Sustainable Food Technology

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: N. T. Flores Jimenez, J. A. Ulloa and J. E. Urías-Silvas, *Sustainable Food Technol.*, 2025, DOI: 10.1039/D5FB00708A.

View Article Online
DOI: 10.1039/D5FB00708A

Sustainability spotlight

Oil extraction from oilseeds such as sunflower and canola is one of the processes that generates the most waste. Among these by-products, de-oiled sunflower and canola meals stand out for their high protein content. This research proposes the application of de-oiled sunflower and canola meals to produce protein coprecipitates with improved physicochemical, functional and structural properties compared to the individual sources. Numerous studies have reported the valorization of proteins from oilseed industry waste as a strategy for their use as food ingredients, the development of new products, or the improvement of existing ones, consistent with this research. The results obtained in this work could be aligned with Sustainable Development Goals 2 (zero hunger), 9 (industry, innovation, and infrastructure), and 12 (responsible consumption and production).

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DOI: 10.1039/D5FB00708A

Valorization of sunflower (*Helianthus annuus* L.) and canola (*Brassica napus* L.) meals through protein coprecipitation: Physicochemical, functional, and structural characterization

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View Article Online
DOI: 10.1039/D5FB00708A

Abstract

Canola-sunflower protein coprecipitates (CSPoCos) were prepared with different flour ratios, and their physicochemical, functional, and structural properties were analyzed. The crude protein content of the CSPoCos ranged from 88.19% to 91.21%, higher than that of canola protein precipitates (CPoPc, 82.25%) and sunflower protein precipitates (SPoPc, 86.19 %). In addition, protein coprecipitation (PoC) increased the apparent and compact densities, Carr and Hausner indices, while decreasing the particle size and modifying the morphology measured through of dynamic light scattering and scanning electron microscopy, respectively. Glutelins (52.71-60.30%) were the major protein fraction in the CSPoCos. In addition, the PoC increased the properties of water holding capacity, oil holding capacity, last gelation concentration, emulsifying activity index, foam capacity and foam stability. The improvement of these functional properties could be due to the formation or combination of different covalent and non-covalent interactions between the individual protein sources. Moreover, PoC also modified the secondary and tertiary protein structures of the CSPoCos, which was manifested by a significant increase ($p<0.05$) on the surface hydrophobicity, in comparison to CPoPc and SPoPc. On the other hand, the values of *in vitro* antioxidant capacity*, in vitro* digestibility, sulfhydryl groups and disulfide bridges of the CSPoCos were higher than CPoPc. Electrophoresis revealed that CSPoCos had subunits with molecular weights ranging from 15 to 49 kDa. Overall, CSPoCos had better functional properties than single canola and sunflower proteins, making the application of PoC an attractive alternative for obtaining protein powders with improved qualities for use as food ingredients.

Keywords: Coprecipitation, Sunflower proteins, Canola proteins, Physicochemical characteristics, Functional characteristics, Structural characteristics

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DOI: 10.1039/D5FB00708A

1 Introduction

The continuous growth of the world population demands a sufficient supply of protein through alternative sources.[1] In this sense, the recovery of proteins from agro-industrial by-products, including oilseed meals, represents an attractive option.[2] Canola (*Brassica napus* L.) and sunflower (*Helianthus annuus* L.) are the second and third most important oilseed crops in the world, respectively, after soybeans.[3,4] According to FAOSTAT[5], 70 and 56 million metric tons of canola and sunflower were produced worldwide, respectively, being Canada and Ukraine the largest producers of these crops. Oilseeds are an interesting source of protein, since the meals obtained after oil extraction are by-products that can be valorized. Typically, residual oilseed meals from oil extraction may contain 35 to 40 % protein on a dry basis. These oilseeds are recognized as an alternative protein source with potential use in human nutrition, although to date they are mainly used for animal consumption.[6,7]

One of the alternatives for the recovery of proteins for human consumption from oilseed meals are protein precipitates (PoPc) such as concentrates or isolates.[8] However, each oilseed meal has limited nutritional value, due to the presence of anti-nutrients or a low functional quality, which restricts its use as a food ingredient.[9,10] Therefore, there is interest in formulating food products by combining proteins to solve these drawbacks, which can be achieved through PoB (protein blending) and PoC (protein coprecipitation) using two or more sources (plant-plant, animal-animal or plant-animal proteins).[11,12,13] The differences between these protein products are based on the preparation method. PoB can be obtained through the direct mixing of protein isolates or concentrates, while PoC can be made by precipitation or by heat.[14]

The formation of PoB and PoCos involves different interactions that give rise to different structures. These structures present in PoB and PoCos can have a synergistic effect on their techno-functional, nutritional and biological properties,[9,11,15,16]depending mainly on various intrinsic properties (type of proteins, protein concentration, amino acid composition, structure, conformation, molecular weight, surface properties) of the protein sources and

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DOI: 10.1039/D5FB00708A

processing conditions such as pH, temperature, ionic force, meal: solvent ratio, etc. [12,17,18,19,20]

For example, Wu et al.[13] studied the effect of mixing soybean and zein proteins. The results of this study revealed that the soybean-zein (5:1; w/w at pH 7) PoB improved solubility, foaming properties and water holding capacity, in contrast to the zein proteins. These researchers point out that the increase in these techno-functional properties was a consequence of the fusion of the two proteins and the modification of their secondary structure. In addition, Zhou et al.[21] observed that PoB obtained from pea and grass carp protein (1:1; w/w at pH 7) isolates enhanced solubility and foaming properties, compared to grass carp protein isolate. These results are due to the fact that the protein molecules of the pea and the grass carp have opposite charges, and the intermolecular electrostatic interactions are strengthened to generate soluble protein aggregates.

On the other hand, in the case of PoC, Zhang et al.[22] demonstrated that the association of Ginseg and Schisandra (6:1; w/w) proteins in the form of PoCo by alkaline extraction (pH 9) and isoelectric precipitation (pH 3.5) increased water and oil holding capacities, compared to individual proteins. This improvement resulted from the increase in hydrogen bonding interactions and hydrophilic groups exposed under alkaline conditions. For his part, Kristensen et al.,[16] reported that the pea and whey (20:80; v/v) PoCo by alkaline extraction (pH 11) and isoelectric precipitation (pH 4.6) produced new electrostatic interactions and disulfide bonds between these proteins, increasing solubility and surface hydrophobicity.

To date, this work constitutes the first report on obtaining PoCos from canola and sunflower as an alternative protein source. Therefore, the objective was to determine the physicochemical, functional, and structural attributes of CSPoCos to evaluate their potential application as a novel food ingredient.

2 Materials and methods

2.1 Material and Chemicals

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Canola meal (CM) was provided by Forrajes y Alimentos San Cayetano, S. de R.L. de C.V. (Tepic, Nayarit, México), while sunflower meal (SM) was obtained from New Country Organics, Inc. (Lubbock, Texas, USA). The contents of crude protein (N x 6.25), moisture, ash, fat, and total carbohydrate contents for the CM, according to the AOAC [19] methods, were 39.72 ± 0.32 %, 9.76 ± 0.14 %, 7.05 ± 0.04 %, 2.96 ± 0.25 %, and 31.74 ± 0.81 %, respectively. The proximal composition of the SM is presented in section 2.2, since it was necessary to remove a significant amount of oil. All chemicals employed in this research were reagent grade and purchased from Sigma-Aldrich, Fermont and J.T. Baker (Ciudad de México, México).

2.2 Preparation of defatted sunflower flour (DSF)

Because the SM contained a considerable amount of lipids (21.23 ± 0.25 %), it was necessary to apply a step to reduce the percentage of this component, according to the following procedure. First, lots of 100 g of SM were placed in 1 L of ethyl ether with stirring at 500 rpm for 1 h, during two successive cycle and replacing the spent solvent with fresh solvent. The material obtained from the second defatting cycle was placed in a plastic container for complete desolventization, in an extractor hood for 12 h to obtain DSF, which was stored in airtight bags at room temperature for subsequent characterization. The contents of crude protein (N × 6.25), moisture, ash, fat, and total carbohydrates of DSF were 33.54 ± 0.89 %, 8.81 ± 0.04 %, 6.76 ± 0.80 %, 0.49 ± 0.18 %, and 50.40 ± 0.61 %, respectively.

2.3 Preparation of canola and sunflower protein precipitates (CPoPc and SPoPc) and coprecipitates (CSPoCos)

CPoPc and SPoPc were obtained using 100% CM and DSF, respectively, as source of proteins. CSPoCos were obtained employing batches of 200 g pastes, with the following proportions: 70%CM:30%DSF, 50%CM:50%DSF, and 30%CM:70%DSF (w/w) by alkaline extraction and isoelectric precipitation, following the method reported by Tian et al.[12] Briefly, CM, DSF and mixtures thereof were suspended in distilled water (1:20, w/v), adjusted to pH 11 with 1 M NaOH by magnetic stirring at 20 °C for 45 min, and the insoluble residue was separated by centrifugation at 8000 × g and 14 °C for 10 min. After,

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144 the pH of the protein suspensions was adjusted to pH 4 (for CM) and 4.5 (for DSF and
145 mixtures) with 1 M HCl by magnetic stirring at 20 ºC for 30 min. Subsequently, the slurries
146 were centrifuged at 8000 × *g* at 14 ºC for 10 min and the PoPc and PoCos were re-
147 suspended in distilled water in a ratio of 1:10 (w/v) at pH 7 with 1M NaOH. Finally, the
148 protein dispersions were lyophilized in a freeze dryer model FreeZone 10 L (Labconco,
149 USA) to obtain the CPoPc, SPoPc and CSPoCos ($CSPoCo_{70/30}$, $CSPoCo_{50/50}$ and
150 $CSPoCo_{30/70}$, where the first subindex in each material represent the CM proportion and the
151 second subindex is the DSF proportion used in this preparation).

152 **2.4 Extraction yield and protein purity**

153 The extraction yield of the samples was calculated using two parameters (weight yield and
154 protein yield). The weight yield was determined as the ratio between the total weight of the
155 recovered CPoPc, SPoPc and/or the CSPoCos and the initial weight of CM and/or DSF
156 used in the extraction process (Eq. 1). The protein yield was estimated by comparing the
157 total protein content in recovered CPoPc, SPoPc and/or the CSPoCos and the initial protein
158 content in CM and/or DSF (Eq. 2). The protein purity was calculated according to the Eq.
159 3:

160 $$\text{Process yield (\%)} = \frac{\text{weight of recovered PoPc or PoCos}}{\text{weight of CM or DSF}} \times 100 \quad \text{(Eq. 1)}$$

161 $$\text{Protein yield (\%)} = \frac{\text{weight of protein in recovered PoPc or PoCos}}{\text{weight of initial protein in CM or DSF}} \quad \text{(Eq. 2)}$$

162 $$\text{Protein purity (\%)} = \frac{\text{weight of protein in PoPc or PoCos}}{\text{weight total of PoPc or PoCos}} \times 100 \quad \text{(Eq. 3)}$$

163 **2.5 Composition and physicochemical characteristics**

164 **2.5.1 Proximate analysis**

165 CM, DSF, PoPc and PoCos samples were subjected to proximate composition following to
166 AOAC methods.[23] The total carbohydrates were estimated by subtraction of the moisture,
167 fat, protein, and ash content. Results were registered as % on moist basis.

168 **2.5.2 Bulk (b_ρ) and compact (c_ρ) density**

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b_ρ was determined by adding protein sample to the 10 mL line of a graduated test tube and noting the weight. Then, test tube was hit 40 times, and the volume was registered to obtain the c_ρ.[24] b_ρ and c_ρ were expressed as g/cm^3.

2.5.3 Carr´s index (CaIn) and Hausner´s ratio (HaRa)

CaIn and HaRa are the two major parameters to quantify powder flowability. CaIn and HaRa were calculated from b_ρ and c_ρ of protein samples as shown below:

$$\text{CaIn (\%)} = \frac{c_\rho - b_\rho}{c_\rho} \times 100 \quad \text{(Eq. 4)}$$

$$\text{HaRa} = \frac{c_\rho}{b_\rho} \quad \text{(Eq. 5)}$$

2.5.4. Water activity (a_w)

The a_w of the samples was analyzed with an AquaLab Serie 4 TEV equip (Decagon Devices Inc., USA).

2.5.5 Turbidity (T_{rb})

T_{rb} was measured by absorbance in 1 % (w/v) protein dispersions at pH 7, using a UV–Vis spectrophotometer model FI-01620 (Thermo Fisher Scientific, Vantaa, Finland) at 600 nm.

2.5.6 Color analysis

Color meter model CR-300 (Konica Minolta Holdings, Inc., Japan) was utilized to evaluate the coloring of the samples. The measurements were represented by means of the CIELAB color scope, determining L^* (lightness), a^* (+redness, -greenness) and b^* (+yellowness, -blueness). The L_0^*, a_0^*, and b_0^* numbers of the white pattern plate employed as reference were 94.44, -0.20 and 3.87, respectively. The total variation of color (ΔE) was estimated as:

$$\Delta E = [(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2]^{1/2} \quad \text{(Eq. 6)}$$

In addition, the hue angle (H_{ab}) was calculated as the arc tangent of the ratio of yellowness to redness, as show in Eq. 7

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$$H_{ab} = Tan^{-1}\left(\frac{b^*}{a^*}\right) \quad \text{(Eq. 7)}$$

Chroma (C^*) is considered as the purity of color or colorfulness of the sample. It was determined as the Euclidean distance of the color vector (L, a, b) for a sample from the lightness axis as show in Eq. 8

$$C^* = \sqrt{a^2 + b^2} \quad \text{(Eq. 8)}$$

2.5.7 Protein fractionation

Albumin (Al), globulin (Gl), prolamin (Pr), and glutelin (Gx) fractions were solubilized from protein samples successively, as was reported by Serrano-Sandoval et al.,[25] with some modifications. First with distilled water at a 1:10 (w/v) ratio for 1 h of stirring at 4 °C to extract the Al, which were recovered in the supernatant obtained by centrifugation for 20 min at 6,000 × g and stored under refrigeration at 6 °C for subsequent analysis. To the resulting precipitate, 30 mL of a 50 mM Tris-HCl + 0.4 M NaCl solution (pH 8) were added for Gl extraction, and the mixture was centrifuged under the same conditions. Next, 30 mL of 70% ethanol were added to the new precipitate to obtain Pr, and subsequently, 30 mL of 0.1 M NaOH were added to the final precipitate to recover Gx. The separation and storage of the supernatant at each extraction stage was performed under the same conditions as for the albumin fraction. The total protein content of each fraction was measured using the Bradford assay [26] using as reference albumin from bovine serum.

2.6 Functional characteristics

2.6.1 Protein solubility (PoSo)

PoSo was performed as described by Balderas-León et al.,[27] with modifications. Samples containing 40 mg of protein were mixed with 20 mL 0.01M sodium phosphate buffer (pH 7) for 60 min. Then, the dispersion was centrifuged at 8000 × g for 10 min at 20 °C, and the supernatant was used for protein quantification by Bradford[22] microplate assay method.

2.6.2 Water holding capacity (WaHC) and oil holding capacity (OiHC)

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To evaluate the WaHC and OiHC, samples containing 0.5 g of protein were dispersed in 10 mL of distilled water or canola oil within a pre-weighed 50 mL centrifuge tube. The dispersions were vigorously vortexed in an MX-S (Avante, Guadalajara, Mexico) and then allowed to stand at room temperature for 30 min before centrifugation (5000× g, 20 min, 25 °C). After centrifugation, the supernatants were discarded and the tubes containing the pellets were weighed.[28] WaHC and OiHC were reported as grams of water or oil absorbed per gram of protein

2.6.3 Last gelation concentration (LaGeCo)

LaGeCo was determined with the procedure used by López-Mártir et al., [28] with some changes. Protein dispersions of 4 to 20 % (w/v) were prepared in volumes of 5 mL at pH 7, which were kept in test tubes and subsequently heated at 90 °C for 1 h. After heating, the tubes containing the samples were quickly cooled and refrigerated at 4 °C for 2 h. LaGeCo was identified as that concentration when the sample from the inverted test tube did not fall down or slip.

2.6.4 Emulsifying characterization

The emulsifying activity index (EAcIn) and emulsifying stability index (EStIn) were measured according to the method of Briceño-Islas et al.[29] For emulsion formation, 5 mL of canola oil and 16 mL of sample solution (0.1% w/v) in 0.01M sodium phosphate buffer (pH 7) were homogenized with a T-25 Ultra-turrax homogenizer (IKA Instruments, Germany) at 12,000 rpm for 1 min. Then, 50 µL of emulsion from the bottom of the tube was taken after 0 min (for EAcIn) and 10 min (for EStIn) to add them to 5 mL of 0.1 % SDoS (sodium dodecyl sulfate) solution. After shaking in a vortex for 5 s, the absorbance of emulsions was recorded at 500 nm. EAcIn and EStIn were calculated using the following equations:

$$\text{EAcIn}\left(\frac{\text{m}^2}{\text{g}}\right) = \frac{2T \times A_0 \times N \times 10^{-4}}{\emptyset \times L \times C} \qquad \text{(Eq. 9)}$$

$$\text{EStIn}\ (\%) = \frac{\text{A}_{10}}{\text{A}_0} \times 100 \qquad \text{(Eq. 10)}$$

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where T = constant value (2.30), A_0 and A_{10} = absorbance of the emulsion at 0 and 10 min, respectively, *N* = dilution factor (100), Ø = oil volume fraction (0.20), *L* = path length of the cuvette (m), and C = protein concentration in aqueous phase (g/m^3).

2.6.5 Foaming characterization

The foam capacity (FmC) and foam stability (FmS) of samples were measured following the procedure of Cháirez-Jiménez et al.,[7] with small variations. Protein suspensions were prepared (5 %) with 0.01M sodium phosphate buffer (pH 7). Then, a sample of 15 mL was mixed for 1 min at 13,000 rpm and poured into the graduated tube. Foam volume at 1 min was recorded as V_{1min}, and the foam volume at 20 min as V_{20min}, and the FmC and FmS calculated as follow:

$$\text{FmC (\%)} = \frac{V_{1min}}{15} \times 100 \quad \text{(Eq. 11)}$$

$$\text{FmS (\%)} = \frac{V_{20min}}{V_{1min}} \times 100 \quad \text{(Eq. 12)}$$

2.7 Biochemical properties

2.7.1 Electrophoretic profile by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The determination of SDS-PAGE of the protein samples was performed according to the method described by Flores-Jiménez et al.,[30] with slight changes, utilizing a Mini-Protean tetra chamber (Bio-Rad Laboratories, Inc., USA). Samples were processed on 4-20 % precast polyacrylamide gel (Cat. #456-1095). Samples were prepared in reducing conditions with β-mercaptoethanol (+β-M), and non-reducing conditions without β-mercaptoethanol (–β-M). A total of 20 µL of each sample with 15 µg of protein were charged into each channel of gel, including the molecular weight marker (MW) from 10 to 250 kDa (Precision Plus Protein All Blue Prestained Protein Standards). Electrophoresis was performed at 140 V to concentrate for 10 min, after that at 110 V to divided gel approximately 2 h. The gel was stained with Coomassie brilliant blue (G-250) for 24 h. Ultimately, the gel was destained until bands were easily seen and then captured using the ImageJ software (National Health Institute, USA) for molecular mass definition.

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2.7.2 *In vitro* protein enzymatic digestibility (IVPoDi)

The IVPoDi method of Flores-Jiménez et al.,[24] was used with slight changes. Briefly, a dispersion was prepared by dissolving 625 mg of protein sample in 100 mL of distiller water, which was fitted with NaOH (0.1 M) to pH 8. The multienzyme preparation was elaborated with trypsin, protease and bovine pancreatin at concentrations of 1.6 mg/mL, 0.05 mg/mL and 1 mg/mL, respectively, adjusting the medium to pH 8 and keeping it in an ice bath until its application. Afterwards, 1.5 mL of the multienzyme preparation was incorporated to the sample and kept agitation for 10 min at 37 °C. The pH fall was determined to the 10 min of reaction. The IVPoDi was estimated as follow:

$$\text{IVPoDi (\%)} = 210.464 - 18.10(Z) \qquad \text{(Eq. 13)}$$

where Z = pH sample dispersion after 10 min of reaction with the multienzyme preparation.

2.7.3 *In vitro* antioxidant capacity (IViAC)

The IViAC of the protein samples was evaluated using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay according to the method described by Mesfin et at.,[31] with modifications. An aliquot of 250 µL of 0.06 mM DPPH was added and mixed vigorously with 30 µL of protein suspension (0.5 mg/mL), and then the mixture was incubated in the dark for 30 min at room temperature. The absorbance was monitored at 515 nm by spectrophotometry after 30 min, using a blank (250 µL of DPPH + 30 µL distiller water). Finally, IViAC was calculated according to the following equation:

$$\text{IViAC (\%)} = 1\text{-}\left(\frac{A_{sa}}{A_{bl}}\right) \times 100 \quad \text{(Eq. 14)}$$

where A_{bl} and A_{sa} are the absorbances of the blank and the sample, respectively.

2.8 Structural properties

2.8.1 Microstructure

A scanning electron microscope (SEM; SNE-3200M, SEC Co., LTD, South Korea) was used to observe the microstructure and shape of the samples. A small amount of the

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samples was taken, coated with gold, and placed in the SEM for suction under a voltage of 20 kV and a magnification of 150×. After the focus became clear under the magnification, the characteristics of the samples were photographed.

2.8.2 Molecular flexibility (MoFl)

MoFl was measured in the samples at a concentration of 1 mg/mL (0.01 M sodium phosphate buffer, pH 7), employing the technique described by Rodríguez-Rivera et al.[32]

2.8.3 Particle size (Pz) and ζ-potential

The Pz and ζ-potential were measured using a Zetasizer Nano-ZS90 (Malvern Instruments, Malvern, UK). The samples were diluted to 0.2 mg/mL with 0.01 M sodium phosphate buffer (pH 7). To measure Pz the samples were placed in a glass cuvette (ZEN0118, Malvern Instruments) at 20 ºC for measurement. The ζ-potential was determined by measuring the velocity and direction of dispersion as the particles moved along the electric field using Smoluchowski's mathematical method. Measurements were performed at 20 ºC, and the samples were placed in disposable folded capillary cells (DTS1060, Malvern Instruments).

2.8.4 Surface hydrophobicity (S_uH_o)

Protein S_uH_o was estimated by the fluorescence technique,[33] utilizing molecular probe, 8-anilino-1-naphthalenesulfonic acid (ANS) on a 200 Pro spectrophotometer (Tecan Infinite, Austria). The samples were diluted to different protein concentrations (0.05-0.01 mg/mL), and the test tubes were brought to a volume of 2 mL according to the solubility of the samples. Then, 25 µL of ANS was added, to which fluorescence was determined at excitation (364 nm) and emission (475 nm) wavelengths. S_uH was considerate as the slope of the graph of fluorescence vs protein concentration (mg/mL).

2.8.5 Intrinsic fluorescence spectra (IFS)

IFS of samples (0.2 mg/mL protein with 0.01 M sodium phosphate buffer at pH 7) were obtained with a 200 Pro fluorescence spectrophotometer (Tecan Infinite 200 Pro, Grödig,

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Austria) at an excitation wavelength of 290 nm, spectral scanning range of 320-450 nm, and a slit = 5 nm.

2.8.6 Free (SH_{Fr}) and total (SH_{To}) sulfhydryl groups and disulfide bridges ($S\text{-}S_{Br}$)

The determination of the content of SH_{Fr}, SH_{To} and $S\text{-}S_{Br}$ of protein samples was carried out according to the methods described by Wu et al.[34] and Ren et al.,[35] with some adaptations. To determine the SH_{Fr} content, 100 µL of the samples (1 mg/mL protein with 0.01 M sodium phosphate buffer at pH 7) was dissolved with 500 µL of Tris-glycine buffer (0.086 M Tris, 0.09 M glycine, 0.04 M EDTA-Na_2, pH 8) and 10 µL of Ellman´s reagent (4 mg of DTNB/mL of Tris-glycine buffer), followed by incubation at 25 º C for 1 h. Samples were then centrifuged at 8000 rpm for 10 min using an Eppendorf Minispin centrifuge (Hamburg, Germany) and the absorbances were measured at 412 nm with sodium phosphate buffer as blank. For the analysis of SH_{To} content, the same process was followed, with the addition of 8 M urea to the Tris-glycine buffer. The concentration of the SH_{Fr}, SH_{To} and $S\text{-}S_{Br}$ was calculated using the following equations:

$$SH_{Fr} \text{ or } SH_{To}\left(\frac{\mu mol}{g \text{ protein}}\right) = \frac{73.53 \times A_{412} \times D}{C} \quad \text{(Eq. 15)}$$

$$S - S_{Br}\left(\frac{\mu mol}{g \text{ protein}}\right) = \frac{SH_{To} - SH_{Fr}}{2} \quad \text{(Eq. 16)}$$

where A_{412} = absorbance of the sample at 412 nm, for both SH_{Fr} and SHT_o, C = sample protein concentration (mg/mL), 73.53 = constant, and D = dilution factor (D = 1).

2.8.7 Attenuated total reflectance Fourier transforms infrared spectra (ATR-FTIR)

Infrared spectra of the protein samples were obtained using an ATR-FTIR spectrometer (Agilent Cary 630, Agilent Technologies, USA). 100 mg of protein samples were placed in the ZnSe cell, using as conditions the range of 650 to 4000 cm^{-1} with a 4 cm^{-1} resoluteness and 21 sweeps at 25 ºC.

2.9 Statistical analysis

The physicochemical, functional, biochemical and structural characteristics of the PoPc and

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CSPoCos were reported as the means ± standard deviations of the three batches. Data analysis involved a one-way ANOVA and comparison of means was performed using Fisher's Protected LSD (statistical significance considered at $p<0.05$) with the Statistica software version 7.1 (TIBCO Software, Inc., USA).

3 Results and discussion

3.1 Extraction yield and protein purity

The yield and purity of protein materials are important factors associated with their production methods that determine their economic feasibility.[36] The results on the product yield, protein recovery, and purity of PoPc and the CSPoCos are presented in Table 1. The product yield ranged from 5.20-17.10 %, following the order of CPoPc < $CSPoCo_{70/30}$ < $CSPoCo_{50/50}$ < $CSPoCo_{30/70}$ < SPoPc. These results are comparable to those of Wintersohle et al.,[35] who found product yield was from 8.3 % to 18.89 % for mung bean PoPc obtained by alkaline extraction and isoelectric precipitation. Meanwhile, the protein recovery reached values from 11.76 % to 53.07 % in the order of CPoPc < $CSPoCo_{70/30}$ < $CSPoCo_{50/50}$ < $CSPoCo_{30/70}$ < SPoPc. The maximum protein recovery obtained in this study was similar to that reported for PoPc of sunflower (55.67 %),[6] *Hermetia illucens* (55 %),[37] and Japanese quince (54 %).[38] In the case of protein purity, PoC significantly increased ($p<0.05$) this parameter from 82.18 % and 86.22 % for CPoPc and SPoPc, respectively, to values that ranging from 88.25 % to 92.20 % for CSPoCos (Table 1). The purity of the protein obtained in the present study for both PoPc (82.18-86.22 %) and PoCos (88.25-92.20 %) ranged between those reported for Japanese quince PoPc (83.88 %).[39]

Table 1. Process yield, protein recovery and protein purity of canola protein precipitate (CPoPc), sunflower protein precipitate (SPoPc) and canola-sunflower protein coprecipitates (CSPoCos).

Samples	Process yield (%)	Protein recovery (%)	Protein purity (%)
CPoPc	5.20 ± 0.26^{e}	11.76 ± 0.60^{e}	82.18 ± 0.02^{e}
SPoPc	17.10 ± 0.36^{a}	53.07 ± 1.16^{a}	86.22 ± 0.06^{d}

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$CSPoCo_{70/30}$	11.40 ± 0.53[d]	29.92 ± 1.37[d]	88.25 ± 0.13[c]
$CSPoCo_{50/50}$	13.27 ± 0.25[c]	37.18 ± 0.70[c]	89.15 ± 0.03[b]
$CSPoCo_{30/70}$	16.23 ± 0.40[b]	50.48 ± 1.27[b]	92.20 ± 0.02[a]

Values are the mean of three determinations of independent batches ± standard deviations. Different letters within the same column indicate significant differences ($p < 0.05$) among the groups. $CSPoCo_{70/30}$ = 70 % canola/30 % sunflower, $CSPoCo_{50/50}$ = 50 % canola/50 % sunflower and $CSPoCo_{30/70}$ = 30 % canola/70 % sunflower. The subscripts for each coprecipitate correspond to the proportions of canola and sunflower pastes, respectively.

3.2 Composition and physicochemical characteristics

3.2.1 Proximate composition

The protein amount of the CSPoCos (88-19-91.21 %) was higher than those obtained from CPoPc (82.25 %) and SPoPc (86.19 %). The variation in the protein content of CSPoCos could be due to the precipitation pH used (4.5), being the same for sunflower proteins, but different from that of canola proteins (4.0), which could also affect the techno-functional properties. In general, the contents of moisture, ash, lipids and total carbohydrates were reduced by effect of PoC, in contrast with the PoPc, except $CSPoCo_{70/30,}$ due its moisture value was higher than SPoPc, but in the case of lipid content all CSPoCos had no significant difference ($p>0.05$), with respect to SPoPc (Table 2).

The protein content of the CSPoCos was higher than pea-grass carp (87.91 %)[19] and soybean-brewers' spent grain (83.67 %)[40] PoCos but lower than soybean-wheat (91.5-94 %)[12] and sheep whey-casein (97.5 %)[41] PoCos.

The proximate composition of PoPc from canola[42] and sunflower[43] was similar to that of CPoPc and SPoPc obtained in this study.

Table 2. Proximate chemical composition of canola protein precipitate (CPoPc), sunflower protein precipitate (SPoPc) and canola-sunflower protein coprecipitates (CSPoCos).

Components (%)	CPoPc	SPoPc	$CSPoCo_{70/30}$	$CSPoCo_{50/50}$	$CSPoCo_{30/70}$

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Protein	82.25 ± 0.56[d]	86.19 ± 0.32[c]	88.19 ± 0.45[b]	89.17 ± 0.54[b]	91.21 ± 0.92[a]
Moisture	4.42 ± 0.13[a]	3.20 ± 0.04[c]	3.63 ± 0.03[b]	2.80 ± 0.09[d]	3.21 ± 0.07[c]
Ash	3.83 ± 0.11[a]	3.67 ± 0.29[a]	2.74 ± 0.08[b]	2.78 ± 0.02[b]	2.40 ± 0.13[c]
Lipids	1.23 ± 0.30[a]	0.95 ± 0.58[ab]	0.91 ± 0.57[b]	0.86 ± 0.02[b]	0.87 ± 0.10[b]
Carbohydrates	8.27 ± 0.55[a]	5.99 ± 0.37[b]	4.53 ± 0.73[c]	4.39 ± 0.52[c]	2.31 ± 0.43[d]

Values are the mean of three determinations of independent batches ± standard deviations. Different letters within the same row indicate significant differences ($p < 0.05$) among the groups. $CSPoCo_{70/30}$ = 70 % canola/30 % sunflower, $CSPoCo_{50/50}$ = 50 % canola/50 % sunflower and $CSPoCo_{30/70}$ = 30 % canola/70 % sunflower. The subscripts for each coprecipitate correspond to the proportions of canola and sunflower pastes, respectively.

3.2.2 b_ρ and c_ρ

The density of food powders is classified into two categories: b_ρ and c_ρ. These densities serve as a reference for evaluating the behavior of powders under external forces.[44] The b_ρ of the CSPoCos (0.366-0.372 g/cm^3) was not significantly ($p>0.05$) different from that of SPoPc (0.358 g/cm^3), but it was higher than that of CPoPc (0.326 g/cm^3) (Table 3).

Regarding c_ρ, significant ($p<0.05$) higher values were observed for the CSPoCos (0.482-0.499 g/cm^3) compared to CPoPc (0.430 g/cm^3) and SPoPc (0.436 g/cm^3) (Table 3). According to various studies, b_ρ and c_ρ are influenced by factors such as particle size, size distribution, shape, surface area, roughness, and particle arrangement of powder products.[45,46] The values obtained in this study of b_ρ and c_ρ for CPoPc and SPoPc of 0.326 g/cm^3 and 0.430 g/cm^3 and 0.358 g/cm^3 and 0.436 g/cm^3, respectively, were higher than those obtained of 0.156 g/cm^3 and 0.200 g/cm^3 for soursop seed PoPc.[28]

3.2.3 CaIn and HaRa

The CaIn is an indirect measure of the b_ρ, size and shape, surface area, moisture content, and cohesion of powder materials.[27] According to Shah et al.,[47] powders can be classified respect to its flow properties as follows: very good (CaIn <15 %), good (CaIn =15–20 %), fair (CaIn =20–35 %), bad (CaIn =35–45 %) or very bad flowing (CaIn >45 %).

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Conforming to this scale CPoPc (24.24 %), $CSPoCo_{70/30}$ (25.69 %), $CSPoCo_{50/50}$ (23.73 %), and $CSPoCo_{30/70}$ (24.05 %) had a fair fluency, while SPoPc had good fluidity (17.74 %) (Table 3).

On the other hand, HaRa measures the cohesion of the particles, and therefore its value determines high or low fluidity in powders.[48] Powders with HaRa of 1.2 are classified as free-flowing, while those with values of 1.2-1.4 or >1.4 are considered to be of intermediate or high cohesion, respectively.[30] The CPoPc, SPoPc, $CSPoCo_{70/30}$, $CSPoCo_{50/50}$, and $CSPoCo_{30/70}$ had values of HaRa of 1.32, 1.22, 1.35, 1.31 and 1.32, respectively, therefore were classified as intermediate cohesion powders (Table 3). Specifically, the optimal CaIn and HaRa values depend on the potential applications of the powdered products. Therefore, in some cases, higher or lower CaIn and HaRa values may be preferable.[48]

López-Mártir et al.[28] reported for soursop seed PoPc fair flowability (CaIn = 22 %) and intermedia cohesiveness (HaRa = 1.28), although Rodríguez-Rivera et al.[32] found that groundnut paste PoPc presented good flowability (CaIn = 18.33 %) and intermediate cohesiveness (HaRa = 1.22).

3.2.4 a_w

a_w is used to determine the critical storage conditions of food. This parameter indicates if powders are susceptible to deterioration changes such as collapse, stickiness and caking.[49] a_w of CPoPc, SPoPc, and CSPoCos varied significantly ($p<0.05$), as shown in Table 3. $CSPoCo_{30/70}$ (0.11) had the smallest value of a_w, followed by $CSPoCo_{50/50}$ (0.14), SPoPc (0.20) and $CSPoCo_{70/30}$ (0.25), while CPoPc presented the highest value (0.41). Flores-Jiménez et al.[30] pointed out that values less than 0.66 for a_w are beneficial in the stability of the powders, due to the reduced availability of water for microbial proliferation and biochemical reactions, which increases the useful life of the food products.

aw values of 0.41, 0.21 and 0.31 have been reported for PoPc of canola,[42] guamuchil,[24] and gourd,[50] respectively.

3.2.5 T_{rb}

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T_{rb} is an important property as a criterion for defining the formation of protein aggregates in macroscopic dimension.[51] Table 3 shows T_{rb} values of PoPc and PoCos. The CSPoCos (0.34-0.48) were more turbid compared to the CPoPc (0.11), although less turbid than the SPoPc (0.90). These results could be due to the variation in particle size (Fig. 6B) of the canola and sunflower proteins, resulting from different interactions between the two proteins during the PoC, which consequently impact their functional properties (Fig. 2A). Malik et al.[52] and Flores-Jiménez et al.[24] reported previous T_{rb} values of 0.97 and 1.05, respectively, in sunflower and guamuchil PoPc, which were higher than those obtained for all the protein materials of this study.

3.2.6 Color

Color is one of the most important characteristics of a food product, generally associated as a quality factor that describes its level of freshness, ripeness, consumer acceptance, and safety.[53] Table 4 shows the color parameters *L**, *a**, *b**, C*, H, and ΔE for PoPc and PoCos. The highest *L** values were observed for CPoPc (51.01) and $CSPoCo_{70/30}$ (54.07), which contain mainly canola proteins, compared than SPoPc (40.45), $CSPoCo_{50/50}$ (47.34), $CSPoCo_{30/70}$ (44.41) that have greater amount of sunflower proteins (Fig.1).

All PoPc and PoCos showed positive values for *a** (except SPoPc and $CSPoCo_{30/70}$), and *b** chromaticity (Table 4). High *b** values indicate a yellow tone with a slight reddish tone due to small *a** values (Fig. 1). SPoPc and $CSPoCo_{30/70}$ exhibited a slightly greenish hue expressed by the negative *a** value, because these protein samples contained a considerable amount of sunflower proteins compared to the other samples (Fig. 1).

Table 3. Physicochemical properties of canola protein precipitate (CPoPc), sunflower protein precipitate (SPoPc) and canola-sunflower protein coprecipitates (CSPoCos).

Properties	CPoPc	SPoPc	$CSPoCo_{70/30}$	$CSPoCo_{50/50}$	$CSPoCo_{30/70}$
Density					
b_ρ (g/cm^3)	0.326 ± 0.01^{b}	0.358 ± 0.01^{a}	0.370 ± 0.01^{a}	0.372 ± 0.01^{a}	0.366 ± 0.00^{a}
c_ρ (g/cm^3)	0.430 ± 0.01^{d}	0.436 ± 0.02^{d}	0.499 ± 0.01^{ab}	0.488 ± 0.01^{bc}	0.482 ± 0.00^{c}

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Flow characteristics					
CaIn (%)	24.24 ± 2.19[a]	17.74 ± 1.72[b]	25.69 ± 1.21[a]	23.73 ± 1.76[a]	24.05 ± 1.02[a]
Flow	Fair	Good	Fair	Fair	Fair
HaRa	1.32 ± 0.04[a]	1.22 ± 0.03[b]	1.35 ± 0.02[a]	1.31 ± 0.03[a]	1.32 ± 0.02[a]
Cohesion	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate
a_w	0.41 ± 0.01[a]	0.20 ± 0.03[c]	0.25 ± 0.01[b]	0.14 ± 0.01[d]	0.11 ± 0.01[e]
T_{rb}	0.11 ± 0.00[e]	0.90 ± 0.01[a]	0.34 ± 0.01[d]	0.41 ± 0.01[c]	0.48 ± 0.01[b]

b_ρ = bulk density, c_ρ = compact density, CaIn = Carr´s index, HaRa = Hausner´s ratio, a_w = water activity, T_{rb} = turbidity. Values are the mean of three determinations of independent batches ± standard deviations. Different letters within the same row indicate significant differences ($p < 0.05$) among the groups. $CSPoCo_{70/30}$ = 70 % canola/30 % sunflower, $CSPoCo_{50/50}$ = 50 % canola/50 % sunflower and $CSPoCo_{30/70}$ = 30 % canola/70 % sunflower. The subscripts for each coprecipitate correspond to the proportions of canola and sunflower pastes, respectively.

In a study with pea-grass carp PoCos[19] were found values for L^*, a^* and b^* of 74.21, -0.44, and 12.92, respectively. For their part, Flores-Jiménez et al.[41] reported L^*, a^*, and b^* values of 51.27, 3.38, and 15.17 for canola PoPc, respectively, while Alexandrino et al.[6] obtained values of 51.66 (L^*), 7.02 (a^*), and 2.21 (b^*) for sunflower PoPc.

For their part, Flores-Jiménez et al.[42] reported L^*, a^*, and b^* values of 51.27, 3.38, and 15.17 for canola PoPc, respectively, while Alexandrino et al.[6] obtained values of 51.66, 7.02, and 2.21

H_{ab} indicates whether an object is red (0°), yellow (90°), green (180°) and (270°) [50]. SPoPc and $CSPoCo_{30/70}$ showed H_{ab} of 100.88º and 93.14º, respectively, confirming the greenish-yellowish tones due to higher proportion of sunflower protein. In change, lower values of H_{ab} for CPoPc (83.19º), $CSPoCo_{50/50}$ (84.13º), and $CSPoCo_{70/30}$ (80.65º) showed reddish-yellowish tones, which can be associated to higher content of canola proteins. Furthermore, CPoPc and $CSPoCo_{30/70}$ presented higher C^* values compared to SPoPc, $CSPoCo_{50/50}$ and $CSPoCo_{70/30}$, indicating greater color intensity/vividity.

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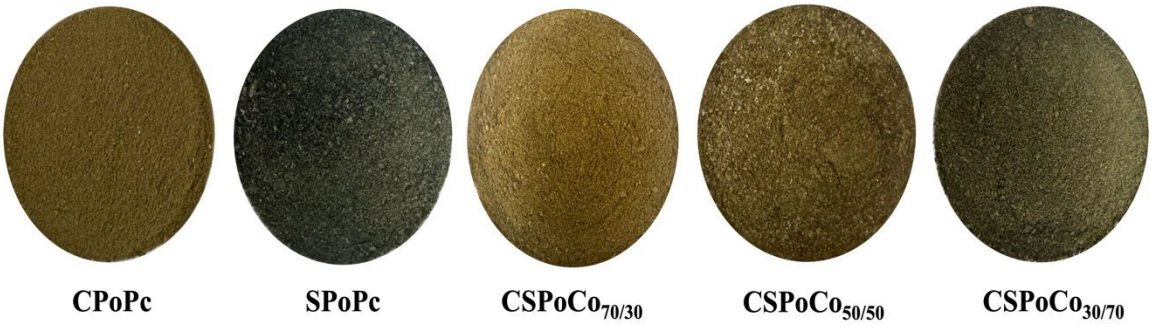

Fig. 1. Effect of the coprecipitation on color appearance. CPoPc = canola protein precipitate. SPoPc = sunflower protein precipitate. CSPoCo = canola-sunflower protein coprecipitate. $CSPoCo_{70/30}$ = 70 % canola/30 % sunflower, $CSPoCo_{50/50}$ = 50 % canola/50 % sunflower and $CSPoCo_{30/70}$ = 30 % canola/70 % sunflower.

Naik et al.[54] and López-Mártir et al.[55] reported previous C^* values of 18.25 and 13.87, respectively, in melon and noni PoPc, which were higher than those obtained for SPoPc, $CSPoCo_{50/50}$ and $CSPoCo_{30/70}$ of this study.

Table 4. Color of canola protein precipitate (CPoPc), sunflower protein precipitate (SPoPc) and canola-sunflower protein coprecipitates (CSPoCos).

Color	CPoPc	SPoPc	$CSPoCo_{70/30}$	$CSPoCo_{50/50}$	$CSPoCo_{30/70}$
L^*	51.01 ± 0.37^b	40.45 ± 0.54^e	54.07 ± 0.38^a	47.34 ± 0.55^c	44.41 ± 0.83^d
a^*	2.11 ± 0.03^b	-0.92 ± 0.07^e	2.84 ± 0.21^a	1.16 ± 0.10^c	-0.45 ± 0.02^d
b^*	18.56 ± 0.16^a	4.72 ± 0.16^e	17.21 ± 0.76^b	11.27 ± 0.18^c	8.32 ± 0.22^d
ΔE	49.25 ± 0.21^d	57.68 ± 0.62^a	46.10 ± 0.56^e	51.10 ± 0.01^c	53.57 ± 0.27^b
C^*	18.19 ± 0.32^a	4.81 ± 0.17^e	17.44 ± 0.79^b	11.33 ± 0.19^c	8.33 ± 0.22^d
H_{ab}	83.19 ± 0.44^d	100.88 ± 0.30^a	80.65 ± 0.37^e	84.13 ± 0.44^c	93.14 ± 0.16^b

L^* = lightness, a^* = +redness, -greenness, b^* = +yellowness, -blueness, ΔE = total variation of color, C^* = chroma, H_{ab} = hue angle. Values are the mean of three determinations of independent batches ± standard deviations. Different letters within the same row indicate significant differences ($p < 0.05$) among the groups. $CSPoCo_{70/30}$ = 70 % canola/30 % sunflower, $CSPoCo_{50/50}$ = 50 % canola/50 % sunflower and $CSPoCo_{30/70}$ = 30 % canola/70 % sunflower. The subscripts for each coprecipitate correspond to the proportions of canola and sunflower pastes, respectively.

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3.2.7 Protein fractionation

The storage proteins are classified according to the Osborne method into Al, Gl, Gx and Pr based on their PoSo in different solvents.[50] Table 5 shows the protein fractionation of PoPc and PoCos from canola and sunflower. The data showed that Gx and Al were the major fractions, while Gl and Pr were the minor fractions in both PoPc and PoCos. In comparison with CPoPc, the CSPoCos increased the Al content due greater inclusion of sunflower protein, while the Gl, Gx and Pr contents diminished. Interestingly, a higher Al content and lower Gx content in the CSPoCos produced an improvement in PoSo (Fig. 2A).

As in this study, Gx and Al were the main protein fractions in PoPc obtained from canola (glutelins 57.18 %, albumins 23.09 %),[42] orange seeds (glutelins 70.13 %, albumins 10.39 %),[56] and noni seeds (glutelins 64.62 %, albumins 24.12 %).[55]

Table 5. Protein fractional composition of canola protein precipitate (CPoPc), sunflower protein precipitate (SPoPc) and canola-sunflower protein coprecipitates (CSPoCos).

Protein fraction (%)	CPoPc	SPoPc	$CSPoCo_{70/30}$	$CSPoCo_{50/50}$	$CSPoCo_{30/70}$
Albumin (Al)	9.33 ± 0.51^{d}	46.22 ± 1.11^{a}	31.28 ± 1.01^{c}	30.57 ± 1.64^{c}	38.74 ± 1.13^{b}
Globulin (Gl)	9.31 ± 0.63^{a}	9.64 ± 0.52^{a}	7.15 ± 0.15^{c}	8.45 ± 0.76^{ab}	7.63 ± 0.53^{bc}
Glutelin (Gx)	78.15 ± 0.69^{a}	43.77 ± 1.09^{d}	60.30 ± 0.43^{b}	59.96 ± 2.04^{b}	52.71 ± 1.13^{c}
Prolamin (Pr)	3.21 ± 0.53^{a}	0.98 ± 0.36^{bc}	1.26 ± 0.07^{b}	1.02 ± 0.09^{bc}	0.92 ± 0.06^{c}

Values are the mean of three determinations of independent batches ± standard deviations. Different letters within the same row indicate significant differences ($p < 0.05$) among the groups. $CSPoCo_{70/30}$ = 70 % canola/30 % sunflower, $CSPoCo_{50/50}$ = 50 % canola/50 % sunflower and $CSPoCo_{30/70}$ = 30 % canola/70 % sunflower. The subscripts for each coprecipitate correspond to the proportions of canola and sunflower pastes, respectively.

3.3 Functional characteristics

3.3.1 PoSo

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PoSo is a function of hydrophilic-hydrophobic balance and electrostatic repulsion and strongly influences other techno-functional properties such as foaming, emulsifying and gelling characteristics.[57] The PoSo values of $CSPoCo_{70/30}$, $CSPoCo_{50/50}$ and $CSPoCo_{30/70}$ were 1.25, 1.58 and 1.50 mg/mL, respectively, higher than those obtained with CPoPc (0.41 mg/mL), but lower than those of SPoPc (1.69 mg/mL); specifically, the PoSo of CSPoCos increased in the range of 204.9% ($CSPoCo_{70/30}$) to 285.4% ($CSPoCo_{50/50}$), compared to CPoPc (Fig. 2A).

These increases in the PSo of CSPoCos were due to a higher inclusion of sunflower proteins that contain a higher proportion of the Al protein fraction (Table 5). Moreover, the fusion of canola and sunflower polymers may have strengthened intermolecular electrostatic interactions to generate soluble protein aggregates as can be observed in Fig. 6B. In a study by Zhou et al.[21] with a PoCo from pea and grass carp using alkaline extraction-isoelectric precipitation, it was observed an augment on PoSo (at pH 7) of 14.3 % and 22.9 %, in contrast to the pea and grass carp proteins, respectively. In addition, Tian et al.[12] reported that PoCo from soybean and wheat proteins had higher value of PoSo (pH 7), than the soybean and wheat protein isolates. This modification in PoCo from such protein sources were due to the secondary and tertiary structural changes, in contrast to soybean and wheat protein isolates.

3.3.2 WaHC and OiHC

WaHC is associated with the formation of gels and texture in food formulation.[58] Oil binding as measured by OiHC is an important functional property for improving palatability and mouthfeel, as well as flavor retention of certain foods such as emulsions, dairy products, sausages, and bread.[24] Fig. 2B shows the WaHC and OiHC of the PoPc and the CSPoCos. The WaHC values of CSPoCos (2.76–2.95 g water/g protein) were higher than those of CPoPc (2.04 g water/g protein) and SPoPc (2.35 g water/g protein), except for $CSPoCo_{30/70}$ (2.54 g water/g protein). Consistent with the results obtained in this study, Zhou et al.[19] reported for a pea-grass carp PoCo an improvement of WaHC of 9.8–13.04 %, compared to individual proteins of pea and grass carp, respectively, which could be due to the interaction between both protein sources promoting a higher affinity with water. In another study, with a PoCo from pea and lentil (1:3; w/w) increases of WaHC by 6.6% and

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9.8% were also observed, in contrast to pea protein isolate and lentil protein isolate, respectively.[59]

On the other hand, the OiHC of CSPoCos (3.88–4.22 g oil/g protein) was significantly higher ($p<0.05$) compared to CPoPc (2.89 g oil/g protein) and SPoPc (3.17 g oil/g protein) (Fig. 2B). These results may be due to PoC inducing greater exposure of the hydrophobic groups (Fig. 6D), which facilitated better interaction with the oil and led to an increase in OiHC.

3.3.3 LaGeCo

Gelation of globular proteins occurs in two stages. In the first stage, partial denaturation or conformational modification of these polymers occurs. Then, in the second stage, gradual association or aggregation occurs in a three-dimensional structure that traps water, fat and other components.[60] Fig. 2C shows the results of the LaGeCo for PoPc and PoCos from canola and sunflower. CSPoCos experimented a significant reduction ($p<0.05$) in LaGeCo with respect to CPoPc and SPoPc. The highest LaGeCo reduction of 83 % was observed with $CSPoCo_{70/30}$, followed by 66.7 % and 50 % for $CSPoCo_{50/50}$ and $CSPoCo_{30/70}$, respectively. The improved gelling characteristics of CSPoCos could be due to interactions such as disulfide bonds (Table 6) and non-covalent bonds (Fig. 6D) between polypeptide chains, thus forming larger aggregates. According to Flores-Jiménez et al.,[61] proteins with a low α-helices content and a high percentage of β-sheet structure form stronger gels (Table 7). Similarly, other researchers have reported that PoC improves the gelation properties of proteins by reducing LaGeCo. For example, Niu et al.[62] demonstrated that a PoPc of soybean and myofibrillar proteins improved gelation properties by up to 20 %, compared to the individual sources. Furthermore, Wu et al.[63] observed an 83.3 % LaGeCo reduction during gel formation of PoPc from soybean and cod, compared to the original protein sources.

3.3.4 Emulsion characteristics

One of the most important types of emulsions in the food industry are those prepared from an oil-water mixture (immiscible liquids), using proteins as interfacial stabilizers to prevent

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coalescence.[64] PoC significantly rose ($p<0.05$) the EAcIn of all CSPoCos, being up to 44.27-63.28 % for $CSPoCo_{70/30}$, in comparison with CPoPc and SPoPc, respectively, followed by 37.4-55.5 % for $CSPoCo_{50/50}$ (Fig. 2D). On the contrary, the PoC damaged the EStIn, provoking a reduction of up to 20.6-28.6 % for $CSPoCo_{50/50,}$ in contrast to CPoPc and SPoPc, respectively. According to Zhou et al.,[21] a PoCo from a pea and grass carp proteins improved EAcIn and EStIn, compared to the pea PoPc. These authors point out that the improved emulsifying properties are due to the fact that the pea and grass carp protein molecules have opposite charges. This situation can cause intermolecular electrostatic interactions and, therefore, the formation of soluble protein aggregates (Fig. 6B), which triggers the reconstruction of hydrophobic (Fig. 6D) and disulfide bonds (Table 6). Conversely, Awal et al.,[65] studied the formation of a PoCo from Bambara groundnut protein isolate and fish collagen. The results of this study showed a decrease in EAcIn (47.6%) and an increase in EStIn (63.9%) of the PoCo compared to the Bambara groundnut protein isolate. The authors concluded that the increase in EStIn is due to the fish proteins, which promote electrostatic interactions, generating more stable emulsions.

3.3.5 Foam characterization

Foams are dispersions of gas bubbles within a continuous phase (usually water), which can be generated by bubbling, stirring or pouring, with relevant applications in food.[60] As shown in Fig. 2E, the FmC of the CSPoCos was significantly improved ($p<0.05$), with the largest increases being 23.42 % and 16.74 % for $CSPoCo_{50/50}$, compared to CPoPc and SPoPc, respectively, except for $CSPoCo_{70/30}$, in which a reduction in this property was observed.

In addition, PoC significantly ($p<0.05$) increased FmS of the CSPoCos with respect to CPoPc and SPoPc. The highest increases in FmS of 47.26 % and 28.44 % were for $CSPoCo_{50/50}$, compared to CPoPc and SPoPc, respectively (Fig. 2E). In general, it was observed that the higher the proportion of sunflower proteins in the PoCos, the better foaming properties were achieved. Furthermore, the increase in FoP of the CSPoCos was possibly due to the interaction of canola and sunflower protein molecules, resulting in smaller particles and increases in S_uH_0 (Fig. 6B and D). In a study by Kristensen et al.[9] with a PoCo of pea and whey, an increase in foaming properties was reported, in contrast to the

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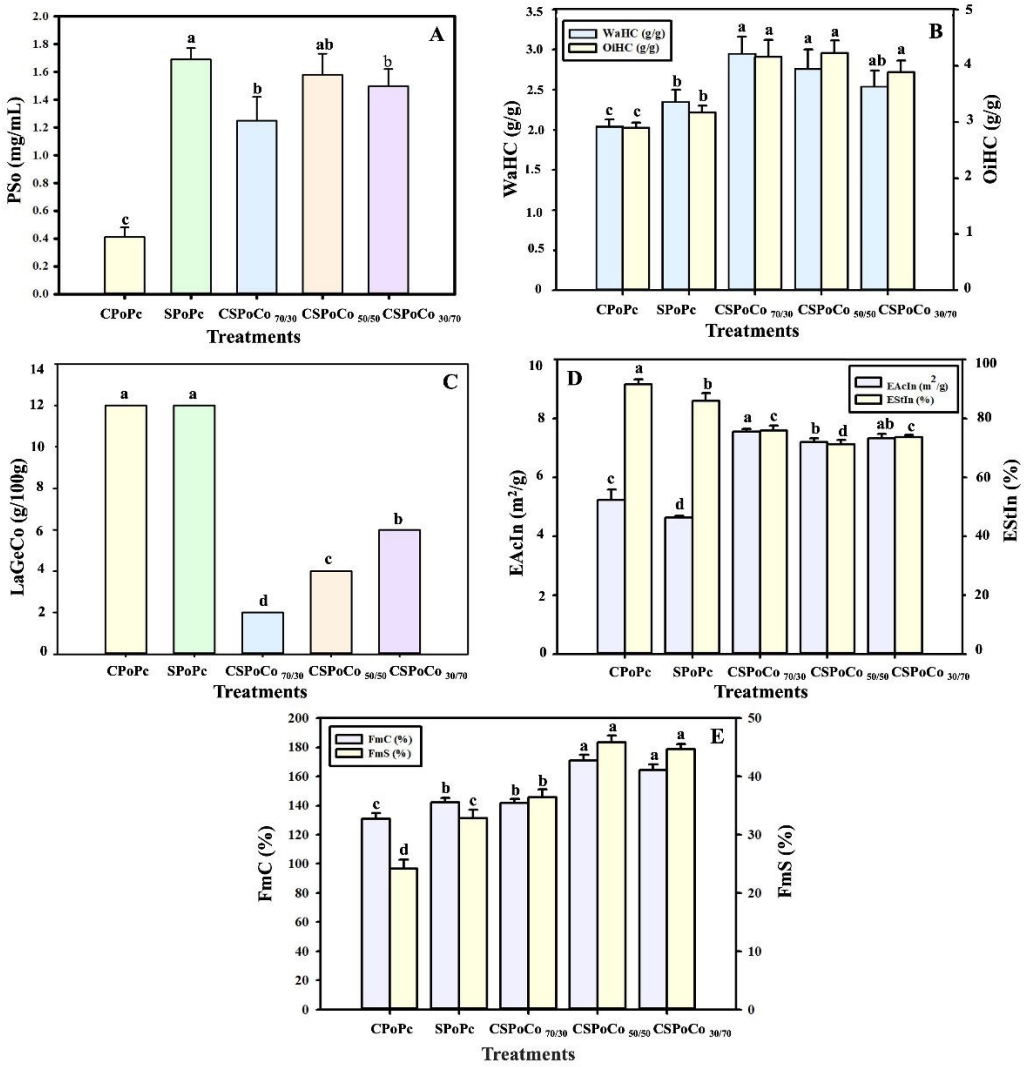
609 pea PoPc, possibly due to the interaction of the polymers used, which generated smaller
610 particles.



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612 **Fig. 2.** Effect of the coprecipitation on; A-Protein solubility (PoSo), B-Water holding
613 capacity (WaHC)/Oil holding capacity (OiHC), C-Last gelation concentration (LaGeCo),
614 D-Emulsifying activity index (EAcIn)/Emulsifying stability index (EStIn) and E-Foam
615 capacity (FmC)/Foam stability (FmS). CPoPc = canola protein precipitate. SPoPc =
616 sunflower protein precipitate. CSPoCo = canola-sunflower protein coprecipitate.
617 $CSPoCo_{70/30}$ = 70 % canola/30 % sunflower, $CSPoCo_{50/50}$ = 50 % canola/50 % sunflower
618 and $CSPoCo_{30/70}$ = 30 % canola/70 % sunflower. Distinct letters on the bars indicate
619 significant ($p<0.05$) difference (average ± SD, n = 3 independent batches).

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3.4 Biochemical properties

3.4.1 SDS-PAGE

SDS-PAGE analysis is used to quantify, compare and characterize proteins based on their molecular weight.[66] The impact of PoC on the electrophoretic patterns of canola and sunflower proteins under non-reducing and reducing conditions is shown in Fig. 3.

Under non-reducing conditions (Fig. 3A), the molecular weight distribution of CPoPc consisted of four main fractions with the following molecular weights: ~49 kDa, 30 kDa, 25 kDa, and 15 kDa, which correspond to the molecular weights of cruciferin (~50-20 kDa) y napin (~7-11 kDa) of the protein fractions reported by Cháirez-Jiménez et al.[7] However, in presence of the reducing agent β-mercaptoethanol (Fig. 3B), the band at ~49 kDa of CPoPc was dissociated into its two polypeptides (~20 kDa and ~30 kDa), as reported by Flores-Jiménez et al.[42] In the case of SPoPc, the main bands detected were: ~49, 38, 35, 29, 25 and 8 kDa under the non-reducing conditions (Fig. 3A). The subunits with a molecular weight between 20 and 50 kDa correspond to helianthinin (11S globulins), while those between 10-19 kDa correspond to 2S albumin. Under reducing conditions (Fig. 3B), 11S globulin dissociated into its two acidic subunits with a molecular weight of ~38-49 kDa and 25–29 kDa, as well as in its basic subunits with a molecular weight of 20–23 kDa.[67]

On the other hand, under non-reducing conditions the CSPoCos presented seven bands with molecular weights of ~49, 38, 35, 29, 25, 18, and 15 kDa, which are related to the 11S globulins and 2S albumins fractions of canola and sunflower proteins (Fig. 3A). Under reducing conditions (Fig. 3B), the molecular weight distribution of the CSPoCos consisted of nine main fractions, with molecular weights of ~49, 38, 35, 29, 25, 23, 20, 18, and 12 kDa. Interestingly, all the protein bands from canola and sunflower were present in all CSPoCos, under both reducing and non-reducing conditions. However, under non-reducing conditions, an additional 18 kDa band appeared in the CSPoCos (Fig. 3A), with the band in $CSPoCos_{70/30}$ being more intense compared to the other coprecipitates, likely due to a higher canola content. According to Guidi et al.,[59] protein interactions can be observed through the appearance or disappearance of bands in the electrophoretic profile. This suggests a strong interaction between the polypeptide chains of both protein sources,

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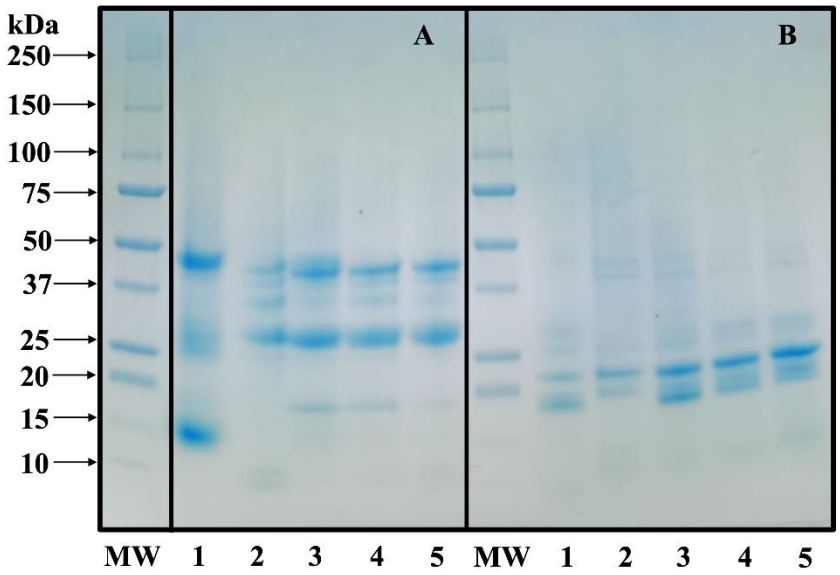
649 directly related to their functional properties (Fig. 2). Similar results were observed in a
650 PoCo of Bambara groundnut protein isolate and fish collagen, which showed the presence
651 of the characteristic bands of both polymers.[65]



652 **Fig. 3.** Effect of the coprecipitation on sodium dodecyl sulfate-polyacrylamide gel
653 electrophoresis; A-Non reducing and B-reducing conditions. Lane MW, molecular weight
654 marker; Lane 1, CPoPc; Lane 2, SPoPc; Lane 3, $CSPoCo_{70/30}$; Lane 4, $CSPoCo_{50/50}$ and
655 Lane 5, $CSPoCo_{30/70}$. CPoPc = canola protein precipitate. SPoPc = sunflower protein
656 precipitate. CSPoCo = canola-sunflower protein coprecipitate. $CSPoCo_{70/30}$ = 70 %
657 canola/30 % sunflower, $CSPoCo_{50/50}$ = 50 % canola/50 % sunflower and $CSPoCo_{30/70}$ = 30
658 % canola/70 % sunflower.

659 **3.4.2 IVPoDi**

660 IVPoDi is a parameter is used to estimate the nutritional quality of proteins, which largely
661 depends on the nature of these biopolymers that can allow or restrict the action of digestive
662 enzymes.[56] The IVPoDi values of $CSPoCo_{70/30}$ (86.64 %), $CSPoCo_{50/50}$ (86.22 %), and
663 $CSPoCo_{30/70}$ (85.25 %) were significantly ($p>0.05$) comparable with those of CPoPc (84.47
664 %), but lower than those of SPoPc (91.65 %) (Fig. 4A). This could be because the IVPoDi
665 of CPoPc is lower than that of SPoPc, and increasing the proportion of CPoPc in CSPoCos
666 significantly decreased this parameter. This suggests that CPoPc contains a higher content

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of antinutritional compounds such as soponins, tannins, lectins, phytates, glucosinolates, phytic acid, and thiocyanate.[68] Therefore, PoC using canola and sunflower as protein sources did not result in any increase in IVPoDi values.

Other studies have revelated IVPoDi values of 83.9 % and 90.7 % for canola[42] and sunflower[6] PoPc, respectively, which are similar to the values obtained for the CSPoCos and PoPc of this study.

3.4.3 IViAC

The IViAC of PoPc and PoCos from canola and sunflower was determined by DPPH, which is a radical that has the capacity to retain an electron or oxygen from an antioxidant substance.[69] The DPPH radical inhibition percentages of $CSPoCo_{70/30}$, $CSPoCo_{50/50}$, and $CSPoCo_{30/70}$ were 72.22, 70.12 and 72.34, respectively, which were higher than that of 67.79 obtained by CPoPc, but lower than the 80.46 % of SPoPc (Fig. 4B). The results obtained in IViAC of the PoPc and PoCos can be assumed as good, possibly due to the existence of phenols, flavonoids and aromatic amino acids with the capacity to give protons to radicals that lack electrons.[30]

Alu'datt et al.[18] found increases up to 50.4 % in IViAC, for a soybean and flaxseed PoCo compared to soybean PoPc. The authors of this study concluded that the increase of the IViAC is due to the fact that PoC increases the proportion of phenolic compounds and certain amino acids with antioxidant activity.

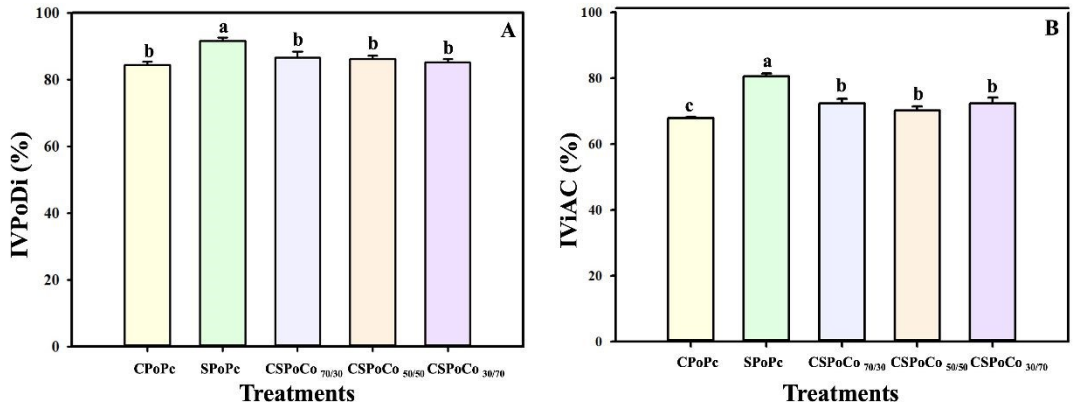

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Fig. 4. Effect of the coprecipitation on; A-In vitro protein enzymatic digestibility (IVPoDi) and B-In vitro antioxidant capacity (IViAC). CPoPc = canola protein precipitate. SPoPc = sunflower protein precipitate. CSPoCo = canola-sunflower protein coprecipitate. $CSPoCo_{70/30}$ = 70 % canola/30 % sunflower, $CSPoCo_{50/50}$ = 50 % canola/50 % sunflower and $CSPoCo_{30/70}$ = 30 % canola/70 % sunflower. Distinct letters on the bars indicate significant ($p<0.05$) difference (average ± SD, n = 3 independent batches).

3.5 Structural characterization

3.5.1 SEM

Morphology is one of the major analyses to characterize powder products, which influences the functional of the protein materials.[70] Fig. 5 shows the granular structure of the PoPc and PoCos from canola and sunflower obtained by SEM (at 150 ×). The surface of CPoPc was irregular with protuberances. On the other hand, SPoPc presented larger aggregates with a flat and smooth structure compared to CPoPc and CSPoCos. In the case of the CSPoCos, more heterogeneous structures with porous and smooth surfaces were presented compared to CPoPc and SPoPc. Furthermore, $CSPoCo_{50/50}$ had larger aggregates compared with the other coprecipitates, which could be due to greater exposure of hydrophobic groups on the surface of the molecules (Fig. 6D), favoring interaction between them to form larger aggregates during lyophilization. Previous studies have reported that protein powders with smoother surfaces tend to have higher solubility,[71,72,73] as observed in this study (Fig. 2A).

According to a previous study, the presence and proportion of proteins fractions in base to solubility can influence the microstructure of proteins seen by SEM.[30] So, Al gives a lamellar appearance, Gx a rough surface with several tiny pores and Gl a structure composed of small globules, which is in line with the amount of the protein fractions of Gx, Al and Gl in CPoPc, SPoPc and CSPoCos. For example, CPoPc had the highest Gx content (Table 5) presenting a more porous and rougher surface, while SPoPc had a higher proportion of Al showing lamellar structures. In addition, CSPoCos observed a greater balance between Gx and Al fractions, so a combination of porous/rough and soft/smooth structures were detected (Table 5 and Fig. 5). Therefore, the surface appearance of the CSPoCos observed through SEM appears to be associated with the protein composition of

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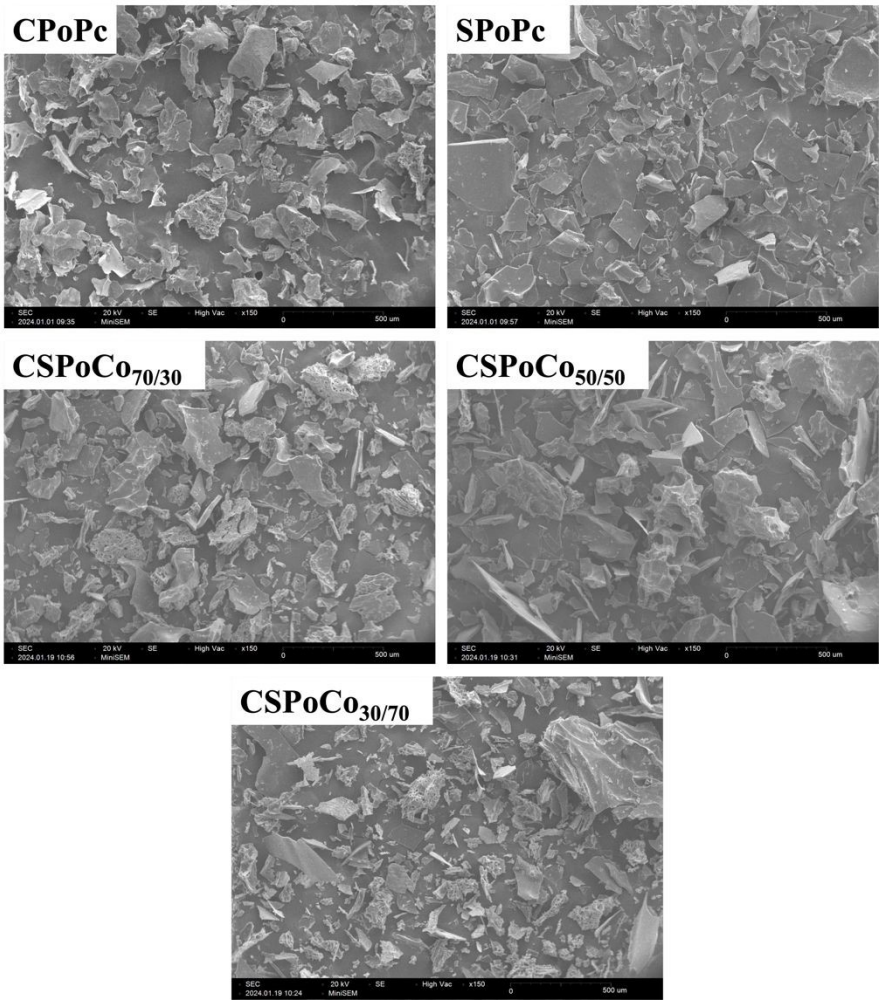
715 canola and sunflower, which in turn could also influence its physicochemical and
716 functional properties.[74]



717 **Fig. 5.** Effect of the coprecipitation on microstructure observed at 150x amplification by
718 scanning electron microscope. CPoPc = canola protein precipitate. SPoPc = sunflower
719 protein precipitate. CSPoCo = canola-sunflower protein coprecipitate. $CSPoCo_{70/30}$ = 70 %
720 canola/30 % sunflower, $CSPoCo_{50/50}$ = 50 % canola/50 % sunflower and $CSPoCo_{30/70}$ = 30
721 % canola/70 % sunflower.

722 **3.5.2 MoFl**

723 MoFl refers to the adaptability of a protein to external environmental changes, which
724 induces a reorientation of its amino acid residues, causing a change in its conformation.[75,76]
725 As shown in Fig. 6A, the MoFl of the CSPoCos was higher than SPoPc (except

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726 $CSPoCo_{30/70}$), but lower compared to CPoPc ($p<0.05$). According to various studies, the
727 increase in MoFl of proteins, as occurred in this research for CSPoCos, causes an
728 improvement in functional properties (Fig. 2) due to the structural reorganization of the
729 polypeptide chain.[75,77]

730 **3.5.3 Particle size (Pz) and ζ-potential**

731 Protein Pz is a characteristic very important that influences functional properties of food
732 systems.[32] Except for $CSPoCo_{70/30}$, all CSPoCos in solution diminished the Pz, compared to
733 CPoPc and SPoPc (Fig. 6B). The higher variation in Pz was observed between CPoPc
734 (199.3 nm) and $CSPoCo_{30/70}$ (86.9 nm). The decrease in Pz of the CSPoCos by PoC may be
735 due to that such phenomenon involves the formation of smaller aggregates or structures
736 more compact by interaction of canola and sunflower proteins.[78] The reduction of Pz by
737 PoC contributed to the increase in PoSo (Fig. 2A), due to a larger surface area and the
738 conservation of electrostatic charges that favor protein-water interactions. Pizones Ruiz-
739 Henestrosa et al.[79] and Chihi et al.[80] reported a reduction in Pz for PoCos of soybean (7S
740 globulins)-whey (β -lactoglobulins) and pea (globulins)-whey (β-lactoglobulin) proteins,
741 respectively, in agreement with the results of this study.

742 ζ-potential is related to the net surface charge and the ionic strength of proteins, affecting
743 the adsorption and unfolding of the polypeptide chain and therefore its functionality.[81] ζ-
744 potential of CPoPc, SPoPc, and CSPoCos were negative (Fig. 6C). The PoC increased the
745 negative value of ζ-potential in the ranges of 8.09 % ($CSPoCo_{70/30}$)–19.94 % ($CSPoCo_{30/70}$),
746 compared with CPoPc. On the contrary, the PoC diminished the negative values for
747 $CSPoCo_{70/30}$ (20.27 %) and $CSPoCo_{50/50}$ (25.22 %) with respect to SPoPc.

748 Changes in ζ-potential may be associated with modifications in the amount of ionizable
749 amino and carboxyl groups exposed on the surface caused by the PoC.[80] Previous research
750 has shown that protein dispersions with high absolute ζ-potentials are characterized by
751 strong intermolecular electrostatic repulsion, which is sufficient to resist agglomeration,
752 thereby improving the dispersibility and stability of the protein system.[10] Therefore, the ζ-
753 potential is related to the improvement of the functional properties of CSPoCos because the
754 interactions between the two polypeptides inhibited structural folding and maintained their

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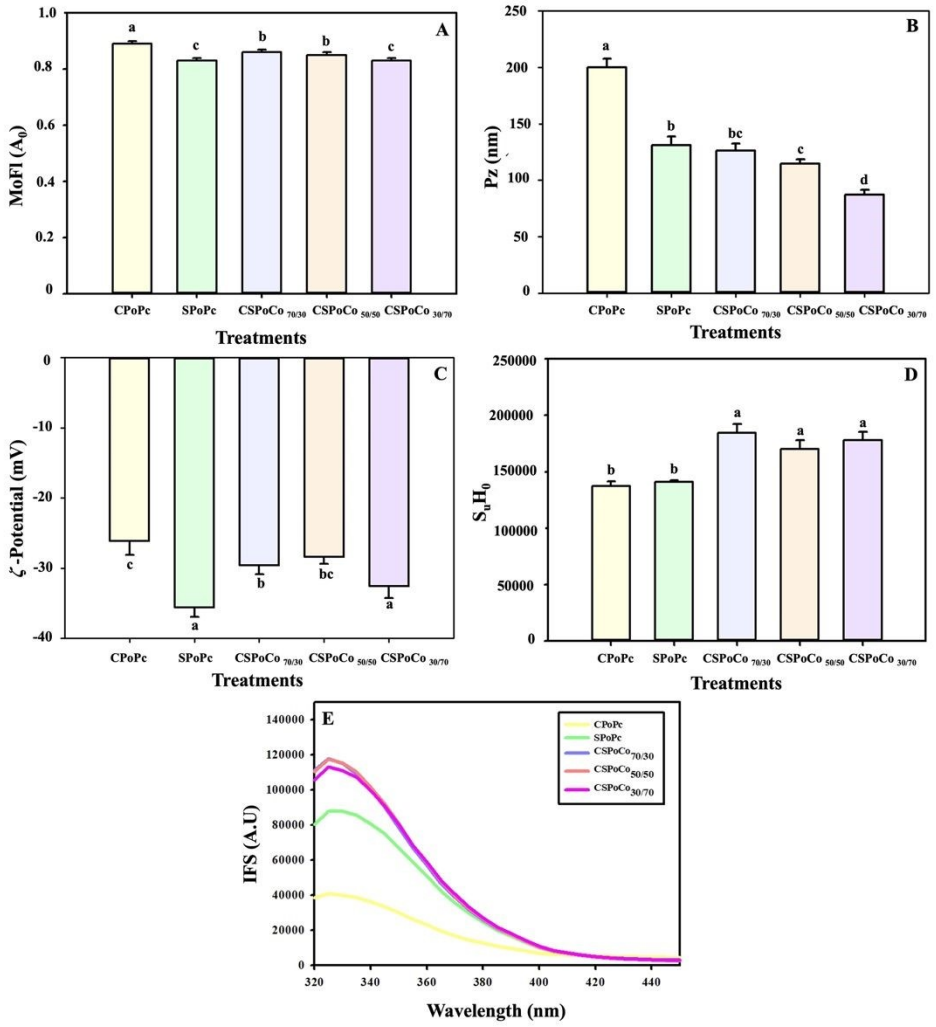
charged surface (Fig. 2). The PoCos of rice and casein[78] and rice and cod[10] proteins also increased the negative value of the ζ-potential, compared to the individual sources.

3.5.4 S_uH_o

S_uH_o indicates the amount of hydrophobic groups distributed along the surface of proteins, whatever has a great impact over the functional characteristics of said polymers.[33] As can be seen in Fig. 6D, $CSPoCo_{70/30}$, $CSPoCo_{50/50}$ and $CSPoCo_{30/70}$ increased S_uH_o by 25.5-23.6 %, 19.3-17.2 %, and 22.8-20.8 % in comparison with CPoPc and SPoPc, respectively. The increase in S_uH_o in CSPoCos could be due to structural changes brought about by the binding of canola and sunflower proteins, which caused the main chains of these polymers to unfold to expose apolar groups on their surface. Kristensen et al.[9] and Kristensen et al.[20]



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demonstrated that the association of pea and whey proteins in the form of PoCo increased S_uH_o, as observed in this study.

Fig. 6. Effect of the coprecipitation on; A-Molecular flexibility (MoFl), B-Particle size (Pz), C- ζ-potential, D-Surface hydrophobicity (S_uH_o) and E-Intrinsic fluorescence spectra (IFS). CPoPc = canola protein precipitate. SPoPc = sunflower protein precipitate. CSPoCo = canola-sunflower protein coprecipitate. $CSPoCo_{70/30}$ = 70 % canola/30 % sunflower, $CSPoCo_{50/50}$ = 50 % canola/50 % sunflower and $CSPoCo_{30/70}$ = 30 % canola/70 % sunflower. Distinct letters on the bars indicate significant ($p<0.05$) difference (average ± SD, n = 3 independent batches).

3.5.5 IFS

Changes in the tertiary structure of proteins can be measured using fluorescence emission spectra when tyrosine, phenylalanine, and tryptophan residues of proteins are excited at a wavelength of 280 to 290 nm.[34] The impact of PoC on the fluorescence spectra of PoPc and PoCos from canola and sunflower is shown in Fig. 6E. The maximum emission wavelength occurred at 325 nm in all treatments. Overall, a higher IFS was observed in the CSPoCos compared to CPoPc and SPoPc. In a study with a soybean and whey PoCo,[12] it was determined that the changes in the tertiary structure by PoC, measured as IFS, are provoked by formation of stronger protein-protein interactions, in congruence with the results of this study. Furthermore, the alteration of the tertiary structure by PoC, determined by IFS, in the CSPoCos is consistent with the results regarding the increase in S_uH_o (Fig. 6D), which is also an indicator of the variation of the protein conformation.

3.5.6 SH_{Fr}, SH_{To} and $S\text{-}S_{Br}$

Sulfhydryl groups, including SH_{Fr}, SH_{To} and $S\text{-}S_{Br}$, are one of the most reactive functional groups in proteins that significantly influence the functional properties of food proteins. SH_{Fr} are those sulfhydryl groups that are located on the surface of the proteins and are easily accessible to react with the environment. SH_{To} includes SH_{Fr} and SH buried within the protein structure.[32] The results of SH_{Fr}, SH_{To} and $S\text{-}S_{Br}$ content of CPoPc, SPoPc and CSPoCos are presented in Table 6.

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Overall, PoC increased the SH_{fr} content (20.13-28.71 %) of the CSPoCos, in contrast to CPoPc, whereas the S-S_{Br} content decreased in the CSPoCos in the ranges of 105.5-503.2 % and 37.4-303.2 %, in contrast with CPoPc and SPoPc, respectively, which could imply an unfolding of the polypeptide chain and thus a modification in the initial proportion of sulfhydryl groups in the new polymeric structures. On the other hand, the SH_{To} content did not change significantly ($p>0.05$) in CSPoCos with respect to CPoPc, but its value was significantly reduced ($p<0.05$) compared to SPoPc (Table 6).

Interestingly, in CSPoCos it was observed that almost all of the sulfhydryl groups corresponded to SH_{Fr}, which also demonstrated a structural rearrangement of its proteins due to the effect of PoC, similar to the effect caused by other physical treatments such as ultrasound[81] and high pressure.[34]

Table 6. Free (SH_{Fr}) and total (SH_{To}) sulfhydryl groups and disulfide bridges (S-S_{Br}) of canola protein precipitate (CPoPc), sunflower protein precipitate (SPoPc) and canola-sunflower protein coprecipitates (CSPoCos)

Sulfhydryl groups (μmol/g protein)	CPoPc	SPoPc	$CSPoCo_{70/30}$	$CSPoCo_{50/50}$	$CSPoCo_{30/70}$
Free (SH_{Fr})	12.47 ± 0.48^{d}	17.87 ± 0.07^{a}	16.05 ± 0.04^{b}	15.02 ± 0.26^{c}	14.98 ± 0.19^{c}
Total (SH_{To})	16.20 ± 0.68^{b}	20.37 ± 0.3^{a}	16.67 ± 0.42^{b}	16.18 ± 0.13^{b}	16.79 ± 0.49^{b}
Bridges (SS_{Br})	1.87 ± 0.60^{b}	1.25 ± 0.28^{a}	0.31 ± 0.40^{c}	0.58 ± 0.17^{c}	0.91 ± 0.41^{bc}

Values are the mean of three determinations of independent batches ± standard deviations. Different letters within the same row indicate significant differences ($p < 0.05$) among the groups. $CSPoCo_{70/30}$ = 70 % canola/30 % sunflower, $CSPoCo_{50/50}$ = 50 % canola/50 % sunflower and $CSPoCo_{30/70}$ = 30 % canola/70 % sunflower. The subscripts for each coprecipitate correspond to the proportions of canola and sunflower pastes, respectively.

3.5.7 ATR-FTIR

The FTIR spectrum of a protein exhibits absorption peaks primarily associated with amide groups. The amide I peak is of great importance, as it allows determining the type of secondary structure (β-sheet, α-helix, β-turn, and random coil) of a protein.[30]

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Table 7 shows the secondary structure content of PoPc and PoCos from canola and sunflower. Compared with CPoPc and SPoPc, the secondary structures of the CSPoCos were significantly different ($p<0.05$). In CPoPc, SPoPc, and CSPoCos, β-sheets were the predominant secondary structure, followed by β-turns, random coils and α-helices. PoC increased the relative β-sheet content from an initial value of 49.55 % for CPoPc to 52.13 %, 51.42 % and 52.58 % for $CSPoCo_{70/30}$, $CSPoCo_{50/50}$ and $CSPoCo_{30/70}$, respectively, however, these values were lower than that 60.96 % obtained by SPoPc. In contrast, α-helices content in the CSPoCos was not significantly ($p>0.05$) different of CPoPc. In the case of β-turn, PoC significantly reduced ($p<0.05$) its content in the range of 4.86 % ($CSPoCo_{50/50}$) to 25.0 % ($CSPoCo_{70/30}$), compared to CPoPc. The reason for these changes could be attributed to the proportion of different protein sources in the PoCos. This composition induces a new equilibrium in the secondary structures by modifying molecular interactions, such as hydrogen bonds, disulfide bonds, and hydrophobic and Van der Waals forces. Consequently, these structural transformations are linked to the enhancement of PoSo, WaHC, OiHC, LaGeCo, EAcIn, FmC, and FmS (Fig. 2). Tan et al.,[11] Tian et al.,[12] and Wang et al.,[78] found that the PoC of proteins from soybean-tilapia, soybean-wheat, and soybean-rice, respectively, modified the secondary structure in contrast to individual protein sources, as resulted in this study.

Secondary structure (%)	CPoPc	SPoPc	$CSPoCo_{70/30}$	$CSPoCo_{50/50}$	$CSPoCo_{30/70}$
β-sheet	49.55 ± 0.68^{c}	60.96 ± 1.10^{a}	52.13 ± 0.45^{b}	51.42 ± 0.28^{b}	52.58 ± 0.69^{b}
α-helix	8.40 ± 0.87^{b}	14.64 ± 0.53^{a}	8.79 ± 0.42^{b}	8.67 ± 0.77^{b}	8.64 ± 0.58^{b}
β-turn	29.77 ± 0.54^{a}	10.72 ± 1.08^{d}	23.81 ± 0.97^{c}	28.39 ± 0.35^{b}	24.11 ± 0.44^{c}
Random coil	11.57 ± 0.39^{b}	13.67 ± 0.54^{a}	14.44 ± 0.32^{a}	12.07 ± 0.15^{b}	14.63 ± 0.45^{a}

Table 7. Secondary structure of canola protein precipitate (CPoPc), sunflower protein precipitate (SPoPc) and canola-sunflower protein coprecipitates (CSPoCo)

Values are the mean of three determinations of independent batches ± standard deviations. Different letters within the same row indicate significant differences ($p < 0.05$) among the groups. $CSPoCo_{70/30}$ = 70 % canola/30 % sunflower, $CSPoCo_{50/50}$ = 50 % canola/50 % sunflower and $CSPoCo_{30/70}$ = 30 % canola/70 %

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sunflower. The subscripts for each coprecipitate correspond to the proportions of canola and sunflower meals, respectively.

4 Conclusions

PoC of canola and sunflower proteins by alkaline extraction-isoelectric precipitation altered the physicochemical, functional and structural characteristics, compared to CPoPc and SPoPc. The protein content, ρb, ρc, CaIn, HaRa, WaHC, OiHC, LaGeCo, EAcIn, FmC, FmS, S_uH_o, IFS values of the CSPoCos increased, with respect to original protein source, while the Pz and EStIn values decreased. In general, the moisture, L^*, a_w, T_{rb}, MoFl, ζ-potential, IVPoDi, IViAc, Al, Gx, Pr, SH_{Fr}, SH_{To}, $S\text{-}S_{Br}$, α-helices, β-sheets, and β-turns values varied between those corresponding to CPoPc and SPoPc. SDS-PAGE results revealed the characteristic bands of the two coprecipitated protein sources, in addition to the appearance of a new band, indicating an interaction between the two polymers. The improvement in the functional characteristics of the CSPoCos was due to the unfolding and interaction of canola and sunflower polypeptide chains that gave rise to new protein structures. Specifically, the values of PoSo, WaHC, FmC and FmS were higher in the $CSPoCo_{50/50}$ compared to the other CSPoCos. Thus, PoC proved to be an effective method for modifying canola and sunflower proteins, which could considerably improve the functional quality of plant proteins, making them a good alternative to animal proteins as food ingredients. Further research into the nutritional and sensory characteristics of the CSPoCos could expand their use in the production of functional foods.

Author contributions

Nitzia Thalía Flores-Jiménez: Investigation; Methodology; Writing-Original draft preparation; Editing. **José Armando Ulloa**: Investigation; Writing-Original draft; Review; Editing; Conceptualization, Supervision; Data curation; Funding acquisition. **Judith Esmeralda Urías-Silvas**: Software; Resources; Supervision; Validation.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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Data availability

Data will be made available on request.

Acknowledgments

To the Secretariat of Science, Humanities, Technology and Innovation (SECIHTI) of Mexico for the postdoctoral scholarship awarded to Nitzia Thalía Flores Jiménez (CVU:601948) and the Patronage to Administer the Special Tax Destined to the Autonomous University of Nayarit (UAN) for the financial support.

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Data availability

Data will be made available on request.