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Evaluation of antioxidant activity and functional properties of protein concentrates from taxo (*Passiflora tripartita*) and granadilla (*Passiflora ligularis*) seeds

Klever Lenin Vàsconez-Barragàn[a] | Isidro Favian Bayas-Morejòn[b] | María Monserrath Morales-Padilla[ac]

[a]Technical University of Cotopaxi, Postgraduate Direction, Faculty of Agricultural Sciences and Natural Resources, P.O. Box 050103, Latacunga, Ecuador.
[b]Bolìvar State University, Faculty of Agricultural Sciences, Natural Resources and Environment, P.O. Box 020150, Campus Laguacoto, Guaranda, Ecuador.
[c]Departamento de Tecnología de Alimentos, Universitat Politècnica de València, Camino de Vera s/n, 46022, Valencia, Spain.

Abstract The growing demand for sustainable sources rich in protein has sparked interest in researching environmentally friendly protein sources, with seeds being a promising alternative to animal proteins. This study evaluated the antioxidant activity and functional properties of protein concentrates from banana, passion fruit and passion fruit seeds. Proximal analysis of the seeds revealed that passion fruit had the highest protein content (21.08%) compared to taxus (10.59%). Protein concentrates were obtained from the seeds by alkaline solubilization at pH 10-12 and precipitation at acidic pH (4-5). Protein quantification was performed using three methods. CPG 12-4 showed the highest protein content according to the Dumas method (65.16%) and 0.69% in Bradford, while CPT 12-5 stood out in BCA with 27.76%. Water retention capacity (WAC) ranged from 1.39 to 242 g water/g sample, oil absorption capacity (AOC) from 1.48 to 2.92 g oil/g sample, and solubility exceeded 100% in CPT at pH 9 and 12. SDS-PAGE revealed high-intensity bands of 29 kDa and 35 kDa. The phenolic compounds content was determined by ABTS, FRAP, and DPPH. It should be noted that the taxo concentrates demonstrated exceptional antioxidant capacity ranging from 7042.44 to 7770.97 µmol ET/g. The FTIR technique revealed specific functional groups as primary amines, amides, and carboxylic acids. The results of this research present the protein concentrates of these seeds as a promising source of vegetable proteins due to their valuable antioxidant compounds and techno-functional properties, contributing to the development of new nutritious and sustainable products.

Keywords: *passiflora tripartita*, *passiflora ligularis*, functional properties, functional groups, protein concentrate, antioxidants

1. Introduction

The fruits of the genus Passiflora are part of the *Passifloraceae* family, a genus comprising more than 600 species, almost all of which are distributed in native areas of South America. Most of these species are climbers, of which we find *Passiflora tripartita* (Fischer et al., 2020) and the passion fruit *Passiflora ligularis*, the second most economically important species of the genus after the passion fruit, with its pleasant flavor (Vardanega et al., 2023). In Ecuador, these fruits are usually consumed as fresh food or processed products (pulp or ice cream); however, agro-industrial processing involves the generation of large quantities of byproducts such as seeds, stems, peels, and leaves (Pérez-Marroquín et al., 2023). These byproducts are not efficiently utilized despite their nutritional value, as they contain dietary fiber, bioactive compounds (phytochemicals, antioxidants, pigments, polyphenols, carotenoids, and fatty acids), and protein (Dey et al., 2021). Macronutrients, which are mainly composed of carbohydrates, lipids, and proteins, are the main components of dry matter. Concentrates obtained from plant byproducts such as seeds, stems, and peels are promising sources of nutrients, standing out as alternatives to animal proteins because of their valuable nutritional composition (Flaibam et al., 2024). According to Loizzo et al. (2019), the pulp contains a high proportion of polyphenols and antioxidants, with the seeds containing the highest amount of these compounds. On the other hand, passion fruit stands out for its potential as a source of polyphenols, carotenoids, anthocyanins, phenolic acids, and amino acids (Angel-Isaza et al., 2021). In addition, among its components, *Passiflora* seeds stand out for their high degree of instauration, which is valuable for their application as a raw material in the food industry (Pereira et al., 2017). Despite previous scientific studies on this taxon and passion fruit, most research has focused on passion fruit (*Passiflora edulis*), which has limited knowledge of other species of the genus. For this reason, an in-depth analysis of these fruits is necessary to broaden their characterization and potential utilization.

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Efficient protein extraction depends largely on the action of pH, as it disrupts the solubility and structural integrity of proteins. Alkaline pH values (10-12) interfere with and promote protein solubilization by increasing the negative charge of protein molecules, promoting electrostatic repulsion between molecules, and interacting with water. On the other hand, acidic pH (4-5) is related to isoelectric precipitation. Research conducted agrees that most plant seed proteins have isoelectric points at acidic pH, where their net charge is close to zero, which reduces electrostatic repulsion and thus promotes protein precipitation.

The selection of acidic pH 4 and 5, in addition to influencing protein precipitation, allows for comparison and understanding of protein behavior when they are close to their isoelectric points, which directly influences extraction yield, as well as functional properties and, eventually, the antioxidant capacity of PCs. Therefore, understanding these pH-dependent characteristics is vital for optimizing the production of protein concentrates.

The objective of this work was to evaluate the functional properties, antioxidant capacity, and protein characteristics of concentrates obtained from banana passionfruit and granadilla passion fruit seeds subjected to alkaline pH extraction and acid pH precipitation conditions, intending to assess their potential use as functional ingredients for the food industry.

2. Materials and Methods

2.1. Obtaining the raw material

The raw material, *Passiflora tripartita* and *Passiflora ligularis* seeds, was purchased from local producers at the wholesale market in the city of Ambato, Ecuador. The fruit was pulped, and the seeds were dried on a drying tray at 40 °C for 8 hours and ground to obtain flour with a particle size of 2 mm.

2.2. Proximate analysis

Passiflora seeds were analyzed for chemical composition according to AOAC 2005, fat content according to AOAC 2005:06:2012, moisture content according to AOAC 925.05:2012, total fiber content according to AOAC 926.09:2005, ash content according to AOAC 942.05.2012, protein content according to the Dumas method, and total carbohydrate content by difference (AOAC: Official Methods of Analysis (Volume 1), 2005).

2.3. Preparation of the protein concentrate

The PC (protein concentrate) was obtained from the seeds according to the methodology of Vilcacundo et al. (Vilcacundo et al., 2020) and Quinteros et al. (Quinteros et al., 2022). We worked with 2.5 kg of banana passionfruit and granadilla seeds. The seeds were ground in a manual corona mill, and 1.5 kg of flour was obtained. For extraction, 30 g of flour was dissolved in distilled water at a ratio of 1:8 (w/v), and the pH was adjusted to 10.0:12.0 with 2 N NaOH. The mixture was subsequently centrifuged for 30 min at 500 rpm at 20.5°C in a centrifuge (Eppendorf 5804 R, Hamburg). The precipitate (fat, starch, fiber, minerals, sugars) was discarded by separating the supernatant (soluble protein) to adjust the pH to 4.0 and 5.0 with 2N HCl. These precipitation pH values were selected to evaluate the behavior of proteins near their isoelectric point and to understand how pH affects the yield and functional properties of PC. The mixture was then centrifuged for 30 minutes at 500 rpm at 20.5 °C to separate the precipitate, frozen at -80 °C, and freeze-dried in a freeze dryer (Christ Alpha 1-4 L Dplus, Germany) at -55 °C for 12 hours. °C, and freeze-dried in a freeze dryer (Christ Alpha 1-4 L Dplus, Germany) at -55 °C for 12 hours. The yield was determined gravimetrically (Kotnala et al., 2024):

$$\%\,\text{yield} = \frac{\text{CP}}{\text{seed flour}} \times 100 \qquad (1)$$

Where: PC: protein concentrate.

2.4. Quantification of protein content

The protein content was determined via the Dumas combustion method with an elemental analyzer (Vario Macro Cube, Elemental, Germany) calibrated with a sulfonamide standard. Small aluminum capsules containing 20 mg of CPG (granadilla protein concentrate) and CPT (banana passionfruit) samples were used and injected for conversion to nitrogen gas by calcination. Analyses were performed in triplicate, and the percentage of protein was calculated via the formula $\%protein = F\ \times \%N$. A conversion factor of 5.5, which is recommended in the literature, was applied to obtain a more accurate estimate on the basis of the amino acid profile of the seeds (Ezeagu et al., 2002).

2.5. Protein quantification methods

2.5.1. Bradford

The Bradford Coomassie assay (Thermo Scientific) was performed with bovine serum albumin (BSA) as a standard, with a range of 75--1000 µg/mL for the calibration curve. Fifty milligrams of freeze-dried protein was diluted in 1000 µL of distilled water, and 50 µL of the sample was mixed with 1500 µL of Coomassie blue solution, shaken in a vortex for 1 min, and then centrifuged for 1 min at 12000 rpm in an Eppendorf Centrifuge 5427 R (Rekowski et al., 2021). The samples were incubated at room temperature for 10 min, and the absorbance was measured with a UV–Vis spectrophotometer (NanoDrop, Thermo Scientific) at 595 nm. This was performed in triplicate, and the results are reported as % protein. If your research manuscript includes large datasets that have been deposited in a publicly available database, please specify the location of the data deposition and provide the relevant accession numbers. If the accession numbers have not been obtained at the time of submission, please indicate that they will be provided during the review process. However, they must be provided before publication.

2.5.2. BCA (bicynconin acid protein assay)

Protein quantification was performed via a BCA assay (SIGMA-ALDRICH) utilizing bovine serum albumin (BSA) as the standard, with a 75 to 1000 µg/mL range for the calibration curve. Fifty milligrams of lyophilized protein was dissolved in 1000 µL of distilled water, and 50 µL of this mixture was mixed with 1000 µL of BCA reagent. The blank was prepared with distilled water. The samples were vortexed for 1 min (Fisher Scientific) and incubated at 37°C for 40 min, and the absorbance was measured at 562 nm with a UV–Vis spectrophotometer (NanoDrop, Thermo Scientific). The results were analyzed in triplicate.

2.6. Functional properties

The functional properties of the banana passionfruit and granadilla passion fruit seed PC that were evaluated were their water absorption capacity, protein solubility, and oil absorption capacity.

2.6.1. Water holding capacity (WAC)

The determination of WAC in the protein concentrates of banana passionfruit (CPT) and granadilla (CPG) was carried out according to the method described by Quinteros et al. (2022) with slight modifications, in which 50 mg of CP was dissolved in 0.5 ml of distilled water in a previously weighed tube. The mixture was homogenized for 30 seconds every 10 minutes 5 times, and then the mixtures were centrifuged at 12,000 rpm for 4 minutes via a centrifuge (Eppendorf 5427 Hamburg). The supernatant was drained at a 45° angle, and the weight was recorded. The ARC was calculated as the absorbed water content per sample weight.

2.6.2. Oil absorption capacity (OAC)

For CPG and CPT, the technique of Deng et al. (2019) was applied with modifications. Fifty mg of lyophilized protein was added to 0.5 mL of commercial virgin olive oil in an Eppendorf tube. The mixture was vortexed for 1 min every 5 min for 30 min, and the mixture was subsequently centrifuged at 10000 rpm for 5 min via a centrifuge (Eppendorf Centrifuge 5427 Hamburg). The oil supernatant was discarded by tilting the tube at 45°C for 10 minutes and weighing. The OAC results are expressed as the percentage of oil content absorbed by a gram of the sample.

2.6.3. Protein solubility

Protein solubility was determined according to the method described by Pazmiño et al. (2018) with some modifications. The protein concentrate (0.5% w/v) of lyophilized banana passionfruit and granadilla was dispersed in distilled water, and the pH was adjusted to 3, 6, 9, and 12 using 2 N HCl and NaOH. The mixture was shaken for 2 minutes at 12000 rpm in a centrifuge (Eppendorf 5804 R). The protein content of the supernatant was determined via the BCA method (Thermo Fisher Scientific, USA), with bovine serum albumin (BSA) used as a standard. The soluble protein content was expressed as the percentage of total protein content in the sample, and the solubility was calculated with the specific formula shown below (Flores-Jiménez et al., 2024):

$$\text{Solubility } (\%) = \frac{\text{protein in the supernatant}}{\text{total protein content in sample}} \times 100 \quad (2)$$

2.7. Protein characterization by SDS–PAGE electrophoresis

Protein concentrates from banana passionfruit, and passion fruit seeds were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) according to the methods of Nazir & Wani (2023). Proteins were separated via 12% resolving or separator gels and 5% stacking or a concentrator in a Miniprotean machine (Bio-Rad, PowerPac, Singapore) at 200 V for 45 min. A 10- to 250-kDa molecular marker (Bio-Rad, CA, USA) was used as a molecular marker. After electrophoresis, the gel was stained with Coomassie Brilliant Blue R250 solution for 12 hours overnight, followed by decolorization with a mixture of acetic acid, methanol, and distilled water. The gels were photographed and processed on a gel analyzer.

2.8. In vitro antioxidant activity and total polyphenols of CP

2.8.1. Obtaining extracts

Free-dried PC from seeds were placed in a 1:10 methanol solution (70%), shaken for 10 min, and then subjected to a Cole Palmer 8892-MTH ultrasonic bath for 10 min. The extracts were centrifuged (Eppendorf 5804 R) for 5 min at 5500 rpm and then separated and stored until analysis (Vilcacundo et al., 2020).

2.8.2. Quantification of total polyphenol content (TPC)

The phenolic compound content was determined via the method of Mihai et al. [18], with some modifications. Briefly, a 0.04 mL aliquot of each extract was added to 0.04 mL of Folin-Ciocalteu's reagent with 1.12 mL of distilled water and 0.8 mL of sodium carbonate (7%), and the samples were incubated at 30°C for 1 h. The absorbance of the samples was analyzed at 750 nm. For TPC quantification, a standard calibration curve for gallic acid (GA) was generated, and the TPC results were expressed as mg GA equivalents (Lucas et al., 2022).

2.8.3. ABTS radical cation decolorization assay

The ABTS assay was performed as described by Vilcacundo et al. (2020) by mixing 200 µL of PC with 3800 µL of 2.45 mM ABTS/potassium sulfate solution (1:1) and diluting with phosphate buffer (pH 7) to an absorbance of 1.10 ± 0.01 at 734 nm. Trolox standard solution was used to determine the antioxidant concentrations. The results are reported as µmol TE/g dry weight (Gupta et al., 2022).

2.8.4. Ferric Reducing Antioxidant Power (FRAP) method

The assay was performed as described by Subbiah et al. (Subbiah et al., 2021); 25 ml of sodium acetate buffer (300 mM, pH 3.60), 5 ml of TPTZ (10 mM in 40 mM HCl), and 5 ml of ferric chloride were mixed. A total of 900 µl of FRAP reagent was combined with 90 µl of distilled water and 30 µl of tax and passion fruit seed concentrates. The mixture was incubated at 37°C for 30 minutes, and the absorbance was measured at 593 nm. Trolox (200–700 µmol) was used as a standard, and the data are expressed as µmol Trolox TE equivalents/g CP.

2.8.5. DPPH method for determination of radical scavenging activity

It was determined by the DPPH assay as described by Nassar et al. (Nassar-Eddin et al., 2021) with minor modifications. A standard solution (0 to 800 µmol Trolox/L) of Trolox was used to obtain the calibration curve. The results obtained are presented as µmol Trolox equivalents (TE)/g sample, dry weight (DW), and all the assays were performed in triplicate.

2.9. FTIR determination and split-peak fitting of samples

The structures and functional groups of taxo and granadilla seeds, as well as those of CPG and CPT, were analyzed via a Fourier transform infrared spectrometer (Perkin Elmer Frontier, Chile) (Wang et al., 2023). The spectra were collected through 30 scans in the absorbance mode in the range of 4000--600 cm-1 with a resolution of 4 cm-1. The CPs were placed under the ATR diamond crystal by pressure setting for all aliquots. A background was collected for each new sample, and measurements were performed in triplicate (Martins et al., 2022).

2.10. Statistical analysis

The assays were performed in triplicate, and the results are presented as the means ± standard deviations. The significance level of the samples in this study was evaluated via ANOVA (p<0.05) and Tukey's test, with significant differences expressed by subscript letters in different columns in the tables. Statistical analysis was performed with the help of Statgraphics Centurion version XVI software.

3. Results and Discussion

3.1. Proximal analysis of the raw material

Table 1 shows the results of the proximate analysis of *Passiflora* seeds. Banana passionfruit seeds are rich in fiber at 55.94%, moisture at 4.93%, fat at 11.08%, protein at 10.59%, and ash at 1.43%. In the same context, Granadilla had 43.38% fiber, 6.81% moisture, fat at 18.09%, protein at 21.08%, and low ash at 2.24%. Andasuryani et al. (Andasuryani et al., 2020) reported a low value of 6.40% protein in passion fruit seeds and 8.25% protein in passion fruit, according to the data in Table 1; however, for fiber, fat, and ash, the data are similar. The protein content reported by Kawakami et al. (Kawakami et al., 2021) was relatively high at 13.07%; however, the fiber and ash contents were relatively low at 48.18% and 1.49%, respectively, in passion fruit because they are within the Passiflora genus.

The protein content of the taxo seeds studied by Romani et al. (2021) is similar to that reported in this study: 10.56% ash, 1.45% moisture, 9.12% moisture, and 15.45% fat. On the other hand, González et al. (González et al., 2019) reported low values for protein (7.29%), fiber (55.7%), and fat (25.5%) in passion fruit seeds. These results differ from those in Table 1, possibly due to the method of analysis used, the place of production, differences between climatic zones for cultivation, and the variety of fruit used in the studies.

Tabla 1 Proximate composition of taxo (*Passiflora tripartita*) and granadilla (*Passiflora ligularis*) seeds.

Parameter	Results %		Method
	Passiflora tripartita (taxo)	*Passiflora ligularis* (granadilla)	
Moisture	4.93 ± 0.04	5.81 ± 0.02	AOAC 925.10
Ash	1.43 ± 0.03	2.24 ± 0.07	AOAC 923.03
Fiber	55.94 ± 1.28	43.38 ± 3.57	WEENDE
Protein	10.59 ± 0.28	21.08 ± 0.54	DUMAS
Fat	11.08 ± 0.04	18.09 ± 0.87	AOAC 2003.06
Carbohydrates	16.02 ± 0.02	9.40 ± 0.05	

The results of the proximal analysis are expressed as the means ± standard deviations (n=3). The ratio was calculated as g/100 g dry matter.

3.2. Percent yields of CPs obtained at different pH values

Table 2 shows the yield percentages of CPT and CPG obtained at different extraction pH values, with the results of the CPT tests at pH 10.0 ranging from 8.57 to 9.51%, whereas at pH 12, they were higher at 9.62 and 9.89%, with CPT 12--5 being the highest. The statistical analysis revealed no significant differences between the treatments. However, the results of the CPG 10-5 test were significantly different from those of the other tests. The results demonstrate that extraction pH and precipitation pH influence the yields of protein concentrates in this study. Tas et al. (2025) reported higher yields than those reported in this study, ranging from 34.1% to 50.3% in pumpkin seed concentrates at alkaline pH values of 11 and 5 for extraction pH, which was attributed to the isoelectric point of proteins likely being between 5 and 6. Additionally, the yields reported for neem seed concentrates at 12.52% are consistent with those reported for the analyzed CPG sample (Arise et al., 2019). For the CPT samples, the highest value was for CPT 12--5, indicating that an alkaline pH of 12 and a precipitation pH of 5 (isoelectric point) significantly influence the yield percentage. The protein yield in neem seeds, studied by Acho et al. (Acho et al., 2023), is 21.31% greater than that reported in this study. Similarly, Fatima et al. (2023) obtained a 39.12% yield when extracting proteins from moringa seeds. The yield through alkaline extraction is influenced by the biomass type, pH, extraction time, temperature, and biomass–solvent ratio (w/v) (Sari et al., 2015). Studies suggest that a temperature increase from 25°C to 50°C could improve yield, as well as pH values far from the isoelectric point (11 to 12), and increase the biomass–solvent (w/v) ratio from 1:10 to 1:15 (Hadinoto et al., 2024).

Table 2 The percent yields of the granadilla seed protein concentrates (CPGs) and taxo (CPT) were obtained at different solubilization pH values, isoelectric precipitation pH values, and protein percentages via the Dumas method.

CP	Yield for 30 g sample
CPT 10 – 4	9.51^{c} ± 0.32
CPT 10 – 5	8.57^{c} ± 0.45
CPT 12 – 4	9.62^{bc} ± 0.26
CPT 12 – 5	9.89^{bc} ± 0.36
CPG 10 – 4	12.53^{a} ± 0.36
CPG 10 – 5	11.98^{b} ± 2.20
CPG 12 – 4	12.42^{a} ± 0.72
CPG 12 - 5	13.74^{a} ± 0.59

All values are expressed as the means ± standard deviations (n=3). Superscript letters (p≤0.05) indicate significant differences in yield based on the extraction pH studied.

3.3. Quantification of the protein content of Cps from Taxo and Granadilla seeds

Table 3 presents the results obtained from the quantification of the protein content of CPTs and PC via the three methods. A conversion factor (kp) of 5.5 was used, which provides a more accurate estimate of the protein content of the seeds, as it is based on the total amino acid profile (Ezeagu et al., 2002). The passion fruit samples stand out in terms of protein content, with CPGs 12--5 accounting for 65.16% and CPGs 10--4 accounting for a lower proportion than the others at 58.69%. Among the CPTs, the lowest protein content was obtained, with the lowest concentration of 27.54% at pH 5 and 12. For quinoa seed protein, Kubba & Al-Obaidi (2023) used a conversion factor of 5.7, resulting in low yields of 24.5% to 38% compared with those of CPG, but these ranges are similar to the yield of CPT. Alpiger et al. (2025) used 5.3 as the conversion factor via the Dumas method for sunflower and Caamaño seeds; the sunflower concentrates ranged between 33.5% and 55.0%, and the hemp concentrates ranged between 34.0% and 42.3%, all of which were subjected to an acid pH of 4.5. These values are higher than the CPT and lower than the CPG.

The CPG treatments at pH 4 presented the highest protein content according to the Bradford method with 0.69% CPG 12--4, and at lower percentages, the CPTs ranged from 0.19% to 0.27%, while according to the BCA method, the CPTs ranged from 18.66% to 27.76%, and the CPGs ranged from 1.53 to 2.03%, with CPG 12--4 being the highest. Studies on chia seed proteins have shown a 57.87% protein content via the BCA method, which is higher than that reported in this study (Cárdenas et al., 2018).

For all the assays, there were significant differences (p<0.05) when the 3 methods were compared; this can be attributed to the reaction principle of each technique. The Bradford method is based on the binding of Coomassie blue dye to basic amino acid residues (arginine, lysine, and histidine), the terminal amino group in the polypeptide chain, and aromatic amino acid residues (Compton & Jones, 1985). The BCA method involves the measurement of Cu+ reduced by peptide bonds and some amino acids (cysteine, tryptophan, and tyrosine) on the basis of the reaction of the sodium salt Bicinchoninic acid with groupuscule ions generated under alkaline conditions (Shen, 2023).

The differences in protein content between the three methods reflect discrepancies in their detection mechanisms. Dumas, which measures total nitrogen, provides an estimate when nitrogen compounds are present. The lower values in Bradford and BCA may suggest that some of the nitrogen detected by Dumas may come from non-protein sources or other compounds that contain nitrogen in seed CP. The low Bradford values compared to Dumas may indicate that passionfruit proteins may have a lower content of basic amino acid residues, which are the main binding sites for Coomassie dye. This difference in amino acid composition between banana passionfruit and passionfruit proteins could explain the differences in their functional properties observed in this study.

Table 3 Quantification of protein content by the Dumas, Bradford, and BCA assays.

CP	Método Dumas	Método Bradford	Método BCA
	%	%	%
CPT 10 - 4	29.43 ± 0.45^{f}	0.19 ± 0.03^{e}	18.66 ± 0.10^{d}
CPT 10 - 5	30.42 ± 0.38^{e}	0.25 ± 0.01^{d}	20.81 ± 0.43^{c}
CPT 12 - 4	29.44 ± 0.11^{f}	0.20 ± 0.02^{e}	23.76 ± 0.63^{b}
CPT 12 - 5	27.54 ± 0.08^{g}	0.27 ± 0.01^{e}	27.76 ± 0.41^{a}
CPG 10 - 4	58.69 ± 0.05^{d}	0.57 ± 0.01^{b}	1.53 ± 0.00^{ef}
CPG 10 - 5	63.98 ± 0.14^{b}	0.38 ± 0.02^{c}	1.24 ± 0.06^{f}
CPG 12 - 4	65.16 ± 0.08^{a}	0.69 ± 0.03^{a}	2.03 ± 0.06^{e}
CPG 12 - 5	63.03 ± 0.06^{c}	0.40 ± 0.03^{c}	1.69 ± 0.04^{ef}

Statistical analysis was performed via one-way ANOVA and Tukey's test, and the results are expressed as the means ± standard deviations (n=3). Letters in the same row in superscripts indicate statistically significant differences (p≤0.05) in protein content according to protein quantification methods.

3.4. Functional properties of protein concentrate

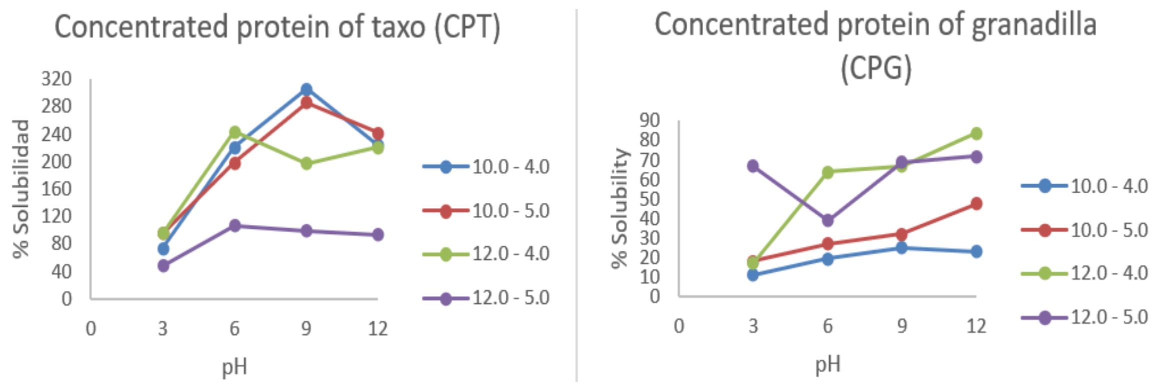

Figure 1 Protein solubilities of taxo seeds (CPT) and granadilla seeds (CPG) at pH 3, 6, 9 and 12.

Solubility is a key functional property, as it directly influences other protein functions, such as emulsification and foam formation, and is a determinant for its application in food formulations (Nissen et al., 2021). Fig. 3 shows the solubilities of taxo (CPT) and passion fruit (CPG) seed proteins at pH 3, 6, 9, and 12.

The protein concentrates exhibited high solubility, exceeding alkaline pH. At pH 9 for the PC (banana passionfruit) 10-4 assays, solubility values of 306.90% were reported, with values of 222% at pH 6 and 224% at pH 12. The results of the CPT 10-5 and CPT 12-4 tests followed the same trend, with values above 100%. Specifically, CPT 10--5 had a solubility of 199.23% at pH 3, 285.81% at pH 6, and 241.69% at pH 12, whereas CPT 12--4 had values of 243.95% at pH 6, 197.21% at pH 6, and 220.75 at pH 12. This high solubility in alkaline media is probably because the concentrates contained high globulin fractions, which are more soluble at alkaline pH than at acidic pH, which was due to an increase in the net protein load as the pH increased; this result is similar to that reported for sesame proteins (Idowu et al., 2021). On the other hand, the solubility at acidic pH 3 remained at values close to 100% in all the assays. These observations indicate that the isoelectric points of the concentrates obtained with pH (12--5) extraction were possibly close to pH 3 and 4 (Liu et al., 2018).

The lowest solubility of protein concentrates of passion fruit seeds (CPGs) was observed in the extraction (10–12 pH) and precipitation (pH 4–5) tests, with 10.95%, 18.37%, and 16.70% of the samples subjected to acid-soluble pH 3 and 6, which could be due to the increase in protein–protein interactions near the isoelectric point of the proteins. On the other hand, in the tests subjected to alkaline pH (9, 12), solubility indices higher than 50% were obtained, with values between 63.90%, 66.86%, 68.67%, 71.71%, and 83.91%. These results agree with the data on watermelon seed concentrates (Hahn et al., 2025) and mango seeds (Saucedo et al., 2021).

As shown in Table 4, the CPG treatments presented higher WAC values in the extraction trials (10--4; 12--5), 2.42 g water/g and 2.10 g water/g. For the CPT, the WAC ranged from 1.51 to 1.81 g water/g. These results are consistent with those of Ruckmangathan et al. (2022), who reported values ranging from 1.4 to 2.3 g/g legume protein, which are higher than those reported for pumpkin seeds, with values ranging from 1.14 to 1.20 g water/g (Habib et al., 2025). Oroumei et al. (2024) noted that high WAC values are due to the ability of proteins to retain water in their three-dimensional structures; furthermore, WAC reflects the interaction between water and protein, which is related to the conformational characteristics, amino acid composition and hydrophilic and hydrophobic balance of the protein (Liu et al., 2018).

While the highest AOC values were found in the CPG and CPT assays subjected to various extraction pH values (12.0--4.0 and 12.0--5.0), the values were 2.05 goil/g, 2.22 and 2.94 goil/g, respectively, which are higher than those presented in yellow pea (0.86--1.43 goil/g) (Ma et al., 2022) and oilseeds (0.86--1.32 g oil/g) (Gao et al., 2020). The high AOC values can be attributed to the presence of several nonpolar amino acids, such as valine, alanine, proline, leucine, tryptophan, methionine, isoleucine, glycine, and phenylamine, which bind to the fatty hydrocarbon chains, resulting in increased oil absorption (Sharma et al., 2016). Statistical analysis of WAC and AOC indicated that all samples were significantly different ($p < 0.05$) from each other.

Table 4 Water holding capacity (WAC) and oil absorption capacity (OAC) of taxo (CPT) and granadilla (CPG) seed protein concentrates and seed flours".

CP	WAC (%)	AOC (%)
CPT		
10.0-4.0	1.74 ± 0.38 ^{bcd}	1.81 ± 0.03 ^{d}
10.0-5.0	1.51 ± 0.15 ^{cd}	1.48 ± 0.13 ^{e}
12.0-4.0	1.81 ± 0.10 ^{bcd}	2.22 ± 0.03 ^{b}
12.0-5.0	1.72± 0.31 ^{bcd}	2.05 ± 0.05 ^{c}
CPG		
10.0-4.0	2.10 ± 0.27^{ab}	2.02 ± 0.11 ^{c}
10.0-5.0	1.62 ± 0.32 ^{cd}	1.63 ± 0.09 ^{e}
12.0-4.0	2.42 ± 0.23 ^{a}	2.92 ± 0.11 ^{a}
12.0-5.0	1.92 ± 0.32 ^{bc}	2.04 ± 0.13 ^{c}
Taxo seed flour	1.39 ± 0.27 ^{d}	1.10 ± 0.16 ^{f}
Granadilla seed flour	1.84 ± 0.07 ^{bcd}	1.20 ± 0.05 ^{f}

The results are expressed as the mean and standard deviation (n=3). CPT: taxo seed protein concentrate; CPG: granadilla seed protein concentrate. Different superscripts with different letters represent statistically significant differences in the WAC AND AOC of taxo- and passion fruit seed proteins as a function of extraction conditions (p≤0.05).

3.5. Characterization of protein by SDS–PAGE electrophoresis

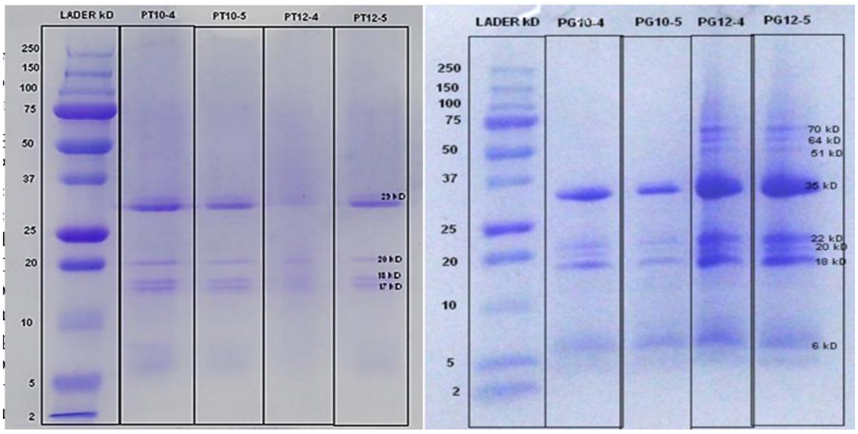

Figure 2 SDS–PAGE of lyophilized banana passionfruit protein concentrate (PT) and granadilla seed protein concentrate (PG) with an extraction pH ranging from 10.0–12.0 and a precipitation pH ranging from 4.0–5.0.

SDS–PAGE analysis revealed variations in molecular weight. In treatments PT10-4, PT10-5, and PT12-5, high-intensity bands with a molecular weight of 29 kDa were obtained, which corresponded to the protein fraction albumin, which was extracted more efficiently at pH 4 and 5. These results are in line with those reported by Torres-Sánchez et al. (2023) for proteins isolated from sacha inchi seed cake extracted at pH 7. Similarly, Villacís-Chiriboga et al. (2023), in their study of Annona muricata seed proteins, obtained bands with molecular weights between 20 and 35 kDa corresponding to globulins and albumins. Additionally, medium-intensity bands with molecular weights of 17, 18, and 20 kDa were obtained, indicating protein degradation, possibly involving oleosins and caleosins (Perera et al., 2022). For the PT12-4 samples, low-intensity and barely visible bands with molecular weights of 20, 27, 28, and 29 kDa were identified, which corresponded to small 2S globulin subunits. These weights demonstrate increased protein hydrolysis due to the extraction pH and incomplete defatting of the samples.

The PG10-4, PG10-5, PG12-4, and PG12-5 treatments resulted in high-intensity molecular weight bands at 35 and 22 kDa, which corresponded to 11 s basic globulin or vicilin (Bojórquez-Velázquez et al., 2024). The low-intensity bands at 22 and 20 kDa are likely globulins and albumins. Moreover, these protein fractions are the primary proteins present in plant storage tissues (Baptista et al., 2017). Additionally, the barely visible low-intensity bands at 6 kDa are proteins degraded by the extraction pH. Habib et al. (2025) reported that the 2 s albumin fraction of pumpkin seeds primarily consists of low-molecular-weight soluble proteins (≤10 kDa) that remain after precipitation.

3.6. Antioxidant activity of taxo- and granadilla seed CP by the ABTS, FRAP, and DPPH methods

This study revealed that the antioxidant activity of banana passion fruit (CPT) and passion fruit (CPG) protein concentrates may be potential antioxidant additives. Statistical analysis revealed that the solubilization pH and precipitation pH influence the antioxidant activity of the concentrates. Significant differences ($p<0.05$) were found among the three treatment methods analyzed. In the ABTS assay, the CPT samples presented high antioxidant activities ranging from 7042.44 to 7770.97 µmol ET/g. CPT 10--5 was the most abundant antioxidant, and the results of the other assays were in the range of 7000 µmol ET/g. On the other hand, the taxo seeds presented 6695 µmol ET/g. Zaky et al. (2020) reported low antioxidant activity in rice concentrates at 45.34 µmol ET/g. Similarly, Vilcacundo et al. (2022) reported low antioxidant data (215.52 to 804.35 µmol TE/g) in purple corn in this study. Compared with granadilla seeds, CPGs have high antioxidant power. The concentration of the CPG 12-4 sample was the highest at 1182.84 µmol TE/g sample, and the lowest concentration of the CPG 12-4 sample was 299.25 µmol TE/g sample. Piñuel et al. (2019), in red bean protein concentrates, reported values between 116.19 and 345.21 µmol TE/g for samples subjected to a pH range of 3--7, which are low values compared with those of CPG. Similarly, the results of Irankunda et al. (Irankunda et al., 2025) revealed low antioxidant activity in elongated pea proteins ranging from 23.75 to 30.12 µmol TE/g sample.

Through the DPPH method, the CPTs presented greater antioxidant activity than the CPGs did, with values ranging from 1052.36 to 908.84 µmol ET/g sample, with the highest value occurring in CPT 10--5, whereas the seeds in a lower proportion presented 354.56 µmol ET/g. Butseekhot et al. (2024) reported 633.55 µmol ET/g sample for black bean protein concentrates, which is low compared with the CPTs in Table 5. Ozgolet et al. (2024), in their study of sea thistle seed proteins, obtained 6.03 g/L antioxidant power. Compared with that of the CPTs, the antioxidant activity of the CPGs and passion fruit flour was lower according to the DPPH method. Table 5 shows values from 18.55 to 157.52 µmol ET/g in the CPG 12--4 assay, which was the highest. Noyola et al. (2022) reported that the protein concentration of Leucaena spp. was 20.83 µmol ET/g lower than that of CPGs. However, the Paeonia arborea seed concentrates had high antioxidant activity at 0.18 mg/ml (456.48 µmol ET/g).

The results of the antioxidant evaluation by FRAP were the same as those of the two methods mentioned above, with the content of CPT being greater than that of the CPGs, unlike that of the seeds, whose antioxidant activity was lower than that of the concentrates. The taxo concentrates (CPT) range from 786.58 to 1175.87 µmol ET/g sample, highlighting the 10--5 CPT. On the other hand, the CPG ranged from 24.29 to 93.30 µmol ET/g of sample. The difference between the CP and seeds can be attributed to the fact that the flours in their structure, as such, are composed of fibers and fat in greater proportions than the CP, which, within the extraction process, were mostly discarded, concentrating only their protein fractions.

In terms of the quantification of the TPC, the content of CPT was greater than that of CPG, whereas seeds presented very low values. The CPTs ranged from 110.74 to 138 mg AG/g sample. Kadiri et al. (2017) reported very low TPC values compared with 0.50 µg/g sample in papaya seed concentrates. The content of CPG was the lowest, ranging from 6.15 to 25.56 mg AG/g. Mota et al. (2022) reported that the TPC content was higher in lupine concentrates than in passion fruit, with 35.19 mg AG/g. The high antioxidant activity in general analyzed by various methods in seed protein concentrates may be associated with the presence, to a greater extent, of some hydrophobic and aromatic amino acids, such as Trp, Pro, and Gl (Khalesi & FitzGerald, 2021).

3.7. Identification of protein concentrate spectra of taxo and passion fruit seeds via Fourier transform infrared spectroscopy (FTIR)

Figure 4 shows the peaks observed in the Fourier transform infrared spectrum, confirming the presence of specific functional groups in the protein concentrates. CPT has characteristic amine groups (primarily) and amides between 1590 and 1650 cm-1, and the peak at 3200–3550 cm-1 corresponds to OH vibrations. The peak at 1650–1580 cm-1 indicates bending of the N–H group, the peak at 1500–1560 cm-1 can be related to C=C vibrations of aromatic rings, and the peak at 1000–1250 cm-1 corresponds to the amide III region, which is due to C=O stretching and the conformation of CN and NH vibrations (Gani et al., 2022). These functional groups coincide with those reported in apple seed proteins, indicating the characteristic presence of amine and amide groups. For CPGs, the FTIR analysis revealed groups similar to those of CPT, with OH and H-linked vibrations at 3200–3550 cm-1, and the peak at 1634.28 cm-1 was the most intense and was assigned to the presence of primary amines and amides (amide I–NH2) governed by the stretching of the C=O and CN groups. The peaks from 1650 to 1750 cm-1 are caused by N–H stretching vibrations, which characterize the secondary structure of proteins (Yang et al., 2021). The findings of this study are similar to those described for protein isolates from basil seeds (Nazir & Wani, 2023).

Table 5 Antioxidant activity of taxo and granadilla seed CP.

CP	ABTS µmol ET/g sample	FRAP µmol ET/g sample	DPPH µmol ET/g sample	TPC mg AG/g sample
CPT 10 - 4	7315.90 ± 60.42^{c}	927.75 ± 11.22^{b}	908.84 ± 20.66^{d}	129.63 ± 1.39^{b}
CPT 10 - 5	$7770.97 \pm 60.\ 44^{a}$	1175.87 ± 7.42^{a}	1052.36 ± 23.67^{a}	138.00 ± 1.39^{a}
CPT 12 - 4	7494.82 ± 0.0^{b}	839.77 ± 9.23^{c}	989.64 ± 13.67^{b}	120.47 ± 0.00^{c}
CPT 12 - 5	7042.44 ± 60.47^{d}	786.58 ± 15.99^{d}	966.15 ± 13.67^{c}	110.74 ± 1.62^{d}
CPG 10 - 4	370.05 ± 5.73^{i}	51.90 ± 0.94^{g}	21.83 ± 0.33^{h}	9.55 ± 0.0^{i}
CPG 10 - 5	299.25 ± 3.31^{j}	24.29 ± 0.48^{h}	18.55 ± 0.88^{h}	6.15 ± 0.35^{j}
CPG 12 - 4	1182.84 ± 18.65^{f}	93.28 ± 1.63^{f}	157.52 ± 3.67^{f}	25.56 ± 0.23^{f}
CPG 12 - 5	805.08 ± 10.94^{g}	93.30 ± 0.0^{f}	144.85 ± 7.34^{fg}	17.83 ± 0.13^{h}
Taxo seed flour	6695 ± 60.48^{e}	439.32 ± 9.72^{e}	354.56 ± 10.34^{e}	74.98 ± 0.80^{e}
Granadilla seed flour	724.13 ± 4.81^{h}	89.60 ± 0.0^{f}	135.76 ± 3.00^{g}	23.31 ± 0.13^{g}

The results are expressed as the mean ± standard deviation (n=3) and were evaluated via ANOVA (p≤0.05). Statistical differences are presented with different superscripts. TPC: total polyphenol content; GAE: gallic acid equivalents; TE: Trolox equivalents.

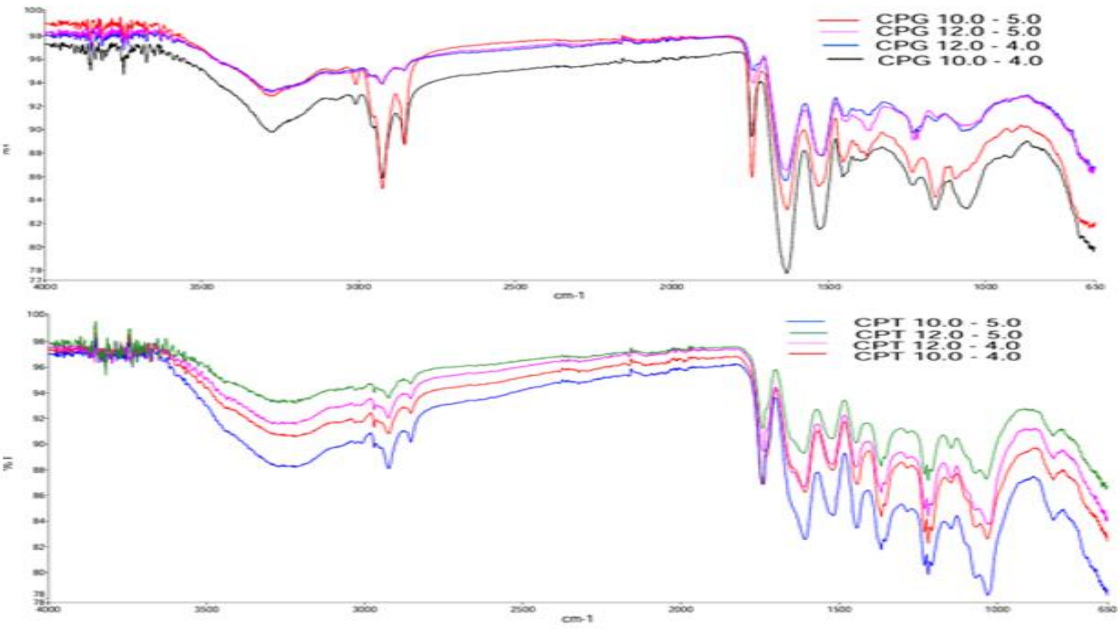

Figure 3 Fourier transform infrared spectroscopy (FTIR) spectra of the granular (CPG) protein concentrates and taxo protein concentrates (CPT).

5. Conclusion

This study demonstrates that banana passion fruit, and granadilla seeds are valuable sources of protein that are not currently exploited for functional applications in new foods.

The extraction pH (10-12) and precipitation pH (4-5) significantly influenced yield and functional properties, and these findings guide industrial-scale protein extraction from these seeds.

The techno-functional properties evaluated in the protein concentrates from taxo and granadilla seeds show good solubility exceeding 100%, which makes them suitable for use in the formulation of innovative foods.

The predominant globulin fraction of SDS-PAGE suggests excellent emulsification potential. Likewise, CPT and CPG exhibited significant antioxidant capacity, with CPT in particular showing high antioxidant activity, making them potential functional ingredients for natural antioxidants, such as ingredients for enriched beverages due to their potential free radical scavenging effect.

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6. Declarations

6.1. Ethical considerations

Not applicable.

6.2. Use of artificial intelligence (AI)

The authors declare that the generative artificial intelligence (AI) tool [Grammarly] was used exclusively for language editing and/or grammatical improvement. The use of AI did not influence the scientific content, study design, data analysis, data interpretation, results, or conclusions of the manuscript. Full responsibility for the content remains with the authors.

6.3. Conflict of Interest

The authors declare no conflicts of interest.

6.4. Funding

This research did not receive any financial support.

References

Acho, M. A., Osemwegie, O. O., Amonsou, E. O., & Arise, R. O. (2023). Physico-chemical and functional characteristics of neem seed protein isolate. *Scientific African*, *21*, e01774. https://doi.org/10.1016/j.sciaf.2023.e01774

Alpiger, S. B., Gregersen, S. B., Ellermann, A. M., Dissing, T. J. J., & Corredig, M. (2025). Extraction of Protein-Enriched Fractions from Sunflower and Hemp Seeds: Composition and Colloidal Stability of Less Refined Fractions as a Function of Initial pH. *Future Foods*, *11*, 100541. https://doi.org/10.1016/j.fufo.2025.100541

Andasuryani, A., Zainal, P. W., & Ifmalinda, I. (2020). Chemical characteristic of sweet passion fruit (Passiflora lingularis Juss) seeds from Indonesia based on maturity levels. *Journal of Physics: Conference Series*, *1469*(1), 012001. https://doi.org/10.1088/1742-6596/1469/1/012001

Angel-Isaza, J., Carmona-Hernandez, J. C., Narváez-Solarte, W., & Gonzalez-Correa, C. H. (2021). Polyphenols from Passiflora ligularis Regulate Inflammatory Markers and Weight Gain. *Biomolecular Concepts*, *12*(1), 36-45. https://doi.org/10.1515/bmc-2021-0005

AOAC: Official Methods of Analysis (Volume 1) (15.ª ed.). (2005). https://law.resource.org/pub/us/cfr/ibr/002/aoac.methods.1.1990.pdf

Arise, R. O., Acho, M. A., Yekeen, A. A., Omokanye, I. A., Sunday-Nwaso, E. O., Akiode, O. S., & Malomo, S. O. (2019). Kinetics of angiotensin -1 converting enzyme inhibition and antioxidative properties of Azadirachta indica seed protein hydrolysates. *Heliyon*, *5*(5). https://doi.org/10.1016/j.heliyon.2019.e01747

Baptista, A. T. A., Silva, M. O., Gomes, R. G., Bergamasco, R., Vieira, M. F., & Vieira, A. M. S. (2017). Protein fractionation of seeds of *Moringa oleifera* lam and its application in superficial water treatment. *Separation and Purification Technology*, *180*, 114-124. https://doi.org/10.1016/j.seppur.2017.02.040

Bojórquez-Velázquez, E., Zamora-Briseño, J. A., Barrera-Pacheco, A., Espitia-Rangel, E., Herrera-Estrella, A., & Barba de la Rosa, A. P. (2024). Comparative Proteomic Analysis of Wild and Cultivated Amaranth Species Seeds by 2-DE and ESI-MS/MS. *Plants*, *13*(19), 2728. https://doi.org/10.3390/plants13192728

Butseekhot, S., Sorndech, W., Ploypetchara, T., Auranwiwat, C., Aluko, R., Sumsakul, W., Siricoon, S., & Chaisuwan, B. (2024). (PDF) Effects of alkaline extraction on the physicochemical and functional properties of protein concentrates obtained from Vigna mungo and Phaseolus vulgaris L. legumes. *ResearchGate*. https://doi.org/10.26656/fr.2017.8(4).207

Cárdenas, M., Carpio, C., Welbaum, J., Vilcacundo, E., & Carrillo, W. (2018). *Chia protein concentrate (Salvia hispanica L.) anti-inflammatory and antioxidant activity. Asian Journal of Pharmaceutical and Clinical Research, 11*(2), 382–386. https://doi.org/10.22159/ajpcr.2018.v11i2.17225

Compton, S. J., & Jones, C. G. (1985). Mechanism of dye response and interference in the Bradford protein assay. *Analytical Biochemistry*, *151*(2), 369-374. https://doi.org/10.1016/0003-2697(85)90190-3

Deng, Y., Huang, L., Zhang, C., Xie, P., Cheng, J., Wang, X., & Li, S. (2019). Physicochemical and functional properties of Chinese quince seed protein isolate. *Food Chemistry*, *283*, 539-548. https://doi.org/10.1016/j.foodchem.2019.01.083

Dey, D., Gu, B.-J., Ek, P., Rangira, I., Saunders, S. R., Kiszonas, A. M., & Ganjyal, G. M. (2021). Apple pomace pretreated with hydrochloric acid exhibited better adherence with the corn starch during extrusion expansion. *Carbohydrate Polymer Technologies and Applications*, *2*, 100089. https://doi.org/10.1016/j.carpta.2021.100089

Ezeagu, I. E., Petzke, J. K., Metges, C. C., Akinsoyinu, A. O., & Ologhobo, A. D. (2002). Seed protein contents and nitrogen-to-protein conversion factors for some uncultivated tropical plant seeds. *Food Chemistry*, *78*(1), 105-109. https://doi.org/10.1016/S0308-8146(02)00105-X

Fatima, K., Imran, M., Ahmad, M. H., Khan, M. K., Khalid, W., AL-Farga, A., Alansari, W. S., Shamlan, G., & Eskandrani, A. A. (2023). Ultrasound-Assisted Extraction of Protein from Moringa oleifera Seeds and Its Impact on Techno-Functional Properties. *Molecules*, *28*(6), Article 6. https://doi.org/10.3390/molecules28062554

Fischer, G., Quintero, O., Téllez, C., & Melgarejo, L. M. (2020). *Curuba: Passiflora tripartita var. mollissima y Passiflora tarminiana* (pp. 105-121).

Flaibam, B., da Silva, M. F., de Mélo, A. H. F., Carvalho, P. H., Galland, F., Pacheco, M. T. B., & Goldbeck, R. (2024). Non-animal protein hydrolysates from agro-industrial wastes: A prospect of alternative inputs for cultured meat. *Food Chemistry*, *443*, 138515. https://doi.org/10.1016/j.foodchem.2024.138515

Flores-Jiménez, N. T., Ulloa, J. A., & Urías-Silvas, J. E. (2024). Assessment of the physicochemical, functional and structural characteristics of a defatted flour from guamuchil (*Pithecellobium dulce* (Roxb.) seeds. *Future Foods*, *9*, 100351. https://doi.org/10.1016/j.fufo.2024.100351

Gani, A., Ashraf, Z. U., Shah, A., Naik, A. S., Wani, I. A., & Gani, A. (2022). Upscaling of Apple By-Product by Utilising Apple Seed Protein as a Novel Wall Material for Encapsulation of Chlorogenic Acid as Model Bioactive Compound. *Foods*, *11*(22), 3702. https://doi.org/10.3390/foods11223702

Gani, A., ul Ashraf, Z., Noor, N., & Ahmed Wani, I. (2022). Ultrasonication as an innovative approach to tailor the apple seed proteins into nanosize: Effect on protein structural and functional properties. *Ultrasonics Sonochemistry*, *86*, 106010. https://doi.org/10.1016/j.ultsonch.2022.106010

Gao, Z., Shen, P., Lan, Y., Cui, L., Ohm, J.-B., Chen, B., & Rao, J. (2020). Effect of alkaline extraction pH on structure properties, solubility, and beany flavor of yellow pea protein isolate. *Food Research International*, *131*, 109045. https://doi.org/10.1016/j.foodres.2020.109045

González, L., Álvarez, A., Murillo, E., Guerra, C., & Méndez, J. (2019). POTENTIAL USES OF THE PEEL AND SEED OF PASSIFLORA EDULIS SIMS F. EDULIS (GULUPA) FROM ITS CHEMICAL CHARACTERIZATION, ANTIOXIDANT, AND ANTIHYPERTENSIVE FUNCTIONALITIES. *Asian Journal of Pharmaceutical and Clinical Research*, 104-112. https://doi.org/10.22159/ajpcr.2019.v12i10.33828

Gupta, M., Dey, A., Majumder, S., Ghosh, S., & Barui, A. (2022). Evaluation of Antioxidant Activity Using by H2O2, DPPH, ABTS Methods and Phytochemical Tests, TPC & TFC of Commonly Used Medicinal Plants of West Bengal. *International Journal of Pharmaceutical Sciences Review and Research*, *72*. https://doi.org/10.47583/ijpsrr.2022.v72i01.018

Habib, M., Singh, S., Alok Sagar, N., Ahmad, S., Qureshi, I., Jan, S., Jan, K., & Bashir, K. (2025). Physicochemical and functional characterization of pumpkin seed protein isolate. *Sustainable Food Technology*. https://doi.org/10.1039/D4FB00268G

Hadinoto, K., Ling, J. K.-U., Pu, S., & Tran, T.-T. (2024). Effects of Alkaline Extraction pH on Amino Acid Compositions, Protein Secondary Structures, Thermal Stability, and Functionalities of Brewer's Spent Grain Proteins. *International Journal of Molecular Sciences*, *25*(12), 6369. https://doi.org/10.3390/ijms25126369

Hahn, A., Liszka, J., Maksym, J., Nemś, A., & Miedzianka, J. (2025). Preliminary Data of the Nutritive, Antioxidative, and Functional Properties of Watermelon (Citrullus lanatus L.) Flour and Seed Protein Concentrate. *Molecules*, *30*(1), Article 1. https://doi.org/10.3390/molecules30010181

Idowu, A. O., Alashi, A. M., Nwachukwu, I. D., Fagbemi, T. N., & Aluko, R. E. (2021). Functional properties of sesame (Sesamum indicum Linn) seed protein fractions. *Food Production, Processing and Nutrition*, *3*(1), 4. https://doi.org/10.1186/s43014-020-00047-5

Irankunda, R., Camaño echavarría, J. A., El hajj, S., Paris, C., Cakir-kiefer, C., Girardet, J.-M., Arnoux, P., Muhr, L., & Canabady-rochelle, L. (2025). *Pisum Sativum* Protein Hydrolysates: Production, Sensitive Screening of Nickel Chelating Peptides and Evaluation of Antioxidant Properties. *Food Bioscience*, 106001. https://doi.org/10.1016/j.fbio.2025.106001

Kadiri, O., Akanbi, C. T., Olawoye, B. T., & Gbadamosi, S. O. (2017). Characterization and antioxidant evaluation of phenolic compounds extracted from the protein concentrate and protein isolate produced from pawpaw (Carica papaya Linn.) seeds. *International Journal of Food Properties*, *20*(11), 2423-2436. https://doi.org/10.1080/10942912.2016.1230874

Kawakami, S., Morinaga, M., Tsukamoto-Sen, S., Mori, S., Matsui, Y., & Kawama, T. (2021). Constituent Characteristics and Functional Properties of Passion Fruit Seed Extract. *Life*, *12*(1), 38. https://doi.org/10.3390/life12010038

Khalesi, M., & FitzGerald, R. J. (2021). In Vitro Digestibility and Antioxidant Activity of Plant Protein Isolate and Milk Protein Concentrate Blends. *Catalysts*, *11*(7), Article 7. https://doi.org/10.3390/catal11070787

Kotnala, B., Panneerselvam, V., & Vijayakumar, A. K. (2024). Physicochemical, structural, and functional characterization of guar meal protein isolate (*Cyamopsis tetragonoloba*). *Heliyon*, *10*(3), e24925. https://doi.org/10.1016/j.heliyon.2024.e24925

Kubba, F., & Al-Obaidi, L. (2023). Preparation of protein concentrate from quinoa seeds and study of its chemical composition and functional properties. *Euphrates Journal of Agricultural Science*, *15*(3), Article 3.

Liu, Y., Ma, X.-Y., Liu, L.-N., Xie, Y.-P., Ke, Y.-J., Cai, Z.-J., & Wu, G.-J. (2018a). Ultrasonic-assisted extraction and functional properties of wampee seed protein. *Food Science and Technology*, *39*, 324-331. https://doi.org/10.1590/fst.03918

Liu, Y., Ma, X.-Y., Liu, L.-N., Xie, Y.-P., Ke, Y.-J., Cai, Z.-J., & Wu, G.-J. (2018b). Ultrasonic-assisted extraction and functional properties of wampee seed protein. *Food Science and Technology*, *39*, 324-331. https://doi.org/10.1590/fst.03918

Loizzo, M. R., Lucci, P., Núñez, O., Tundis, R., Balzano, M., Frega, N. G., Conte, L., Moret, S., Filatova, D., Moyano, E., & Pacetti, D. (2019). Native Colombian Fruits and Their by-Products: Phenolic Profile, Antioxidant Activity and Hypoglycaemic Potential. *Foods*, *8*(3), 89. https://doi.org/10.3390/foods8030089

Lucas, B. N., DALLA NORA, Boeira, C. P., Verruck, S., & Rosa, C. S. da. (2022). Determination of total phenolic compounds in plant extracts via Folin-Ciocalteu's method adapted to the usage of digital images. *Food Science and Technology*, *42*, e35122. https://doi.org/10.1590/fst.35122

Ma, K. K., Grossmann, L., Nolden, A. A., McClements, D. J., & Kinchla, A. J. (2022). Functional and physical properties of commercial pulse proteins compared to soy derived protein. *Future Foods*, *6*, 100155. https://doi.org/10.1016/j.fufo.2022.100155

Martins, M. S., Nascimento, M. H., Barbosa, L. L., Campos, L. C. G., Singh, M. N., Martin, F. L., Romão, W., Filgueiras, P. R., & Barauna, V. G. (2022). Detection and quantification using ATR-FTIR spectroscopy of whey protein concentrate adulteration with wheat flour. *LWT*, *172*, 114161. https://doi.org/10.1016/j.lwt.2022.114161

Mihai, R. A., Guerra, N. J. L., & Catana, R. D. (2024). Assessment of phenolic composition and antioxidant activity of fermented Andean blackberry beverage enriched with medicinal plants of Ecuador. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *52*(3), 13423-13423. https://doi.org/10.15835/nbha52313423

Mota, J., Casimiro, S., Fernandes, J., Hartmann, R. M., Schemitt, E., Picada, J., Costa, L., Marroni, N., Raymundo, A., Lima, A., & Ferreira, R. B. (2022). Lupin Protein Concentrate as a Novel Functional Food Additive That Can Reduce Colitis-Induced Inflammation and Oxidative Stress. *Nutrients*, *14*(10), 2102. https://doi.org/10.3390/nu14102102

Nassar-Eddin, G., Zheleva-Dimitrova, D., Danchev, N., & Vitanska-Simeonova, R. (2021). Antioxidant and enzyme-inhibiting activity of lyophilized extract from Clinopodium vulgare L. (Lamiaceae). *Pharmacia*, *68*, 259-263. https://doi.org/10.3897/pharmacia.68.e61911

Nazir, S., & Wani, I. A. (2023). Protein isolate from basil seeds (*Ocimum basilicum* L.): Physicochemical and functional characterisation. *Food Chemistry Advances*, *3*, 100424. https://doi.org/10.1016/j.focha.2023.100424

Nissen, S. H., Schmidt, J. M., Gregersen, S., Hammershøj, M., Møller, A. H., Danielsen, M., Stødkilde, L., Nebel, C., & Dalsgaard, T. K. (2021). Increased solubility and functional properties of precipitated Alfalfa protein concentrate subjected to pH shift processes. *Food Hydrocolloids*, *119*, 106874. https://doi.org/10.1016/j.foodhyd.2021.106874

Noyola, B., Méndez-Lagunas, L. L., Rodríguez-Ramírez, J., Sandoval-Torres, S., Aquino-González, L. V., & Barriada-Bernal, L. G. (2022). Techno-functional properties and antioxidant capacity of the concentrate and protein fractions of Leucaena spp. Seeds. *Arch. latinoam. nutr*, 196-204.

Oroumei, S., Rezaei, K., & Chodar Moghadas, H. (2024). *Pomegranate seed as a novel source of plant protein: Optimization of protein extraction and evaluation of in vitro digestibility, functional, and thermal properties*. https://doi.org/10.1002/fsn3.4242

Ozgolet, M., Cakmak, Z. H. T., Bozkurt, F., Sagdic, O., & Karasu, S. (2024). Optimization of extraction parameters of protein isolate from milk thistle seed: Physicochemical and functional characteristics. *Food Science & Nutrition*, *12*(5), 3346-3359. https://doi.org/10.1002/fsn3.4001

Pazmiño, A., Vasquez, G., & Carrillo, W. (2018). PIGEON PEA PROTEIN CONCENTRATE (CAJANUS CAJAN) SEEDS GROWN IN ECUADOR FUNCTIONAL PROPERTIES. *Asian Journal of Pharmaceutical and Clinical Research*, 430-435. https://doi.org/10.22159/ajpcr.2018.v11i6.24966

Pereira, M. G., Hamerski, F., Andrade, E. F., Scheer, A. de P., & Corazza, M. L. (2017). Assessment of subcritical propane, ultrasound-assisted and Soxhlet extraction of oil from sweet passion fruit (*Passiflora alata* Curtis) seeds. *The Journal of Supercritical Fluids*, *128*, 338-348. https://doi.org/10.1016/j.supflu.2017.03.021

Perera, S. P., McIntosh, T., Coutu, C., Tyler, R. T., Hegedus, D. D., & Wanasundara, J. P. D. (2022). Profiling and characterization of (L.) Crantz meal proteins. *Journal of the American Oil Chemists' Society*, *99*(10), 873-889. https://doi.org/10.1002/aocs.12634

Pérez-Marroquín, X. A., Estrada-Fernández, A. G., García-Ceja, A., Aguirre-Álvarez, G., & León-López, A. (2023). Agro-Food Waste as an Ingredient in Functional Beverage Processing: Sources, Functionality, Market and Regulation. *Foods*, *12*(8), Article 8. https://doi.org/10.3390/foods12081583

Piñuel, L., Vilcacundo, E., Boeri, P., Barrio, D. A., Morales, D., Pinto, A., Moran, R., Samaniego, I., & Carrillo, W. (2019). Extraction of protein concentrate from red bean (Phaseolus vulgaris L.): Antioxidant activity and inhibition of lipid peroxidation. *Journal of Applied Pharmaceutical Science*, *9,*(9), 045-058. https://doi.org/10.7324/JAPS.2019.90804

Quinteros, M. F., Martínez, J., Barrionuevo, A., Rojas, M., & Carrillo, W. (2022). Functional, Antioxidant, and Anti-Inflammatory Properties of Cricket Protein Concentrate (Gryllus assimilis). *Biology*, *11*(5), 776. https://doi.org/10.3390/biology11050776

Rekowski, A., Langenkämper, G., Dier, M., Wimmer, M. A., Scherf, K. A., & Zörb, C. (2021). Determination of soluble wheat protein fractions using the Bradford assay. *Cereal Chemistry*, *98*(5), 1059-1065. https://doi.org/10.1002/cche.10447

Romaní, D. R., Calixto-Cotos, M. R., & Apaza, F. S. (2021). Aprovechamiento de los residuos del fruto de Passiflora tripartita. *Scientia Agropecuaria*, *12*(3), Article 3. https://doi.org/10.17268/sci.agropecu.2021.049

Ruckmangathan, S., Ganapathyswamy, H., Sundararajan, A., Thiyagamoorthy, U., Green, R., & Subramani, T. (2022). Physico-chemical, structural, and functional properties of protein concentrate from selected pulses: A comparative study. *Journal of Food Processing and Preservation*, *46*(12), e17169. https://doi.org/10.1111/jfpp.17169

Sari, Y. W., Mulder, W. J., Sanders, J. P. M., & Bruins, M. E. (2015). Towards plant protein refinery: Review on protein extraction using alkali and potential enzymatic assistance. *Biotechnology Journal*, *10*(8), 1138-1157. https://doi.org/10.1002/biot.201400569

Saucedo, M. del R. P., Ulloa, J. A., Ulloa, P. R., Ramírez, J. C. R., & Silva-Carrillo, Y. (2021). Caracterización tecno-funcional de un concentrado proteínico obtenido de la semilla de mango (Mangifera indica L.). *Biotecnia*, *23*(1), Article 1. https://doi.org/10.18633/biotecnia.v23i1.1306

Sharma, L., Singh, C., & Sharma, H. K. (2016). Assessment of functionality of sesame meal and sesame protein isolate from Indian cultivar. *Journal of Food Measurement and Characterization*, *10*(3), 520-526. https://doi.org/10.1007/s11694-016-9330-3

Shen, C.-H. (2023). Chapter 9—Quantification and analysis of proteins. En C.-H. Shen (Ed.), *Diagnostic Molecular Biology (Second Edition)* (pp. 231-257). Academic Press. https://doi.org/10.1016/B978-0-323-91788-9.00002-8

Subbiah, V., Zhong, B., Nawaz, M. A., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. R. (2021). Screening of Phenolic Compounds in Australian Grown Berries by LC-ESI-QTOF-MS/MS and Determination of Their Antioxidant Potential. *Antioxidants*, *10*(1), Article 1. https://doi.org/10.3390/antiox10010026

Tas, O., Sumnu, S. G., & Oztop, M. H. (2025). Effect of Extraction Methods and Preheat Treatments on the Functional Properties of Pumpkin Seed Protein Concentrate. *ACS Food Science & Technology*, *5*(1), 105-117. https://doi.org/10.1021/acsfoodscitech.4c00601

Torres-Sánchez, E., Hernández-Ledesma, B., & Gutiérrez, L.-F. (2023). Isolation and Characterization of Protein Fractions for Valorization of Sacha Inchi Oil Press-Cake. *Foods*, *12*(12), Article 12. https://doi.org/10.3390/foods12122401

Vardanega, R., Fuentes, F. S., Palma, J., Bugueño-Muñoz, W., Cerezal-Mezquita, P., & Ruiz-Domínguez, M. C. (2023). Valorization of granadilla waste (*Passiflora ligularis,* Juss.) by sequential green extraction processes based on pressurized fluids to obtain bioactive compounds. *The Journal of Supercritical Fluids*, *194*, 105833. https://doi.org/10.1016/j.supflu.2022.105833

Vilcacundo, E., García, A., Vilcacundo, M., Morán, R., Samaniego, I., & Carrillo, W. (2020). Antioxidant Purple Corn Protein Concentrate from Germinated Andean Purple Corn Seeds. *Agronomy*, *10*(9), Article 9. https://doi.org/10.3390/agronomy10091282

Vilcacundo, E., Montalvo, V., Sanaguano, H., Moran, R., Carrillo, W., & García, A. (2022). Identification of Phytochemical Compounds, Functional Properties and Antioxidant Activity of Germinated Purple Corn Protein Concentrate and Its Gastrointestinal Hydrolysates. *Agronomy*, *12*(9), Article 9. https://doi.org/10.3390/agronomy12092217

Villacís-Chiriboga, J., Prandi, B., Ruales, J., Van Camp, J., Sforza, S., & Elst, K. (2023). Valorization of soursop (*Annona muricata*) seeds as alternative oil and protein source using novel de-oiling and protein extraction techniques. *LWT*, *182*, 114777. https://doi.org/10.1016/j.lwt.2023.114777

Wang, X., He, X., & Wang, X. (2023). FTIR Analysis of the Functional Group Composition of Coal Tar Residue Extracts and Extractive Residues. *Applied Sciences*, *13*(8), Article 8. https://doi.org/10.3390/app13085162

Yang, K., Xu, T.-R., Fu, Y.-H., Cai, M., Xia, Q.-L., Guan, R.-F., Zou, X.-G., & Sun, P.-L. (2021). Effects of ultrasonic pre-treatment on physicochemical properties of proteins extracted from cold-pressed sesame cake. *Food Research International*, *139*, 109907. https://doi.org/10.1016/j.foodres.2020.109907

Zaky, A. A., Chen, Z., Qin, M., Wang, M., & Jia, Y. (2020). Assessment of antioxidant activity, amino acids, phenolic acids and functional attributes in defatted rice bran and rice bran protein concentrate. *Progress in Nutrition*, *22*(4), e2020069-e2020069. https://doi.org/10.23751/pn.v22i4.8971