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

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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.

22 **Abstract**

23 This study investigated different methods of extraction of protein from *Ganxet* beans
24 (*Phaseolus vulgaris* L. var. Ganxet) and evaluated the functional properties of these
25 valuable proteins. Overall, ultrasound processing (40 kHz, 250 W) resulted in higher
26 yields and increased percentages of material solubilized and proteins recovered. The
27 highest percentage of recovered protein was obtained after extraction using 0.4 M
28 NaOH followed by ultrasound processing for 60 min and was calculated as
29 $78.73 \pm 4.88\%$ ($p < 0.05$). Extraction using 0.4 M NaOH followed by sonication for 60
30 min resulted in the highest yield and percentage of solubilized material calculated as
31 37.98 ± 0.02 and $54.58 \pm 0.19\%$, respectively ($p < 0.05$). The water- and oil-holding
32 capacities of the *Ganxet* protein concentrate were calculated as 2.33 ± 0.12 and
33 2.69 ± 0.32 g of water or oil per g of protein concentrate, respectively. The highest
34 emulsifying capacity was observed at pH 8.0 and was calculated as $69.4 \pm 0.8\%$.

35 **Keywords:** functional properties, vegetable proteins, protein extraction, protein
36 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
39 nitrogen and are included among pulses. Dry seeds of common beans are excellent
40 protein sources. Indeed their protein content can be up to three fold higher than in
41 cereals (Rivera, Roselló, & Casañas, 2015) and contribute to approximately 6 g of
42 protein per capita and per day in several developing countries (Luna-Vital, Mojica,
43 González de Mejía, Mendoza, & Loarca-Piña, 2015). *Ganxet* beans (*Phaseolus vulgaris*
44 L. var. *Ganxet*) are easily recognized for the squashed and hooked shape of their seeds
45 and are one of the most prestigious bean landraces cultivated in Europe. Indeed, the
46 *Ganxet* bean has Protected Designation of Origin status from the EU (EU, 2011) and is
47 known for its high protein content, which ranges between 24 and 29% (Rivera et al.,
48 2015).

49 Plant-derived proteins are cheaper to produce, when compared to animal-derived
50 proteins, and an increase in their utilization replacing the latter could be beneficial in
51 terms of preventing the effects of climate change (Garcia-Vaquero, Lopez-Alonso, &
52 Hayes, 2017). Isoelectric solubilisation-precipitation (ISP) enabled protein recovery
53 from a variety of sources including seaweed (Kadam, Álvarez, Tiwari, & O'Donnell,
54 2017) and fish (Álvarez, Lélou, Lynch, & Tiwari, 2018). When applied to vegetable
55 sources, this strategy is generally used in combination with enzymatic hydrolysis, which
56 involves the use of enzymes that degrade the cell wall, facilitating the release of
57 proteins and other strategies such as chemical hydrolysis or subcritical water hydrolysis
58 (Kadam et al., 2017). Non-conventional strategies such as the use of high pressure
59 processing, pressurized liquid extraction, microwaves, or sonication can also be used to
60 enhance the extraction rate and yield of the process. Sonication has shown to be
61 efficient in facilitating the extraction of several compounds from plant sources such as

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

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

84 2. Materials and methods

85 2.1 Chemicals and reagents

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

94 2.2 Protein extraction and determination

95 Dried *Ganxet* beans were kindly provided by Fundació Miquel Agustí, Barcelona,
96 Spain. Sample processing was performed at the pilot plant of the IRTA Fruitcentre in
97 Lleida, Spain. Dried *Ganxet* seeds were milled to a thin powder using a MINIMOKA
98 GR-020 grinder (Taurus Group, Barcelona, Spain) and passed through a sieve of 1 mm.
99 Sieved samples were mixed with alkaline solutions of different concentrations, listed in
100 Table 1, at a sample:solvent ratio of 1:10 (w/v). The mixture was homogenized for 30 s
101 using a T-25 digital ULTRA-TURRAX® homogenizer (IKA, Staufen, Germany) at
102 14,000 rpm. Homogenized samples were immediately placed in a stirrer allocated in a
103 cold room at 4 °C for 15 min. After this period samples were either left untreated or
104 processed using an ultrasonic bath (JP Selecta S.A., Barcelona, Spain) operating at 4 °C,
105 40 kHz, and 250 W for 30 or 60 min. Samples were then centrifuged using a Sigma 3-
106 18 KS centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at
107 10,000 × g for 20 min. Proteins in the supernatant were precipitated by shifting the pH

108 value to 5.5 (value selected based on preliminary trials in which we obtained the highest
109 recoveries). Precipitates obtained were separated by centrifugation at $10,000 \times g$ for 20
110 min, frozen, freeze-dried using a Crydos-50 freeze-dryer (Telstar, Barcelona, Spain),
111 and stored at $-20\text{ }^{\circ}\text{C}$ until further analysis.

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

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

120 where W_{GPC} is the amount of GPC obtained expressed in g, PC_{GPC} is the protein content
121 in the precipitated GPC expressed as g of protein per 100 g of GPC, W_G is the amount
122 of milled and sieved *Ganxet* beans used for protein precipitation expressed in g, and
123 PC_G is the protein content of *Ganxet* beans expressed as g of protein per 100 g of
124 *Ganxet* bean.

125 The water activity (a_w) of the isolated dried proteins was measured in triplicate using an
126 AquaLab meter (Decagon Devices Inc., WA, USA) at $22.0 \pm 0.9\text{ }^{\circ}\text{C}$.

127 **2.4 SDS-PAGE profile**

128 MW profile of proteins extracted from *Ganxet* bean was determined by means of SDS-
129 PAGE electrophoresis following Laemmli's methodology (Laemmli, 1970). Freeze-
130 dried samples were solved in 0.25 M NaOH solution to a final concentration of 6

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

141 **2.5 Colour evaluation**

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

149 **2.6 Assessment of functional properties**

150 The water- (WHC) and oil-holding capacity (OHC) of the *Ganxet* protein concentrates
151 were determined following the method of Raikos, Neacsu, Russell, and Duthie (2014).
152 Determinations were carried out in triplicate for each sample and replicate and results
153 were expressed either as g of water or sunflower oil per g of protein concentrate.

154 The foaming capacity (FC) of the isolated proteins was determined as described by
155 Poole, West, and Walters (1984) with some modifications. Briefly, *Ganxet* proteins
156 were re-suspended in ultrapure water at a concentration of 1.5% (w/v) and the pH was
157 adjusted to either 2, 4, 6, 8, or 10. Protein suspensions were homogenized using a T-25
158 digital ULTRA-TURRAX[®] homogenizer (IKA, Staufen, Germany) at 10,000 rpm for 1
159 min and the volume of foam generated was measured in a graduated cylinder. FC was
160 measured as the volume of foam generated as a percentage of the initial volume and
161 foaming stability (FS) was expressed as the percentage of decrease of foam volume over
162 time as described by Garcia-Vaquero et al. (2017).

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

178 **2.7 Statistical analysis**

179 Results are expressed as mean \pm standard deviation (S.D.). Differences between samples
180 were analysed using analysis of variance (ANOVA) with JMP 13 (SAS Institute Inc.,
181 Cary, USA). Where significant differences were present, a Tukey pairwise comparison
182 of the means was conducted to identify where the sample differences occurred. The
183 criterion for statistical significance was $p < 0.05$. To identify relationships between
184 parameters, bivariate Pearson's correlation analysis was carried out.

185 3. Results and discussion

186 3.1 Protein extraction

187 The total protein content of *Ganxet* beans was calculated as $24.7 \pm 0.4\%$ which is in the
188 range 24 - 29% previously reported by Rivera et al. (2015). Similar protein contents
189 were reported previously for other pulses including faba beans, lentils, and lupins
190 (Boye, Zare, & Pletch, 2010; Lizarazo et al., 2015).

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

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

234 Previous studies reported higher protein yields after alkaline extraction when compared
235 to acid extraction (Chen & Jaczynski, 2007; Taskaya, Chen, & Jaczynski, 2009). A
236 recent study published by Álvarez et al. (2018) suggested that sequential alkaline/acid
237 extraction assisted by US was efficient in recovering approximately 100% of total
238 protein in mackerel whole fish and this strategy could be useful in increasing the protein
239 yield and optimizing the extraction process.

240 **3.2 SDS-PAGE protein profile**

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

259 was highly similar to those reported by Mirali, El-Khouri, and Rizq (2007) and Nikolić,
260 Đorđević, Torbica, and Mikić (2012), which were found to be highly dependent on the
261 variety of *Vicia faba* employed. These same authors reported how the polymorphism
262 present in *Vicia* species could alter the profile of the extracted proteins.

263 **3.3 Functional properties**

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

267 **3.3.1 Colour**

268 Table 2 lists the colour parameters of both protein-rich powders. The L^* parameter
269 which denotes lightness and varies from 0 (black) to 100 (white) was measured as 91.40
270 ± 1.63 . This value was high when compared to that obtained for other vegetable-derived
271 proteins such as hempseed meal protein isolates prepared by either micellization (82.80
272 ± 0.31) or isoelectric precipitation (56.39 ± 0.29) (Hadnadev et al., 2018) or kidney
273 bean, field pea, or amaranth protein isolates which showed L^* values of 79.6 ± 0.1 , 88.1
274 ± 0.2 , or 78.0 ± 0.8 , respectively (Shevkani & Singh, 2015). This denotes a lighter
275 appearance of *Ganxet* proteins when compared to proteins derived from other
276 vegetables. The GPC showed a similar C^*_{ab} value, a quantitative indicator of the
277 intensity of a distinctive hue, when compared to other vegetable-derived proteins
278 (Garcia-Vaquero et al., 2017; Hadnadev et al., 2018), suggesting that the colour
279 intensity of the precipitated *Ganxet* proteins was similar to that of previously isolated
280 vegetable-derived proteins. In addition, the δE combines the change in L^* , a^* , and b^*
281 values to quantify the colour deviation from a standard reference sample, in this case,
282 previously reported protein-rich powders derived from vegetables. Those samples with
283 $\delta E > 3$ display a visible colour deviation (Wibowo et al., 2015). The δE was higher than

284 3 when comparing GPC with proteins derived from kidney bean, field pea, amaranth
285 (Shevkani & Singh, 2015), hemp seed (Hadnadev et al., 2018), soybean, pigeon, and
286 cowpea (Garcia-Vaquero et al., 2017) and other beans (Wani, Sogi, Shivhare, & Gill,
287 2015). To the best of the authors' knowledge, there are no studies on the colour of
288 *Ganxet* protein extracts and the colour of the protein concentrate obtained herein was
289 perceptually different to that of other vegetable-derived proteins. However, no visible
290 colour deviation ($\delta E > 3$) was observed between GPC and powders derived from milk
291 such as a whey protein isolate and sodium caseinate (Krupa-Kozak, Bączek, & Rosell,
292 2013) which are currently commercialized protein-rich powders.

293 **3.3.2 Water activity**

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

302 **3.3.3 Water- and oil-holding capacity**

303 Interactions of water and oil with proteins are important for the food industry because of
304 their effects on the flavour and texture of foods (Kumar, Ganesan, Selvaraj, & Rao,
305 2014). The WHC of the extracted GPC was 0.98 ± 0.10 g of water per g of GPC. These
306 values were lower when compared to those obtained for different varieties of kidney
307 beans including *French*, *Yellow*, and *Master* beans which varied from 5.34 to 5.85 g of
308 water per g of sample (Wani et al., 2015). However, results obtained herein were in line

309 with those reported previously for cowpea proteins and within the range of the
310 commercial values of protein concentrates (Ragab, Babiker, & Eltinay, 2004). Similar
311 WHC values were also reported recently for hemp seed protein isolates prepared by
312 micellization, calculated as 0.80 ± 0.03 g/g (Hadnadev et al., 2018). High WHC values
313 help to maintain freshness and moist mouth feel of foods and have been associated with
314 reduced moisture loss in packed bakery goods (Garcia-Vaquero et al., 2017). High
315 WHC values are desirable in viscous foods such as sausages, custards, or baked
316 products as this would help to hold water without dissolution of protein, providing
317 thickening and viscosity (Seena & Sridhar, 2005). Differences in WHC values could be
318 attributed to different protein structures and low availability of polar amino acids which
319 have been shown to be primary sites for water interaction of proteins (Li, Shu, Yan, &
320 Shen, 2010).

321 In addition, the OHC of the GPC generated herein was calculated as and 2.33 ± 0.12 g
322 of sunflower oil per g of GPC. This value was low when compared to that obtained
323 previously for kidney bean which ranged from 5.8 to 6.9 g of oil per g of sample (Wani
324 et al., 2015) and *Bambara* groundnut protein, which ranged from 6.7 to 7.2 g of oil per
325 g of sample (Adebowale, Schwarzenbolz, & Henle, 2011). The OHC value of GPC was
326 higher than that of hemp seed proteins, which ranged between 1.62 ± 0.06 and 1.79
327 ± 0.02 g of oil per g of protein isolate, depending on the isolation method (Hadnadev et
328 al., 2018). The use of different vegetable oils between the current paper and the above
329 mentioned studies could partially explain the observed differences in OHC values, as
330 functional properties can be affected by using different vegetable oils (Garcia-Vaquero
331 et al., 2017). Similar OHC values were reported for mung beans, which ranged from
332 1.00 to 3.38 mL of oil per g of sample (Li et al., 2010), and chickpea isolates which
333 varied from 2.08 to 2.96 mL/g (Kaur & Singh, 2007), depending on the cultivar.

334 3.3.4 Foaming capacity and foam stability

335 Figure 2 shows the FC and FS of *Ganxet* bean proteins. The highest FC, calculated as
336 $65.0 \pm 3.5\%$, was observed at pH 2.0 ($p < 0.05$). Results were in line with those obtained
337 for other protein sources such as seaweed (Kumar et al., 2014) or cowpea (Ragab et al.,
338 2004). Results were also comparable to those reported by Li et al. (2010), who
339 calculated the FC of several mung beans which ranged between 33.00 ± 2.20 and 67.50
340 $\pm 1.04\%$, depending on the cultivar. In the current study, an increase was observed in
341 the FC at pH 10 when compared to pH 4-8 ($p < 0.05$) and no statistically significant
342 differences were observed in the FC when assessed at pHs 4, 6, or 8. Proteins in foams
343 contribute to the uniform distribution of fine air cells in food matrices promoting
344 smoothness and lightness of foods (Garcia-Vaquero et al., 2017). Previous studies also
345 observed higher FC of proteins at extreme pH values such as 2.0 (Kumar et al., 2014)
346 and 10.0 (Garcia-Vaquero et al., 2017) probably caused by increased net charges on the
347 protein, which weakened the hydrophobic interactions but increased the flexibility of
348 the protein (Ragab et al., 2004). Protein structural properties can also affect FC. Good
349 foaming abilities have been related with flexible proteins that can reduce surface tension
350 and globular proteins, which are difficult to surface denature, give low foaming
351 properties (Kaur & Singh, 2007). FS represents the percentage volume of foam
352 remaining after a specified time as the initial foam volume and is an important
353 parameter in, for example, whipping agents which need to maintain the whip as long as
354 possible (Li et al., 2010). The FS was significantly affected by time ($p < 0.001$), pH
355 ($p < 0.001$), and the interaction between both factors ($p < 0.001$). The GPC showed lower
356 FS at pHs 6.0 and 8.0 being statistically different to the rest of the groups after 30 min,
357 except for the FS assessed at pH 8.0 after 120 min. Similar results were obtained at pHs

358 6.0 and 8.0 for seaweed (Garcia-Vaquero et al., 2017), cowpea (Ragab et al., 2004), and
359 sesame (Khalid, Babiker, & Tinay, 2003) proteins.

360 **3.3.5 Emulsifying capacity and emulsion stability**

361 The manufacture and commercialization of vegetable-derived beverages or meat
362 analogs are a hot trend in the food industry. Good emulsifying properties are highly
363 desired in the manufacture of these foods (Tiwari, Tiwari, Jagan Mohan, &
364 Alagusundaram, 2008). Emulsifying properties of *Ganxet* bean proteins are shown in
365 Figure 3. The EC and ES were found to be pH-dependent ($p < 0.05$). Dependence of EC
366 on pH was observed previously and it is caused because the emulsion capacity of
367 proteins depend on the hydrophilic-lipophilic balance, which is affected by the pH
368 (Ragab et al., 2004). The highest EC was observed at pH 8.0 and was calculated as 69.4
369 $\pm 0.8\%$. Similar results were obtained previously. Indeed, Ragab et al. (2004) observed
370 that alkaline pH improved the emulsion capacity more than acidic pH did. Moreover,
371 Wani et al. (2015) reported significantly higher emulsifying activities in several kidney
372 bean varieties when assessed at pH 7.0 in comparison to pH 3.0 and 5.0. Results were
373 lower than those obtained for seaweed-derived proteins which showed EC values
374 ranging from 70-95% when using sunflower oil (Garcia-Vaquero et al., 2017). No
375 differences were observed between the EC when assessed at pHs 2.0, 6.0, and 10.0. In
376 addition, the ES was also pH-dependent ($p < 0.05$). The generated emulsions were found
377 to be very stable and the ES was significantly higher at pHs 4.0, 6.0, and 10.0 ($p < 0.05$).
378 Wani et al. (2015) reported higher stability of emulsions created at pH 5.0 when
379 compared to those made at lower pHs (pH 3.0). This could be caused by the dissociation
380 of some proteins during heating at those pHs which resulted in the formation of subunits
381 with more hydrophobic groups and this stronger interactions with the lipid phase. The
382 lowest ES value was calculated as $78.7 \pm 1.0\%$ and was observed at pH 2.0 ($p < 0.05$).

383 This value was still comparable to that of other emulsions created using vegetable-
384 derived proteins (Ragab et al., 2004). In the current study, the emulsifying properties of
385 *Ganxet* derived proteins were assessed using sunflower oil. Previous studies
386 demonstrated different EC and ES for different oils (Garcia-Vaquero et al., 2017).
387 Therefore, the EC of these proteins should be re-assessed depending on the vegetable oil
388 utilized in each process. Moreover, the presence of salts could affect the emulsifying
389 properties of proteins (Ragab et al., 2004).

390 4. Conclusions

391 Overall, US processing resulted in increased yields, and percentages of material
392 solubilized and proteins recovered, especially when the extraction was performed at
393 higher sodium hydroxide concentrations (0.3 or 0.4 M). The highest percentage of
394 recovered protein was obtained after extraction using sodium hydroxide at a
395 concentration of 0.4 M followed by US processing for 60 min and was calculated as
396 $78.73 \pm 4.88\%$ ($p < 0.05$). These conditions also resulted in the highest yield and
397 percentage of solubilized material, which were 37.98 ± 0.02 and $54.58 \pm 0.19\%$,
398 respectively ($p < 0.05$). The colour parameters of the generated protein concentrate were
399 comparable to those of other protein-rich powders currently commercialized, especially
400 when compared to those derived from milk. In addition, functional properties including
401 WHC, OHC, FC, and EC were comparable to those of other vegetable-derived proteins.
402 However, further studies are needed to develop potential applications from *Ganxet*
403 beans and their derived proteins.

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414 **Conflict of interests**

415 The authors declare no conflict of interests

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416 **Figure legends**

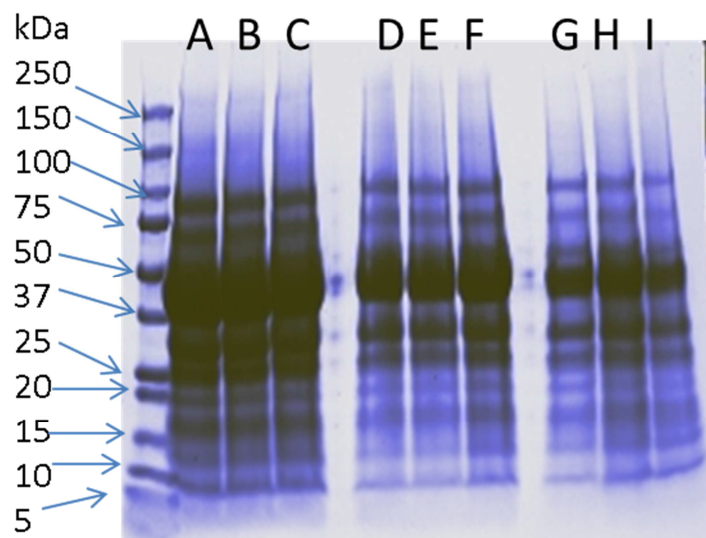
417 **Figure 1. SDS-PAGE profile of proteins extracted under different processing**
418 **conditions: (A), (B), and (C), NaOH 0.2 M at 0, 30 or 60 min US, respectively; (D),**
419 **(E), and (F), NaOH 0.3 M at 0, 30 and 60 min US, respectively; and (G), (H), and**
420 **(I), NaOH 0.4 M at 0, 30 and 60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

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

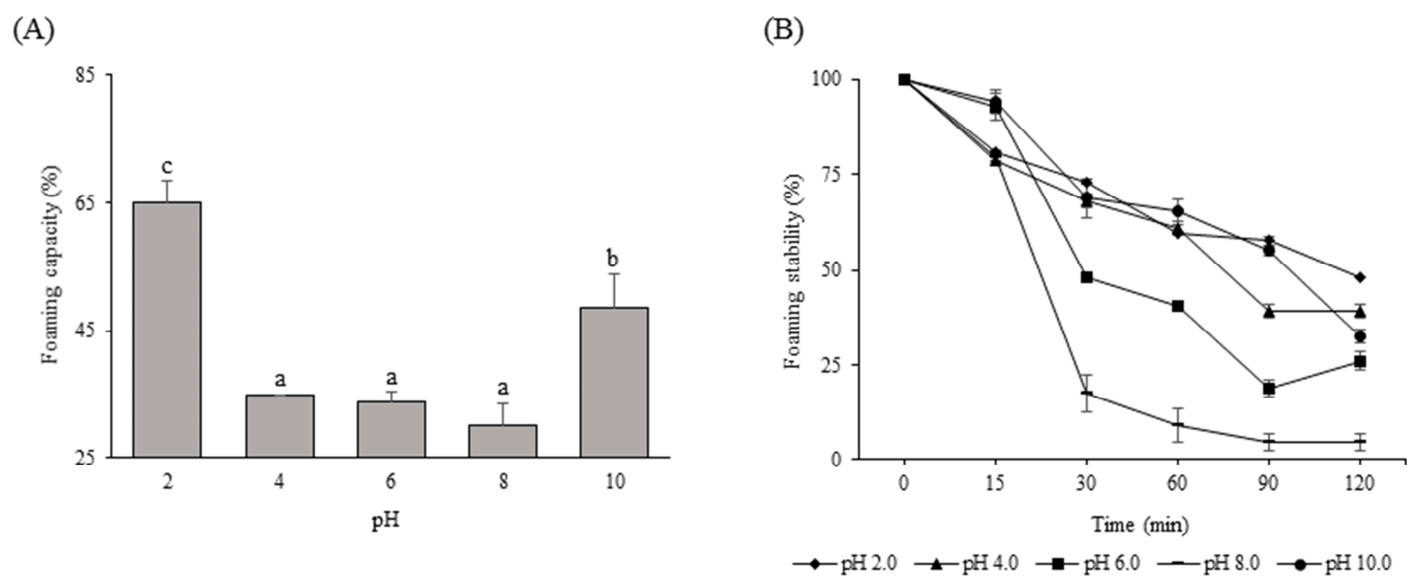
423 Values represent the mean of three independent experiments \pm S.D. Different letters
424 indicate significant differences in foaming capacity. The criterion for statistical
425 significance was $p < 0.05$. Foam stability was significantly affected by time ($p < 0.001$),
426 pH ($p < 0.001$), and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

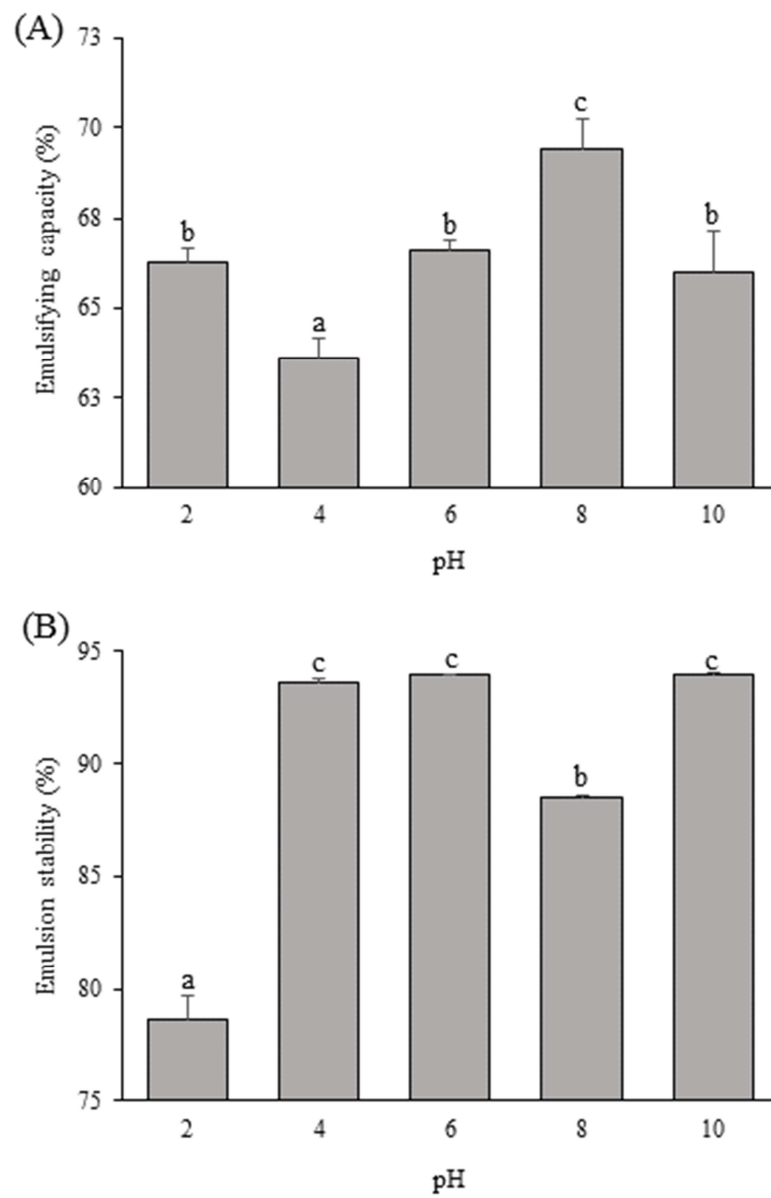
427 **Figure 3. (A) Emulsifying capacity and (B) stability of *Ganxet* bean proteins**
428 **extracted using NaOH 0.4 M assisted by ultrasounds**

429 Values represent the mean of three independent experiments \pm S.D. Different letters
430 indicate significant differences in emulsifying capacity or stability assessed at different
431 pHs. The criterion for statistical significance was $p < 0.05$.

432 **Figure 1**

433

434 **Figure 2**

436 **Figure 3**

437

438 **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

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

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

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Highlights

- Ultrasound processing for 30 or 60 min resulted in higher protein recovery yields
- Water- and oil-holding capacities were 0.98 ± 0.10 and 2.33 ± 0.12 g/g respectively
- Highest foaming capacity and foam stability were observed at pH 2.0
- Highest emulsifying capacity was observed at pH 8.0