

1 Optimization of sunflower albumin extraction from oleaginous meal and 2 characterization of their structure and properties

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8

9 Abstract

10 This article proposes an alternative extraction strategy for the valorization of the sunflower meal leading to the
11 production of an albumin isolate for human nutrition and a valuable **residual meal** for feed application. For this
12 purpose, a response surface methodology was employed to study the effect of pH (3–6) and NaCl (0–0.5 mol.L⁻¹
13 ¹) on albumin extraction (yield, **content**, and phenolic contamination). Total phytate and nitrogen content in
14 **residual meal** were also considered. **As a result, the significant influence (p-value<0.05) of both pH and NaCl**
15 **concentration on investigated responses was demonstrated.** The analysis of regression and variance confirmed
16 a high accuracy of developed models for process prediction. Based on generated equations, a multi-objective
17 optimization was applied for simultaneous maximization of albumin extraction performances and the **residual**
18 **meal** value. The best trade-off for sunflower albumin extraction was found at pH 4.1 and 0.25 mol.L⁻¹ of NaCl
19 concentration. Under this condition, good albumin recovery was achieved (>70%), whereas extract was enriched
20 in sunflower albumin fraction (>90% of total proteins) and phenolic contamination of albumins was considerably
21 reduced (<1.6 mg of covalently bound chlorogenic acid per gram of proteins). The selected condition **produced**
22 value-added **residual meal** with low phytate level (<4% on dry matter basis) and high protein content (>40% on
23 dry matter basis). **Further** purification of sunflower albumins yielded **a** white color product characterized by
24 excellent solubility (**approximately 100% at pH 2–11**), **stable conformation of structure against pH and**
25 **temperature, and beneficial functional properties (foaming and emulsifying).**

26

27 **Keywords:** Sunflower albumin; Solid/liquid extraction; Response surface methodology; Multi-objective
28 optimization; Structure; Functional

29 1. Introduction

30 Global demand for production of protein isolates from alternative plant sources is expected to rise at a fast pace
31 in the coming years (Gonzalez-Perez & Vereijken, 2007; Pickardt, Eisner, Kammerer, & Carle, 2015; Ivanova,
32 Chalova, & Koleva, 2014; Wildermuth, Young, & Were, 2016). Sunflower (*Helianthus annuus* L.) is the fourth
33 worldwide oilseed with the global output reaching the value over 47 million tons in 2017 (FAO, 2019). The solid
34 residue remaining after oil extraction process (sunflower meal) is a valuable source of proteins (30–50% on dry
35 matter basis, dm). The two major classes of sunflower proteins are helianthins (60–80%) and sunflower
36 albumins (SFA) (25–35%) (Gonzalez-Perez & Vereijken, 2007; Kortt, & Caldwell, 1990; Raymond, Robin, & Azanza,
37 1995; Sosulski & Bakal, 1969; Wildermuth et al., 2016). Helianthins have an oligomeric structure of 300–350
38 kDa. The predominant hexameric form (11S) is composed of six monomers composed of basic (21–27 kDa) and
39 acidic polypeptide chain (32–44 kDa) linked by disulphide bridge. The isoelectric point (IP) of helianthins was
40 reported in the pH range between 4 and 6. SFA (2S) are polymorphic proteins constituted of a single basic
41 polypeptide chain (10–18 kDa, average IP 8.8). Several individual forms of SFA (8–13) have been identified
42 including 2S albumin storage proteins (16 kDa) and methionine-rich 2S proteins (12 kDa) (Gonzalez-Perez &
43 Vereijken, 2007).

44 Many reports describe sunflower protein isolate production processes. For many of the plant protein sources,
45 alkali extraction conditions were employed to reach high protein extraction yields. According to study on
46 sunflower proteins undertaken by Sosulski *et al.* (1969) their extractability at basic pH (in 0.2% NaOH) could
47 reach even 90%. Later works of Ordonez *et al.* (2001) and Gonzalez *et al.* (2002) demonstrate maximal protein
48 extraction yields ranging 50–70% at pH close to 10. In these conditions, both helianthins and SFA are extracted.
49 Nonetheless, at high pH values phenolic compounds, notably chlorogenic acids (CQA) (1–4% of dm) are largely
50 co-solubilized. The basic pH is in favor of CQA oxidation leading to phenol-protein covalent bonding (Bongartz
51 et al., 2016; Gonzalez-Perez & Vereijken, 2007; Ozdal, Capanoglu, & Altay, 2013; Wildermuth et al., 2016). This
52 yields protein isolates with a characteristic greenish color that are unsuitable for food applications. Furthermore,
53 it is well known that phenolic binding can decrease protein solubility, digestibility, nutritive value and functional
54 properties (Gonzalez-Perez & Vereijken, 2007; Ozdal et al., 2013; Wildermuth et al., 2016).

55 Different process approaches have been tried to limit protein-phenol interactions, while keeping a satisfying
56 protein extraction yield. The first one was to implement high ionic strength extraction. This efficiently increased
57 protein recovery at pH around neutrality. Indeed, Pickardt *et al.* (2009) and Ivanova *et al.* (2012) showed that
58 pH around 6 and 1.6–2.1 mol.L⁻¹ NaCl allowed a high extraction yield (50% vs 20% without salt). This resulted in
59 light-colored protein product. However, such high salt concentration is unsuitable due to the cost of effluent
60 treatments and the corrosive risk toward stainless steel. Other authors tried to limit the phenol oxidation by
61 adding reducing agent such as Na₂SO₃, NaHSO₃ or ascorbic acid (Kuijpers et al., 2012; Richard-Forget & Gaillard,
62 1997; Salgado et al., 2012). Nevertheless, this method still yielded greenish isolate solutions at basic pH or after
63 protein storage (Yoruk & Marshall, 2003; Wildermuth et al., 2016). Another approach was to carry out a
64 polyphenol extraction with organic or organic-aqueous mixtures such acidified butanol (Saeed & Cheryan, 1988),
65 60% methanol (Malik & Saini, 2017), 80% methanol (González-Pérez et al., 2002) or 95% ethanol (Carter,

66 Gheyasuddin, & Mattil, 1972). **This alcohol treatment** revealed to be efficient in term of color but an important
67 loss in low-molecular proteins was observed (Rahma & Rao, 1979). Furthermore, the use of organic solvents for
68 food processing is more often questioned.

69 **Therefore, the conventional strategy that consists of searching extraction conditions that maximize total protein**
70 **extraction yield from sunflower meal needs to be reconsidered.** Moreover, the value of the remaining **residual**
71 **meal** after extraction has never been addressed to our knowledge. Hence, an alternative production way that
72 integer protein quality, recovery and remaining meal value has to be searched.

73 Among two principal protein fractions, only SFA presents completely balanced amino acid profile and usually
74 high amount of sulphur-containing amino acids (Gonzalez-Perez & Vereijken, 2007; Kortt & Caldwell, 1990; Kortt,
75 Caldwell, Lilley, & Higgins, 1991) in accordance to the requirement pattern of the FAO/WHO/UNU (2007). It is
76 also well known, that SFA exhibit interesting foaming and emulsifying properties (Burnett et al. 2002; Gueguen,
77 Walrand, & Bourgeois, 2016). The excellent nutritional and **functional** properties make SFA attractive for various
78 food applications. Interestingly, selective extraction of albumins from oleaginous meals has been previously
79 **noted** upon acidic conditions (Gonzalez-Perez, Vereijken, Van Koningsveld, Gruppen, & Voragen, 2005a; Nioi,
80 Kapel, Rondags, & Marc, 2012). This low pH of extraction **prevents** the oxidation of phenolic compounds **and**
81 **allows to obtain** the light-colored proteins. Another important benefit of acidic extraction is by-production of
82 valuable **residual meal** remaining after extraction process. This is because, SFA fraction represents only 25–35%
83 of total sunflower proteins and consequently the selective isolation of albumins would keep a high amount of
84 nitrogen in the solid phase after protein extraction. Furthermore, antinutritional compound such as phytic acid
85 would be removed efficiently under acidic conditions (Cheryan & Rackis, 1980; Kumar, Sinha, Makkar, & Becker,
86 2010; Nissar, Ahad, Naik, & Hussain, 2017). To the best of our knowledge, neither the impact of **extraction**
87 **conditions** on SFA yield, selectivity, phenolic complexation and composition of the remaining **residual meal** has
88 ever been studied.

89 **In this work, the effect of pH (3–6) and NaCl (0–0.5 mol.L⁻¹) on the selective extraction of SFA from sunflower**
90 **meal was investigated by design of experiments (DoE). Albumin extraction yield, albumin content and amount**
91 **of CQA covalently bound to albumin, and phytic acid/nitrogen content in the residual meal (after protein**
92 **extraction) were considered as performance criteria. Then, a multi-objective optimization strategy was applied**
93 **in order to find the best trade-off between all considered criteria. Eventually, the structure and functional**
94 **properties of SFA obtained in the optimal conditions were characterized.**

95

96 **2. Materials and Methods**

97 2.1. Materials and chemicals

98 The ground sunflower cold press meal was provided by Olead (Pessac, France). The protein, fat and phytic acid
99 content in the meal were 37.9, 14.6, and 6.6%/dm, respectively. Sodium chloride (NaCl, CAS 7647-14-201),
100 sodium hydroxide pellets (NaOH, CAS 1310-73-2) ethylenediaminetetraacetic acid (EDTA, CAS 6381-92-6) were

101 obtained from VWR (Darmstadt, Germany). Hydrochloric acid (HCl, CAS 7647-01-0) was from Carlo Erba (Milan,
102 Italy). Tris(hydroxymethyl)aminomethane (Tris, CAS 77-86-1), glycine (CAS 56-40-6), iron (III) chloride (FeCl₃, CAS
103 7705-08-0), sodium sulfate (Na₂SO₄, CAS 7757-82-6), 5-sulfosalicylic acid hydrate (CAS 304851-84-1) was from
104 Fisher Scientific (Hampton, USA). Soy protein isolate with a purity of 90%/dm was purchased from Solae (St.
105 Louis, MO, USA)

106 2.2. Effect of pH on extraction performances

107 In the preliminary study, the effect of pH (3–7) on helianthinin and SFA extraction yield as well SFA content in
108 aqueous extract was assessed. For this purpose, sunflower meal was extracted at 1:9 of the solid/liquid ratio
109 (w/w). The pH was adjusted to given values (3–7) using 1 mol.L⁻¹ NaOH or HCl solution. The mixture was stirred
110 at 600 rpm during 60 min at 20 °C. If necessary, pH was readjusted. Then, the slurry was centrifuged (15 000×g,
111 30 min, 20 °C) and partly clarified on a Whatman filter paper to get sunflower extract.

112 2.3. Experimental design

113 The design of experiment and statistical analysis were based on response surface methodology (RSM). The
114 influence of two factors: pH (3–6) and NaCl concentration (0–0.5 mol.L⁻¹) on five responses: SFA extraction yield
115 (SFA_{YIELD}%), SFA content (C_{SFA}%) and CQA covalently bound to SFA (SFA_{PHEN} mg.g⁻¹) in liquid extract, as well as
116 phytic acid (C_{PHYT}%) and nitrogen/protein (C_{PROT}%) content in remaining residual meal, were studied using face-
117 centered central composite design. For design of experiments, Modde 9.1.1.0 software from Sartorius Stedim
118 Biotech (Gottingen, Germany) was used. According to generated experimental matrix, 11 experiments were
119 performed containing three replications at the central point (Table A.1). The solid/liquid extraction was
120 performed as described in the section 2.2. From each extraction, about 1600 mL of liquid was obtained. The
121 remaining residual meal (pellet from solid/liquid separation) was collected, stored at -80 °C and freeze-dried. As
122 a result of modelling, polynomial equation, response surface curve and observed/predicted plot were achieved.
123 The mathematical relationship between factors and response was described by second-degree polynomial
124 equation (Eq. 1) as follow:

$$y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{12}x_1x_2 \quad (\text{Eq. 1})$$

125 where y is the response, β_0 is the constant, β_1 and β_2 are the coefficients of linear effect, β_{11} and β_{22} are the
126 coefficients of cubic effect, β_{12} is the coefficient of interaction effect, x_1 and x_2 are the coded independents of
127 pH and NaCl concentration, respectively.

128 Analyze of regression and variance test was performed. Three statistical indicators were evaluated: coefficient
129 of determination (percent of the variation of the response explained by the model, R²), p-value of regression
130 (comparison between the variance of regression and residual error) and lack-of-fit (comparison between the
131 variance of model error and pure error). Good fitting is characterized by R² close to 1, regression p-value<0.05
132 and lack of fit>0.05.

133 2.4. Multi-objective optimization

134 The multi-objective optimization of extraction process was carried out with using the predictive equations of
135 RSM. The genetic-evolutionary algorithm was employed to identify the optimum of experimental parameters in
136 terms of pH and NaCl concentration. The aim of optimization was to maximize SFA_{YIELD} , C_{SFA} and C_{PROT} and to
137 minimize SFA_{PHEN} and C_{PHYT} . The non-dominated solutions were also calculated including the set of constraints:
138 $SFA_{YIELD}>70\%$, $C_{SFA}>90\%$, $SFA_{PHEN}<1.6$ mg of CQA covalently bound per gram of proteins, $C_{PHYT}<4\%$ and $C_{PROT}>40\%$.
139 The optimization runs (n=2 000) and all data exploitation were carried out using MATLAB software from
140 MathWorks (Natick, MA, USA). The selected optimal condition was validated on additional experimental batch
141 extraction by comparing the observed responses with prediction intervals of models (PI) and calculation of
142 relative error (RE).

143 2.5. Protein purification

144 Protein purification performed to obtain SFA isolate was divided into three principal stages: extract clarification
145 by microfiltration, protein precipitation from extract using ammonium sulphate and protein desalting by size
146 exclusion chromatography. The microfiltration step was carried out on Akta system from GE Healthcare
147 (Chicago, IL, USA) using Hydrosart membrane system (0.2 μm , 200 cm^2) from Sartorius (Gottingen, Germany).
148 The 4 L of collected liquid extract was concentrated by a volumetric factor of 8 and then the retentate was
149 washed with 2 diafiltration volumes of 0.5 $\text{mol}\cdot\text{L}^{-1}$ NaCl. The total microfiltration permeates were pooled for next
150 step. Ammonium sulfate was added to proteins of microfiltration permeates were precipitated a 65% of
151 saturation of ammonium sulfate (or 429.88g of ammonium sulfate added to 1L of protein solution at 20 °C) and
152 stirred for 20 min at a room temperature. After protein precipitation and centrifugation step (15 000 \times g for 30
153 min at 20 °C) obtained pellet was dissolved in 750 mL of deionized water. The desalting of proteins was carried
154 out on low-pressure chromatography system of Akta Pure from GE Healthcare. The sample volume of 20% bed
155 volume of column (10 cm of height, 5 cm of diameter) was injected into G-25 Fine silica gel (also from GE
156 Healthcare). The elution was performed at 10 $\text{mL}\cdot\text{min}^{-1}$ with deionized water and the peak corresponding to
157 protein recorded at 280 nm was collected and freeze-dried.

158 2.6. Analytical methods

159

160 2.6.1. Protein characterization by gel electrophoresis

161 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli
162 method (1970). Sunflower aqueous extract was diluted in 0.1 $\text{mol}\cdot\text{L}^{-1}$ of pH 7 sodium phosphate buffer to obtain
163 a final concentration of 2 $\text{g}\cdot\text{L}^{-1}$. Then, the sample was solubilized in 50 μL of Laemmli buffer containing 2% β -
164 mercaptoethanol (v/v) and heated at 95 °C for 5 min. Molecular weight markers ranging 250–10 kDa (Precision
165 Plus Protein Standards) and 26.6–6.5 kDa (Polypeptide SDS-PAGE Standard) were used (Bio-Rad, Hercules, USA).
166 10 μL of sample and markers were deposited to a 5% of polyacrylamide stacking gel and separated in a 17%
167 polyacrylamide resolving gel. The migration step was carried out at 20 mA per gel. The gel was subsequently
168 stained with Coomassie Brilliant Blue and destained overnight in 10% acetic acid solution (v/v).

169 2.6.2. Helianthinin and albumin content in extract

170 SE-HPLC analysis was performed according to the method of Defaix *et al.* (2019). The analyses were carried out
 171 on a HPLC Shimadzu LC30 system coupled with photodiode array detector (PDA) and operated by LabSolutions
 172 software, all from Shimadzu Corporation (Kyoto, Japan). 5–20 µL of sample was injected into a Biosep SEC s-
 173 2000 column (300×7.8 mm; 5 µm) from Phenomenex (Torrance, CA, USA). The exclusion range of molecular
 174 weight was between 1 and 300 kDa. During analysis the autosampler and column compartment were maintained
 175 at 20 and at 35 °C, respectively. The mobile phase consisted of acetonitrile/water/trifluoroacetic acid (45:54.9:0.1
 176 v/v). The elution flow rate was set at 0.6 mL.min⁻¹. All solvents were HPLC grade and were supplied from Fisher
 177 Scientific (Hampton, USA). The ultrapure water (H₂O) with resistivity ≥ 18.2 MΩ.cm⁻¹ was used. The PDA signal
 178 was recorded between 190 and 400 nm with maximal absorption at 214 nm for protein. Assuming the same
 179 value of molar extinction coefficient for helianthinins and sunflower albumins [according to Defaix *et al.* (2019)],
 180 the content of helianthinins (C_{HEL}) and sunflower albumins (C_{SFA}) in extracts were calculated as follows:

$$C_{HEL} = \frac{A_{HEL}}{A_{HEL} + A_{SFA}} \times 100\% \quad (\text{Eq. 2})$$

$$C_{SFA} = \frac{A_{SFA}}{A_{HEL} + A_{SFA}} \times 100\% \quad (\text{Eq. 3})$$

181 where A_{HEL} and A_{SFA} were peak surface at 214 nm corresponding to helianthinins or sunflower albumins,
 182 respectively. All measurements were performed in triplicate and a mean value was calculated.

183 2.6.3. Protein and free chlorogenic acid isomer concentration in extract, chlorogenic acid bound to 184 proteins

185 Sunflower protein and free chlorogenic acid isomer (3-CQA, 5-CQA et 4-CQA) content in liquid extract were
 186 determined using the SE-HPLC method of Albe Slabi *et al.* (2019). The same HPLC system and chromatographic
 187 as presented in section 2.6.2 was used except for mobile phase composition [acetonitrile/water/formic acid
 188 (10:89.9:0.1 v/v)]. The amount of covalently fixed CQAs (mg of CQAs bound per gram of SFA) were quantified
 189 considering the surface of peak at 325 nm eluted at the retention time of SFA and the average calibration slop
 190 of 3-CQA, 5-CQA and 4-CQA. The mass of soluble SFA in liquid extract was determined based on the
 191 concentration of total sunflower proteins and the content of SFA fraction in aqueous extract (section 2.6.2.). In
 192 the calculations, the same value of molar attenuation coefficient for helianthinins and SFA was assumed, as it
 193 has been confirmed by Defaix *et al.* (2019). All analysis was carried out in triplicate and an average value was
 194 calculated.

195 2.6.4. Kjeldahl method

196 Total nitrogen content in sample was carried out in accordance to Kjeldahl method procedures described in
 197 AOAC method 991.20 (1995). 0.5–2 mL of sample was mineralized in a digestion flask with 4 mL of 96% H₂SO₄
 198 (v/v) and approximately 10 mg of catalyst. The mineralization step was achieved at 450 °C during 150 min. After
 199 this time, the solution was distilled with 32% NaOH (w/v) and the mixture was titrated against 0.01 mol.L⁻¹ HCl.
 200 Blank contained all reagents without the sample. The non-protein nitrogen in sample was determined in
 201 supernatant after protein precipitation using 50% trichloroacetic acid (w/v). A conversion factor of N×5.6 was

202 used according to Defaix *et al.* (2019), Pickardt *et al.* (2011; 2015). All analyzes were repeated in triplicate.
203 Average values of concentration and standard deviation were calculated.

204 2.6.5. Helianthinin and albumin extraction yield

205 Extraction yield of helianthinins (HEL_{YIELD}) and sunflower albumins (SFA_{YIELD}) were calculated, as follows:

$$HEL_{YIELD} = \frac{C_{Pe} \times V_e \times C_{HEL}}{C_{Pm} \times m_m \times 70\%} \times 100\% \quad (\text{Eq. 4})$$

$$SFA_{YIELD} = \frac{C_{Pe} \times V_e \times C_{SFA}}{C_{Pm} \times m_m \times 30\%} \times 100\% \quad (\text{Eq. 5})$$

206 where C_{Pe} and C_{Pm} were protein concentrations (measured by Kjeldahl method) in the extract or in the starting
207 sunflower meal, V_e was the extract volume, m_m was the amount of sunflower meal, C_{HEL} and C_{SFA} were
208 helianthinin and albumin content (calculated Eq. 2 or 3), respectively. 70% and 30% represented helianthinin
209 and SFA content in the starting meal according to previous reports (Baudet & Mosse, 1977; Rahma & Rao, 1979;
210 Raymond *et al.*, 1995; Sosulski, 1979).

211 2.6.6. Quantification of phytic acid

212 Phytic acid determination was adapted from the method described by Garcia-Esteva *et al.* (1999). For this
213 purpose, 250–300 mg of solid sample was stirred with 20 mL of 0.4 mol.L⁻¹ HCl/10% Na₂SO₄ (w/v) for about 120
214 min at room temperature. The blank consisted of deionized water. Then, the mixture was centrifuged (10 000×g,
215 30 min, 20 °C) and supernatant was additionally clarified using 0.22 μm filter. In centrifuge tubes 2.5 mL of 20
216 mmol.L⁻¹ FeCl₃, 0.4 mol.L⁻¹ HCl/10% Na₂SO₄ (w/v), 20% sulfosalicylic acid (w/v) and filtered supernatant were
217 mixed and heated at 100 °C for 20 min. After cooling at room temperature, the sample was centrifuged
218 (10 000×g, 30 min, 20 °C), filtered (0.22 μm) and transferred into 50 mL volumetric flask. The pellet was washed
219 with 4 mL of deionized water, centrifuged (10 000×g, 10 min, 20 °C) and supernatant was added after filtration
220 (0.22 μm) into 50 mL volumetric flask. This step was repeated in triplicate. The volumetric flask was filled with
221 deionized water. This solution (20 mL) was adjusted to pH 2.5 ± 0.5 with glycine, heated at 70–80 °C and tittered
222 against 2 mmol.L⁻¹ EDTA solution. The equivalent volume was reached when the solution changes color from
223 burgundy to yellow green. The results were expressed as phytic acid content on dry matter basis (Ac Phyt/dm%).
224 All measurements were performed in triplicate and average value was calculated.

225 2.6.7. Sunflower albumin solubility

226 The SFA isolate was suspended in deionized water at 5.0 g.L⁻¹ (room temperature). The pH was adjusted to a
227 given value by adding 0.1 mol.L⁻¹ NaOH or 0.1 mol.L⁻¹ HCl and kept constant (pH ± 0.05) during 30 min under
228 agitation (100 rpm). Then, the slurries were centrifuged (15 000×g, 10 min, 20 °C). The protein concentration in
229 supernatant was measured by SE-HPLC according to Albe Slabi *et al.* (2019). All analyzes were repeated in
230 triplicate and an average value of concentration and standard deviation were calculated.

231 2.6.8. Color measurement

232 The solution of protein powder in deionized water was prepared at a concentration of 1% (w/v) and clarified
233 through a 0.22 μm membrane filter. The color was recorded in CIE $L^*a^*b^*$ scale using Lovibond PFX195
234 Tintometer at room temperature. The measure was performed in ten repetitions and average value of L^* , a^* ,
235 b^* parameters with standard deviation were calculated.

236 2.6.9. Amino acid composition

237 Amino acid profile was determined for purified sunflower albumins according to ISO 13903:2005 and EU
238 152/2009 protocols. For this purpose, about 10 mg of sample was hydrolyzed. Depending of analyzed amino
239 acid, three different hydrolysis methods was performed: acidic hydrolysis for le, Leu, Lys, Phe, Tyr, Thr, Val;
240 alkaline hydrolysis for Trp; and oxidative hydrolysis for Cys and Met. Then, the hydrolysate was injected into C18
241 column of HPLC system). Composition in amino acids was determined by reaction with ninhydrin using
242 photometric detection at 570 nm. For quantification of tryptophan an HPLC system coupled with fluorescence
243 detector (excitation 280 nm, emission 231 nm) was used. Amino acid profile was expressed as mg of amino acid
244 per g of sunflower albumins.

245 2.6.10. Sunflower albumin structure study

246 SFA proteins were analyzed by Dynamic Light Scattering (DLS), Circular Dichroism (CD) and Differential Scanning
247 Calorimetry (DSC).

248 DLS measurement was recorded on Zeta Sizer Nano-S from Malvern Instruments (Worcestershire, UK). 20 μL of
249 filtered (0.22 μm) SFA solution at a concentration of 1 $\text{g}\cdot\text{L}^{-1}$ at pH 4 (10 $\text{mmol}\cdot\text{L}^{-1}$ sodium phosphate buffer), pH
250 7 (10 $\text{mmol}\cdot\text{L}^{-1}$ sodium phosphate buffer) and pH 9 (10 $\text{mmol}\cdot\text{L}^{-1}$ borate buffer) were used. Samples were
251 maintained at 25 $^{\circ}\text{C}$ during measurement. The number of acquisitions varied between 13 and 17 scans.
252 Volumetric distribution of particle size was determined.

253 CD was carried out using a Chirascan Plus device from Applied Photophysics (Leatherhead, UK). The far-UV
254 spectra were obtained in the UV region of 180–280 nm. CD spectrophotometer was kept under constant flow
255 of nitrogen gas and the temperature was maintained at 20 $^{\circ}\text{C}$. Samples were prepared in the same way as for
256 DLS analysis and blanks corresponding to appropriate buffer solution was subtracted. All spectra were repeated
257 at least in triplicate and mean measurement was calculated. The far-UV spectra were converted into mean
258 residue ellipticity $[\Theta_{\text{MRE}}]$ using the number of amino acids set at 130. The content of α -helix, β -sheet structures
259 and random coils were obtained after spectrum deconvolution using CDNN software version 2.1.

260 DSC determination was carried out by Microcal VP-DSC from Malvern Panalytical (Worcestershire, UK) using the
261 SFA solution at a concentration of 2 $\text{g}\cdot\text{L}^{-1}$ in 10 $\text{mmol}\cdot\text{L}^{-1}$ pH 7 sodium phosphate buffer. The solution was filtered
262 (0.22 μm) prior analysis. The blank consisted of protein-free phosphate buffer. Thermogram was acquired over
263 a temperature range from 20 to 130 $^{\circ}\text{C}$ at the rate of 1 $^{\circ}\text{C}\cdot\text{min}^{-1}$. Thermal properties of SFA were expressed as
264 the temperature of denaturation (T_d) and the enthalpy calorimetry ΔH_{cal} , that represents the total amount of
265 energy emitted during the denaturation process.

266 2.6.11. Functional properties of sunflower albumins

267 For foaming **properties**, 20 mL of 1% (w/v) SFA isolate solution in 10 mmol.L⁻¹ pH 7 sodium phosphate buffer
268 was mixed at 10 000 rpm during 5 min at room temperature using an Ultra-Turrax® T25 digital homogenizer
269 from IKA (Staufen im Breisgau, Germany). Foaming capacity was calculated as ratio (in %) foam volume (mL) into
270 initial volume of SFA solution (mL). The foaming stability was expressed as the percentage of remaining foam
271 volume (mL) after 5, 15, 30, 60 and 120 min.

272 The emulsifying capacity were evaluated using 5% (w/v) SFA isolate solution in 10 mmol.L⁻¹ pH 7 sodium
273 phosphate buffer. For this purpose, 5 mL of above mentioned SFA solution was vigorously stirred with 2.5 mL of
274 sunflower oil using an Ultra-Turrax® homogenizer at 10 000 rpm at room temperature during 30 s. After this
275 time, 2.5 mL of sunflower oil was additionally mixed at the same speed during 90 s. The mixture was then
276 centrifuged at 1 100×g during 5 min. The ratio (in %) of emulsion volume (mL) after centrifugation and initial
277 volume of mixture (mL) represents the emulsifying capacity. The stability of emulsion was determined after
278 heating at 85 °C during 15 min and additional centrifugation (the same parameters). The stability of emulsion
279 represents the ratio (in %) of emulsion volume (mL) after and before heating.

280 All measurement of **functional** properties was carried out in triplicate and average value with standard deviation
281 were calculated. The functional properties of soy protein isolate measured in the same way as those of SFA were
282 used as a reference.

283 3. Results and Discussion

284

285 3.1. Effect of pH on sunflower proteins extraction

286 Fig. 1A presents the extraction yield of helianthinins and SFA at pH ranging from 3 to 7. Under acidic conditions
287 (pH 3–6) helianthinins were poorly extracted (around 3%). Their extraction yield increased significantly at pH 7
288 ($23.64 \pm 0.90\%$). Helianthinin isoelectric point (IP) is given at pH around 4–6 (Gonzalez-Perez & Vereijken, 2007).
289 This was consistent with their poor extractability in this pH range. The increase of their extractability at pH 7 is
290 explained by the fact that the more a protein is charged, the greater is their solubility in water. Interestingly,
291 helianthinin extractability did not increase below their IP in spite of its expected net positive charge. Gonzalez-
292 Perez *et al.* (2007) have reported the denaturation/unfolding of helianthinins occurred under strong acidic pH.
293 Hence, insoluble helianthinin aggregates were probably formed under strong acidic conditions.

294 Besides, extraction yield of sunflower albumins (SFA) was around 50% under acidic conditions (pH 3–5). It started
295 to decrease at pH 6 to reach a minimum at pH 7 ($25.87 \pm 2.38\%$). SFA have isoelectric pH around 9 and are known
296 to be highly soluble in aqueous solution in a broad pH range (Gonzalez-Perez *et al.*, 2005a; Gonzalez-Perez &
297 Vereijken, 2007). Hence, this behavior is rather unusual. At pH 7, SFA were positively charged, while negatively
298 charged helianthinins started to precipitate. Interestingly, a co-precipitation of total sunflower proteins between
299 pH 6 and 7 was observed by Gonzalez *et al.* (2002) and Pickardt *et al.* (2009). Thus, the observed limitation in
300 SFA extraction at pH close to neutrality might be related to electrostatic interactions between soluble SFA and
301 the aggregated fraction of helianthinins. At pH 6 and below helianthinin net charge is zero or positive like SFA.
302 This probably led to the increase of SFA extraction yield. Authors showed that the lowest extraction yield of total
303 sunflower proteins was between pH 4–6 (15–20%) (Gheyasuddin, Cater, & Mattil, 1970; Pawar, Patil, Sakhale, &
304 Agarkar, 2001; Pickardt *et al.*, 2009) which was in line with the present results.

305 Fig. 1B shows the content of soluble SFA in relation to total sunflower proteins determined in aqueous extract
306 by SE-HPLC (Defaix *et al.*, 2019). As expected, SFA largely dominated at pH 3–5 (>85%), while their content
307 decreased dramatically at pH 7 (about 30%). The protein composition was additionally evaluated by SDS-PAGE
308 (Fig. 1C). The gel clearly showed the selective extraction of a band between 10–18 kDa corresponding to SFA
309 molar weight in this pH range. Interestingly, the gel also revealed traces of helianthinin acidic polypeptide (26.6–
310 37 kDa). Acidic conditions are known to partly break disulfide bridges (Gonzalez-Perez & Vereijken, 2007). Hence,
311 it is possible that a small proportion of acidic polypeptides remained soluble at pH 2 to 4 while basic polypeptides
312 was in an aggregated and insoluble form.

313 Gonzalez *et al.* (2005a) had already reported a selective extraction of SFA from sunflower seeds at pH 5.0.
314 Likewise, selective extraction of rapeseed albumins was also noted by Nioi *et al.* (2012) under acidic conditions.
315 The present study provided further evidence that this behavior takes place also for sunflower proteins in similar
316 pH range and indicated a generic behavior of oilseed albumins and globulins.

317 3.2. Simultaneous effect of pH and NaCl on solid/liquid extraction performances

318 As it was shown in section 3.1, SFA extraction yield under acidic condition was only around 50%. However, NaCl
319 is known to improve sunflower protein extractability around neutrality. Therefore, simultaneous effect of pH
320 (x_1 , 3–6) and mild NaCl concentration (x_2 , 0–0.5 mol.L⁻¹) on SFA extraction was studied by design of experiments
321 (DoE). Responses related to the liquid extract and the residual meal after protein extraction were considered.
322 Besides SFA extraction yield (SFA_{YIELD}%), SFA content in extracted proteins (C_{SFA}%) and irreversible phenol-protein
323 interaction (expressed as CQA equivalent covalently bound to SFA, SFA_{PHEN}%) were investigated. Phenol-protein
324 interactions were included in the study because they are strongly related to protein color. Furthermore, the
325 impact of extraction conditions was also evaluated on the residual meal composition in phytate (C_{PHYT}%) and
326 protein (C_{PROT}%). Indeed, these two criteria are important indicators for assessing the value of meal for feed
327 applications. High protein and low phytate content are in favor of high meal value.

328 As a result, the coefficients of models (constant terms, cubic effects, interaction) claiming significant level of p-
329 value<0.05 were found (Table 1). According to data analysis, good correlation between predicted and observed
330 values ($R^2 \geq 0.802$) was obtained for all developed models. Likewise, regression p-value (≤ 0.027) and lack of fit
331 (≥ 0.051) confirmed the high performance of models for predictive purposes.

332 3.2.1. Sunflower albumin yield, content and phenolic contamination

333 The response surface describing the combined effect of pH and NaCl on albumin extraction yield (SFA_{YIELD}%) is
334 presented in Fig. 2A. From these results, an antagonist effect of pH and ionic strength could be deduced. As
335 observed before (section 3.1), DoE showed also that the SFA extraction yield was negatively impacted without
336 salt addition by increasing pH (minimum around 6). Interestingly, the contour plot also demonstrated that the
337 SFA extraction can be considerably increased by NaCl in the whole studied pH range. The maximal extractability
338 of SFA ($\geq 75\%$) was found at pH from 3.25 to 4.35 and salt addition above 0.33 mol.L⁻¹. It is commonly assumed
339 that SFA belong to water-soluble family. Therefore, the observed increase of extraction yield under mild salt
340 concentration would be rather due to a decrease of affinity with the solid than an increase of affinity in the liquid
341 phase. Thus, it is possible that these mild NaCl concentration shields electrostatic interactions that retains SFA
342 in helianthinin insoluble aggregates.

343 Fig. 2B shows the impact of pH and NaCl on SFA content in the liquid phase (C_{SFA}%). In the pH range between 3
344 and 5 the content of SFA was high (80–90%) and relatively independent of NaCl. Since SFA extraction yield was
345 improved by salts, the constant content of SFA indirectly indicated an improvement in helianthinin extractability
346 as well. This suggested a partial protective effect of ionic strength against helianthinin denaturation under acidic
347 conditions. A negative effect of pH and NaCl was observed between pH 5 and 6. The lowest SFA content (<40%)
348 was observed at pH 6 and 0.5 mol.L⁻¹. This is most probably due to the increase of helianthinin extraction yield
349 by NaCl addition, which has previously been suggested by others (Gonzalez-Perez & Vereijken, 2007; Ivanova et
350 al., 2012; Pickardt et al., 2009).

351 The third response was the amount of CQA covalently bound to SFA (SFA_{PHEN} mg.g⁻¹). Fig. 2C shows an
352 antagonistic effect of pH and NaCl on the irreversible phenolic association with SFA. Low NaCl concentration and
353 high pH increased phenolic complexation. The maximum binding was observed between pH 5 and 6 (>2 mg CQA

354 per gram of SFA). CQA-SFA covalent binding was limited by NaCl. This effect was most pronounced under strong
355 acidic pH. CQA has a negative net charge ($pK_a=3-3.5$) in this pH range, while SFA are positively charged. Hence,
356 electrostatic interactions probably increase local concentration of CQA at protein surface, increasing covalent
357 binding. On the other hand, these electrostatic interactions are probably shielded by salt protecting from the
358 irreversible association. This observation is in good agreement with previous reports (Ozdal et al., 2013; Sastry
359 & Rao, 1990) indicating a negative effect of ionic strength on phenol-protein bonding studied in model
360 interaction systems. Therefore, these results clearly demonstrate that lower pH and higher NaCl concentration
361 should to be applied during solid/liquid extraction to reduce the covalent phenol attachment to SFA.

362 3.2.2. Phytic acid and protein content in residual meal after acidic extraction

363 The level of phytic acid and the nitrogen content are known to be the most important indicators, for determining
364 the value of oilseed meal for feed application. Phytic acid (3–10%/dm of sunflower meals) is particularly pointed
365 out due to decrease of bioavailability of some minerals (Ca^{2+} , Mg^{2+} , Zn^{2+} , Fe^{2+} , Mn^{2+} , Cu^{2+}) and proteins in
366 digestive tract by forming unabsorbable complexes (Cheryan & Rackis, 1980; Kumar et al., 2010; Nissar et al.,
367 2017). Thus, the extraction condition allowing simultaneously minimizing the amount of phytic acid and
368 maintaining the nitrogen content in residual meal of solid/liquid extraction was searched to increase its
369 suitability for animal nutrition.

370 The contour plot illustrating the effect of extraction conditions on phytic acid content in residual meal after SFA
371 extraction ($C_{PHYT\%}$) is presented in Fig. 3A. The generated response surface indicated that the content of phytic
372 acid in the residual meal decreased considerably in a pH range between 4 and 5. At pH lower than 5.5, NaCl also
373 contributed to lower the phytic acid in meal. The similar effect of the pH on phytic acid content in residual meal
374 has already been observed by others (Blancher, Elstner, Stein, & Mukherjee, 1983; Cheryan & Rackis, 1980;
375 Gandhi, Jha, & Gupta, 2008; Kumar et al., 2010; Nissar et al., 2017; Serraino, & Thompson, 1984). Interestingly,
376 Saeed *et al.* (1988) and Bulmaga *et al.* (1989) have also showed that the maximal phytate extractability from
377 sunflower meal is around pH 5. This probably explains the observed effect of the pH on phytate content in the
378 meal after extraction. NaCl might probably help lowering phytate in residual meal also by improving phytate
379 solubility in the aqueous phase.

380 The protein content remaining after solid/liquid extraction ($C_{PROT\%}$) plotted against pH and NaCl concentration
381 is displayed in Fig. 3B. Generally, protein concentration in residual meal decreased with the increase of pH and
382 ionic strength. In accordance to the above-mentioned conclusion of protein extractability, minimum residual
383 protein content in meal (30%) was achieved with high value of pH (about 6) and high NaCl concentration (about
384 0.5 mol.L^{-1}). These conditions enhanced probably the extraction of helianthinins that is the main protein fraction.
385 In contrast, protein rich residual meal was by-produced using pH ranging from 4 to 5.5 without salt addition.

386 3.3. Multi-objective optimization

387 The polynomial equations of models were used to identify the most suitable scenario for solid/liquid extraction.
388 The objective of the optimization was to maximize extraction yield and content of SFA, while minimize their

389 phenolic contamination. Simultaneously, a value-added **residual meal** characterized by reduced content of
390 phytic acid and high protein level was desired. To reach the targets, the following constraints were selected:
391 $SFA_{YIELD} > 70\%$, $C_{SFA} > 90\%$, $SFA_{PHEN} < 1.6 \text{ mg.g}^{-1}$, $C_{PHYT} < 4\%$ and $C_{PROT} > 40\%$. The set of non-dominated solutions from
392 multi-objective optimization is presented in Fig. 4 (grey points). According to these results, solutions region was
393 found in the design area between pH 3.8–4.2 and 0.25–0.5 mol.L⁻¹ NaCl. Since reduction of salt concentration
394 leads to decrease the **cost** of industrial process, the lowest ionic strength was privileged. Additionally, lower pH
395 values resulting in more selected extraction of SFA were also considered. Considering these aspects, the
396 extraction at pH of 4.1 and NaCl concentration of 0.25 mol.L⁻¹ was found to be the best trade-off. The proposed
397 solution **allowed** to achieve simultaneously satisfying theoretically responses for aqueous extract
398 ($SFA_{YIELD} = 71.82\%$, $C_{SFA} = 90.80\%$, $SFA_{PHEN} = 1.52 \text{ mg.g}^{-1}$) and remained after extraction **residual meal** ($C_{PHYT} = 3.98\%$,
399 $C_{PROT} = 42.18\%$).

400 The accuracy of models to predict the process performance in the selected condition was verified by comparing
401 **the** predicted and actual values. The results of model validation including prediction interval (PI), experimental
402 value and **relative error (RE)** are summarized in Table 2. The experimental values obtained for C_{SFA} (88.78%),
403 SFA_{PHEN} (1.55 mg.g⁻¹), C_{PHYT} (3.74%) and C_{PROT} (40.97%) was included in the 95% probably prediction intervals.
404 Only SFA_{YIELD} (61.62%) was slight lower (**RE** = -14.20%) in relation to expected value. This is probably due to
405 extremely good replicates in the central point of DoE (reproducibility = 99.3%) resulting in narrow PI ($\pm 3.61\%$).
406 However, all experimental data of SFA extraction fitted to predicted values within an acceptable limit of $RE \leq 15\%$
407 that is commonly used for simulation of complex process. Therefore, the selected condition **guaranteed** the
408 most sustainable SFA extraction from meal and the high value-added of residual meal.

409 3.4. Characterization of sunflower albumins obtained from acid extraction

410 Based on the results of multi-objective optimization, SFA were extracted at pH 4.1 and 0.25 mol.L⁻¹ NaCl and
411 purified by size exclusion chromatographic technique. The chemical composition of the obtained SFA product is
412 shown in Table 3. The purified proteins (93.3% proteins/dm) revealed rich content in sunflower albumins (90.3
413 $\pm 0.1\%$). In addition, they were free of CQA isomers (not detected), low in phytic acid (0.88%/dm) and their color
414 was close to white ($L^* = 92.2 \pm 2.9$, $a^* = 1.3 \pm 0.7$, $b^* = 11.0 \pm 1.4$). Regarding the composition of amino acids (Table
415 A.2), almost all amino acids exceed required content in comparison with FAO/WHO/UNU pattern (2007). Only
416 tryptophan and isoleucine concentrations were lower (69.67% and 98.82%, respectively). However, as expected,
417 SFA were unusually rich in cysteine ($39.23 \pm 5.47 \text{ mg.g}^{-1}$ protein) and methionine ($34.08 \pm 4.82 \text{ mg.g}^{-1}$ protein).
418 These amounts correspond to 333.24% of recommended by FOA/WHO/UNU the protein input of sulphur-
419 containing amino acids.

420 The structural and functional properties of isolated SFA were also studied. The graph in Fig. A.1 shows the
421 solubility of SFA as a function of pH ranging from 2 to 11. According to these data, SFA revealed highly soluble
422 (close or equal to 100%) regardless the pH solution. The obtained solubility curve coincided well with those
423 found in the recent works of Gonzalez *et al.* (2005a and 2007).

424 In order to assess information about size distribution of SFA under various pH values (4, 7, 9) DLS analysis were
425 performed. As presented in Fig. 5A, a single peak with size between 3.8–4.0 d. nm (99.5–100% of total sample
426 volume) was observed regardless pH value. The previously measured size of plant 2S albumins was around 4
427 d.nm (Kaszyńska et al., 2002). Thus, the observed peak was correlated to the presence of SFA. Furthermore, no
428 protein aggregation was detected.

429 On the other hand, the stability of SFA structure against various pH and thermal treatment was investigated.
430 The far-UV CD spectra (Fig. 5B) indicated that there is no change in SFA conformation state across variable pH
431 values (4, 7 and 9). As shown in the internal table of Fig. 5B, the deconvolution of CD spectra revealed similar
432 proportion of α -helix (36–38%) and β -sheet (36–37%) elements, while random coil forms were found at slightly
433 lower level (26–27%). These results agreed with previous observations (Berezcz et al., 2010; Burnett et al. 2002;
434 Pandya et al., 2000) demonstrating a comparable content of α -helix (10–40%), β -sheet (30–45%) and random
435 coil (27–30%) in native SFA structure. Also, DSC thermogram of SFA solution at pH 7 (Fig. 5C) recording the peak
436 of denaturation at the temperature of 120.8 °C with a ΔH_{cal} of 73.6 kJ.mol⁻¹ indicated extremely high
437 thermoresistance of produced SFA. This finding fits well with earlier reports (Berezcz et al., 2010; Burnett et al.
438 2002; Gonzalez-Perez et al., 2005a) describing denaturation of SFA far above 100 °C ($T_d \approx 110$ –120 °C).

439 The graph in Fig. 6 displays foaming (A and A') and emulsifying proprieties (B) of SFA compared with
440 commercially available soy proteins used as a reference. Generally, SFA presented higher foaming capacity (359
441 \pm 14% of initial solution volume, Fig. 6A) in relation to value obtained for soy proteins (297 \pm 19% of initial
442 solution volume). Also, the foam formed by SFA (about 50% over 120 min from mixing) turned out to be more
443 stable compared to the more labile foam of soybean proteins (24 \pm 14% over 120 min from mixing). On the other
444 hand, as shown at Fig. 6B, the two proteins evaluated exhibited comparable ability to form emulsion (about 40–
445 45% of initial solution volume) and the thermal stability at 85 °C of emulsions obtained by SFA and soy isolate
446 were similar and very high (about 95–100% of initial emulsion volume). Altogether, the obtained results meet
447 the principal conclusions (Gonzalez-Perez et al., 2005a; Gonzalez-Perez et al., 2005b; Gonzalez-Perez, Vereijken,
448 van Koningsveld, Gruppen, & Voragen, 2005c; Gonzalez-Perez & Vereijken, 2007; Gueguen et al., 2016) that SFA
449 have an important potential as an alternative plant-based ingredient.

450 4. Conclusion

451 To this date, the precise impact of classically applied experimental conditions on SFA solid/liquid extraction from
452 meal was not known. The present study provided an essential fundamental knowledge, which enhances the
453 overall understanding of protein extraction from sunflower meal. For the first time, selective extraction of SFA
454 was demonstrated in the range of pH from 3 to 6. Further complete and rational approach using DoE and multi-
455 objective optimization allowed establishing the best compromise of extraction conditions in terms of pH and
456 NaCl concentration. Based on these results, the solid/liquid extraction at pH 4.1 and 0.25 mol.L⁻¹ NaCl resulted
457 in selective production of SFA (88.78%) with reduced phenolic contamination (1.55 mg of CQA per g of SFA) and
458 good SFA extraction yield (61.62%). Simultaneously, the optimal extraction condition allowed obtaining the
459 protein-rich residual meal (40.97%/dm) with reduced amount of antinutritional phytate (3.74%/dm) and other

460 non-protein compounds. The white-colored SFA isolated in the optimum of extraction conditions were perfectly
461 soluble in water (about 100% in all pH range 2–11) and highly resistant on both pH and temperature treatments.
462 Likewise, comparable functional properties (foaming and emulsifying) of SFA to the routinely used protein
463 products from soybean were shown. These results supported the idea that SFA are very **promising valuable**
464 protein-based ingredient which could be successfully used in various food applications. Therefore, proposed
465 alternative process for preparation of SFA and **residual meal** could be an answer for sustainable valorization of
466 sunflower meal in food industry.

467

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473 **References**

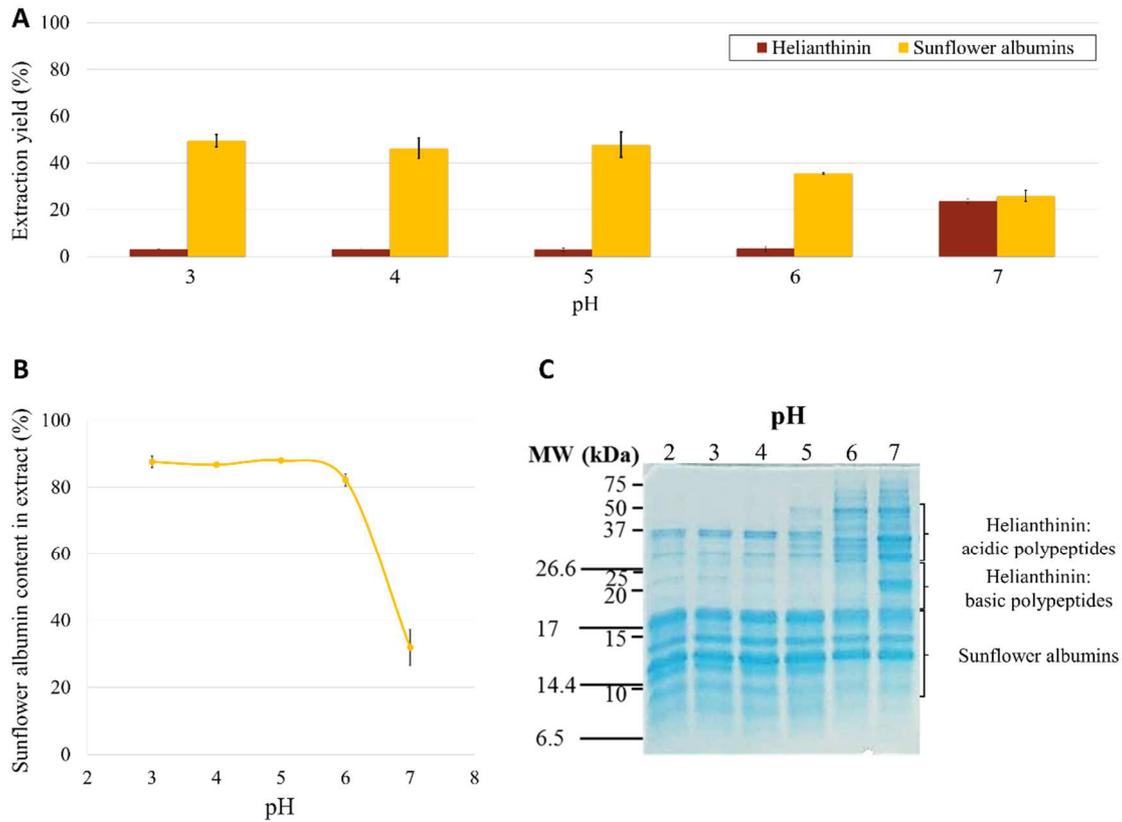
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597 **Figures**



598

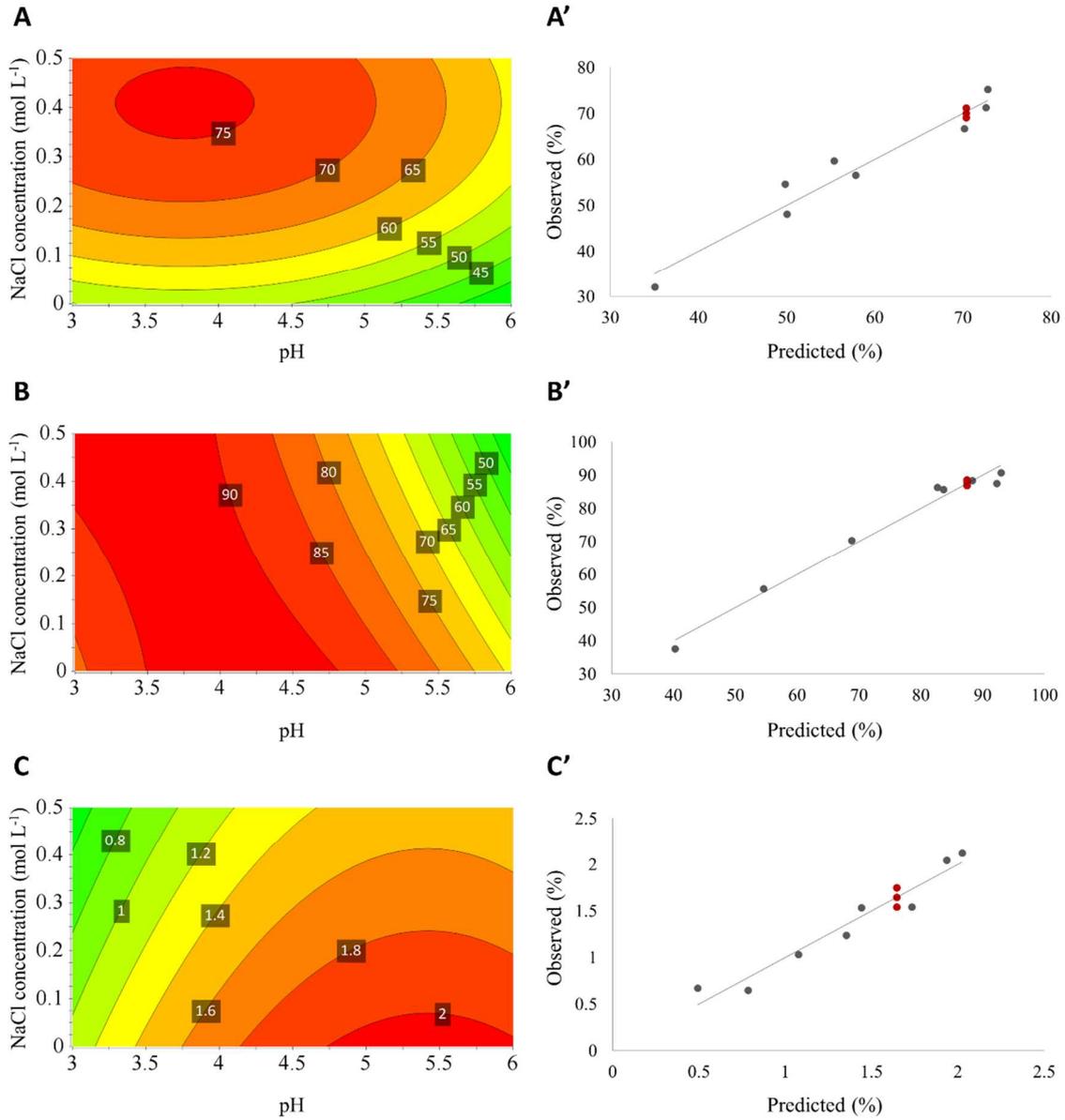
599 **Fig. 1.** Influence of pH (3–7) on extraction yields of helianthinins and **sunflower albumins** (A). The composition
 600 of extracted sunflower proteins analyzed by SE-HPLC (B) or visualized by reducing SDS-PAGE at the same pH
 601 range (C).

602

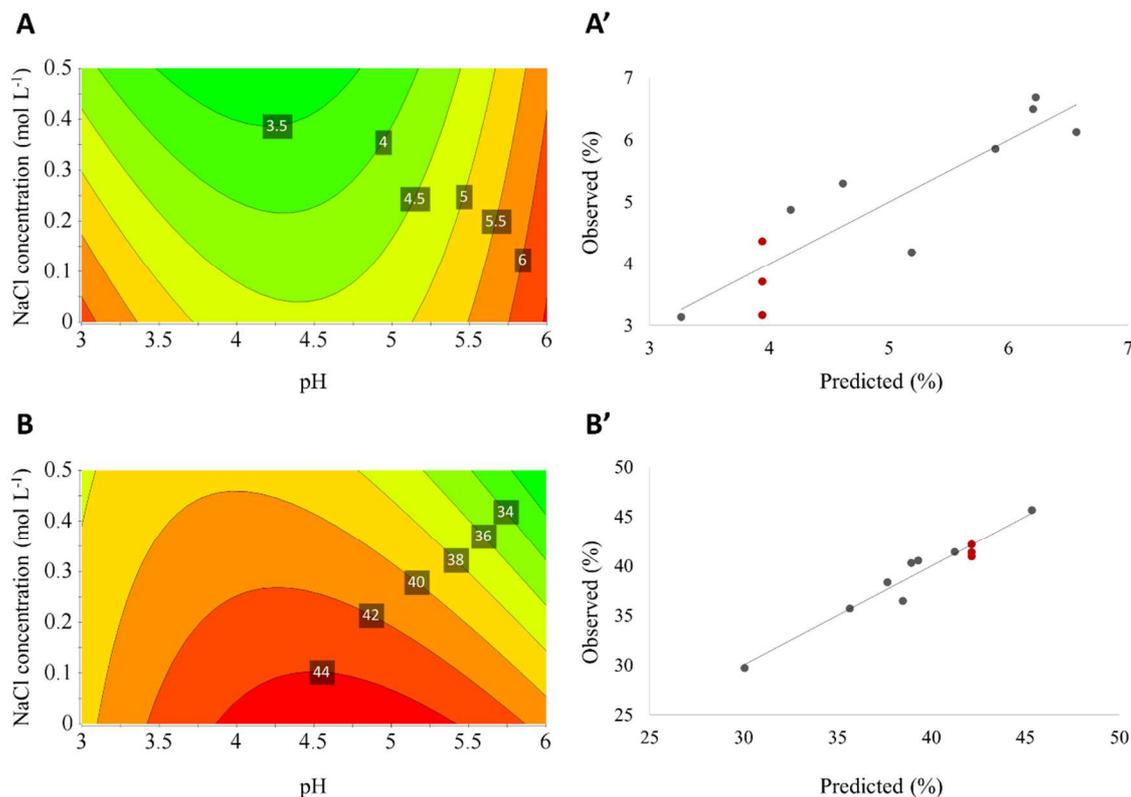
603 **Table 1.** Regression coefficients of the predicted polynomial models for **sunflower albumin** extraction yield
 604 (SFA_{YIELD}), albumin content in liquid extract (C_{SFA}), phenolic contamination of albumins (SFA_{PHEN}) and phytic acid
 605 (C_{PHYT}) and protein (C_{PROT}) content in residual meal.

Response	Regression coefficients						Statistic model parameters			
	β_0^a	β_1^b	β_2^c	β_{11}^d	β_{22}^e	β_{12}^f	R ²	RSD ^g	Regression p-value	Lack of fit
SFA _{YIELD} ^h	3.92	25.42	117.0	-3.37	-142.8	n.s. ⁱ	0.953	3.61	0.000	0.056
C _{SFA} ^j	-29.89	59.23	94.43	-7.13	n.s.	-25.27	0.981	3.10	0.000	0.051
SFA _{PHEN} ^k	-2.96	1.86	-1.17	-0.17	n.s.	n.s.	0.937	0.15	0.000	0.311
C _{PHYT} ^l	20.00	-6.96	-6.75	0.79	n.s.	0.90	0.802	0.74	0.027	0.390
C _{PROT} ^m	-3.20	20.92	15.77	-2.25	n.s.	-6.35	0.942	1.31	0.001	0.154

606 ^a constant; ^b coefficient of linear effect of pH; ^c coefficient of linear effect of NaCl concentration; ^d coefficient of cubic effect of pH; ^e coefficient
 607 of cubic effect of NaCl concentration; ^f interaction coefficient of pH and NaCl concentration; ^g residual standard deviation; ^h sunflower
 608 albumin extraction yield (%); ⁱ not significant; ^j sunflower albumin content in extract (%); ^k phenolic bonding to sunflower albumins in extract
 609 (mg.g⁻¹); ^l acid phytic content in residual meal (%); ^m protein content in residual meal (%).

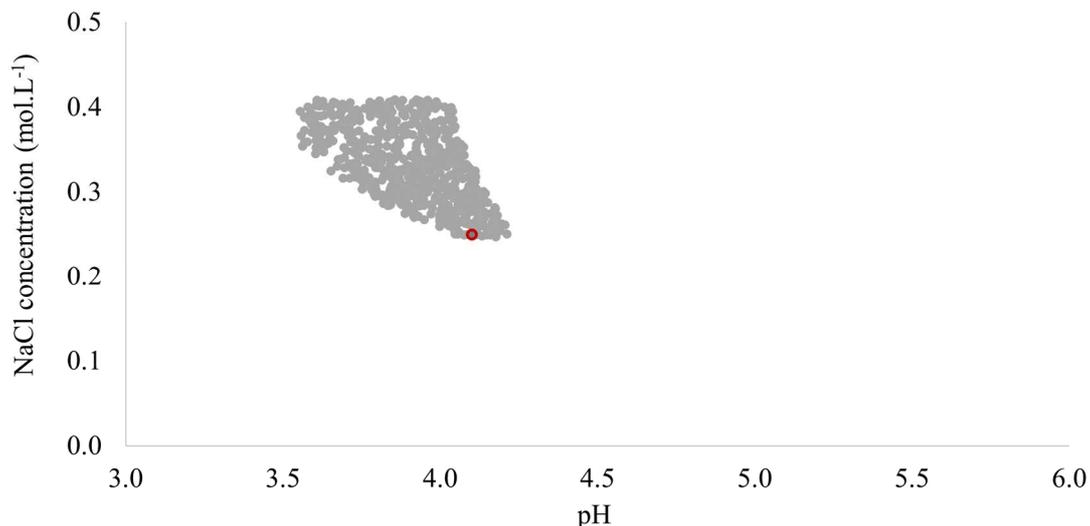


610
 611 **Fig. 2.** The contour plot of **sunflower albumin** extraction yield (A), **sunflower albumin** content in extract (B) and
 612 **sunflower albumin** phenolic contamination (C) as a function of pH (3–6) and NaCl concentration (0–0.5 mol.L⁻¹).
 613 Comparison the predicted by model and actual values for SFA_{YIELD} (A'), C_{SFA} (B') and SFA_{PHEN} (C') responses. The
 614 central experiments of design matrix correspond to red point on the graph.



615
 616 **Fig. 3.** The contour plot of phytate (A) and protein content (B) in residual meal from protein extraction as a
 617 function of pH (3–6) and NaCl concentration (0–0.5 mol.L⁻¹). Comparison the predicted by model and actual
 618 values for C_{PHYT} (A') and C_{PROT} (B') responses. The central experiments of design matrix correspond to red point
 619 on the graph.

620



621
 622 **Fig. 4.** Non-dominated responses (grey points) in multi-objective optimization of solid/liquid extraction taking
 623 into consideration sunflower albumin extraction yield (SFA_{YIELD}>70%), sunflower albumin content (C_{SFA}>90%),
 624 sunflower albumin phenolic contamination (SFA_{PHEN}<1.6 mg.g⁻¹) in aqueous extract and phytate (C_{PHYT}<4%),
 625 protein content (C_{PROT}>40%) in residual meal. Red-framed point correspond to the optimal condition selected
 626 for solid/liquid extraction.

627

628 **Table 2.** Results of model validation for the extraction in the optimum of parameters.

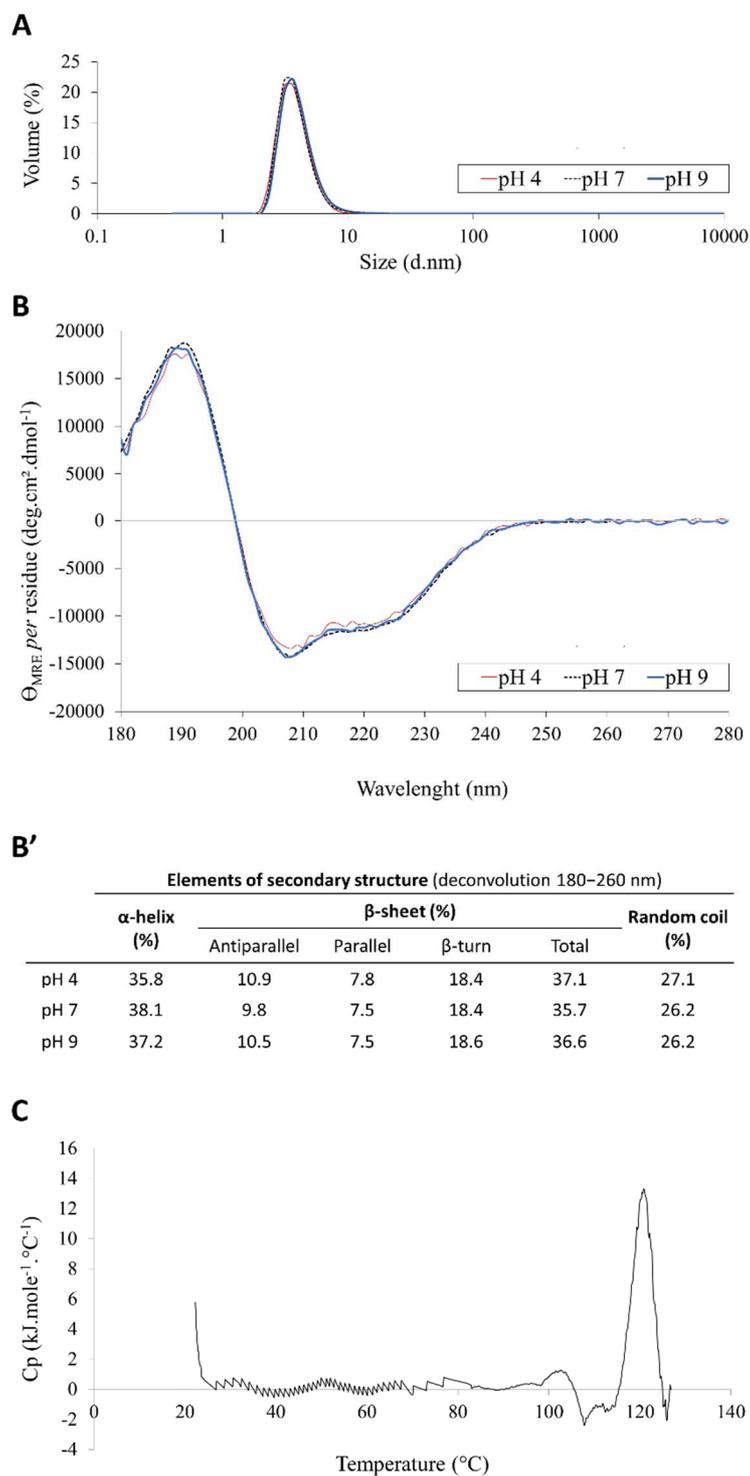
Factor		Response	PI ^a	Experimental value	RE ^b
pH	C _{NaCl} (mol.L ⁻¹)				
4.1	0.25	SFA _{YIELD} ^c	68.21–75.43	61.62	-14.20
		C _{SFA} ^d	87.70–93.90	88.78	-2.23
		SFA _{PHEN} ^e	1.37–1.67	1.55	+2.02
		C _{PHYT} ^f	3.24–4.72	3.74	-6.03
		C _{PROT} ^g	40.87–43.49	40.97	-2.88

629 ^a prediction interval at a probability level of 95%; ^b relative error between predicted and actual values (%); ^c sunflower albumin extraction
 630 yield (%); ^d sunflower albumin content in extract (%); ^e phenolic bonding to sunflower albumins in extract (mg.g⁻¹); ^f acid phytic content in
 631 residual meal (%); ^g protein content in residual meal (%).
 632

633 **Table 3.** Characterization of **sunflower albumins** produced under optimal extraction condition (pH 4.1 and 0.25
 634 mol.L⁻¹ NaCl).

Dry matter content (%)		94.8
Protein content/dm (%)		93.3
Albumin content (%)		90.3 ± 0.1
Chlorogenic acid monoisomers content/dm (%)	3-CQA	n.d. ^a
	5-CQA	n.d.
	4-CQA	n.d.
Phytic acid content/dm (%)		0.88
Color	L*	92.2 ± 2.9
	a*	1.3 ± 0.7
	b*	11.0 ± 1.4
Sulfur-containing amino acids (mg.g ⁻¹ protein)		73.31

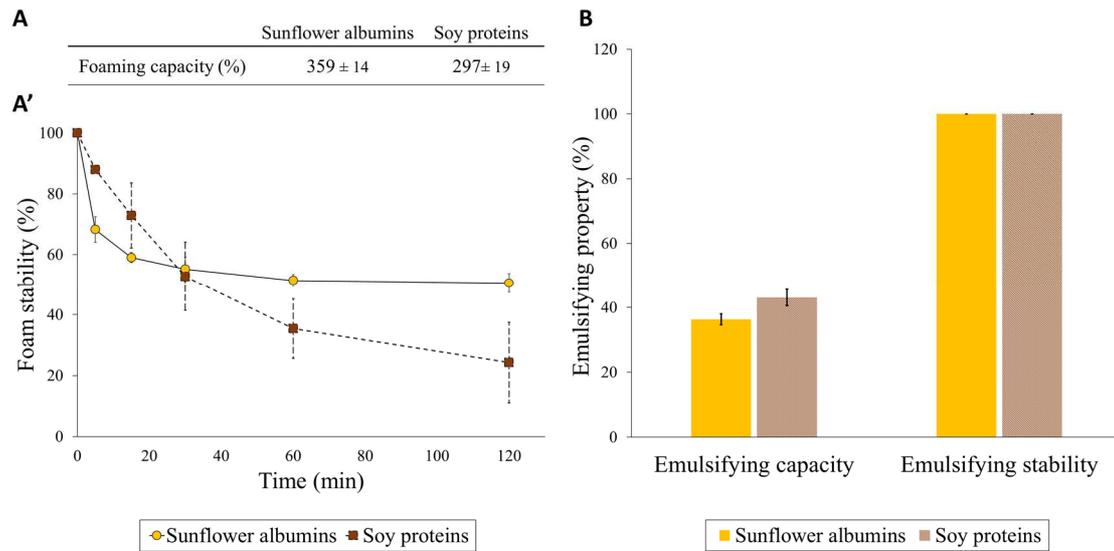
635 ^a not detected
 636



637

638 **Fig. 5.** The volume size distribution of **purified sunflower albumins** at pH 4, 7 and 9 analyzed by DSL at 20 °C (A).
 639 The far-UV CD spectra (180–280 nm) of the same protein solutions at 20 °C (B) expressed in mean residual
 640 ellipticity (deg.cm².dmol⁻¹ *per residue*) and the results of spectral deconvolution (internal table). The repetitive
 641 DSC scan obtained for **purified sunflower albumins** at pH 7 at temperature ranging from 20 to 130 °C (C).

642



643

644 **Fig. 6.** Foaming capacity (**A**) foaming stability (**A'**) and emulsifying properties (**B**) of **purified sunflower albumins**
 645 compared to reference soy proteins. Each point represents the average of three independent measurements.

646

647 **Appendix**

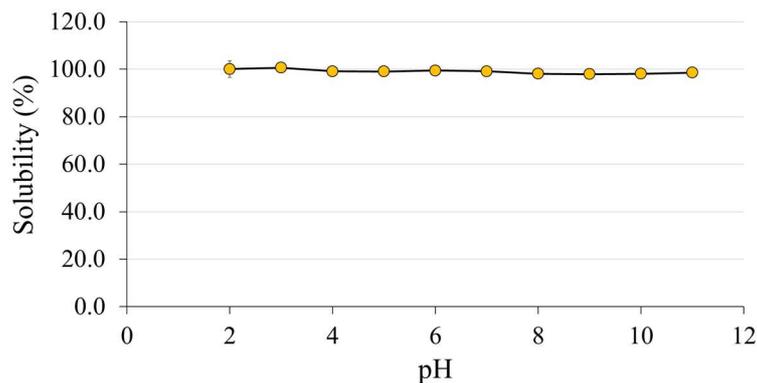
648 **Table A.1.** Experimental matrix (coded and uncoded) of 3² full factorial design with three replications in the
 649 central point. Investigated variables were pH (x₁) ranging from 3–6 and NaCl concentration (x₂) from 0–0.5.mol.L⁻¹
 650 ¹.

No.	Coded factors		Uncoded factors	
	x ₁ ^a	x ₂ ^b	x ₁	x ₂
1	0	-1	4.5	0
2	0	0	4.5	0.25
3	1	1	6.0	0.5
4	0	0	4.5	0.25
5	-1	1	3.0	0.5
6	-1	-1	3.0	0
7	-1	0	3.0	0.25
8	0	0	4.5	0.25
9	0	1	4.5	0.5
10	1	-1	6.0	0
11	1	0	6.0	0.25
1	0	-1	4.5	0

^a pH; ^b NaCl concentration

651

652



653

654 **Fig. A.1.** Solubility of **purified sunflower albumins** as a function of pH (2–11). Each point represents the average
 655 of three independent measurements.

656 **Table A.2.** Amino acid composition of **purified sunflower albumins** in comparison to the FAO/WHO/UNU
 657 requirement pattern.

Amino acid	Amino acid composition of sunflower albumins (mg.g ⁻¹ protein)	FAO/ WHO/UNU amino acid pattern (mg.g ⁻¹ protein)
Histidine	20.26	15.00
Isoleucine	40.19	30.00
Leucine	58.31	59.00
Lysine	58.95	45.00
Methionine	34.08	16.00
Cystine	39.23	6.00
Methionine and cysteine	73.31	22.00
Phenylalanine and tyrosine	42.55	38.00
Threonine	41.80	23.00
Tryptophan	4.18	6.00
Valine	42.55	39.00

658

