Biochemical and techno-functional investigation of argan press-cake proteins foreseeing food application

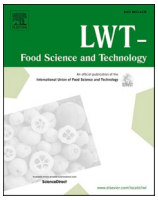
Noamane Taarji, Karim Lyamlouli, Abdellatif Barakat, Kenichi Tominaga, Hiroko Isoda, Mitsutoshi Nakajima

LWT - Food Science and Technology 192 (2024) 115734

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt



Biochemical and techno-functional investigation of argan press-cake proteins foreseeing food application

Noamane Taarji [a,b,*], Karim Lyamlouli [b], Abdellatif Barakat [c], Kenichi Tominaga [a], Hiroko Isoda [a,d], Mitsutoshi Nakajima [a,d]

[a] Food and Medicinal Resource Engineering Open Innovation Laboratory, National Institute of Advanced Industrial Science and Technology (AIST), AIST Tsukuba Central 5-2, Tsukuba City, Ibaraki, 305-8565, Japan
[b] College of Sustainable Agriculture and Environmental Sciences, AgroBiosciences Program, Mohammed 6 Polytechnic University (UM6P), Benguerir, 43150, Morocco
[c] IATE, University of Montpellier, INRAE, Agro Institute, Montpellier 34060, France
[d] Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8572, Japan

ARTICLE INFO

Keywords:
Argania spinosa
By-product
Albumins
Globulins
Techno-functions

ABSTRACT

Argan press-cake (APC) is reported to contain a significant content of proteins, comparable to conventional sources of protein isolates, such as soybean. In this regard, the present study evaluated the biochemical and techno-functional properties of major APC proteins fractions, foreseeing food application. Albumins and globulins represented approximately 41% and 50% of total extractable proteins, respectively. Globulins were prone to aggregation, potentially by hydrophobic interactions, resulting in limited solubility in water in the range of pH 3 to pH 11. Nevertheless, the limited solubility of globulins in water at pH $\leq$ 9 could be improved, up to 8-fold, by pH_{12}-shifting treatment, that is, unfolding the proteins structure at pH 12, followed by refolding at pH 9 or lower. pH_{12}-shifted albumins and globulins presented similar surface activity in the range of pH 3 to pH 11 and were able to form and stabilize foams and emulsions. Comprehensively, both fractions reduced the interfacial tension and surface tension up to 8 mN/m and 48 mN/m, respectively, and formed stable emulsions, with a mean droplet volume diameter ($d_{4,3}$) of approximately 16 μm, using rotor-stator homogenization. These results indicate that albumins and globulins extracted from argan by-products could have potential applications as techno-functional food ingredients.

1. Introduction

The global demand on purified forms of dietary proteins is increasing, while the need for natural and sustainable sources of food ingredients also escalates. Protein isolates are the most purified forms of protein-based products in food industry. They can be obtained by solvent-assisted extraction from various materials, including multiple steps of separation/fractionation to achieve maximum concentrations (Mondor & Hernández-Álvarez, 2022). Animal protein isolates (API), which are usually obtained from dairy products such as milk and whey are relatively easy to prepare. Their downstream processing steps include mild physicochemical treatments, such as micro- and ultra-filtration, which results in good quality proteins and high purity isolates (Wang & Guo, 2019). By contrast, plant proteins isolates (PPI) are more difficult to prepare and often require intensive separation/fractionation steps to achieve desirable purity. Particularly, the presence of multiple impurities in protein-rich plant materials and the sturdy fibers of their cell walls implies the use of additional disruption and refining steps to enhance protein extraction (Kumar et al., 2021).

Upstream processing of PPI is much more encouraging compared to API. The carbon footprint of PPI is 3–80 times less than API, depending on the type of proteins and the applied life cycle assessment methodology (Thrane, Paulsen, Orcutt, & Krieger, 2017). PPI isolates also have a lower water footprint in comparison to API and often require less energy and land area for large-scale production (Detzel et al., 2022). Soybeans represent nowadays the major source of PPI in food industry. Other important sources include cereals, nuts and beans such as wheat, pea and lupin (Sá, Moreno, & Carciofi, 2020). Soy protein isolates, which are generally obtained from extruded soybean meals after oil

* Corresponding author. College of Sustainable Agriculture and Environmental Sciences, AgroBiosciences Program, Mohammed 6 Polytechnic University (UM6P), Benguerir, 43150, Morocco.
E-mail address: noamane.taarji@um6p.ma (N. Taarji).

https://doi.org/10.1016/j.lwt.2024.115734
Received 1 September 2023; Received in revised form 4 January 2024; Accepted 5 January 2024
Available online 5 January 2024

extraction, represent a good example of sustainably viable types of protein isolates in the limited sense of the aforementioned discussion. Ever-growing remarks about their preparation, however, suggest that soy protein isolates are not necessarily sustainable as generally regarded. Soybean production, for example, is indicated to contribute to a large extent to Amazon deforestation (Delaroche, Dias & Massoca, 2023). It also depends strongly on genetically modified varieties, which raises additional concerns about its sustainability (Sharma & Saini, 2022). Other sources of PPI, including rice, pea and lupin, have been the source of highly optimistic forecasts because of this situation. These crops are also abundant and inherently rich in proteins, which makes them suitable for large scale production (Sá et al., 2020). However, the continuous increase of human population and the growing concerns about nutritional security have long raised probing calls about the need of exploring alternative sources of PPI, as protein-rich crops could be consumed preferably as whole foods instead of purified ingredients (van der Goot et al., 2016).

Diversifying the sources of protein isolates, while improving their technology of production are both important to maintain a sustainable supply for food production. Food by-products, which are generally regarded as low quality materials unsuitable for human consumption, can contain important concentrations of these components (Gençdağ, Görgüç & Yılmaz, 2020). Moreover, non-timber forest products, including wild varieties of legume and oilseed plants, can be explored as naturally occurring sources of dietary protein isolates. Koteck-a-Majchrzak, Sumara, Fornal, and Montowska (2020) reviews the structural and functional properties of various oilseed cakes, with emphasis on proteins' fractional composition. Proteins isolated from pumpkin seeds (Vinayashree & Vasu, 2021) or prickly pear seeds cakes (Borchani et al., 2021), for example, are predominantly rich in glutelins, while those prepared from camellia seed cake (Ningxiang et al., 2022) are rich in albumins. The fractional composition of plant proteins and the physicochemical properties of each fraction are important to determine the techno-functional properties of PPI and hence their application. Globulins isolated from flaxseed press-cake, for example, have a higher foaming capacity than albumins as attributed to higher surface hydrophobicity (Nwachukwu & Aluko, 2018). In contrast, albumins isolated from African yam bean have better foaming and emulsifying properties than globulins (Ajibola, Malomo, Fagbemi, & Aluko, 2016).

Argan (*Argania spinosa*) is an endemic plant species of Morocco. It thrives naturally in the southwestern parts of the country over an estimated area of 800.000 ha (Mechqoq et al., 2021). Argan oil is the main product of argan tree. It is extracted from the fruit kernels, and it is marketed throughout the world as a dietary and cosmetic ingredient. Particularly, it is used as an active ingredient in many mainstream beauty products to treat skin infections and hair loss and as a generic moisturizer for the whole body (Gharby & Charrouf, 2021). Argan press-cake (APC) is the residue obtained after argan oil extraction. It is rich in phenolic compounds and saponins and contains approximately 500 g/kg of proteins (Mirpoor et al., 2022). APC saponins and polyphenols have been well characterized and evaluated for multiple applications. In their respective works, for example, Taarji et al. (2018) and Taarji et al. (2020), indicate that the surface-active and emulsifying performance of various saponins-rich extracts from this material and suggested their utilization as food emulsifiers. APC proteins, however, have not been characterized previously and only two patents about the skin care and hair growth properties of their enzymatic hydrolysates are available (Charrouf, Henry, Moser, & Pauly, 2005; Lydie, Fabre & Charveron, 1999).

APC protein content is comparable to soybean and exceeds that of conventional sources of plant protein isolates, such pea and lupin. Nevertheless, the limited information on their isolation conditions and biochemical and techno-functional characteristics may restrain their application for nutritional and food functionality purposes. Therefore, the aim of this study was to isolate the major fractions of APC proteins and to evaluate their potential as food ingredients by studying their techno-functional properties, as affected by their physicochemical and structural characteristics.

2. Materials and methods

2.1. Materials

Argan press-cake (APC) was provided by an argan oil producing cooperative in Marrakech, Morocco. All other products were purchased from Fujifilm Wako Pure Chemical Co. (Osaka, Japan), unless stated differently. APC was stored at 4 °C until utilization and was grinded (<500 μm) using an ultracentrifuge hammer mill (ZM 200, Retsch Inc., Haan, Germany) and defatted by n-hexane before protein extraction. Defatting was performed by triple hexane extraction at a 1:3 press-cake powder to hexane ratio (mg/mL) for 2 h each, followed by air-drying in a fume hood at room temperature for 24 h.

2.2. Protein isolation

APC proteins were isolated according to Osborne classification (Osborne, 1924). Briefly, 25 g of the defatted sample was added to 500 mL of distilled water and stirred for 2 h at room temperature. The suspension was then centrifuged at 2000 *g* for 30 min (Kubota 8420, Kubota Corp., Osaka, Japan) and filtered through a Whatman filter paper (11 μm) to remove suspended particles. The supernatant was next adjusted to pH 4.25, using 1 mol/L HCl, for albumins precipitation, while the pellet was suspended in 1 mol/L NaCl solution (500 mL) for globulins extraction. After extraction, centrifugation, and filtration, at similar conditions, the supernatant was adjusted to pH 3.25, using 1 mol/L HCl, for globulins precipitation, and the pellet was resuspended in 500 mL of a 50 mmol/L NaOH solution and adjusted to pH 10. Glutelins were then precipitated by adjusting the pH to 5, using 1 mol/L HCl, to obtain the corresponding fraction. After each precipitation step, the isolates were centrifuged at 2000 *g* for 30 min (Kubota 8420, Kubota Corp., Osaka, Japan), resuspended in distilled water (100 mL) and re-adjusted to pH 7 by 1 mol/L NaOH solution. The samples were then frozen at −20 °C for 12 h and lyophilized at −80 °C and 5 Pa for 48 h.

2.3. Isoelectric point determination (pI)

The protein solubility of the different extracts (i.e., water, NaCl, NaOH), was determined at various pH (2–12) to determine the pI of each protein fraction: the pH providing the lowest solubility was considered as pI and was used for precipitating the appropriate protein fraction as described in the previous section (2.3). Briefly, the extracts were adjusted to various pH by using 1 mol/L HCl or 1 mol/L NaOH solutions, followed by stirring for 30 min to allow precipitation. Each aliquot was then centrifuged at 10000 *g* for 30 min (MX-307, Tomy Seiko Co., Ltd., Tokyo, Japan), filtered using a low protein-binding syringe filter (SY25VF, PVDF 0.2 μm, MDI membrane technologies Inc., Camp Hill, USA) and analyzed for protein concentration using the Bradford reagent (section 2.4). The relative protein solubility (mg/g) was determined by dividing the protein concentration at each pH on the maximum protein concentration obtained at pH 12.

2.4. Proteins and saponins quantification

The total protein content was determined using a CHN elemental analyzer (PerkinElmer 2400 II, Waltham, USA) based on the original Dumas method (PerkinElmer, 2002), using a combustion and reduction temperatures of 925 and 650 °C, respectively, and a standard nitrogen-to-protein conversion factor of 6.25. The soluble protein concentration was determined following the Bradford's method (Bradford, 1976). Briefly, 3 mL of Bradford reagent was added to 0.1 mL of extract solution and left to incubate for 5 min at room temperature. The

absorbance was then measured at 595 nm and the amount of soluble proteins was expressed as Bovine Serum Albumin (BSA) equivalent using a BSA standard curve (0–1.4 mg/mL). Saponin content was spectrophotometrically (V-570, Jasco Co., Hachioji, Japan) determined according to the method of Xiang, Tang, Chen, and Shi (2001), using oleanolic acid (0–1.4 mg) as standard. Briefly, 0.1 ml of sample was mixed with 0.1 mL of 50 g/L vanillin-acetic acid solution and 1.2 mL of a 600 mg/g perchloric acid and then incubated for 20 min at 70 °C. After cooling down at room temperature, 5 mL ethyl acetate was added, and the absorbance was measured at 550 nm.

2.5. Solubility in water

The protein isolate was dispersed in 10 mL distilled water and hydrated for 15 min at room temperature. The dispersion was then adjusted to the target pH (3–11) with 1 mol/L HCl or 1 mol/L NaOH solutions and stirred magnetically at room temperature for 60 min. The dispersion was next centrifuged at 10000 *g* for 30 min (MX-307, Tomy Seiko Co., Ltd., Tokyo, Japan), and the protein concentration of supernatant was measured using the Bradford reagent (Section 2.4.). Protein solubility was also evaluated following pH_{12}-shifting treatment. In this set of experiments, the protein isolate dispersion was initially adjusted to pH 12 for 15 min and then readjusted to the target pH (3–11) for 60 min. Centrifugation and protein quantification were then performed as described for original samples (i.e., without pH shifting).

2.6. Interfacial/surface activities and dynamic light scattering measurements

Interfacial activity was evaluated at the sunflower oil/water interface, using an automatic interfacial tensiometer (PD-W, Kyowa Interface Science Co., Ltd., Saitama, Japan). The samples (10 mg/mL) were prepared by pH_{12}-shifting treatment as described in section 2.5. They were then injected into sunflower oil through a 22-gauge syringe needle and analyzed for 200 s at 25 °C. Surface tension was measured at the air/water interface using the Wilhelmy-plate method (CBVP-Z, Kyowa Interface Science Co., Ltd., Saitama, Japan). The samples were placed at 5–10 mm distance from the plate surface and then analyzed at equilibrium conditions at 25 °C. Hydrodynamic diameter and ζ-potential were measured using a dynamic light scattering particle analyzer at 25 °C (Zetasizer, Nano ZS, Malvern Instruments Ltd., Malvern, Worcestershire). The refractive indexes of the samples (0.1 mg/mL) were initially determined using an automatic refractometer and used for the measurements.

2.7. Emulsion preparation

Sunflower oil-in-water emulsions were prepared by rotor-stator (RSH) and high-pressure homogenization (HPH), using a protein isolate concentration of 10 mg/mL and a protein isolate-to-oil mixing ratio of 1:10 (g:g). The aqueous phases were prepared by pH_{12}-shifting treatment of the protein fractions followed by readjustment to pH 9 (section 2.5.). RSH emulsions (coarse emulsions) were prepared by homogenizing the two phases at 10000 rpm for 5 min (Polytron, PT-3000 Kinematica-AG, Littace, Switzerland). HPH emulsions were prepared by re-homogenizing the coarse emulsions for 4 consecutive cycles at 100 MPa (NanoVater, NV200, Yoshida Kikai, Nagoya, Japan). The volume mean droplet diameter ($d_{4,3}$) and droplet size distribution were determined using a laser diffraction particle size analyzer (LS 13,320, Beckman Coulter, Brea, USA). The microstructures were observed using an optical microscope at ×40 magnification (DM IRM, Leica Microsystems Pty., Ltd., Wetzlar, Germany).

2.8. Foam preparation

Protein samples (200 mg) were dispersed in 20 mL deionized water and maintained at pH 9, after pH_{12}-shifting treatment (section 2.5.). They were then homogenized at 10000 rpm for 5 min (Polytron, PT-3000 Kinematica-AG, Littace, Switzerland) and evaluated for foaming ability using the following equation:

$$\text{Relative foam volume} = (V2 - V1)/V1$$

where V_2 is the volume of the protein dispersion plus the foam and V_1 is the initial volume of the protein dispersion.

2.9. Thermogravimetry - differential thermal analysis (TG-DTA)

TG-DTA measurements were performed using a DTG 60H thermogravimetric analyzer (Shimadzu Co., Ltd., Kyoto, Japan). Briefly, 5–15 mg of protein isolate was placed into an aluminum pan and heated under a nitrogen atmosphere from 25 to 600 °C, at a heating rate of 10 °C/minutes; an empty pan was used as reference. The first weight loss was assigned to moisture loss, and the subsequent weight losses were assigned for protein denaturation and degradation.

2.10. Fluorescence spectroscopy (FS)

The samples (1 mg/mL) were adjusted to pH 9 after pH_{12}-shifting treatment (section 2.5.). They were then vortexed for 30 min at room temperature, centrifuged at 4 °C to remove undissolved particles and protein concentration was adjusted to 50 μg/mL. The fluorescence spectra were obtained at an emission wavelength range of 300–400 nm, following specific excitation at 280 or 295 nm for tryptophan + tyrosine or tryptophan amino acids, respectively (FP-6500, Jasco, Inc., Tokyo, Japan). The quantum of fluorescence attributed to tyrosine was obtained by subtracting fluorescence quantum obtained at 295 nm from the one obtained at 280 nm.

2.11. Surface hydrophobicity

The concentration of the proteins in the supernatant (section 2.10) was determined by the Bradford (section 2.4.), and the stock solutions were diluted with distilled water (pH 9) to achieve a final protein concentration of 0–80 μg/mL. Freshly prepared 8 mmol/L Anilino naphthalene-8-sulfonate (ANS) solution (20 μL), dissolved in 10 mmol/L phosphate buffer (pH 7.0), was then added to 4 mL of the diluted protein solutions, and the mixture was incubated in the dark for 15 min at room temperature. The fluorescence intensity (FI) was measured at excitation and emission wavelengths of 390 and 470 nm, respectively, using excitation and emission slit widths of 3 nm and photomultiplier tube (PMT) intensity of 500 V (FP-6500, Jasco, Inc., Tokyo, Japan). The slope of ANS FI versus protein concentration was considered as surface hydrophobicity (H_0) index.

2.12. Amino acids analysis

Approximately 4 mg of each protein powder was hydrolyzed in 1 mL of 6 mol/L HCl for 22 h at 110 °C under vacuum conditions. The hydrolyzed samples were cooled down to 4 °C to stop the hydrolyzation process, then they were filtered using a 0.22 PTFE membrane to remove suspended particles. Amino acids profile was determined using high-performance liquid chromatography tandem mass spectrometry (LCMS-8050, Schimadzu, Kyoyo, Japan). Liquid chromatography was performed at 37 °C using an Intact Intrada Amino Acid, 100 × 3.0 mm column (Imtakt Co., Kyoto, Japan) and a gradient system with the mobile phase consisting of solvent A (Acetonitrile/Formic acid, 100/0.3, mL/mL) and solvent B (Acetonitrile/100 mmol/L Ammonium formate, 20/80, mL/mL) at a flow rate of 600 μL/min, and injection volume of 5 μL. The mass spectrometer was used in the ESI-positive mode. The gradient program used was initial 20% B and 80% A, hold for 4 min, linear gradient to 100% B in 10 min, and hold for 2 min.

2.13. Statistical analysis

All measurements were repeated in triplicate, and data were expressed as mean and standard deviation. Statistical differences among groups were evaluated using analysis of variance (ANOVA) followed by Sidaki post-hoc test. The data were considered statistically significant at $p < 0.05$. All statistical analysis were carried out using GraphPad Prism 8.0 software.

3. Results and discussion

3.1. Protein content and amino acids profile

The fractional composition of APC proteins is presented in Table 1. APC extractable proteins consisted of 414.8 g/kg albumins, 500.9 g/kg globulins and 84.1 g/kg glutelins (Table 1). The yield of albumins was significantly ($p < 0.05$) lower than globulins. Moreover, the protein content of the albumins fraction (847.1 g/kg) was slightly lower than globulins (877.5 g/kg). These results are comparable with chia seeds (Julio, Ruiz-Ruiz, Tomás, & Segura-Campos, 2019) and bitter melon seeds (Horax, Hettiarachchy, Over, Chen, & Gbur, 2010), where globulins and/or albumins represent the predominant fractions of proteins. They are different, however, from the data obtained for pumpkin seeds (Vinayashree & Vasu, 2021) or prickly pear seeds (Borchani et al., 2021), which are richer in glutelins. Albumins can be extracted using water, salt or alkaline solutions, glutelins can be extracted using alkaline water (pH ≥ 10), while globulins are exclusively extracted using salt solutions, thus the possibility to co-extract them with albumins (Adebiyi, Adebiyi, Hasegawa, Ogawa, & Muramoto, 2009). Based on these results, the use of diluted salt solutions may be preferred for maximum extraction of APC proteins. The use of alkaline conditions may be unnecessary as only a minor fraction of glutelins was identified in APC. Moreover, extended increase of pH during proteins extraction can result in the loss of certain amino acids and/or the formation of toxic compounds, such as lysinoalanine (Zhang, Wang, Dai, He, & Ma, 2018).

The amino acid composition of the major fractions of APC proteins (i.e., albumins, globulins) is presented in Table 2. The total amount of amino acids was slightly higher for both fractions, compared to the total protein content determined by N analysis and using a nitrogen-to-protein conversion factor of 6.25 (Table 1). Similar findings are reported by Feyzi, Varidi, Zare, and Varidi (2015) and Horax et al. (2010) who found that the total amino acid contents of fenugreek protein isolate, soy protein isolate, and bitter melon seeds isolate are relatively higher than their respective protein contents as determined by N analysis. A nitrogen-to-protein conversion factor of 6.25 is often used to estimate the total protein content of foodstuff. However, the N content of amino acids can vary considerably from one amino acid to another, leading to estimation errors of protein content using N analysis, depending on amino acid composition (Mariotti, Tomé & Mirand, 2008).

Table 1
Yield, saponins, proteins and moisture contents of argan press-cake protein isolates. Yield was presented as g/kg of defatted argan press-cake. Proteins, saponins and moisture contents were presented as g/kg of protein isolate. The relative protein fraction (% of total extractible proteins) was calculated based on the total amount of extractible proteins (albumins + globulins + glutelins). ND = Not determined. Number of replications (n) = 3. p-value <0.05.

	Yield	Saponins	Proteins	Moisture	Relative protein fraction
Albumins	124.6 ± 5.7^{b}	29.5 ± 1.9^{a}	847.1 ± 0.4^{b}	38.4 ± 0.2^{b}	41.5 ± 0.9^{b}
Globulins	145.2 ± 8.9^{a}	11.3 ± 0.7^{b}	877.5 ± 37.1^{ab}	41.7 ± 1.8^{a}	50.1 ± 1.0^{a}
Glutelins	23.8 ± 3.3^{c}	ND	903.1 ± 6.1^{a}	39.7 ± 0.6^{a}	8.4 ± 0.1^{c}

Table 2
Amino acid profile of argan press-cake albumins and globulins isolates. All values are presented as g/kg of protein isolate. ND = Not detected. Number of replications (n) = 3. p-value <0.05.

Amino acid	Albumins	Globulins
Histidine	75 ± 1^{b}	84 ± 2^{a}
Isoleucine	109 ± 8^{a}	111 ± 3^{a}
Leucine	3 ± 0^{a}	4 ± 0^{a}
Lysine	ND	ND
Methionine	30 ± 1^{a}	29 ± 0^{a}
Phenylalanine	28 ± 0^{b}	32 ± 0^{a}
Threonine	14 ± 2^{a}	14 ± 0^{a}
Valine	25 ± 3^{a}	30 ± 3^{a}
Alanine	13 ± 0^{a}	12 ± 2^{a}
Arginine	77 ± 0^{b}	84 ± 4^{a}
Aspartic acid	9 ± 1^{b}	14 ± 0^{a}
Cysteine	42 ± 1^{a}	48 ± 3^{a}
Glutamic acid	306 ± 2^{b}	332 ± 7^{a}
Glycine	88 ± 9^{a}	93 ± 0^{a}
Proline	12 ± 0^{a}	6 ± 0^{a}
Serine	21 ± 4^{a}	25 ± 2^{a}
Tyrosine	58 ± 4^{a}	45 ± 0^{b}
AAA	160 ± 2^{a}	163 ± 2^{a}
HAA	321 ± 4^{a}	318 ± 7^{a}
EAA	209 ± 0^{a}	220 ± 0^{b}
TAA	910 ± 1^{b}	963 ± 1^{a}

AAA (Aromatic amino acids): Histidine, Phenylalanine, Tyrosine. HAA (Non-polar amino acids): Alanine, Cysteine, Valine, Methionine, Isoleucine, Leucine, Proline, Phenylalanine, Tyrosine. EAA (Essential amino acids): Leucine, Isoleucine, Lysine, Valine, Threonine, Phenylalanine, Methionine. TAA: Total amino acids.

APC albumins and globulins contained a remarkable amount of glutamic acid (306 and 332 g/kg, respectively). Moreover, both fractions were rich in isoleucine and glycine, but poor in sulfur-containing amino acids methionine and cysteine. APC albumins and globulins also contained comparable amounts of hydrophobic amino acids, including fluorescent amino acids phenylalanine and tyrosine. The total amount of hydrophobic amino acids was relatively lower than in proteins isolated from various sources, such as flaxseed cake (Sharma & Saini, 2022) and pumpkin seeds (Vinayashree & Vasu, 2021) and in the same range of other sources, such as soybean (Feyzi et al., 2015). Finally, the essential amino acids contents of APC albumins and globulins accounted for 209 and 220 g/kg, respectively, which is relatively lower than conventional sources of protein isolates, such as soybean and sunflower (Kaur & Ghoshal, 2022).

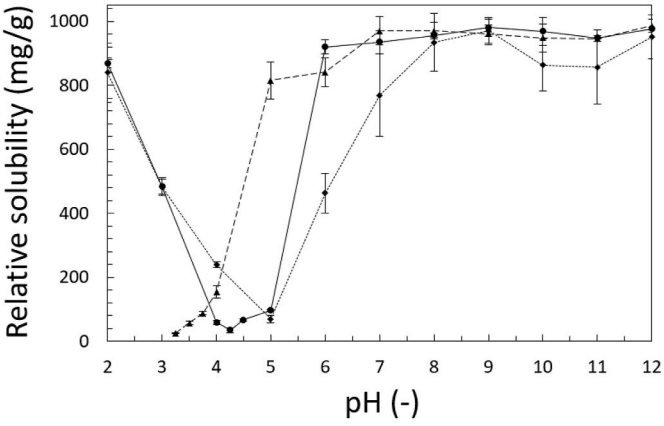


Fig. 1. Relative protein solubility of argan press-cake albumins and globulins extracts (before isoelectric precipitation) as a function of pH (2–12). Protein solubility (mg/g) is presented as related to the maximum solubility obtained at pH 12. Albumins (full line); globulins (dashed line); glutelins (dotted line).

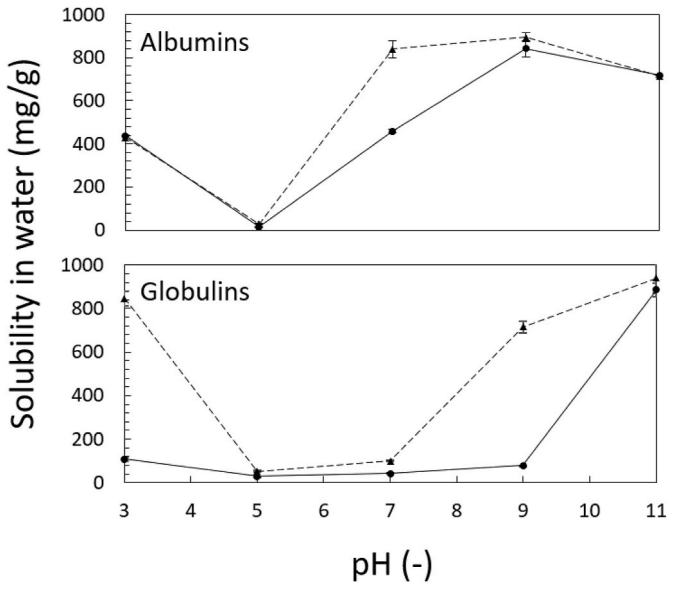


Fig. 2. Solubility in water of isoelectrically precipitated argan press-cake albumins and globulins isolates as a function of pH (3–11), before and after pH_{12}-shifting treatment. Before pH_{12}-shifting (full line); after pH_{12}-shifting (dashed line).

3.2. Isoelectric points

Following extraction, the solubilized proteins were recovered from the protein solution by isoelectric precipitation. Albumins and glutelins exhibited a typical U-shaped precipitation profile as a function of pH, whereas globulins showed a characteristic step-shape profile, as camelina meal globulins (Li et al., 2014). The lowest protein solubilities were observed at pH 4.25, 3.25 and 5 for albumins, globulins and glutelins, respectively (Fig. 1), and were then used for APC proteins isolation.

3.3. Aqueous solubility

The solubility profile of albumins was similar before and after isoelectric precipitation (the proteins extremely aggregated at pH 4–5 and then considerably dissolved with further decrease or increase of pH), while globulins solubility was appreciably decreased at pH 3–9, following isoelectric precipitation (Fig. 2). Globulins were extracted using concentrated NaCl solutions (1 mol/L), which can explain their limited solubility in distilled water (Adebiyi et al., 2009). Moreover, isoelectric precipitation of globulins during protein isolation could have resulted in important conformational changes, particularly surface exposure of hydrophobic amino acids, that reduced their affinity to water and promoted protein-protein interaction (Malomo & Aluko, 2015). The higher solubility of albumins over globulins could improve their other techno-functional properties (e.g., emulsification) that require sufficient initial solubilization of proteins. Moreover, the higher solubility of albumins at pH 3 indicated that these proteins could be used as a valuable ingredient in acidic beverages and food formulations.

Various methods can be used to enhance the aqueous solubility of plant proteins, including pH shifting treatment. This technique consists of adjusting the pH to extreme alkaline or acidic conditions, followed by rapid readjustment to appropriate pH to cause partial structural alteration. Jiang, Xiong, and Chen (2010) indicate that alkaline pH shifting treatment of soy protein isolate considerably improves its aqueous solubility at pH 2–3 and 6–8. The same authors also indicate in a previous study that this treatment decreases the solubility of soy protein isolates at pH 7 due to extensive denaturation (Jiang et al., 2010). The solubility of APC globulins was appreciably improved upon pH_{12}-shifting treatment. Particularly, the solubility at pH 3 and 9 was improved by up to 8-fold, indicating the effectiveness of the applied method at enhancing protein solubility (Fig. 2). Albumins' solubility was also improved appreciably at pH 7, following pH shifting treatment; no significant changes were observed, however, beyond that pH, indicating the limited effect of this treatment on albumins' solubility (Fig. 2).

Globular proteins can undergo important conformational changes when exposed to extreme alkaline conditions. Particularly, the increased net charge of the protein at high alkaline pH increases its interaction

Table 3
Interfacial activity (sunflower oil/water), surface activity (air/water), hydrodynamic diameter and ζ-potential of pH_{12}-shifting treated argan press-cake albumins and globulins isolates. The static interfacial tension between sunflower oil and water was 26.5 mN/m. The equilibrium surface tension between air and water was 72.1 mN/m (IT: Interfacial tension; ST: Surface tension; d_{av}: Hydrodynamic diameter; ζ-pot: ζ-potential; ND: Not determined). Number of replications (n) ≥ 3. p-value <0.05.

	pH (−)	IT (mN/m)	ST (mN/m)	d_{av} (nm)	ζ-pot. (mV)
Albumins	3	11.2 ± 0.1[a]	49.4 ± 0.2[a]	285.5 ± 4.2[a]	19.8 ± 0.8[a]
	5	12.9 ± 0.4[a]	50.4 ± 0.2[a]	ND	−16.8 ± 1.0[b]
	7	12.2 ± 0.5[a]	47.9 ± 0.1[a]	157.9 ± 1.7[b]	−26.8 ± 0.3[c]
	9	11.1 ± 0.3[a]	48.8 ± 0.2[a]	101.7 ± 2.3[c]	−32.2 ± 1.4[d]
	11	8.2 ± 0.6[b]	48.0 ± 0.2[a]	108.2 ± 2.9[c]	31.0 ± 2.3[a]
Globulins	3	13.3 ± 0.5[a]	50.9 ± 0.1[a]	113.7 ± 5.3[a]	28.5 ± 1.9[a]
	5	12.9 ± 0.2[a]	52.9 ± 0.2[a]	ND	−11.6 ± 1.4[b]
	7	12.9 ± 0.3[a]	50.0 ± 0.1[a]	ND	−25.5 ± 0.9[c]
	9	11.2 ± 0.2[a]	50.2 ± 0.2[a]	84.3 ± 1.6[c]	−24.7 ± 1.0[c]
	11	8.1 ± 0.2[b]	48.2 ± 0.1[a]	103.4 ± 6.5[b]	−36.6 ± 1.8[d]

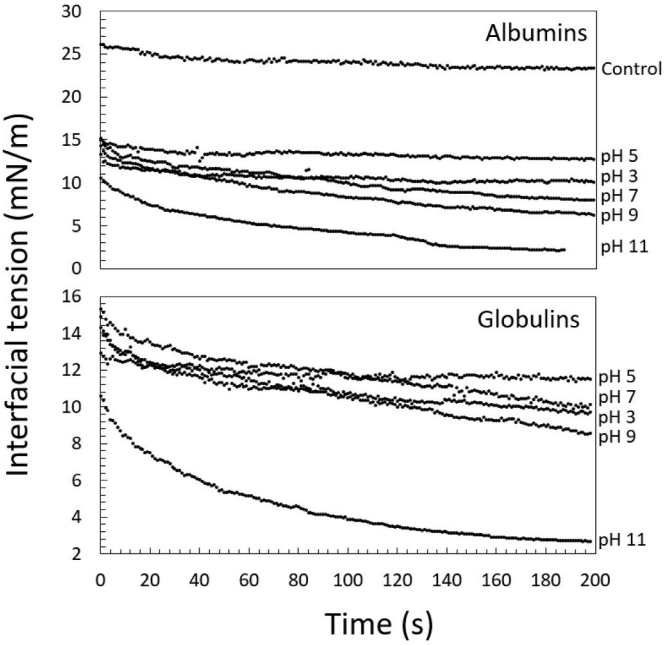


Fig. 3. Dynamic interfacial activity of 10 mg/mL pH_{12}-shifting treated argan press-cake albumins and globulins isolates at the sunflower oil/water interface as a function of pH. The control refers to interfacial tension between distilled water and sunflower oil.

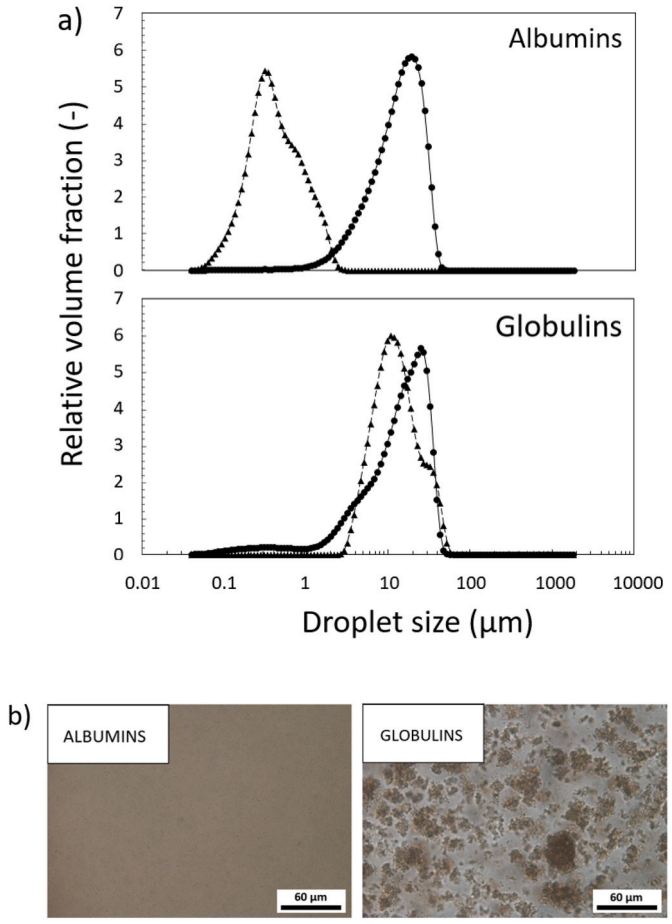


Fig. 4. Preparation characteristics of sunflower oil-in-water emulsions prepared using 10 mg/mL solutions of pH_{12}-shifting treated argan press-cake albumins or globulins isolates, by rotor-stator homogenizer (RSH) or high-pressure homogenizer (HPH). (a) Droplet size distributions. RSH (full line); HPH (dashed line). (b) Microstructures of emulsions prepared using HPH. bar = 60 μm.

with water and favors the movement of hydrophobic amino acids into the inner core of the protein, reducing intermolecular hydrophobic interactions (Ajibola et al., 2016). Reducing the pH after alkaline pH-shifting treatment re-alters the protein structure, but often results in reduced aggregation compared to the original structures at the same pH and, therefore, better solubility in water (Jiang et al., 2010; Yildiz, Andrade, Engeseth, & Feng, 2017). In general, the use of alkaline pH_{12}-shifting treatment could be an option to improve the solubility of APC globulins in water, at certain pH conditions, following isoelectric precipitation. Nevertheless, it would be helpful to evaluate other methods for the recovery of APC globulins after extraction, such as membrane filtration, as it may induce less conformational alteration (Malomo & Aluko, 2015).

3.4. Surface-active properties

APC albumins and globulins isolates appreciably reduced the surface and interfacial tensions at all pH conditions, compared to the air/water (72.1 mN/m) and oil/water (26.5 mN/m) interfaces. The interfacial tension remained fairly the same from pH 3 to 9 and was significantly ($p < 0.05$) reduced at pH 11. In contrast, the surface tension remained stable over the entire range of pH. Even at pH 5, where the protein solubility was negligible, the surface and interfacial tensions were reduced to approximately 50 and 13 mN/m, respectively (Table 3). Albumins and globulins fractions prepared in this study contained 29.5 and 11.3 g/kg of saponins, respectively (Table 1). The content of saponins was significantly ($p < 0.05$) reduced upon sequential extraction of proteins and no saponins were detected in glutelins. Saponins have a relatively small molecular size and tend to rapidly adsorb at the oil/water and air/water interfaces. Moreover, they can effectively reduce the surface and interfacial tensions at very low concentrations, thus their popular use as surfactants (Yang, Leser, Sher, & McClements, 2013). Soy protein isolates contain approximately 7.6 g/kg of saponins according to Ireland, Dziedzic, and Kearsley (1986). The content of saponins in soy protein isolates, however, can vary considerably due to genetic and environmental factors and/or processing conditions (Lin, Krishnan & Wang, 2006). To get a better insight into the surface-active properties of APC proteins, the dynamic interfacial tension of albumins and globulins fractions was evaluated as a function of pH. As shown in Fig. 3, the precipitation of albumins and globulins at pH 5 and 7 appreciably affected their dynamic interfacial activity. In the absence of proteins, the interfacial tension remained stable through the entire period of measurement because saponins quickly adsorb on the interface in the first seconds of measurements due to their small molecular size and small interfacial activity. As the content of soluble proteins was increased, however, the interfacial tension was gradually decreased over time, indicating substantial adsorption of proteins at the oil/water interface. Static and dynamic interfacial tension measurements indicated, therefore, that both proteins and residual surface-active components (i.e., saponins) contributed to interfacial tension reduction. The foams and emulsions were then prepared using the entire compositions without elimination of any specific components.

Surface-active ingredients are important for emulsion and foams stabilization, indicating that APC albumins and globulins fractions could be used as a emulsifiers, co-emulsifiers, or foaming agents in various food and beverage products. Comprehensively, they can promote droplet disruption during homogenization by reducing interfacial or surface tension and create a protective barrier around the newly generated droplets, which prevents their coalescence or aggregation (Ozturk & McClements, 2016).

3.5. Hydrodynamic diameter and ζ-potential

The hydrodynamic diameter (d_{av}) of albumins and globulins fractions decreased significantly ($p < 0.05$) upon increasing the pH from 3 to 11. The proteins extremely aggregated at pH 5 and 7, thus the irrelevance of measuring d_{av}. Moreover, the d_{av} of albumins was significantly ($p < 0.05$) increased at pH 3, due to lower solubility in water (Table 3). In contrast, the ζ-potential profile of albumins and globulins was similar and comparable to most PPI. The proteins had a positive charge below their isoelectric point and then charged negatively upon further increase of pH (Table 3). These results agreed with the data obtained from the solubility profiles of the prepared fractions and were also consistent with the d_{av} measurements. Interestingly, at pH 5 and 7, where both albumins and globulins showed negligible solubility in water, the samples presented appreciable negative charge after protein elimination. This confirmed the presence of residual surface-active components that can form negatively charged micellar particles at low concentrations, such as saponins.

3.6. Emulsifying and foaming properties

The emulsifying properties of albumins and globulins were evaluated by RSH and HPH. RSH consists of applying a combination of moderate longitudinal, rotational and radial velocity gradients to disrupt oil droplets, while HPH consist of applying a rapid turbulent flow inside of a narrower homogenization chamber to enhance droplet size reduction (McClements, 2015). As shown in Fig. 4a, the preparation characteristics of albumins and globulins emulsions were similar when using RSH; the emulsions had a $d_{4,3}$ of approximately 16 μm and presented closely overlapped droplet size distributions. Globulin's emulsions were

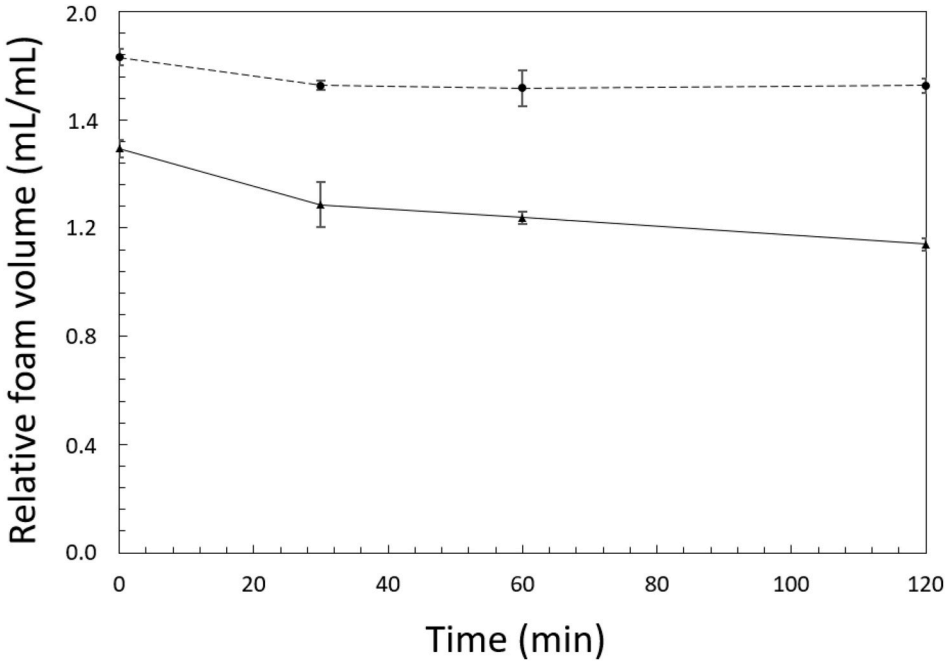


Fig. 5. Foaming capacity (0 min) and foam stability of 10 mg/mL solutions of pH_{12}-shifting treated argan press-cake albumins and globulins isolates. Albumins (full line); globulins (dashed line).

sensitive, however, to HPH with larger particles upon HPH treatment (Fig. 4b), which may limit their application in fine emulsion systems (e.g., nano/microemulsions) that require strong homogenization conditions to reduce the droplet size. The droplet size of emulsions prepared using albumins, on the other hand, appreciably decreased upon HPH (Fig. 4a). The emulsions also presented limited aggregation in comparison to globulins emulsions, indicating limited droplet-droplet interaction (Fig. 4b). Hence, they can be suitable as emulsifiers or co-emulsifiers for nano/microemulsions systems.

The foaming capacity of globulins was higher than that of albumins at relatively moderate homogenization conditions (Fig. 5). Both fractions presented, however, stronger foaming abilities in comparison to conventional PPI, including soybean, pea, and rapeseed protein isolates (Dong et al., 2011; Kim & Kim, 2015; Taherian et al., 2011). The foam stability of globulins was also better than that albumins at the specified homogenization conditions; approximately 1.73 mL/mL of foam was maintained by globulins after 120 min of storage, while 1.14 mL/mL was preserved by albumins (Fig. 5).

The emulsifying and foaming abilities of proteins are generally attributed to interfacial and surface tensions reduction. Proteins can also form cohesive viscoelastic films around the newly generated interfaces, via intermolecular interactions, which enhances droplet stabilization (Makri, Papalamprou & Doxastakis, 2005). The adsorption conditions of proteins, however, may vary considerably during foam or emulsion formation. Particularly, they may gain or lose interfacial affinity upon homogenization and undergo important conformational changes that modulate their intermolecular interaction. Kuhn and Cunha (2012) indicate that HPH (80 MPa) of whey protein isolate can lead to the formation of high molecular weight aggregates with reduced emulsifying capacity below denaturation temperature. Other studies indicate, otherwise, the beneficial effect of HPH on the emulsifying performance of proteins by dissociating molecular subunits and enhancing their adsorption (Chen et al., 2019; Wang et al., 2008). The effect of HPH treatment on proteins aggregation can be attributed to the rupture of intramolecular hydrophobic and electrostatic bonds, leading to unfolded structures that are prone to intermolecular interaction (Kuhn & Cunha, 2012). Such behavior is observed for both adsorbed and non-adsorbed 11S soy proteins, for example, indicating that regardless of the location of the proteins (aqueous phase or interface), the use of HPH could induce aggregation (Puppo et al., 2011). Globulins seemed to undergo, therefore, important conformational changes upon HPH, which intensified intermolecular interaction. Globulins seemed also to form stronger interfacial films during foam formation, explaining their better foaming ability at relatively moderate homogenization conditions. In general, both APC albumins and globulins fractions can be suitable as foaming ingredients. Isoelectric-precipitated APC globulins, however, may be less efficient as emulsifier or co-emulsifier agents when HPH is included.

3.7. Thermal degradation

The thermal stability of APC proteins was evaluated by TG-DTA. TG represents the weight loss as a function of temperature, while DTA is the curve of heat flux versus temperature. As shown in Fig. 6a, the weight of albumins and globulins samples decreased slightly from 25 to 200 °C. It decreased sharply between 200 and 400 °C and then fairly stabilized from 400 to 600 °C. The minor weight loss observed before 200 °C was marked by a single endothermic peak at about 80–90 °C. The main loss observed between 200 and 400 °C was preceded by an endothermic peak at approximately 230 °C, followed by a series of endothermic and exothermic peaks from 300 to 400 °C, while the final weight loss above 400 °C was mainly described as an endothermic process (Fig. 6b). The total weight loss was 74.8% and 63.9% for albumins and globulins, respectively.

Similar TG fingerprints are described for soybean (Qu, Huang, Wu, Sun, & Chang, 2015) and Guava seed (Fontanari et al., 2006) protein isolates. The first step, observed from room temperature to 200 °C, is mainly attributed to free water and bound water evaporation (protein denaturation can also occur at this stage but less likely contribute to weight loss). The actual weight loss, involving the scission of intramolecular and intermolecular hydrogen bonds, electrostatic bonds and the random cleavage of peptide bonds in the protein backbone leading to fragment evaporation is due to occur at higher temperatures (Fontanari et al., 2006; Qu et al., 2015). Since the water loss of both samples was

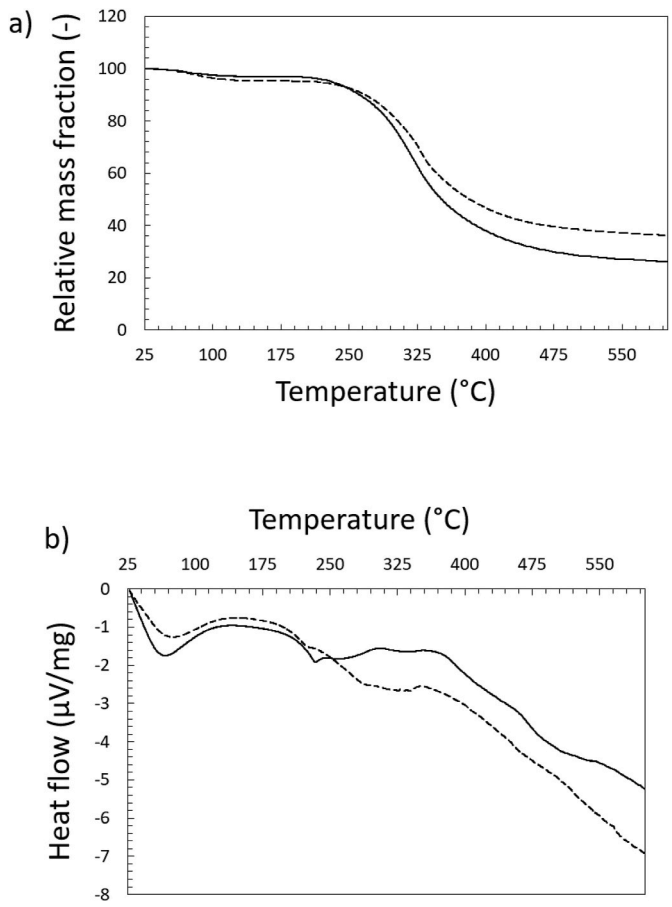


Fig. 6. Thermal analysis of argan press-cake albumins and globulins isolates obtained by isoelectric precipitation. (a) Thermogravimetric analysis. (b) Differential thermal analysis. Albumins (full line); globulins (dashed line).

similar, the lower weigh loss of globulins, compared to albumins over the entire range of temperature was attributed to their stronger intra-molecular and/or intermolecular interactions (natively present or formed upon extraction and isoelectric precipitation). Comprehensively, Qu et al. (2015) indicate that the formation of strong Van der Waals forces, intermolecular hydrogen bonds, and hydrophobic interactions among hydrolyzed soy proteins reduces their segmental motion compared to native soy proteins, creating a barrier that delays heat diffusion.

The amino acids profile of both protein fractions can also lead to their differential stability at elevated temperatures. For example, non-polar and aromatic amino acids, such as phenylalanine and tyrosine can contribute to increasing interaction between the polypeptide chains (Ahmed, Zulfiqar, Tang, & Lin, 2022). In addition, selected amino acids, such as cysteine ang glutamine tend to degrade more rapidly compared to other amino acids, such as aspartate and histidine (Weiss, Muth, Drumm, & Kirchner, 2018). The amino acids compositions of albumins and globulins were then evaluated and compared it in terms of aromatic, polar and non-polar amino acids. As shown in Table 2, both samples had a comparable amino acid composition with only slight differences, thus it was difficult to draw conclusive remarks about the role of amino acid composition on thermal degradation. For example, globulins had slightly higher sulfur-containing amino acids, especially cysteine when compared to albumins. In contrast, albumins had slightly more hydrophobic and aromatic tyrosine than albumins (Table 2).

3.8. Surface hydrophobicity

The location of hydrophobic amino acids in the 3-dimensional configuration of proteins plays an important role in determining their physicochemical and techno-functional characteristics. This location can be estimated using fluorescence spectroscopy by measuring the fluorescence emission of hydrophobic amino acids, such as tryptophan and tyrosine. Albumins showed greater fluorescence emission than globulins, following tryptophan excitation at 295 nm (Fig. 7). The fluorescence maximum was about 335 nm, which is characteristic for tryptophan residues that are partially buried in the hydrophobic core of the protein (Arogundade, Mu, & Akinhanmi, 2016). The fluorescence emission of albumins was intensified upon dual excitation of tryptophan and tyrosine at 280 nm, indicating the presence of compact tertiary conformations, with significant contribution of tyrosine. In contrast, the fluorescence emission of globulins was slightly affected by tyrosine excitation, which could be attributed to the higher exposure of this residue to the surface of the protein and/or to its lower content in globulins (Table 2). Overall, in undenatured proteins, tyrosine emission is not usually seen (Lacowicz, 2006) in opposition to APC albumins. Both albumins and globulins were denatured, therefore, following extraction and isoelectric precipitation, but more pronounced changes occurred potentially in globulins.

The exposure of hydrophobic amino-acids to the surface of albumins and globulins was confirmed by ANS-Fluorescence assay. ANS tends to bind to the hydrophobic regions of proteins, resulting in stronger fluorescence intensity (Arogundade et al., 2016). As shown in Fig. 8, the surface hydrophobicity (H_0) of globulins was higher than that of albumins, indicating that globulins had potentially more hydrophobic groups in contact with the aqueous environment. Protein denaturation promotes the exposure of hydrophobic amino acids that are buried inside the native structure of proteins, increasing H_0 (Arogundade et al.,

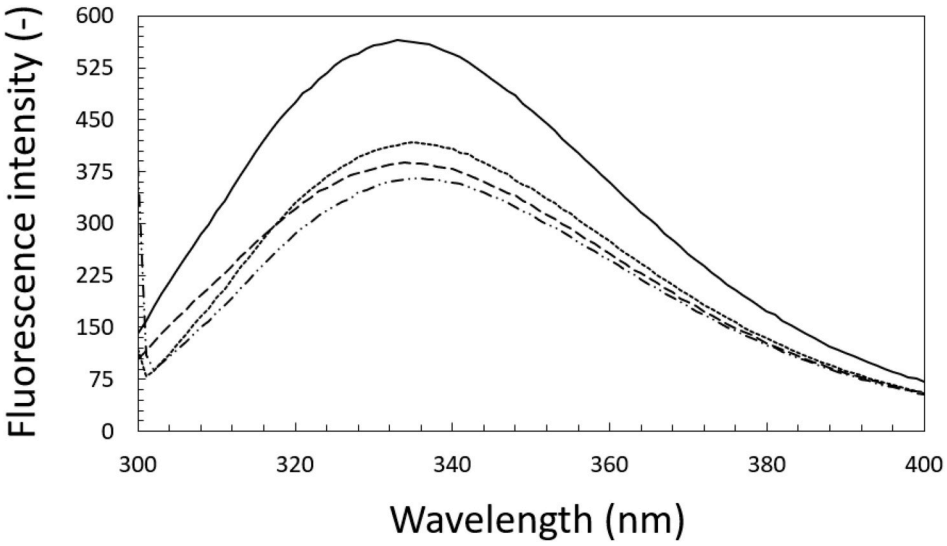


Fig. 7. Intrinsic fluorescence spectra of pH_{12}-shifting treated argan press-cake albumins and globulins isolates. Albumins (Tyrosine + Tryptophane): full line; albumins (Tryptophane): dotted line; globulins (Tyrosine + Tryptophane): dashed line; globulins (Tryptophane): dash-dotted line.

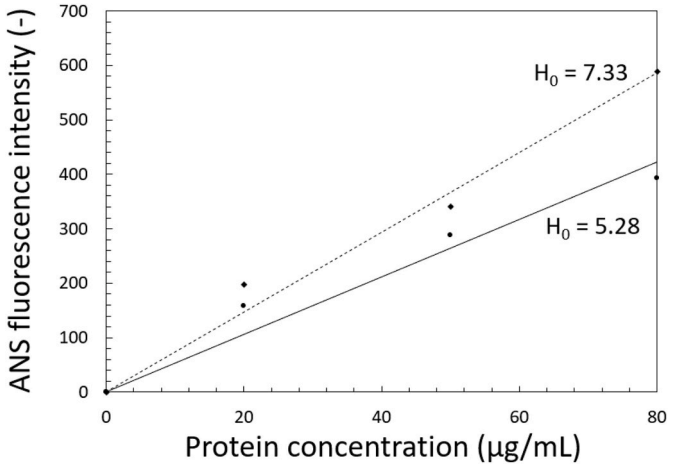


Fig. 8. Surface hydrophobicity of pH_{12}-shifting treated argan press-cake albumins and globulins isolates obtained by isoelectric precipitation. Albumins (full line); globulins (dashed line).

2016). Hence the higher H_0 observed with globulins compared to albumins can be attributed to their higher extent of denaturation, following isoelectric precipitation.

4. Conclusions

The major fractions of APC proteins were albumins and globulins. APC globulins were prone to aggregation, potentially by hydrophobic interactions, resulting in lower solubility in water. Thus, enhancing their solubility by unfolding-refolding using pH-shifting was suggested to promote their application as techno-functional food ingredients. pH-shifted albumins and globulins had good surface activities at the air/water and oil/water interfaces and presented better foaming capacity and foam stability, compared to conventional PPI, such as soybean, pea, and rapeseed protein isolates. APC globulins, however, were highly sensitive to high-pressure emulsification, resulting in extensive aggregation, which may limit their application in fine emulsion systems (e.g., nano/microemulsions) that require strong homogenization conditions. The results of this study could offer a useful guide for the extraction and isolation of APC proteins. Moreover, it provided insights on the utilization of argan oil extraction by-products foreseeing food application, by providing better knowledge about the fractional, biochemical, and techno-functional properties of APC proteins.

CRediT authorship contribution statement

Noamane Taarji: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Karim Lyamlouli:** Writing – review & editing, Validation. **Abdellatif Barakat:** Writing – review & editing, Validation. **Kenichi Tominaga:** Writing – review & editing, Validation, Supervision, Investigation, Funding acquisition, Conceptualization. **Hiroko Isoda:** Validation, Project administration, Funding acquisition. **Mitsutoshi Nakajima:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing 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 paper.

Data availability

Data will be made available on request.

References

Adebiyi, A. P., Adebiyi, A. O., Hasegawa, Y., Ogawa, T., & Muramoto, K. (2009). Isolation and characterization of protein fractions from deoiled rice bran. *European Food Research and Technology, 228*(3), 391–401. https://doi.org/10.1007/s00217-008-0945-4

Ahmed, Z., Zulfiqar, H., Tang, L., & Lin, H. (2022). A statistical analysis of the sequence and structure of thermophilic and non-thermophilic proteins. *International Journal of Molecular Sciences, 23*(17), Article 10116. https://doi.org/10.3390/ijms231710116

Ajibola, C. F., Malomo, S. A., Fagbemi, T. N., & Aluko, R. E. (2016). Polypeptide composition and functional properties of African yam bean seed (*Sphenostylis stenocarpa*) albumin, globulin and protein concentrate. *Food Hydrocolloids, 56*, 189–200. https://doi.org/10.1016/j.foodhyd.2015.12.013

Arogundade, L. A., Mu, T.-H., & Akinhanmi, T. F. (2016). Structural, physicochemical and interfacial stabilisation properties of ultrafiltered African yam bean (*Sphenostylis stenocarpa*) protein isolate compared with those of isoelectric protein isolate. *LWT - Food Science and Technology, 69*, 400–408. https://doi.org/10.1016/j.lwt.2016.01.049

Borchani, M., Yaich, H., Abbès, F., Blecker, C., Besbes, S., Attia, H., et al. (2021). Physicochemical, functional and antioxidant properties of the major protein fractions extracted from prickly pear (*Opuntia ficus indica*) seed cake. *Waste and Biomass Valorization, 12*(4), 1749–1760. https://doi.org/10.1007/s12649-020-01111-4

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1–2), 248–254.

Charrouf, Z., Henry, F., Moser, P., & Pauly, G. (2005). *Cosmetic and/or dermopharmaceutical preparations containing native proteins of the spinosa argania plant.* US20040042996A1. United States Patent.

Chen, S., Wang, X., Xu, Y., Zhang, X., Wang, X., & Jiang, L. (2019). Effect of high pressure treatment on interfacial properties, structure and oxidative stability of soy protein isolate-stabilized emulsions. *Journal of Oleo Science, 68*(5), 409–418. https://doi.org/10.5650/jos.ess18228

Delaroche, M., Dias, V. M., & Massoca, P. E. (2023). The intertemporal governance challenges of Brazil's Amazon: Managing soybean expansion, deforestation rates, and urban floods. *Sustainability Science, 18*(1), 43–58. https://doi.org/10.1007/s11625-022-01149-4

Detzel, A., Krüger, M., Busch, M., Blanco-Gutiérrez, I., Varela, C., Manners, R., et al. (2022). Life cycle assessment of animal-based foods and plant-based protein-rich alternatives: An environmental perspective. *Journal of the Science of Food and Agriculture, 102*(12), 5098–5110. https://doi.org/10.1002/jsfa.11417

Dong, X. Y., Guo, L. L., Wei, F., Li, J. F., Jiang, M. L., Li, G. M., et al. (2011). Some characteristics and functional properties of rapeseed protein prepared by ultrasonication, ultrafiltration and isoelectric precipitation. *Journal of the Science of Food and Agriculture, 91*, 1488–1498. https://doi.org/10.1002/jsfa.4339

Feyzi, S., Varidi, M., Zare, F., & Varidi, M. J. (2015). Fenugreek (*Trigonella foenum-graecum*) seed protein isolate: Extraction optimization, amino acid composition, thermo and functional properties. *Journal of the Science of Food and Agriculture, 95*(15), 3165–3176. https://doi.org/10.1002/jsfa.7056

Fontanari, G. G., Batistuti, J. P., Bannach, G., Pastre, I. A., Ionashiro, E. Y., & Fertonani, F. L. (2006). Thermalstudy and physico-chemical characterization of some functional properties of guava seeds protein isolate (*Psidium guajava*). *Journal of Thermal Analysis and Calorimetry, 83*(3), 709–713. https://doi.org/10.1007/s10973-005-6802-9

Gençdağ, E., Görgüç, A., & Yılmaz, F. M. (2020). Recent advances in the recovery techniques of plant-based proteins from agro-industrial by-products. *Food Reviews International, 37*(4), 447–468. https://doi.org/10.1080/87559129.2019.1709203

Gharby, S., & Charrouf, Z. (2021). Argan oil: Chemical composition, extraction process, and quality control. *Frontiers in Nutrition, 8*, Article 804587. https://doi.org/10.3389/fnut.2021.804587

Horax, R., Hettiarachchy, N., Over, K., Chen, P., & Gbur, E. (2010). Extraction, fractionation and characterization of bitter melon seed proteins. *Journal of Agricultural and Food Chemistry, 58*(3), 1892–1897. https://doi.org/10.1021/jf902903s

Ireland, P. A., Dziedzic, S. Z., & Kearsley, M. W. (1986). Saponin content of soya and some commercial soya products by means of high-performance liquid chromatography of the sapogenins. *Journal of the Science of Food and Agriculture, 37*(7), 694–698. https://doi.org/10.1002/jsfa.2740370715

Jiang, J., Xiong, Y. L., & Chen, J. (2010). pH shifting alters solubility characteristics and thermal stability of soy protein isolate and its globulin fractions in different pH, salt concentration, and temperature conditions. *Journal of Agricultural and Food Chemistry, 58*(13), 8035–8042. https://doi.org/10.1021/jf101045b

Julio, L. M., Ruiz-Ruiz, J. C., Tomás, M. C., & Segura-Campos, M. R. (2019). Chia (*Salvia hispanica*) protein fractions: Characterization and emulsifying properties. *Journal of Food Measurement and Characterization, 13*(4), 3318–3328. https://doi.org/10.1007/s11694-019-00254-w

Kaur, R., & Ghoshal, G. (2022). Sunflower protein isolates-composition, extraction and functional properties. *Advances in Colloid and Interface Science, 306*, Article 102725. https://doi.org/10.1016/j.cis.2022.102725

Kim, H. J., & Kim, B. K. (2015). Comparison of soy protein concentrates produced using membrane ultrafiltration and acid precipitation. *Food Science and Biotechnology, 24*, 67–73. https://doi.org/10.1007/s10068-015-0011-5

Kotecka-Majchrzak, K., Sumara, A., Fornal, E., & Montowska, M. (2020). Oilseed proteins – properties and application as a food ingredient. *Trends in Food Science & Technology, 106*, 160–170. https://doi.org/10.1016/j.tifs.2020.10.004

Kuhn, K. R., & Cunha, R. L. (2012). Flaxseed oil – whey protein isolate emulsions: Effect of high pressure homogenization. *Journal of Food Engineering, 111*(2), 449–457. https://doi.org/10.1016/j.jfoodeng.2012.01.016

Kumar, M., Tomar, M., Potkule, J., Verma, R., Punia, S., Mahapatra, A., et al. (2021). Advances in the plant protein extraction: Mechanism and recommendations. *Food Hydrocolloids, 115*, Article 106595. https://doi.org/10.1016/j.foodhyd.2021.106595

Lacowicz, J. R. (2006). *Principles of fluorescence spectroscopy* (3rd ed.). Berlin: Springer. https://doi.org/10.1007/978-0-387-46312-4

Li, N., Qi, G., Susan Sun, X., Wang, D., Bean, S., & Blackwell, D. (2014). Isolation and characterization of protein fractions isolated from camelina meal. *Transactions of the ASABE, 57*(1), 169–178. https://doi.org/10.13031/trans.57.10455

Lin, J., Krishnan, P. G., & Wang, C. (2006). Retention of isoflavones and saponins during the processing of soy protein isolates. *Journal of the American Oil Chemists' Society, 83*(1), 59–63. https://doi.org/10.1007/s11746-006-1176-0

Lydie, L., Fabre, B., & Charveron, M. (1999). *Synergistic composition containing argan oil and argan peptide. FR2756183A1.* France Patent.

Makri, E., Papalamprou, E., & Doxastakis, G. (2005). Study of functional properties of seed storage proteins from indigenous European legume crops (lupin, pea, broad bean) in admixture with polysaccharides. *Food Hydrocolloids, 19*(3), 583–594. https://doi.org/10.1016/j.foodhyd.2004.10.028

Malomo, S. A., & Aluko, R. E. (2015). A comparative study of the structural and functional properties of isolated hemp seed (*Cannabis sativa* L.) albumin and globulin fractions. *Food Hydrocolloids, 43*, 743–752. https://doi.org/10.1016/j.foodhyd.2014.08.001

Mariotti, F., Tomé, D., & Mirand, P. P. (2008). Converting nitrogen into protein—beyond 6.25 and jones' factors. *Critical Reviews in Food Science and Nutrition, 48*(2), 177–184. https://doi.org/10.1080/10408390701279749

McClements, D. J. (2015). *Food emulsions: Principles, practices, and techniques* (3rd ed.). Boca Raton: CRC Press. https://doi.org/10.1201/b18868

Mechqoq, H., El Yaagoubi, M., El Hamdaoui, A., Momchilova, S., Guedes da Silva Almeida, J. R., Msanda, F., et al. (2021). Ethnobotany, phytochemistry and biological properties of Argan tree (*Argania spinosa* (L.) Skeels) (Sapotaceae) - a review. *Journal of Ethnopharmacology, 281*, Article 114528. https://doi.org/10.1016/j.jep.2021.114528

Mirpoor, S. F., Giosafatto, C. V. L., Mariniello, L., D'Agostino, A., D'Agostino, M., Cammarota, M., et al. (2022). Argan (*Argania spinosa* l.) seed oil cake as a potential source of protein-based film matrix for pharmaco-cosmetic applications. *International Journal of Molecular Sciences, 23*(5), 8478. https://doi.org/10.3390/ijms23158478

Mondor, M., & Hernández-Álvarez, A. J. (2022). Processing technologies to produce plant protein concentrates and isolates. In A. Manickavasagan, L. T. Lim, & A. Ali (Eds.), *Plant protein foods* (pp. 61–108). Cham: Springer. https://doi.org/10.1007/978-3-030-91206-2_3.

Ningxiang, Y., Shengxin, S., Weiwei, H., Qin, Y., Xiaohua, N., Yuanchao, L., et al. (2022). Preparation of novel self-assembled albumin nanoparticles from Camellia seed cake waste for lutein delivery. *Food Chemistry, 389*, Article 133032. https://doi.org/10.1016/j.foodchem.2022.133032

Nwachukwu, I. D., & Aluko, R. E. (2018). Physicochemical and emulsification properties of flaxseed (*Linum usitatissimum*) albumin and globulin fractions. *Food Chemistry, 255*, 216–225. https://doi.org/10.1016/j.foodchem.2018.02.068

Osborne, T. B. (1924). *The vegetable proteins* (2nd ed.). London: Longmans, green and Co. https://doi.org/10.1002/jctb.5000431704

Ozturk, B., & McClements, D. J. (2016). Progress in natural emulsifiers for utilization in food emulsions. *Current Opinion in Food Science, 7*, 1–6. https://doi.org/10.1016/j.cofs.2015.07.008

PerkinElmer Inc. (2002). 2400 series II CHNS/O analyzer. https://resources.perkinelmer.com/corporate/cmsresources/images/44-74656prd_2400seriesiichnsoanalyzer.pdf Accessed on 14/12/2023.

Puppo, M. C., Beaumal, V., Speroni, F., de Lamballerie, M., Añón, M. C., & Anton, M. (2011). β-Conglycinin and glycinin soybean protein emulsions treated by combined temperature–high-pressure treatment. *Food Hydrocolloids, 25*(3), 389–397. https://doi.org/10.1016/j.foodhyd.2010.07.005

Qu, P., Huang, H., Wu, G., Sun, E., & Chang, Z. (2015). The effect of hydrolyzed soy protein isolate on the structure and biodegradability of urea–formaldehyde adhesives. *Journal of Adhesion Science and Technology, 29*(6), 502–517. https://doi.org/10.1080/01694243.2014.995909

Sá, A. G. A., Moreno, Y. M. F., & Carciofi, B. A. M. (2020). Plant proteins as high-quality nutritional source for human diet. *Trends in Food Science & Technology, 97*, 170–184. https://doi.org/10.1016/j.tifs.2020.01.011

Sharma, M., & Saini, C. S. (2022). Amino acid composition, nutritional profiling, mineral content and physicochemical properties of protein isolate from flaxseeds (*Linum usitatissimum*). *Journal of Food Measurement and Characterization, 16*(1), 829–839. https://doi.org/10.1007/s11694-021-01221-0

Taarji, N., Rabelo da Silva, C. A., Khalid, N., Gadhi, C., Hafidi, A., Kobayashi, I., et al. (2018). Formulation and stabilization of oil-in-water nanoemulsions using a saponins-rich extract from argan oil press-cake. *Food Chemistry, 246*, 457–463. https://doi.org/10.1016/j.foodchem.2017.12.008

Taarji, N., Vodo, S., Bouhoute, M., Khalid, N., Hafidi, A., Kobayashi, I., et al. (2020). Preparation of monodisperse O/W emulsions using a crude surface-active extract from argan by-products in microchannel emulsification. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 585*, Article 124050. https://doi.org/10.1016/j.colsurfa.2019.124050

Taherian, A. R., Mondor, M., Labranche, J., Drolet, H., Ippersiel, D., & Lamarche, F. (2011). Comparative study of functional properties of commercial and membrane processed yellow pea protein isolates. *Food Research International, 44*, 2505–2514. https://doi.org/10.1016/j.foodres.2011.01.030

Thrane, M., Paulsen, P. V., Orcutt, M. W., & Krieger, T. M. (2017). Chapter 2 - soy protein: Impacts, production, and applications. In R. N. Sudarshan, P. D. W. Janitha, & S. Laurie (Eds.), *Sustainable protein sources* (pp. 23–45). London: Academic Press. https://doi.org/10.1016/B978-0-12-802778-3.00002-0.

van der Goot, A. J., Pelgrom, P. J. M., Berghout, J. A. M., Geerts, M. E. J., Jankowiak, L., Hardt, N. A., et al. (2016). Concepts for further sustainable production of foods. *Journal of Food Engineering, 168*, 42–51. https://doi.org/10.1016/j.jfoodeng.2015.07.010

Vinayashree, S., & Vasu, P. (2021). Biochemical, nutritional and functional properties of protein isolate and fractions from pumpkin (*Cucurbita moschata* var. Kashi Harit) seeds. *Food Chemistry, 340*, Article 128177. https://doi.org/10.1016/j.foodchem.2020.128177

Wang, G., & Guo, M. (2019). Manufacturing technologies of whey protein products. In M. Guo (Ed.), *Whey protein production, chemistry, functionality, and applications* (pp. 13–37). John Wiley & Sons Ltd. https://doi.org/10.1002/9781119256052.ch2.

Wang, X.-S., Tang, C.-H., Li, B.-S., Yang, X.-Q., Li, L., & Ma, C.-Y. (2008). Effects of high-pressure treatment on some physicochemical and functional properties of soy protein isolates. *Food Hydrocolloids, 22*(4), 560–567. https://doi.org/10.1016/j.foodhyd.2007.01.027

Weiss, I. M., Muth, C., Drumm, R., & Kirchner, H. O. K. (2018). Thermal decomposition of the amino acids glycine, cysteine, aspartic acid, asparagine, glutamic acid, glutamine, arginine and histidine. *BMC Biophysics, 11*(1), 2. https://doi.org/10.1186/s13628-018-0042-4

Xiang, Z., Tang, C., Chen, G., & Shi, Z. (2001). Studied on corlorimetric determination of oleanolic acid in Chinese quince. *Natural Product Research and Development, 13*(4), 23–26.

Yang, Y., Leser, M. E., Sher, A. A., & McClements, D. J. (2013). Formation and stability of emulsions using a natural small molecule surfactant: Quillaja saponin (Q-Naturale®). *Food Hydrocolloids, 30*(2), 589–596. https://doi.org/10.1016/j.foodhyd.2012.08.008

Yildiz, G., Andrade, J., Engeseth, N. E., & Feng, H. (2017). Functionalizing soy protein nano-aggregates with pH-shifting and mano-thermo-sonication. *Journal of Colloid and Interface Science, 505*, 836–846. https://doi.org/10.1016/j.jcis.2017.06.088

Zhang, Z., Wang, Y., Dai, C., He, R., & Ma, H. (2018). Alkali extraction of rice residue protein isolates: Effects of alkali treatment conditions on lysinoalanine formation and structural characterization of lysinoalanine-containing protein. *Food Chemistry, 261*, 176–183. https://doi.org/10.1016/j.foodchem.2018.04.027