaquero et al., 2017). Previous studies also observed higher FC of
339 proteins at extreme pH values such as 2.0 (Kumar et al., 2014) and 10.0 (Garcia-Vaquero
340 et al., 2017) probably caused by increased net charges on the protein, which weakened
341 the hydrophobic interactions but increased the flexibility of the protein (Ragab et al.,
342 2004). Protein structural properties can also affect FC. Good foaming abilities have been
343 related with flexible proteins that can reduce surface tension and globular proteins, which
344 are difficult to surface denature, give low foaming properties (Kaur & Singh, 2007). FS
345 represents the percentage volume of foam remaining after a specified time as the initial
346 foam volume and is an important parameter in, for example, whipping agents which need
347 to maintain the whip as long as possible (Li et al., 2010). The FS was significantly affected
348 by time ($p<0.001$), pH ($p<0.001$), and the interaction between both factors ($p<0.001$). The
349 GPC showed lower FS at pHs 6.0 and 8.0 being statistically different to the rest of the
350 groups after 30 min, except for the FS assessed at pH 8.0 after 120 min. Similar results
351 were obtained at pHs 6.0 and 8.0 for seaweed (Garcia-Vaquero et al., 2017), cowpea
352 (Ragab et al., 2004), and sesame (Khalid, Babiker, & Tinay, 2003) proteins.

353 **3.3.5 Emulsifying capacity and emulsion stability**

354 The manufacture and commercialization of vegetable-derived beverages or meat analogs
355 are a hot trend in the food industry. Good emulsifying properties are highly desired in the
356 manufacture of these foods (Tiwari, Tiwari, Jagan Mohan, & Alagusundaram, 2008).

357 Emulsifying properties of *Ganxet* bean proteins are shown in Figure 3. The EC and ES
358 were found to be pH-dependent (p<0.05). Dependence of EC on pH was observed
359 previously and it is caused because the emulsion capacity of proteins depend on the
360 hydrophilic-lipophilic balance, which is affected by the pH (Ragab et al., 2004). The
361 highest EC was observed at pH 8.0 and was calculated as 69.4 ± 0.8%. Similar results
362 were obtained previously. Indeed, Ragab et al. (2004) observed that alkaline pH improved
363 the emulsion capacity more than acidic pH did. Moreover, Wani et al. (2015) reported
364 significantly higher emulsifying activities in several kidney bean varieties when assessed
365 at pH 7.0 in comparison to pH 3.0 and 5.0. Results were lower than those obtained for
366 seaweed-derived proteins which showed EC values ranging from 70-95% when using
367 sunflower oil (Garcia-Vaquero et al., 2017). No differences were observed between the
368 EC when assessed at pHs 2.0, 6.0, and 10.0. In addition, the ES was also pH-dependent
369 (p<0.05). The generated emulsions were found to be very stable and the ES was
370 significantly higher at pHs 4.0, 6.0, and 10.0 (p<0.05). Wani et al. (2015) reported higher
371 stability of emulsions created at pH 5.0 when compared to those made at lower pHs (pH
372 3.0). This could be caused by the dissociation of some proteins during heating at those
373 pHs which resulted in the formation of subunits with more hydrophobic groups and this
374 stronger interactions with the lipid phase. The lowest ES value was calculated as 78.7 ±
375 1.0% and was observed at pH 2.0 (p<0.05). This value was still comparable to that of
376 other emulsions created using vegetable-derived proteins (Ragab et al., 2004). In the
377 current study, the emulsifying properties of *Ganxet* derived proteins were assessed using
378 sunflower oil. Previous studies demonstrated different EC and ES for different oils
379 (Garcia-Vaquero et al., 2017). Therefore, the EC of these proteins should be re-assessed
380 depending on the vegetable oil utilized in each process. Moreover, the presence of salts
381 could affect the emulsifying properties of proteins (Ragab et al., 2004).

382 **4. Conclusions**

383 Overall, US processing resulted in increased yields, and percentages of material
384 solubilized and proteins recovered, especially when the extraction was performed at
385 higher sodium hydroxide concentrations (0.3 or 0.4 M). The highest percentage of
386 recovered protein was obtained after extraction using sodium hydroxide at a
387 concentration of 0.4 M followed by US processing for 60 min and was calculated as 78.73
388 ± 4.88% (p<0.05). These conditions also resulted in the highest yield and percentage of
389 solubilized material, which were 37.98 ± 0.02 and 54.58 ± 0.19%, respectively (p<0.05).
390 The colour parameters of the generated protein concentrate were comparable to those of
391 other protein-rich powders currently commercialized, especially when compared to those
392 derived from milk. In addition, functional properties including WHC, OHC, FC, and EC
393 were comparable to those of other vegetable-derived proteins. However, further studies
394 are needed to develop potential applications from *Ganxet* beans and their derived proteins.

395 **Acknowledgements**

396 This work was supported by the CERCA Programme of *Generalitat de Catalunya*. T.
397 Lafarga is in receipt of a *Juan de la Cierva* contract awarded by the Spanish Ministry of
398 Economy, Industry, and Competitiveness (FJCI-2016-29541). I. Aguiló-Aguayo thanks
399 the National Programme for the Promotion of Talent and its Employability of the Spanish
400 Ministry of Economy, Industry, and Competitiveness and to the European Social Fund
401 for the Postdoctoral Senior Grant *Ramon y Cajal* (RYC-2016-19949). The authors also
402 thank Fundació Miquel Agustí (Barcelona, Spain) for supplying *Ganxet* beans for use in
403 this study and Silvia Villaró and Ariadna Bademunt for their technical assistance.

404 **Conflict of interests**

405 The authors declare no conflict of interests

406 **Figure legends**

407 **Figure 1. SDS-PAGE profile of proteins extracted under different processing**
408 **conditions: (A), (B), and (C), NaOH 0.2 M at 0, 30 or 60 min US, respectively; (D),**
409 **(E), and (F), NaOH 0.3 M at 0, 30 and 60 min US, respectively; and (G), (H), and (I),**
410 **NaOH 0.4 M at 0, 30 and 60 min US, respectively.**

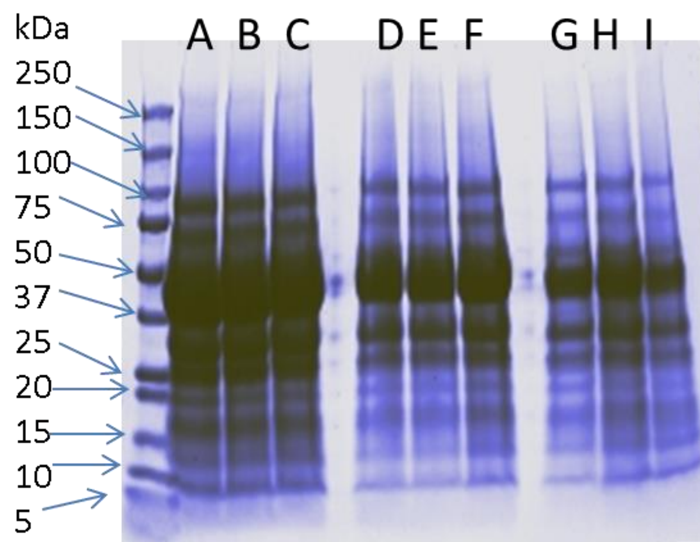
411 **Figure 2. (A) Foam capacity and (B) stability of *Ganxet* bean proteins extracted**
412 **using NaOH 0.4M assisted by ultrasounds**

413 Values represent the mean of three independent experiments ± S.D. Different letters
414 indicate significant differences in foaming capacity. The criterion for statistical
415 significance was $p<0.05$. Foam stability was significantly affected by time ($p<0.001$), pH
416 ($p<0.001$), and the interaction between both factors time*pH ($p<0.001$).

417 **Figure 3. (A) Emulsifying capacity and (B) stability of *Ganxet* bean proteins**
418 **extracted using NaOH 0.4 M assisted by ultrasounds**

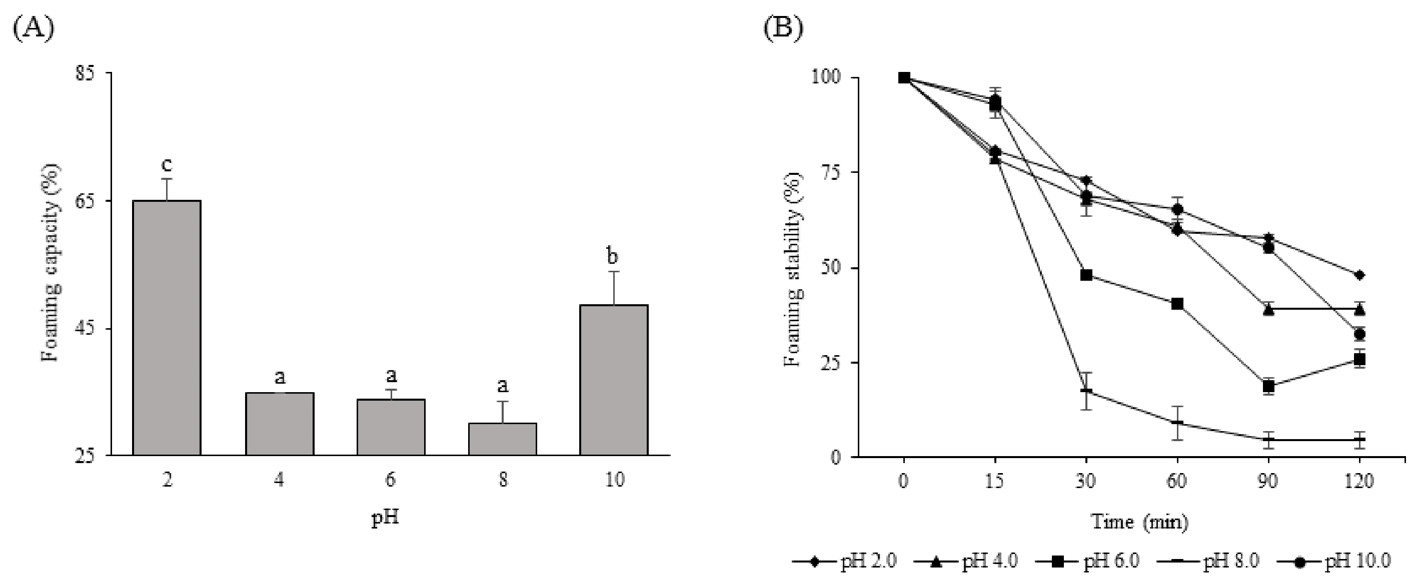
419 Values represent the mean of three independent experiments ± S.D. Different letters
420 indicate significant differences in emulsifying capacity or stability assessed at different
421 pHs. The criterion for statistical significance was $p<0.05$.

422 **Figure 1**

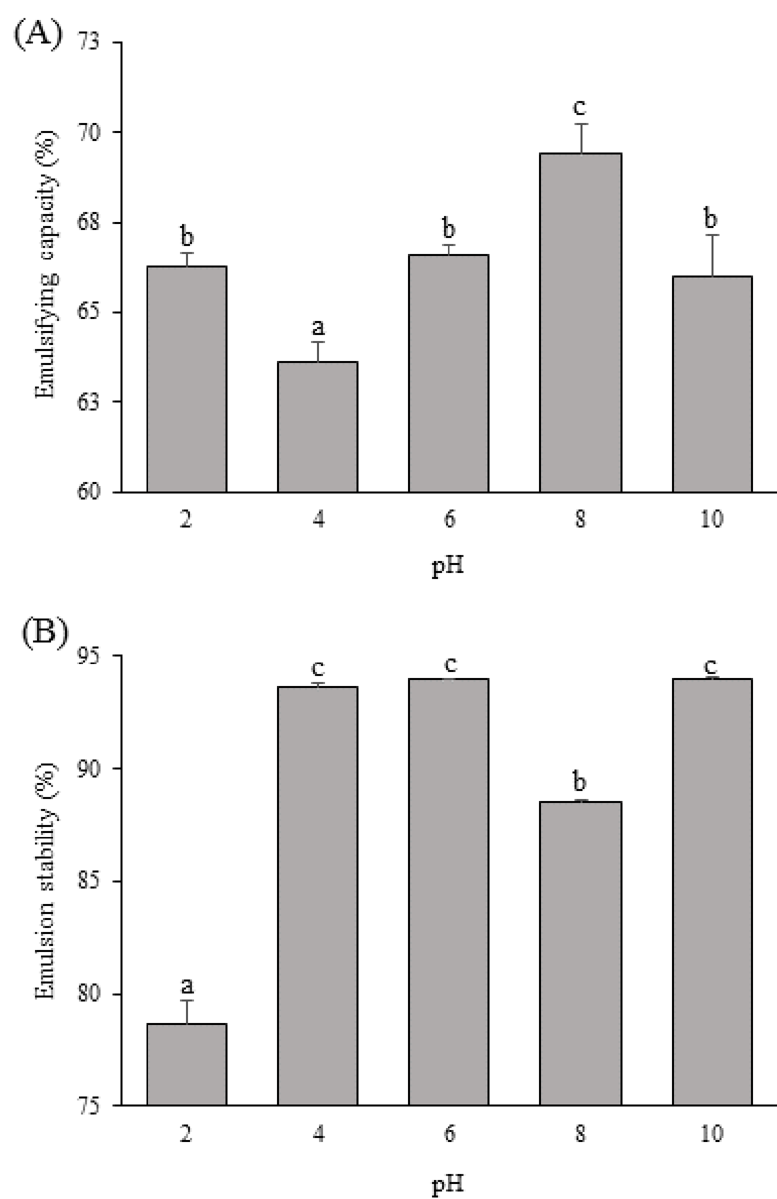


423

424 **Figure 2**



426 **Figure 3**



427

428 **Table 1. Yield, percentage of solubilized material, and recovered protein after ultrasound-assisted protein extraction.**

Treatment	Solvent	pH	US (min)*	% Solubilized material	Yield (g of GPC per 100 g of raw material)	% Precipitated material (pH 5.5)	% Recovered protein
T1	0.1 M NaOH	12.06 ± 0.05	0	41.77 ± 0.75 A a	13.59 ± 1.23 A a	32.59 ± 3.54 A b	45.61 ± 3.41 A ab
T2	0.1 M NaOH	12.04 ± 0.02	30	47.37 ± 2.02 B a	14.23 ± 0.94 A a	30.03 ± 0.71 A c	51.73 ± 3.17 AB a
T3	0.1 M NaOH	12.04 ± 0.03	60	46.85 ± 0.20 AB a	14.54 ± 0.66 A a	30.84 ±1.29 A c	54.87 ± 3.04 B b
T4	0.2 M NaOH	12.55 ± 0.06	0	41.31 ± 0.37 A a	14.57 ± 0.03 A b	35.29 ± 0.23 B b	43.95 ± 0.33 A a
T5	0.2 M NaOH	12.55 ± 0.03	30	47.45 ± 0.49 B a	14.55 ± 0.10 A a	30.67 ± 0.09 A c	45.11 ± 0.76 B a
T6	0.2 M NaOH	12.52 ± 0.02	60	48.25 ± 0.97 B a	14.83 ± 0.08 A a	30.75 ± 0.80 A c	48.79 ± 1.17 C a
T7	0.3 M NaOH	12.77 ± 0.04	0	45.50 ± 2.19 A ab	22.87 ± 0.05 A c	50.30 ± 1.50 B a	47.30 ± 0.39 A b
T8	0.3 M NaOH	12.71 ± 0.04	30	51.17 ± 0.33 B ab	23.96 ± 0.05 B b	46.82 ± 0.41 A b	56.22 ± 1.80 B b
T9	0.3 M NaOH	12.74 ± 0.03	60	54.62 ± 0.27 C b	24.97 ± 0.21 C b	45.73 ± 0.62 A b	58.41 ± 3.39 B b
T10	0.4 M NaOH	12.94 ± 0.03	0	46.58 ± 0.24 A b	24.02 ± 0.45 A c	51.56 ± 0.71 C a	50.17 ± 4.52 A b
T11	0.4 M NaOH	12.97 ± 0.02	30	52.66 ± 0.09 B b	35.40 ± 0.46 B c	67.22 ± 0.77 B a	73.88 ± 2.19 B c
T12	0.4 M NaOH	12.95 ± 0.02	60	54.58 ± 0.19 C b	37.98 ± 0.02 C c	69.59 ± 0.23 A a	78.73 ± 4.88 B c

429 * Abbreviations: US, ultrasound; GPC: *Ganxet* protein concentrate. Samples were homogenized for 30 s at 14,000 rpm prior to US processing.

430 Different capital letters indicate significant differences between samples extracted at the same pH but using different US treatments. Lower case
431 letters indicate significant differences between proteins extracted using the same US conditions but different solvents. The criterion for statistical
432 significance was $p<0.05$.

433 **References**

434 Adebowale, Y. A., Schwarzenbolz, U., & Henle, T. (2011). Protein isolates from
435 Bambara groundnut (Voandzeia Subterranean L.): Chemical characterization and
436 functional properties. *International Journal of Food Properties, 14*(4), 758-775.
437 Álvarez, C., Lélu, P., Lynch, S. A., & Tiwari, B. K. (2018). Optimised protein recovery
438 from mackerel whole fish by using sequential acid/alkaline isoelectric
439 solubilization precipitation (ISP) extraction assisted by ultrasound. *LWT-Food*
440 *Science and Technology, 88*, 210-216.
441 Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: Processing, characterization,
442 functional properties and applications in food and feed. *Food Research*
443 *International, 43*(2), 414-431.
444 Chen, C., You, L.-J., Abbasi, A. M., Fu, X., & Liu, R. H. (2015). Optimization for
445 ultrasound extraction of polysaccharides from mulberry fruits with antioxidant
446 and hyperglycemic activity in vitro. *Carbohydrate polymers, 130*, 122-132.
447 Chen, Y.-C., & Jaczynski, J. (2007). Protein recovery from rainbow trout (Oncorhynchus
448 mykiss) processing byproducts via isoelectric solubilization/precipitation and its
449 gelation properties as affected by functional additives. *Journal of Agricultural and*
450 *Food Chemistry, 55*(22), 9079-9088.
451 EU. (2011). Commission implementig regulation (EU) No 1376/2011 of 20 December
452 2011 entering a name in the register of protected designations of origin and
453 protected geographical indications, Mongeta del Ganxet (PDO). *Official Journal*
454 *of the European Union, L343*, 14-15.
455 Garcia-Vaquero, M., Lopez-Alonso, M., & Hayes, M. (2017). Assessment of the
456 functional properties of protein extracted from the brown seaweed Himanthalia
457 elongata (Linnaeus) SF Gray. *Food Research International, 99*, 971-978.
458 Hadnađev, M., Dapčević-Hadnađev, T., Lazaridou, A., Moschakis, T., Michaelidou, A.-
459 M., Popović, S., & Biliaderis, C. G. (2018). Hempseed meal protein isolates
460 prepared by different isolation techniques. Part I. Physicochemical properties.
461 *Food Hydrocolloids, 79*, 526-533.
462 Kadam, S. U., Álvarez, C., Tiwari, B. K., & O'Donnell, C. P. (2017). Extraction and
463 characterization of protein from Irish brown seaweed Ascophyllum nodosum.
464 *Food Research International, 99*, 1021-1027.
465 Kaur, M., & Singh, N. (2007). Characterization of protein isolates from different Indian
466 chickpea (Cicer arietinum L.) cultivars. *Food Chemistry, 102*(1), 366-374.
467 Khalid, E., Babiker, E., & Tinay, A. E. (2003). Solubility and functional properties of
468 sesame seed proteins as influenced by pH and/or salt concentration. *Food*
469 *Chemistry, 82*(3), 361-366.
470 Krupa-Kozak, U., Bączek, N., & Rosell, C. M. (2013). Application of dairy proteins as
471 technological and nutritional improvers of calcium-supplemented gluten-free
472 bread. *Nutrients, 5*(11), 4503-4520.
473 Kumar, K. S., Ganesan, K., Selvaraj, K., & Rao, P. S. (2014). Studies on the functional
474 properties of protein concentrate of Kappaphycus alvarezii (Doty) Doty–An
475 edible seaweed. *Food chemistry, 153*, 353-360.
476 Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head
477 of bacteriophage T4. *Nature, 227*(5259), 680-685.
478 Lafarga, T., Rai, D. K., O'connor, P., & Hayes, M. (2016). Generation of Bioactive
479 Hydrolysates and Peptides from Bovine Hemoglobin with In Vitro Renin,

480 Angiotensin-I-Converting Enzyme and Dipeptidyl Peptidase-IV Inhibitory
481 Activities. *Journal of Food Biochemistry, 40*(5), 673-685.
482 Li, W., Shu, C., Yan, S., & Shen, Q. (2010). Characteristics of sixteen mung bean
483 cultivars and their protein isolates. *International journal of food science &*
484 *technology, 45*(6), 1205-1211.
485 Lizarazo, C. I., Lampi, A. M., Liu, J., Sontag-Strohm, T., Piironen, V., & Stoddard, F. L.
486 (2015). Nutritive quality and protein production from grain legumes in a boreal
487 climate. *Journal of the Science of Food and Agriculture, 95*(10), 2053-2064.
488 Luna-Vital, D., Mojica, L., González de Mejía, E., Mendoza, S., & Loarca-Piña, G.
489 (2015). Biological potential of protein hydrolysates and peptides from common
490 bean (Phaseolus vulgaris L.): A review. *Food Research International, 76*, 39-50.
491 Mirali, N., El-Khouri, S., & Rizq, F. (2007). Genetic diversity and relationships in some
492 Vicia species as determined by SDS-PAGE of seed proteins. *Biologia Plantarum,*
493 *51*(4), 660-666.
494 Naczk, M., Diosady, L., & Rubin, L. (1985). Functional Properties of Canola Meals
495 Produced by a Two-phase Solvent Extraction System. *Journal of Food Science,*
496 *50*(6), 1685-1688.
497 Nikolić, Z., Đorđević, V., Torbica, A., & Mikić, A. (2012). Legumes seed storage proteins
498 characterization by SDS-PAGE and Lab-on-a-Chip electrophoresis. *Journal of*
499 *food composition and analysis, 28*(2), 75-80.
500 Ochoa-Rivas, A., Nava-Valdez, Y., Serna-Saldívar, S. O., & Chuck-Hernández, C.
501 (2017). Microwave and ultrasound to enhance protein extraction from peanut
502 flour under alkaline conditions: effects in yield and functional properties of
503 protein isolates. *Food and Bioprocess Technology, 10*(3), 543-555.
504 Parniakov, O., Barba, F. J., Grimi, N., Marchal, L., Jubeau, S., Lebovka, N., & Vorobiev,
505 E. (2015). Pulsed electric field and pH assisted selective extraction of intracellular
506 components from microalgae Nannochloropsis. *Algal Research, 8*, 128-134.
507 Poole, S., West, S. I., & Walters, C. L. (1984). Protein–protein interactions: their
508 importance in the foaming of heterogeneous protein systems. *Journal of the*
509 *Science of Food and Agriculture, 35*(6), 701-711.
510 Radnitz, C., Beezhold, B., & DiMatteo, J. (2015). Investigation of lifestyle choices of
511 individuals following a vegan diet for health and ethical reasons. *Appetite, 90*, 31-
512 36.
513 Ragab, D. M., Babiker, E. E., & Eltinay, A. H. (2004). Fractionation, solubility and
514 functional properties of cowpea (Vigna unguiculata) proteins as affected by pH
515 and/or salt concentration. *Food Chemistry, 84*(2), 207-212.
516 Raikos, V., Neacsu, M., Russell, W., & Duthie, G. (2014). Comparative study of the
517 functional properties of lupin, green pea, fava bean, hemp, and buckwheat flours
518 as affected by pH. *Food science & nutrition, 2*(6), 802-810.
519 Rivera, A., Roselló, S., & Casañas, F. (2015). Seed curvature as a useful marker to
520 transfer morphologic, agronomic, chemical and sensory traits from Ganxet
521 common bean (Phaseolus vulgaris L.). *Scientia Horticulturae, 197*, 476-482.
522 Rodrigues, S., Fernandes, F. A., de Brito, E. S., Sousa, A. D., & Narain, N. (2015).
523 Ultrasound extraction of phenolics and anthocyanins from jabuticaba peel.
524 *Industrial Crops and Products, 69*, 400-407.
525 Roselló-Soto, E., Barba, F. J., Parniakov, O., Galanakis, C. M., Lebovka, N., Grimi, N.,
526 & Vorobiev, E. (2015). High voltage electrical discharges, pulsed electric field,
527 and ultrasound assisted extraction of protein and phenolic compounds from olive
528 kernel. *Food and Bioprocess Technology, 8*(4), 885-894.

529 Samaram, S., Mirhosseini, H., Tan, C. P., Ghazali, H. M., Bordbar, S., & Serjouie, A.
530 (2015). Optimisation of ultrasound-assisted extraction of oil from papaya seed by
531 response surface methodology: Oil recovery, radical scavenging antioxidant
532 activity, and oxidation stability. *Food Chemistry, 172*, 7-17.
533 Sarkis, J. R., Boussetta, N., Blouet, C., Tessaro, I. C., Marczak, L. D. F., & Vorobiev, E.
534 (2015). Effect of pulsed electric fields and high voltage electrical discharges on
535 polyphenol and protein extraction from sesame cake. *Innovative Food Science &*
536 *Emerging Technologies, 29*, 170-177.
537 Schmidt, S., & Fontana, A. (2007). Water Activity Values of Select Food Ingredients and
538 Products. In G. Barbosa-Cánovas, F. AJ, S. Schmidt & T. Labuza (Eds.), *Water*
539 *Activity in Foods-Fundamentals and Applications* (pp. 413-414). USA: Blackwell
540 Publishing and the Institute of Food Technologists.
541 Seena, S., & Sridhar, K. (2005). Physicochemical, functional and cooking properties of
542 under explored legumes, Canavalia of the southwest coast of India. *Food*
543 *Research International, 38*(7), 803-814.
544 Shevkani, K., & Singh, N. (2015). Relationship between protein characteristics and film-
545 forming properties of kidney bean, field pea and amaranth protein isolates.
546 *International Journal of Food Science & Technology, 50*(4), 1033-1043.
547 Taskaya, L., Chen, Y.-C., & Jaczynski, J. (2009). Functional properties of proteins
548 recovered from silver carp (Hypophthalmichthys molitrix) by isoelectric
549 solubilization/precipitation. *LWT-Food Science and Technology, 42*(6), 1082-
550 1089.
551 Tiwari, B., Tiwari, U., Jagan Mohan, R., & Alagusundaram, K. (2008). Effect of various
552 pre-treatments on functional, physiochemical, and cooking properties of pigeon
553 pea (Cajanus cajan L). *Food Science and Technology International, 14*(6), 487-
554 495.
555 Wani, I. A., Sogi, D. S., Shivhare, U. S., & Gill, B. S. (2015). Physico-chemical and
556 functional properties of native and hydrolyzed kidney bean (Phaseolus vulgaris
557 L.) protein isolates. *Food Research International, 76*, 11-18.
558 Wibowo, S., Grauwet, T., Santiago, J. S., Tomic, J., Vervoort, L., Hendrickx, M., & Van
559 Loey, A. (2015). Quality changes of pasteurised orange juice during storage: A
560 kinetic study of specific parameters and their relation to colour instability. *Food*
561 *chemistry, 187*, 140-151.