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

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Abstract

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

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

1. Introduction

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

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

Different process approaches have been tried to limit protein-phenol interactions, while keeping a satisfying protein extraction yield. The first one was to implement high ionic strength extraction. This efficiently increased protein recovery at pH around neutrality. Indeed, Pickardt *et al.* (2009) and Ivanova *et al.* (2012) showed that 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 light-colored protein product. However, such high salt concentration is unsuitable due to the cost of effluent treatments and the corrosive risk toward stainless steel. Other authors tried to limit the phenol oxidation by adding reducing agent such as Na₂SO₃, NaHSO₃ or ascorbic acid (Kuijpers et al., 2012; Richard-Forget & Gauillard, 1997; Salgado et al., 2012). Nevertheless, this method still yielded greenish isolate solutions at basic pH or after protein storage (Yoruk & Marshall, 2003; Wildermuth et al., 2016). Another approach was to carry out a polyphenol extraction with organic or organic-aqueous mixtures such acidified butanol (Saeed & Cheryan, 1988), 60% methanol (Malik & Saini, 2017), 80% methanol (González-Pérez et al., 2002) or 95% ethanol (Carter,

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

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

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

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 meal was investigated by design of experiments (DoE). Albumin extraction yield, albumin content and amount of CQA covalently bound to albumin, and phytic acid/nitrogen content in the residual meal (after protein extraction) were considered as performance criteria. Then, a multi-objective optimization strategy was applied in order to find the best trade-off between all considered criteria. Eventually, the structure and functional properties of SFA obtained in the optimal conditions were characterized.

2. Materials and Methods

2.1. Materials and chemicals

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

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

2.2. Effect of pH on extraction performances

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

2.3. Experimental design

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

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

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 coefficients of cubic effect, β_{12} is the coefficient of interaction effect, x_1 and x_2 are the coded independents of pH and NaCl concentration, respectively.

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

2.4. Multi-objective optimization

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

2.5. Protein purification

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

2.6. Analytical methods

2.6.1. Protein characterization by gel electrophoresis

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

2.6.2. Helianthinin and albumin content in extract

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

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

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

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

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

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

2.6.4. Kjeldahl method

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

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

2.6.5. Helianthinin and albumin extraction yield

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

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

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

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

2.6.6. Quantification of phytic acid

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

2.6.7. Sunflower albumin solubility

The SFA isolate was suspended in deionized water at 5.0 g.L⁻¹ (room temperature). The pH was adjusted to a 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 agitation (100 rpm). Then, the slurries were centrifuged (15 000×g, 10 min, 20 °C). The protein concentration in supernatant was measured by SE-HPLC according to Albe Slabi *et al.* (2019). All analyzes were repeated in triplicate and an average value of concentration and standard deviation were calculated.

2.6.8. Color measurement

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

2.6.9. Amino acid composition

Amino acid profile was determined for purified sunflower albumins according to ISO 13903:2005 and EU 152/2009 protocols. For this purpose, about 10 mg of sample was hydrolyzed. Depending of analyzed amino acid, three different hydrolysis methods was performed: acidic hydrolysis for le, Leu, Lys, Phe, Tyr, Thr, Val; alkaline hydrolysis for Trp; and oxidative hydrolysis for Cys and Met. Then, the hydrolysate was injected into C18 column of HPLC system). Composition in amino acids was determined by reaction with ninhydrin using photometric detection at 570 nm. For quantification of tryptophan an HPLC system coupled with fluorescence detector (excitation 280 nm, emission 331 nm) was used. Amino acid profile was expressed as mg of amino acid per g of sunflower albumins.

2.6.10. Sunflower albumin structure study

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

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

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

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

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.

3. Results and Discussion

3.1. Effect of pH on sunflower proteins extraction

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

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

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

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

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

As it was shown in section 3.1, SFA extraction yield under acidic condition was only around 50%. However, NaCl is known to improve sunflower protein extractability around neutrality. Therefore, simultaneous effect of pH (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 (DoE). Responses related to the liquid extract and the residual meal after protein extraction were considered. Besides SFA extraction yield (SFA_{YIELD}%), SFA content in extracted proteins (C_{SFA}%) and irreversible phenol-protein interaction (expressed as CQA equivalent covalently bound to SFA, SFA_{PHEN}%) were investigated. Phenol-protein interactions were included in the study because they are strongly related to protein color. Furthermore, the impact of extraction conditions was also evaluated on the residual meal composition in phytate (C_{PHYT}%) and protein (C_{PROT}%). Indeed, these two criteria are important indicators for assessing the value of meal for feed applications. High protein and low phytate content are in favor of high meal value.

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

3.2.1. Sunflower albumin yield, content and phenolic contamination

The response surface describing the combined effect of pH and NaCl on albumin extraction yield (SFA_{YIELD}%) is presented in Fig. 2A. From these results, an antagonist effect of pH and ionic strength could be deduced. As observed before (section 3.1), DoE showed also that the SFA extraction yield was negatively impacted without salt addition by increasing pH (minimum around 6). Interestingly, the contour plot also demonstrated that the SFA extraction can be considerably increased by NaCl in the whole studied pH range. The maximal extractability 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 that SFA belong to water-soluble family. Therefore, the observed increase of extraction yield under mild salt concentration would be rather due to a decrease of affinity with the solid than an increase of affinity in the liquid phase. Thus, it is possible that these mild NaCl concentration shields electrostatic interactions that retains SFA in helianthinin insoluble aggregates.

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

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

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

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

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

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

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

3.3. Multi-objective optimization

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

phenolic contamination. Simultaneously, a value-added **residual meal** characterized by reduced content of phytic acid and high protein level was desired. To reach the targets, the following constraints were selected: $SFA_{YIELD} > 70\%$, $C_{SFA} > 90\%$, $SFA_{PHEN} < 1.6 \text{ mg.g}^{-1}$, $C_{PHYT} < 4\%$ and $C_{PROT} > 40\%$. The set of non-dominated solutions from multi-objective optimization is presented in Fig. 4 (grey points). According to these results, solutions region was 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 leads to decrease the **cost** of industrial process, the lowest ionic strength was privileged. Additionally, lower pH values resulting in more selected extraction of SFA were also considered. Considering these aspects, the 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 solution **allowed** to achieve simultaneously satisfying theoretically responses for aqueous extract ($SFA_{YIELD} = 71.82\%$, $C_{SFA} = 90.80\%$, $SFA_{PHEN} = 1.52 \text{ mg.g}^{-1}$) and remained after extraction **residual meal** ($C_{PHYT} = 3.98\%$, $C_{PROT} = 42.18\%$).

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

3.4. Characterization of sunflower albumins obtained from acid extraction

Based on the results of multi-objective optimization, SFA were extracted at pH 4.1 and 0.25 mol.L⁻¹ NaCl and purified by size exclusion chromatographic technique. The chemical composition of the obtained SFA product is shown in Table 3. The purified proteins (93.3% proteins/dm) revealed rich content in sunflower albumins ($90.3 \pm 0.1\%$). In addition, they were free of CQA isomers (not detected), low in phytic acid (0.88%/dm) and their color 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 A.2), almost all amino acids exceed required content in comparison with FAO/WHO/UNU pattern (2007). Only tryptophan and isoleucine concentrations were lower (69.67% and 98.82%, respectively). However, as expected, 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). These amounts correspond to 333.24% of recommended by FAO/WHO/UNU the protein input of sulphur-containing amino acids.

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

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

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

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

4. Conclusion

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

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

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Figures

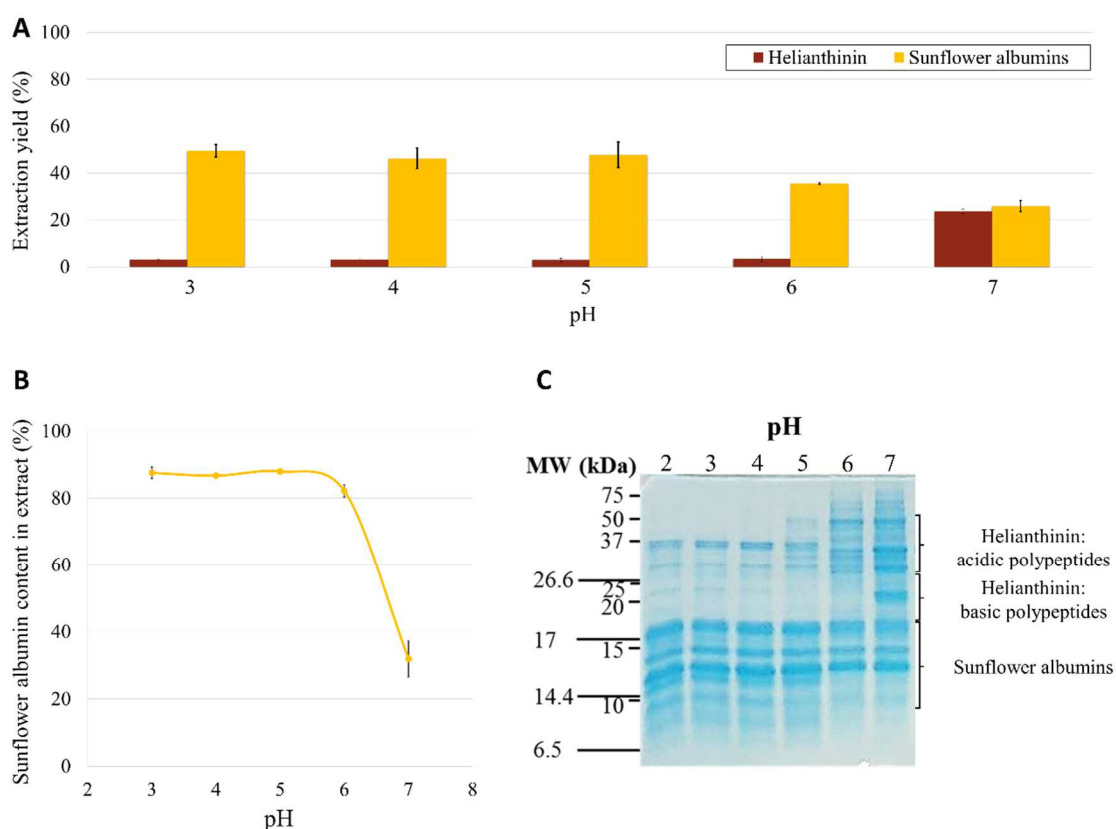


Fig. 1. Influence of pH (3–7) on extraction yields of helianthinins and sunflower albumins (A). The composition of extracted sunflower proteins analyzed by SE-HPLC (B) or visualized by reducing SDS-PAGE at the same pH range (C).

Table 1. Regression coefficients of the predicted polynomial models for sunflower albumin extraction yield (SFA_{YIELD}), albumin content in liquid extract (C_{SFA}), phenolic contamination of albumins (SFA_{PHEN}) and phytic acid (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

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

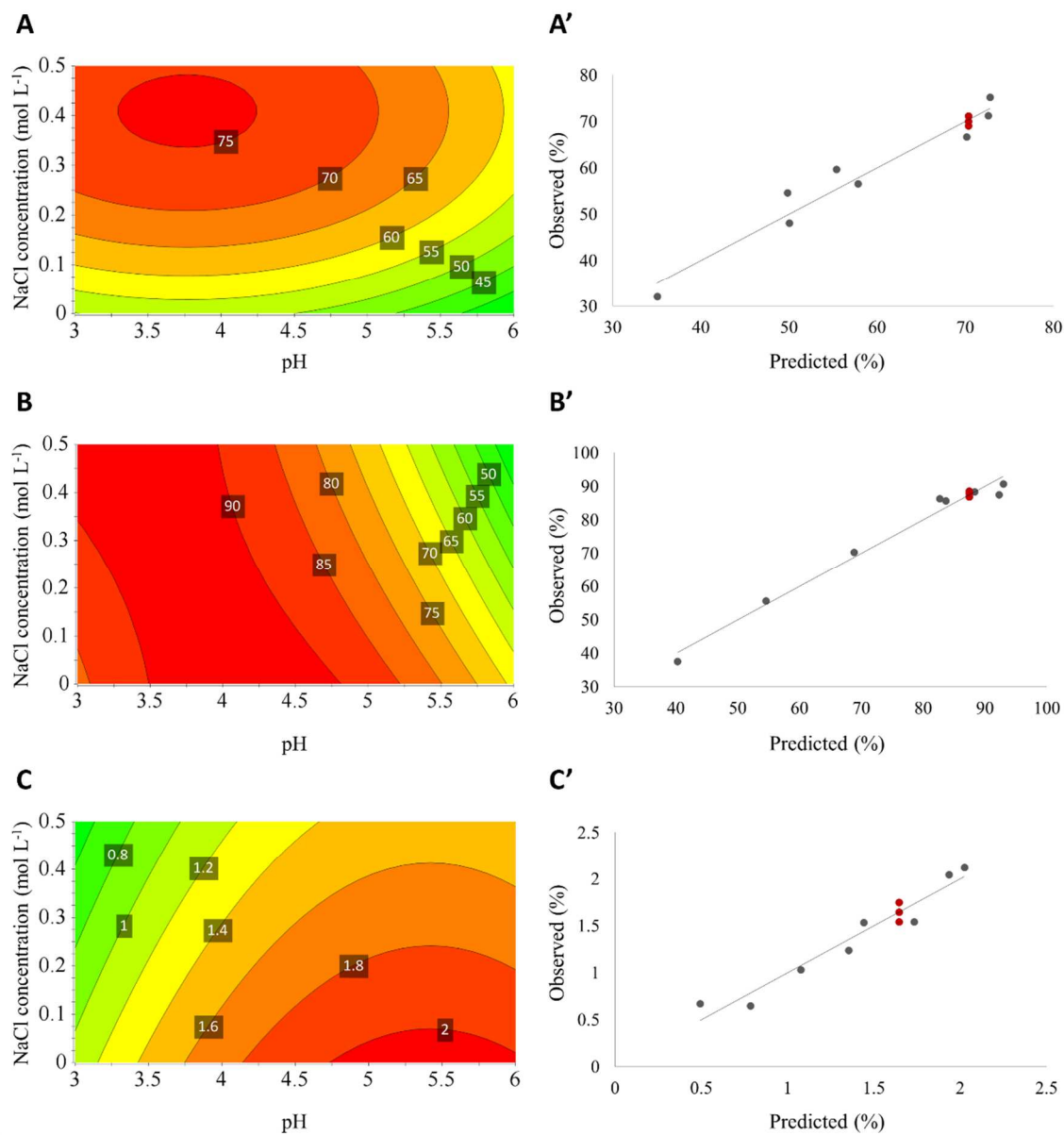


Fig. 2. The contour plot of **sunflower albumin** extraction yield (A), **sunflower albumin** content in extract (B) and **sunflower albumin** phenolic contamination (C) as a function of pH (3–6) and NaCl concentration (0–0.5 mol.L⁻¹). Comparison the predicted by model and actual values for SFA_{YIELD}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

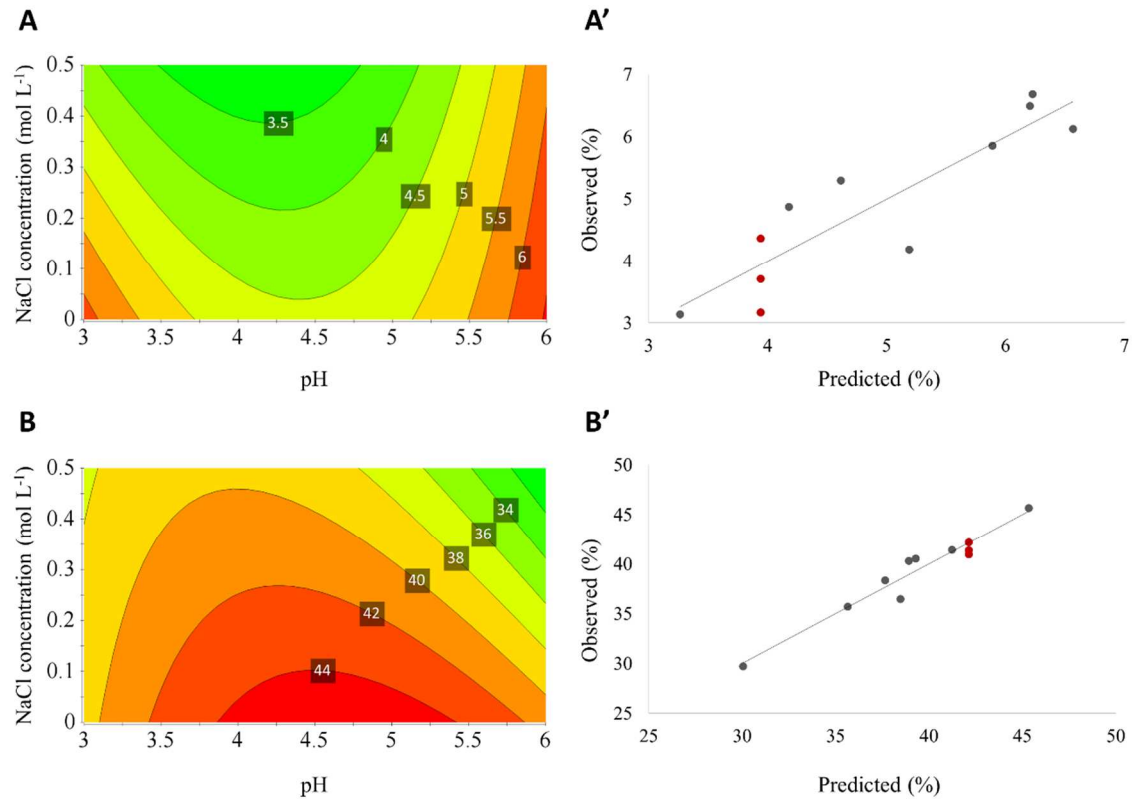


Fig. 3. The contour plot of phytate (A) and protein content (B) in residual meal from protein extraction as a function of pH (3–6) and NaCl concentration (0–0.5 mol.L⁻¹). Comparison the predicted by model and actual values for C_{PHYT} (A') and C_{PROT} (B') responses. The central experiments of design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

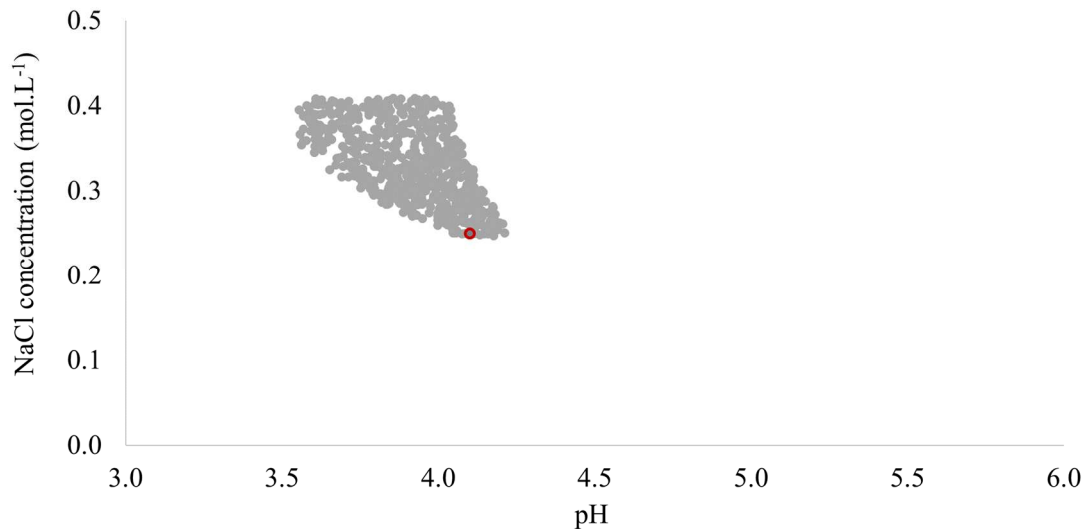


Fig. 4. Non-dominated responses (grey points) in multi-objective optimization of solid/liquid extraction taking into consideration sunflower albumin extraction yield ($SFA_{YIELD} > 70\%$), sunflower albumin content ($C_{SFA} > 90\%$), sunflower albumin phenolic contamination ($SFA_{PHEN} < 1.6 \text{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

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

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

Table 3. Characterization of **sunflower albumins** produced under optimal extraction condition (pH 4.1 and 0.25 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

^a not detected

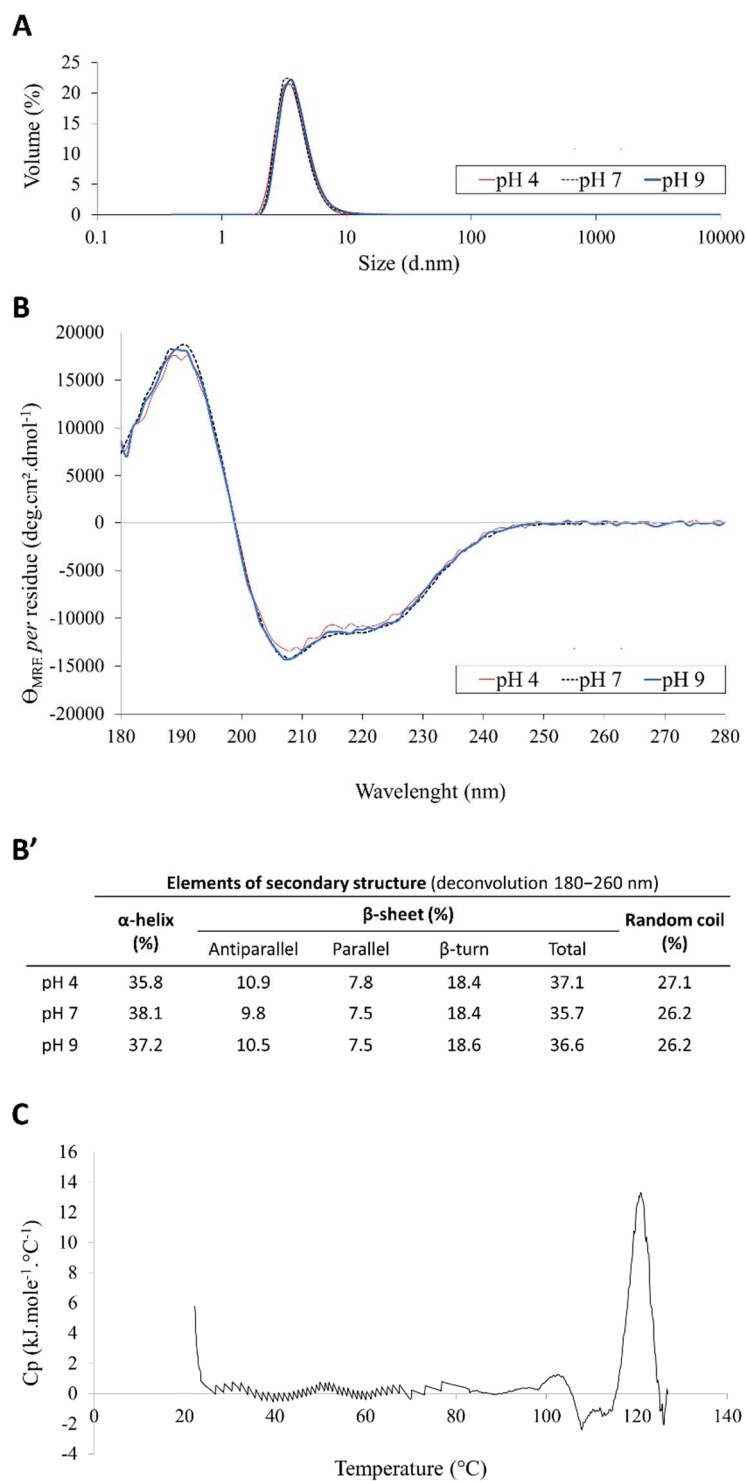


Fig. 5. The volume size distribution of **purified sunflower albumins** at pH 4, 7 and 9 analyzed by DSL at 20 °C (**A**). The far-UV CD spectra (180–280 nm) of the same protein solutions at 20 °C (**B**) expressed in mean residual ellipticity (deg.cm².dmol⁻¹ *per residue*) and the results of spectral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

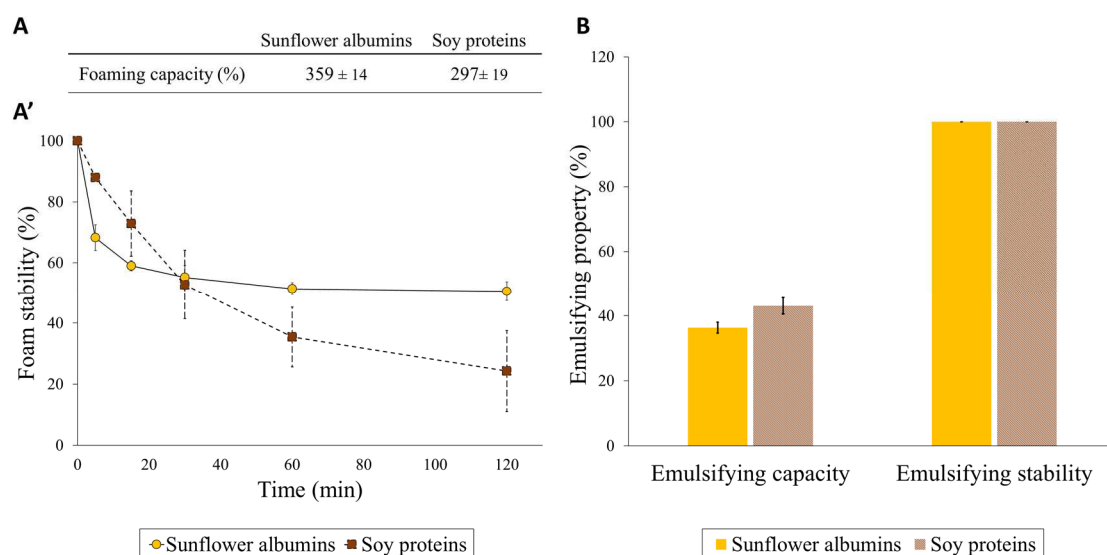


Fig. 6. Foaming capacity (**A**) foaming stability (**A'**) and emulsifying properties (**B**) of **purified sunflower albumins** compared to reference soy proteins. Each point represents the average of three independent measurements.

Appendix

Table A.1. Experimental matrix (coded and uncoded) of 3^2 full factorial design with three replications in the central point. Investigated variables were pH (x_1) ranging from 3–6 and NaCl concentration (x_2) from 0–0.5.mol.L⁻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
12	0	-1	4.5	0

^a pH; ^b NaCl concentration

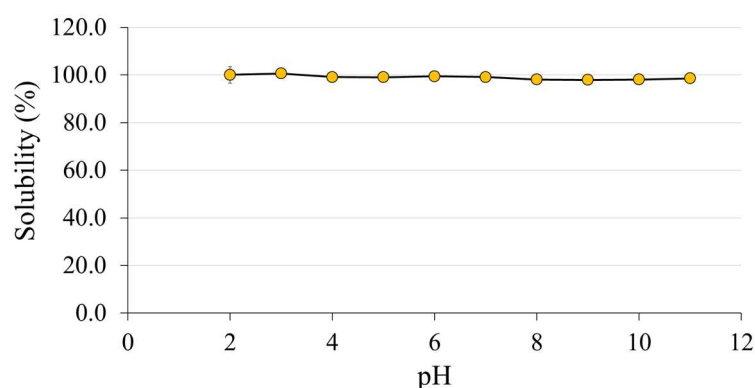


Fig. A.1. Solubility of purified sunflower albumins as a function of pH (2–11). Each point represents the average of three independent measurements.

Table A.2. Amino acid composition of purified sunflower albumins in comparison to the FAO/WHO/UNU 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

