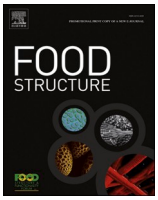
Food Structure 31 (2022) 100254

Contents lists available at ScienceDirect

Food Structure

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



Rethinking plant protein extraction: Albumin—From side stream to an excellent foaming ingredient

Jack Yang [a,b,c,*], Remco Kornet [a,b], Claudine F. Diedericks [b,d], Qiuhuizi Yang [b], Claire C. Berton-Carabin [e,f], Constantinos V. Nikiforidis [c], Paul Venema [b], Erik van der Linden [a,b], Leonard M.C. Sagis [b,**]

[a] TiFN, Nieuwe Kanaal 9 A, 6709 PA Wageningen, the Netherlands
[b] Laboratory of Physics and Physical Chemistry of Foods, Wageningen University, Bornse Weilanden 9, 6708 WG Wageningen, the Netherlands
[c] Laboratory of Biobased Chemistry and Technology, Wageningen University, Bornse Weilanden 9, 6708 WG Wageningen, the Netherlands
[d] Royal Bel Leerdammer B.V., Steenovenweg 4, 4145 KK Schoonrewoerd, the Netherlands
[e] Laboratory of Food Process Engineering, Wageningen University, Bornse Weilanden 9, 6708 WG Wageningen, the Netherlands
[f] INRAE, UR BIA, F-44316 Nantes, France

ARTICLE INFO

Keywords:
Plant protein
Globulin
Albumin
Foam
Interface
Protein extraction

ABSTRACT

The transition from animal- to plant-derived dietary proteins is of global importance. Plant proteins are normally processed into extracts, and due to the type of process, proteins from the globulin class are mainly extracted. Such extractions have several waste streams, containing another protein class: albumins. Here, we show that plant albumins have good functionality. We compared interfacial and foaming properties of albumins and globulins from mung bean, Bambara groundnut and yellow pea. The foaming properties of albumins were good, similar or even superior to those of whey or egg white proteins, while globulin-based foams showed low stability. Albumins form strong cohesive interfacial layers around air bubbles. Globulins are unable to create such layers, mainly due to their aggregated structures. Additionally, we provide a mild extraction method, allowing the co-extraction of albumin and globulin. This protein mixture is able to form foam with half-life times up to 450 min. Though currently underutilized, plant albumins can substitute animal proteins, especially in foaming applications, where they outcompete globulins. Their utilization could be an important contribution to the food protein transition.

1. Introduction

The world population is growing rapidly with a projected 9.6 billion inhabitants in 2050 (Godfray et al., 2010; Tripathi, Mishra, Maurya, Singh, & Wilson, 2018). In response to sustainability concerns in terms of food security and environmental impact, there is currently a global trend to replace animal- with plant-derived proteins in our diets. By doing so, the output of food production could be increased, while reducing its environmental and ecological footprint (Aiking & de Boer, 2018). Most studies on the techno-functional properties of plant proteins focus on protein-rich sources, such as soybean and pea, which mainly contain storage proteins (Barać, Pešić, Stanojević, Kostić, & Čabrilo, 2015; Lam, Can Karaca, Tyler, & Nickerson, 2018; Loveday, 2020; Sari, Mulder, Sanders, & Bruins, 2015; Yong, Sim, Srv, & Chiang, 2021). Storage proteins are commonly classified using the Osborne classification method (Osborne, 1924), as water-soluble (albumins), dilute saline solution-soluble (globulins), alcohol-soluble (prolamins) and dilute acid or alkali solution-soluble (glutelins). In the most commonly utilized plant protein sources (pulses and oilseeds), albumins (10–25%) and globulins (60–80% of total storage protein) are the most abundant proteins (Chéreau et al., 2016; Kim, Wang, & Selomulya, 2020; Yi-Shen, Shuai, & Fitzgerald, 2018). Both globulins and albumins can be extracted with conventional wet extraction methods (Fig. 1) (Sari et al., 2015).

In such extraction methods, pre-processed plant material is dispersed at an alkaline pH (8−13), which solubilizes globulins and albumins (Sari et al., 2015). The next step is the removal of insoluble material, through e.g. centrifugation. Next, the pH of the soluble fraction is adjusted to the

* Corresponding author at: TiFN, Nieuwe Kanaal 9 A, 6709 PA Wageningen, the Netherlands.
** Corresponding author.
E-mail addresses: jack.yang@wur.nl (J. Yang), leonard.sagis@wur.nl (L.M.C. Sagis).

https://doi.org/10.1016/j.foostr.2022.100254
Received 7 October 2021; Received in revised form 11 January 2022; Accepted 11 January 2022
Available online 13 January 2022
2213-3291/© 2022 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

isoelectric point of the globulins, which typically ranges between pH 4–5 (Karaca, Low, & Nickerson, 2011; Shevkani, Singh, Kaur, & Rana, 2015). This causes the precipitation of globulins, while albumins remain soluble. The proteins are then separated by another separation step, often centrifugation. The insoluble fraction is high in protein and mainly contains globulins, while the soluble fraction contains albumins with other solutes (i.e. phenols, sugars and minerals) (Chua & Liu, 2019; Kornet et al., 2020). The globulin-rich fraction is processed into a protein concentrate or isolate, based on the amount of protein in the final extract. This extraction method is most commonly used to extract plant proteins in industry and academia; and implies that the main focus of plant protein research and its utilization is on plant globulins.

The conventional wet extraction process yields a large side stream of the soluble fraction containing albumins. This side stream is generally discarded due to the presence of other solutes and anti-nutritional components (i.e., lipoxygenases, phytic acids and tannins) (Chua & Liu, 2019). The latter can be removed (Lu, Quillien, & Popineau, 2000) (by filtration) or inactivated (Samtiya, Aluko, & Dhewa, 2020) (by heating, fermentation or germination) to obtain an albumin concentrate. Volume-wise, it is worth utilizing this side stream, as shown in the production of soy protein isolate. The production of 1 ton soy protein isolate yields 20 tons of side stream (known as soy whey) containing around 0.3% (w/v) (albumin) protein (Wang, Wu, Zhao, Liu, & Gao, 2013). Based on the storage proteins' composition of various plant sources, we estimate that for each kg of globulins extracted, about 0.1–0.4 kg of albumins is produced.

As the most commonly extracted plant proteins are globulins, a smaller number of studies were performed on the techno-functional properties (e.g. foaming, emulsifying and gelling properties) of plant-based albumins (Yang & Sagis, 2021). Interestingly, several of these studies reported good properties with respect to emulsion and foam stabilization (Cheung, Wanasundara, & Nickerson, 2014; Kapp & Bamforth, 2002; Lu et al., 2000; Wong, Pitts, Jayasena, & Johnson, 2013). Particularly the foaming properties are highly promising, in contrast to globulins which typically exhibit poor foaming properties and generally require additional processing, such as heating or enzymatic hydrolysis, to improve these properties (Amagliani & Schmitt, 2017; Ercili-Cura et al., 2015; Malabat, nchez-Vioque, Rabiller, & Gu guen, 2001; Peng et al., 2020). As a result of this relatively poor performance of the globulin fraction, many food products (e.g., cappuccino foam, ice cream and meringue) are currently still stabilized by animal-derived ingredients with remarkable foaming properties, such as dairy and egg proteins. A potential explanation for the poor globulin functionality might be related to aggregate formation, as during the isoelectric point precipitation large aggregates are formed, which were found to remain (partly) intact when returning to a neutral pH (Kornet et al., 2020). The high potential of albumins for foam stabilization could imply a major role in substituting these animal-derived stabilizers with plant-derived albumin fractions.

Therefore, this study aimed to compare the foam stabilization properties of globulins and albumins extracted from three plant (legume) protein sources. Combined, these sources are cultivated in the majority of the world, and were chosen to translate our findings to a global scale, thus allowing the formulation of general properties of plant proteins. Such generic protein properties are crucial in developing novel protein extraction methodologies to obtain functional properties, especially with regard to foaming properties. The sources are mung bean (Asia), Bambara groundnut (Africa), and yellow pea (Europe and North America). Albumins from pea have received attention in a few works (Djemaoune, Cases, & Saurel, 2019; Lu et al., 2000), but a proper direct comparison with globulins, and especially a comparison to conventional animal-based stabilizers, is lacking. Therefore, the foaming and interfacial properties of these plant globulin and albumins were characterized and compared to those of whey protein isolate (WPI) and of egg white protein isolate (EPI), which are commonly used stabilizers in model and applied protein-based foams. Here, we provide evidence that these albumins from three protein sources possess excellent foaming properties, which we relate to their molecular and interfacial properties. Additionally, we suggest a milder purification method, which consists of fewer steps, is less resource-demanding, and allows for co-extraction of globulins and albumins. With this new insight, an undervalued and underutilized food protein fraction could be redirected from a side stream to a high-end functional ingredient.

2. Experimental section

2.1. Materials

Mung bean (*Vigna radiata*)(Golden Chef, Thailand), Bambara groundnut (*Vigna subterranea* L. Verdc.)(Thusano Products, Louis Trichardt, South Africa) and yellow pea (*Pisum sativum* L.)(Alimex Europe BV, Sint Kruis, The Netherlands) were used as received. Whey protein isolate (Lot nr. JE 099-2-420 - purity 98%)(Davisco Foods international, Le Sueur, USA) and egg white protein isolate (albumin from chicken egg white, A5503, purity 98%)(Merck, Darmstadt, Germany) were included as a dairy and egg white reference protein, respectively. Materials for SDS-PAGE (Invitrogen Novex, ThermoFischer Scientific, Waltham, USA) were used as received. All chemicals and reagents were of analytical grade and were obtained from Merck (Darmstadt, Germany). All samples were prepared in ultrapure water (MilliQ Purelab Ultra, Germany).

2.2. Sample preparation

2.2.1. Protein purification of Mung bean and yellow pea

Mung bean was milled and air-classified using an ZP S50 Alpine Multimill Hosokawa coupled to an ATP50 air classifier (Hosokawa Micron BV, Doetinchem, the Netherlands) to remove most of the starch granules and cell wall material, and resulted in a dry-fractionated mung bean flour (flour particle size, $d_{4,3}$ of 135.0 ± 5.0 μm). Yellow pea was milled into flour (flour particle size, $d_{4,3}$ of 113 ± 15 μm). Mung bean and pea proteins were extracted from the flour using a previously described method (Kornet et al., 2020; Kornet, Yang, Venema, van der Linden, & Sagis, 2021) with several modifications. The flour was dispersed in ultrapure water to obtain a 10% (w/w) flour dispersion. The

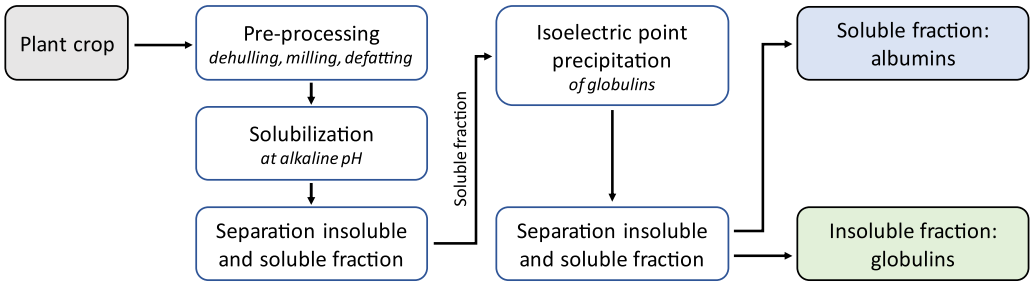


Fig. 1. A schematic overview of a conventional plant protein extraction process.

sample was stirred for 2 hrs, while continuously adjusting the pH to 8.0 with 1 M NaOH to increase protein solubility. Afterwards, the dispersion was centrifuged at 10,000xg for 30 min (20 °C) to remove solids. The supernatant was separated from the pellet, and was stirred for 1 hr, while continuously adjusting the pH to 4.5 with 1 M HCl to precipitate the globulins. The globulins were centrifuged into the pellet at 10,000xg for 30 min (20 °C). The pellet was re-dispersed in ultrapure water, and stirred for two hrs, while constantly adjusting the pH to 7.0, and finally freeze-dried. Freeze-drying for all samples was performed using a Alpha 2–4 LD plus freeze-dryer (Christ, Osterode am Harz, Germany). All samples were lyophilized at < 10 °C and < 1mbar. The supernatant containing albumins was further purified by diafiltration over a 5 kDa membrane, until the conductivity remained constant. The filtered sample containing albumins was freeze-dried. A dissolution pH higher than 8.0 could lead to higher protein yields, however, this was not the focus of this study. Therefore, this extraction pH was sufficient to obtain globulins and albumins.

2.2.2. Protein purification of Bambara groundnut

Bambara groundnut proteins were purified using the method of Diedericks et al. with several modifications (Diedericks, de Koning, Jideani, Venema, & van der Linden, 2019; Yang et al., 2022). The Bambara groundnut seeds were soaked in ultrapure water for 16 hrs to weaken the hull, and the seeds were dried in an oven at 40 °C for 24 hrs. The seeds were dehulled and coarse-milled using a LV 15 M pin mill (Condux-Werk, Selb, Germany). The milled seeds were dispersed in hexane for defatting at a 1:3 (w/v) ratio, stirred for 2 hrs, and afterwards hexane was decanted. These defatting steps were repeated twice, and finally, the mixture was passed through a paper filter to separate the hexane from the milled seeds, which were dried overnight under a stream of nitrogen gas. The defatted and milled seeds were frozen with liquid nitrogen and fine-milled with a 0.5 mm mesh sieve ring equipped on a Pulverisette 14 rotor mill (Fritsch GmbH, Idar-Obsertein, Germany). The flour was sieved through a 0.315 mm mesh sieve on an E200 LS air jet sieve (Hosokawa Alpine, Augsburg, Germany). This yielded the defatted flour. Globulins were extracted from the defatted flour with a similar method as described for mung bean and pea protein extraction with several modifications: the protein extraction from defatted flour was performed at pH 9.5; the precipitation of the globulins was performed at pH 4.0; and the centrifugation speed was 4000xg for 30 min (20 °C). The extraction pH of 9.5 for Bambara groundnut is higher than the pH of 8.0 used for pea and mung bean. A higher pH lead to higher charges of the globulins, thus higher solubility of the globulins. Differences in yield for the globulins are expected to be the main difference between the various globulin extracts. The albumins were extracted from the defatted flour by dispersing the flour in 0.5 M NaCl at a 1:10 (w/w) ratio and stirred for 1 hr. Afterwards, the sample was centrifuged at 10,000xg for 20 min (20 °C). These extraction steps were repeated twice on the pellet, and the final washing step was performed with ultrapure water. This resulted in three supernatants containing globulins and albumins, which were pooled, and the sample was stirred for 30 min, while adjusting the pH to 4.6 with glacial acetic acid to precipitate the globulin, legumin. The sample was centrifuged at 10,000xg for 30 min, the supernatant was recovered, and dialyzed in dialysis tubes with a 3.5 kDa cut-off against demineralized water. The dialysate was exchanged five times until the conductivity was constant. The removal of salts caused aggregation of vicilin, which were centrifuged out at 10,000xg for 30 min. The supernatant containing albumins was adjusted to pH 7.0, and freeze-dried.

2.2.3. Production of protein mixture

Intact Bambara groundnut seeds were coarse-milled with a LV 15 M pin mill (Condux-Werk, Selb, Germany), and were stirred in ultrapure water at a ratio of 1:7 (w/w) for 1 hr. Detached hulls due to soaking and stirring were removed, and the pH was adjusted to 9.5 using 1 M NaOH. The sample was stirred for another 2.5 hr, and the mixture was blended (Vita-Prep blender, Vitamix, Cleveland, USA) for 2 min at max speed. The slurry was stirred for 30 min, while adjusting the pH to 9.5. Afterwards, the solids were removed using a twin-screw press (Angelia 7500, Angel Juicer, Naarden, The Netherlands), and the supernatant was centrifuged at 10,000xg for 30 min, resulting in three layers: a pellet with solids, a top layer with cream, and the middle layer containing (soluble) proteins. The middle layer was recovered and dialyzed at a 3.5 kDa cut-off against demineralized water at 4 °C to remove small solutes. The dialysate was exchanged five times, and the dialyzed sample containing albumins and globulins was freeze-dried.

2.2.4. Protein content

The nitrogen content of the protein extracts was determined using a Flash EA 1112 Series Dumas (Interscience, Breda, The Netherlands). The obtained nitrogen content was converted into a protein content with a conversion factor of Nx5.7 (Fetzer, Herfellner, & Eisner, 2019). The protein content was expressed based on dry matter.

2.2.5. Sample dissolution

All protein samples were dissolved based on protein content (% w/w) in a 20 mM sodium phosphate buffer at pH 7.0. The samples were freshly prepared for all measurements by stirring at room temperature for 4 hrs and discarded 24 hrs after dissolution.

2.3. Determination of protein fractions

The protein fractions of the extracts was determined using sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. Protein solutions of 0.1% (w/w) were prepared in ultrapure water and allowed to hydrate for 4 hrs. The samples were mixed with 500 mM DTT and NuPAGE LDS sample buffer, and heated at 70 °C for 10 min. The samples were loaded on a 4–12% (w/w) BisTris gel together with a marker with a molecular weight ranging from 2.5 to 200 kDa. The electrophoresis was performed at 200 V for about 30 min, and the gel was stained with SimplyBlue Safestain. Finally, the gel was scanned in a gel scanner.

2.4. Protein zeta-potential

The zeta-potential of 0.01% (w/w) protein solutions was measured using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The refractive indices for the water phase and the proteins were set at 1.33 and 1.45, respectively (Ercili-Cura et al., 2015). At least 12 consecutive measurements were performed to ensure an accurate fit in one replicate, and total of three replicates were done at 20 °C.

2.5. Air-water interfacial properties

The interfacial properties were studied using a drop tensiometer PAT-1 M (Sinterface Technologies, Berlin, Germany). Protein solutions at 0.1% (w/w) were pumped through a hollow needle (diameter 1.98 mm) to create a hanging droplet at the tip of the needle. The droplet area was kept constant at 20 mm^2 and the shape of the droplet was fitted using the Young-Laplace equation to calculate the surface tension (Sagis, Humblet-Hua, & van Kempen, 2014). Dilatational deformations on the surface were performed after 3 hrs of equilibration time. The interface was subjected to amplitude sweeps. The amplitude sweeps were performed by increasing the amplitude from 3% to 30% at a constant frequency of 0.02 Hz. A set of five oscillations was performed for each amplitude, which was always followed by a pause phase for the duration of one oscillation. These measurements were performed at least in triplicate at 20 °C on two independently prepared samples.

2.6. Analysis of non-linear behavior

Non-linear behavior in the stress response in the amplitude sweeps

was analyzed using Lissajous plots by plotting the surface pressure ($\Pi(t) = \gamma(t)-\gamma_0$) over the deformation ($(A(t)-A_0)/A_0$). Here, $\gamma(t)$ and $A(t)$ are the surface tension and area of the deformed interface of the droplet, γ_0 and A_0 are the surface tension and area of the non-deformed interface. The middle three oscillations were taken from the set of five oscillation cycles of each amplitude.

2.7. Interfacial microstructure analysis

The interfacial microstructure was analyzed by performing atomic force microscopy (AFM) on Langmuir-Blodgett films. Langmuir-Blodgett films of the protein interfaces were produced in a Langmuir trough (Langmuir-Blodgett Trough KN 2002, KSV NIMA/Biolin Scientific Oy, Espoo, Finland) with an area of 243 mm^2. The trough was filled with about 200 mL of 20 mM sodium phosphate buffer at pH 7.0, and 200 μL of 0.04% (w/w) protein solution was spread on top of the surface using a gas-tight syringe. The interface was equilibrated for 30 min, and the surface pressure was monitored with a Wilhelmy plate (platinum, perimeter 20 mm, height 10 mm). After the equilibrium time, the interfacial layer was compressed by Teflon barriers at a moving speed of 5 mm/min. Surface pressure isotherms were created to select two surface pressures of interest to perform extraction of the Langmuir-Blodgett films, which were 15 and 25 mN/m. The interfacial layer was transferred on a freshly cleaved mica sheet (Highest Grade V1 Mica, Ted Pella, Redding, USA) at a withdrawal speed of 1 mm/min, while maintaining a constant target surface pressure. The duplicate films were prepared from two independently prepared samples at room temperature and dried overnight in a desiccator. After the drying step, the topography of the Langmuir-Blodgett films was analyzed using a Multimode 8-HR AFM (Bruker, Billerica USA). The measurement was performed in tapping mode with a Scanasyst-air model non-conductive pyramidal silicon nitride probe (Bruker, Billerica, USA) with a normal spring constant of 0.40 mN/m at a lateral frequency of 0.977 Hz. At least two 2 × 2 μm areas with a resolution of 512 × 512 pixels were analyzed for each replicate to ensure a good representation. The raw data was processed and analyzed using Nanoscope Analysis software v1.5 (Bruker, Billerica, USA).

2.8. Foaming properties

2.8.1. Foamability

The foamability was determined by whipping 15 mL of 0.1%, 0.18% or 1.0% (w/w) protein solution in a plastic tube (diameter 3.4 cm) for 2 min at 2000 rpm by an aerolatte froth (Aerolatte, Radlett, UK) connected to an overhead stirrer. The top and bottom of the foams were directly marked on the tubes, and the distance was measured with a ruler to obtain the foam height, which was converted into the foam volume using the diameter of the tube. The foam volume was used to determine the foam overrun (%) using Eq. (1). These measurements were performed at room temperature on independently prepared samples. Foam stability could not be studied using the whipping method, as the initial foam volumes were markedly different. As a result, a sparging method was used to create foams with similar foam volumes, which we described in Section 2.8.2.

$$\textit{Foam overrun}\ (\%) = \frac{\textit{Foam volume directly after whipping}\ (mL)}{\textit{Initial liquid volume}\ (15\ mL)}\ x\ 100 \quad (1)$$

2.8.2. Foam stability

The foam stability was determined using an automated foam analyzer Foamscan (Teclis IT-Concept, Civrieux-d'Azergues, France). A glass cylinder (diameter 60 mm) was filled with 40 mL of 0.1%, 0.18% or 1.0% (w/w) protein solution. Foam was formed by sparging nitrogen gas through a metal frit (27 μm pore size, 100 μm distance between centers of pores, square lattice) at a gas flow rate of 400 mL/min to a foam volume of 400 mL. A camera monitored the foam volume until a decay of 50%, also known as the foam volume half-life time. Detailed pictures of the air bubbles were recorded with a SLR lens, which were analyzed using DIPlip and DIPimage image analysis (TU Delft, Delft, the Netherlands) to obtain an average bubble size. These measurements were performed at 20 °C on independently prepared samples.

2.9. Statistical analysis

Experimental results were reported as mean and standard deviations of at least three replicate measurements ($N \geq 3$). The statistical analysis was performed using one-way ANOVA in SPSS 25.0 software (SPSS Inc., Chicago, USA). When significant differences ($p < 0.05$) were found, the results were compared using a post-hoc test (Duncan's).

3. Results and discussion

3.1. Protein extraction

We extracted mung bean (MB), Bambara groundnut (BGN) and yellow pea (PEA) proteins, which yielded globulin (MB-GLOB, BGN-GLOB and PEA-GLOB) and albumin (MB-ALB, BGN-ALB and PEA-ALB) protein extracts. The globulin extracts had protein contents ranging from 78.8% to 86.3% (w/w), while the albumin extracts had lower protein purities ranging from 47.6% to 57.7% (w/w) (Table 1). The protein purities of the albumin-rich extracts are in a similar range as those obtained for lupin, using a comparable extraction method, with a protein content of 57.0% (Wong et al., 2013). In the same work, the diafiltration step increased the protein content from 26.1% to 57.0%, suggesting the removal of substantial amount of non-protein components. The lower protein purity in the albumin extracts is due to the presence of non-proteinaceous components, such as minerals and polysaccharides, as shown previously for yellow pea (Kornet et al., 2020). The protein recovery (% of recovered protein expressed over the initial total protein content of the starting material) was 54.4–79.8% for globulins and 10.5–13.5% for albumins (Table 1), which is in line with the globulin and albumin composition in the crops.

3.2. Protein properties

We evaluated the effectiveness of the protein purification by characterizing the protein fractions using SDS-PAGE (Fig. 2), which showed a successful separation of albumins and globulins. The SDS-PAGE was performed under reducing conditions (breakage of disulfide bridges by adding a reducing agent), leading to the formation of protein subunits and monomers. The albumins of MB and BGN have not been extensively studied, but several albumins were identified for pea albumins ranging from 14 to 53 kDa (Lu et al., 2000), thereby facilitating the interpretation of Fig. 2. The albumin extracts had dark bands at molecular weights between 23 and 26 kDa. This most likely resembles the subunits of pea albumin known as pea albumin 2 (PA2). Another major albumin in pea is pea albumin 1 (PA1), with sizes of the intact protein and subunits ranging from 4 to 18 kDa, as observed for our albumin samples (Lu et al., 2000). The globulins had low-intensity bands resembling those of the albumin fractions, indicating the minor presence of albumins, while

Table 1
The protein content (% w/w) (Nx5.7) based on total dry matter and protein recovery (percentage of protein extracted from starting material) of the albumin and globulin extracts.

	Protein content (% w/w)		Protein recovery (%)	
	Albumin	Globulin	Albumin	Globulin
Mung bean	47.6 ± 1.3	79.1 ± 4.1	10.5	66.6
Bambara groundnut	57.7 ± 2.6	78.8 ± 0.4	13.5	79.8
Pea	52.0 ± 1.7	86.3 ± 1.4	12.3	54.4

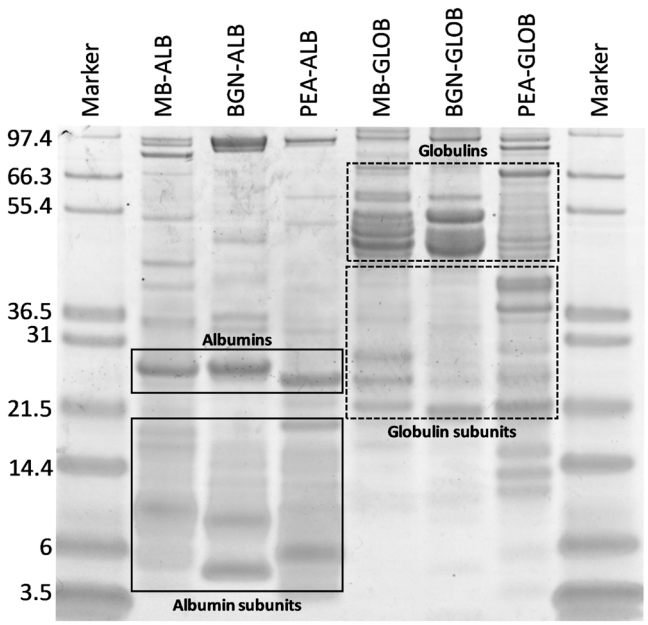


Fig. 2. SDS-PAGE profiles under reducing conditions of different albumin and globulin extracts as indicated on top. Abbreviations: MB-ALB (mung bean albumins), BGN-ALB (Bambara groundnut albumins), PEA-ALB (pea albumins), MB-GLOB (mung bean globulins, BGN-GLOB (Bambara groundnut globulins), and PEA-GLOB (pea globulins). A molecular weight marker is included and the corresponding molecular weights (kDa) are indicated on the outer lanes.

darker bands at higher molecular weights (20–70 kDa) correspond to globulins. The plant globulins are comprised of two sub-groups, known as vicilin and legumin, which were found to be in trimeric and hexameric form with molecular weights ranging from 170 to 385 kDa (Barać et al., 2015; Diedericks et al., 2019). Their quaternary protein structure breaks up into monomers/subunits under reducing conditions, and correspond to the bands between 50 and 60 kDa. The bands representing globulins were substantially less present on the lanes of albumin extracts, demonstrating isolation of a major fraction of globulins. The zeta-potential of the proteins was also evaluated (Table 2). A generic difference is present between the albumins and globulins at pH 7.0, as the albumins had zeta-potentials ranging from − 3.3 to − 2.0 mV, while globulins have more negative values ranging from − 14.8 to − 10.3 mV.

3.3. Foaming properties

We evaluated the foaming properties of the albumins and globulins by determining the foamability and stability in terms of foam overrun (% volume of foam formed, expressed over the initial liquid volume), average air bubble size (images are shown in Fig. S1 in the SI), and the foam half-life time (time required for half of the initial foam volume to collapse) (Fig. 3). Whey protein isolate (WPI) and egg white protein isolate (EPI) were included as animal-derived protein sources, which are commonly applied foam stabilizers in the food industry (Campbell & Mougeot, 1999). The albumin extracts created foams with a high overrun that ranged from 257% to 281%, while globulin extract-stabilized foams had overruns ranging from 12% to 61%, showing a significantly better foamability for albumins, as albumins formed 4–23 times higher foam volumes. Albumins were also better in creating smaller air bubbles with sizes around 0.06 mm, while those obtained with globulins were larger than 0.20 mm. The BGN-GLOB-stabilized foams even had air bubbles that were too large for accurate analysis. Foams stabilized by albumins exhibited exceptionally high foam stability with foam half-life times between 240 and 314 min, while foams stabilized by globulins had half-life times under 70 min. The albumins thus possess significantly better foamability and stability compared to globulins, as mentioned in studies for single sources, such as lupin, rapeseed and pea (Cheung et al., 2014; Lu et al., 2000; Wong et al., 2013).

In addition, albumins had foaming properties similar to those of WPI, and even better foaming properties than EPI. Albumins were able to form foams with smaller air bubbles sizes and higher stability than proteins from egg white. The comparable foaming properties of whey proteins and plant albumins could be related to similarities in protein structure. Both types of proteins are highly water-soluble (Kornet et al., 2020; Mavropoulou & Kosikowski, 1973), and the main proteins in whey, i.e., β-lactoglobulin and α-lactalbumin (14–55 kDa) (Butré, Wierenga, & Gruppen, 2012), are in a similar molecular weight range as the albumins (14–53 kDa, shown in Fig. 2). The main protein in EPI is ovalbumin and is known to be highly hydrophobic (Wierenga, Meinders, Egmond, Voragen, & Jongh, 2003), which impacts the interfacial properties, as shown in the following section.

3.4. Interfacial properties

The foaming properties of proteins are often related to the rheological properties they impart to the air-water interfacial film surrounding the air bubbles (Dickinson, 1999; Narsimhan & Xiang, 2018). First, we studied the adsorption behavior of the proteins by measuring the surface pressure (surface tension of ultrapure water minus surface tension of the protein solution) over time (Fig. 4a & b) at a 0.1% (w/w) protein content, which is similar to the protein content in the protein-stabilized foams (Fig. 3). The albumin extracts immediately increased the surface pressure to between 5 and 8 mN/m, followed by a gradual increase up to 15–22 mN/m, very similar to the surface pressure development with WPI. When comparing the albumins and globulins from MB and pea, a slower initial adsorption phase (first 10 s) was found for the globulins. The initial adsorption phase is important for the formation rate of interfaces in a foam prepared by sparging gas through small pores (as used here to determine foam stability). A rapid diffusion to the interface followed by adsorption leads to the detachment of an air bubble with a smaller size, as observed for albumin-stabilized foams in Fig. 3b. EPI led to a slow increase in surface pressure, probably due to a high surface hydrophobicity of ovalbumin, leading to aggregation (Wierenga et al., 2003). The adsorption behavior of EPI was similar to that observed with globulins, and resulted in larger air bubbles in comparison to albumin- and WPI-stabilized foams (Fig. 3b). The BGN-GLOB led to a fast increase in surface pressure, which seems inconsistent with the limited foam formation, but the reason for this discrepancy is related to the rheological properties of the protein layers at the interface.

The protein-stabilized interfaces were subjected to oscillatory dilatational deformations to obtain surface dilatational moduli (i.e., the dilatational storage, E_d', and loss modulus, E_d''), which provide information on the rheological properties of the interfacial layer, such as the stiffness of the interfacial layer (Fig. 4c & d). The albumin-stabilized interfaces had storage moduli values ranging from 81 to 91 mN/m at a low deformation amplitude of 3%, and these values gradually decreased when increasing the deformation amplitude up to 30%. A

Table 2
Zeta-potential (mV) of albumin and globulin extracts at pH 7.0 (20 mM phosphate buffer). The average and standard deviation was obtained from three replicates.

Albumins			Globulins		
MB	BGN	PEA	MB	BGN	PEA
-2.4 ± 1.4^{c}	-3.3 ± 1.5^{c}	-2.0 ± 1.4^{c}	-14.8 ± 0.9^{a}	-10.5 ± 3.7^{b}	-10.3 ± 2.2^{b}

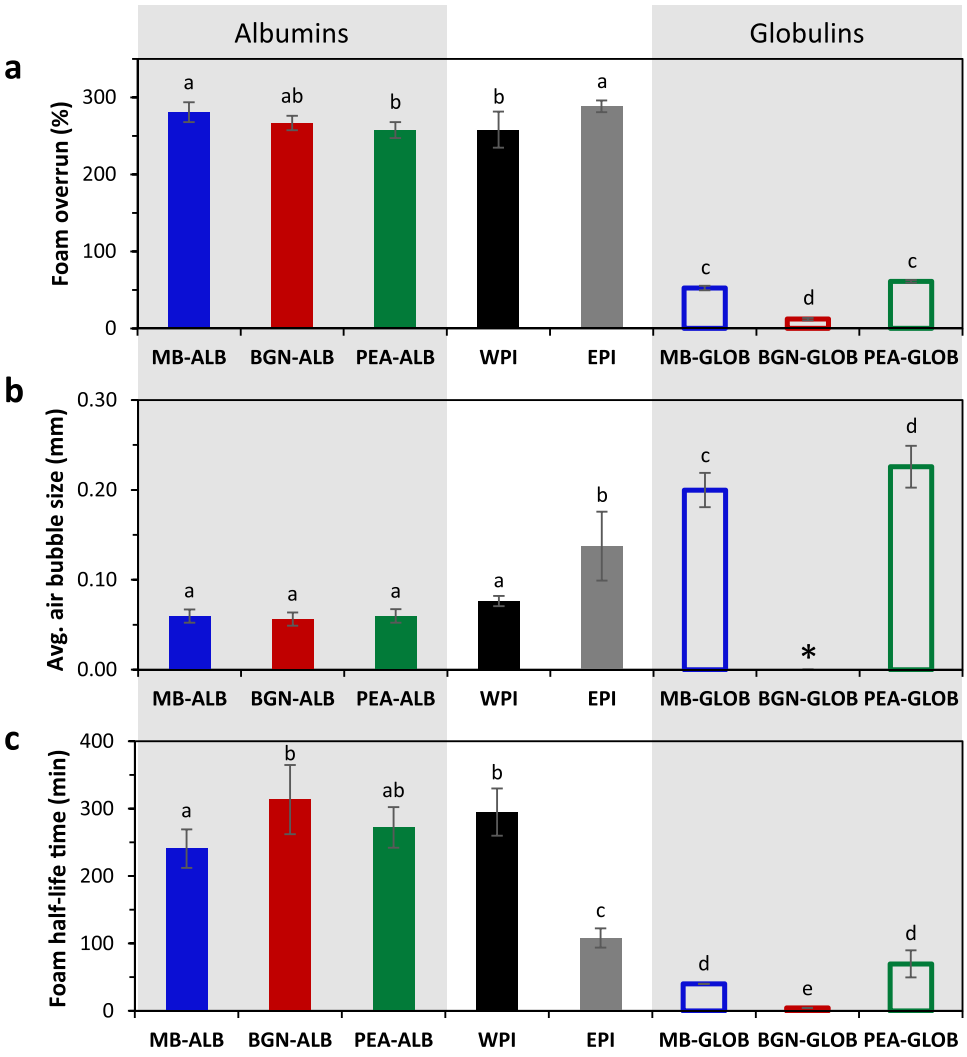


Fig. 3. The overrun (a), average air bubble size (b) and half-life time (c) of foams prepared with albumin and globulin extract solutions. Abbreviations: MB-ALB (mung bean albumins), BGN-ALB (Bambara groundnut albumins), PEA-ALB (pea albumins), MB-GLOB (mung bean globulins, BGN-GLOB (Bambara groundnut globulins), and PEA-GLOB (pea globulins). WPI (whey protein isolate) and egg white protein isolate (EPI) were included as references. The protein concentration of all samples was 0.1% (w/w), and samples were dissolved in a 20 mM phosphate buffer, pH 7.0. * The air bubbles in BGN-GLOB-stabilized foams were too large for accurate recording. The averages and standard deviations were calculated from at least three replicates.

similar decrease was also observed for WPI- or EPI-stabilized interfaces. Whey and egg white proteins are known to form interfaces with a strong and cohesive microstructure due to the strong (in-plane) interactions between protein molecules at the interface (Davis & Foegeding, 2007; Yang, Berton-Carabin, Nikiforidis, van der Linden, & Sagis, 2021). Large deformations disrupt the interfacial structure, and this explains the decrease of moduli at higher deformations. The presence of strong in-plane interactions between albumins at the interface could also be observed in Lissajous plots that were constructed from the oscillatory measurements (see SI Fig. S2 for details and explanation). The globulin-stabilized interfaces had much lower moduli compared to the albumin-stabilized ones. BGN-GLOB and PEA-GLOB showed surface moduli that were nearly independent of the deformation amplitude. This suggests the formation of a weaker interfacial layer, indicating the absence of strong in-plane interactions between globulins. This is confirmed by Lissajous plots (see SI Fig. S3), which also showed a more easily stretchable layer.

Weaker interfacial films formed by globulin proteins were also found by previous studies for sources, such as pea, sunflower, and rapeseed. Elastic moduli values (*Ed'*) around 16 mN/m were found for a commercial pea protein-stabilized air-water interfacial film (Hinderink, Sagis, Schroën, & Berton-Carabin, 2020). Additionally, these values were independent of the deformation amplitude, indicating the formation of weak and easily stretchable layers. For a soy protein isolate, moduli < 10 mN/m were obtained at 15% deformation for an air-water interface (Martinez, Carrera Sanchez, Pizones Ruiz-Henestrosa, Rodríguez Patino, & Pilosof, 2007). The low values for these pea and soy protein-stabilized interfaces are most likely related to the presence of mainly globulin proteins. For sunflower protein-stabilized air-water films, *Ed'* values between 10 and 55 mN/m were observed at a deformation amplitude of 5% (Poirier, Stocco, Kapel, In, & Ramos, 2021). Comparable values between 25 and 60 mN/m were shown at deformation amplitudes between 3% and 50% for rapeseed proteins (Yang et al., 2021). The latter two examples had moduli, which were generally higher than the moduli of interfaces stabilized by the globulins in this study. Perhaps the protein composition of these studies played a role, as the albumins were still present in both sunflower and rapeseed protein extracts. The presence of both albumins and globulins might have increased the interfacial stiffness, which again is an indication of the better performance of albumins compared to globulins in stabilizing interfacial films.

The formation of stiff interfacial layers by whey proteins was previously argued to be responsible for the high foamability and stability of WPI-stabilized foams (Yang et al., 2021). A stiff interfacial layer would reduce the coalescence rate (merging of air bubbles after interfacial film rupture) of air bubbles during foam formation and aging. If the air bubbles show slow or limited coalescence during foam creation, more and smaller bubbles are obtained, thus resulting in a high foam overrun (as observed for albumins, Fig. 3a). Egg white proteins formed stiff interfaces, similar to those obtained with albumins, but showed slower adsorption behavior towards the interface. As a result, the air bubbles were larger, leading to lower foam stability than with albumins. On the

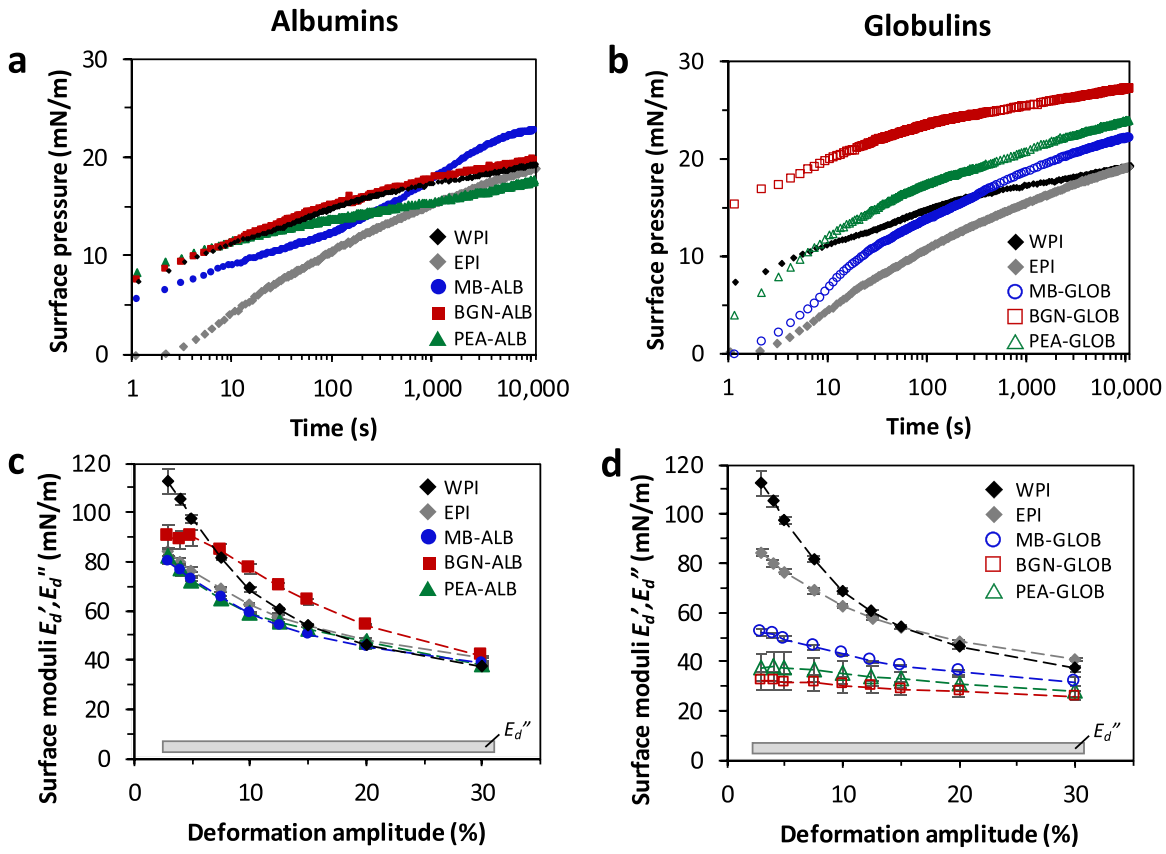


Fig. 4. The surface pressure against time for air-water interfacial films made of albumin (a) and globulin (b) extracts, and the surface dilatational modulus against deformation amplitude of albumin (c) and globulin (d) extracts (ω = 0.02 Hz). In panels c and d, the dilatational storage moduli (E_d') are shown as symbols with dotted lines as a guide for the eye. The dilatational loss moduli (E_d'') of all interfaces were between 2 and 9 mN/m, which is graphically shown with the gray bar. Abbreviations: MB-ALB (mung bean albumins), BGN-ALB (Bambara groundnut albumins), PEA-ALB (pea albumins), MB-GLOB (mung bean globulins, BGN-GLOB (Bambara groundnut globulins), and PEA-GLOB (pea globulins). WPI (whey protein isolate) and egg white protein isolate (EPI) were included as references. The protein concentration of all samples was 0.1% (w/w), and samples were dissolved in a 20 mM phosphate buffer, pH 7.0. The legend in the graphs describes the markers used. The curves in panels a and b are averages obtained from at least three replicates, and the standard deviations were below 5%. Averages and standard deviations on panels c and d were calculated from at least three replicates.

other hand, the stability of EPI-stabilized foams was higher than that obtained with globulins due to the stiffer interfacial layers formed by egg white proteins. Globulins form weaker interfaces, which facilitate faster coalescence of the air bubbles during the foam formation, causing low foam overrun. A lower interfacial stiffness could increase the rate of coalescence and disproportionation after foam formation (Narsimhan & Xiang, 2018), which explains the lower foam stability for globulins compared to albumins. Another point of attention is the low protein purity of the albumin-rich extracts (47.6 – 57.7%, Table 1). Interface and foam formation can be largely affected by non-proteinaceous components, such as phenols and phospholipids (Keppler et al., 2021; Rodríguez Patino, Carrera Sánchez, & Rodríguez Niño, 2008). We expect a major removal of these small surfactants during the filtration step. As shown by Kornet et al., the majority of the non-proteinaceous part comprises of saccharides (Kornet et al., 2020), which are probably too small for removal by centrifugation and too large in size to remove by filtration. These components are most likely not surface active. The potential composition of the protein layer can be further studied by interfacial microstructure visualization, as shown in the next section.

3.5. Interfacial microstructure

The difference between albumin and globulins-stabilized interfaces was further evaluated by visualization of the interfacial microstructure. We used Langmuir-Blodgett (LB) deposition to transfer the protein-stabilized interfacial layers onto a solid substrate. The topography of the films was accurately analyzed using atomic force microscopy (AFM) to obtain detailed insights into the interfacial microstructure (Fig. 5). On an LB-film created at a surface pressure of 15 mN/m, both PEA-ALB and PEA-GLOB showed structures, which were previously identified as protein clusters (Sagis et al., 2019), and which most likely consist of multiple proteins clustered into a larger structure. These clusters were found to be slightly larger on the PEA-GLOB films, which is in line with the fact that globulins also form large aggregates in bulk solutions (Kornet et al., 2020). According to Kornet et al., a large number of aggregates remain when returning to neutral pH after isoelectric point precipitation. A major difference can be observed when increasing the compression state of the film to a surface pressure of 25 mN/m. For the PEA-ALB films, a higher compression resulted in a denser film, while the PEA-GLOB film remained similarly dense. These findings are related to the type of interfacial layer formed: albumins form stiff and cohesive layers that can be compressed into a denser state, due to strong in-plane interactions. Similar rheological behavior at the interface was also observed for the WPI-stabilized films. The globulins at the interface had limited in-plane interactions between the proteins, thus a weaker binding to the interface. As a result, the proteins might be pushed out of the surface into the bulk or just below the surface (undetectable for AFM measurements) upon compression, which would result in a similar interfacial microstructure to that formed with PEA-GLOB at both a low (15 mN/m) and high (25 mN/m) compression.

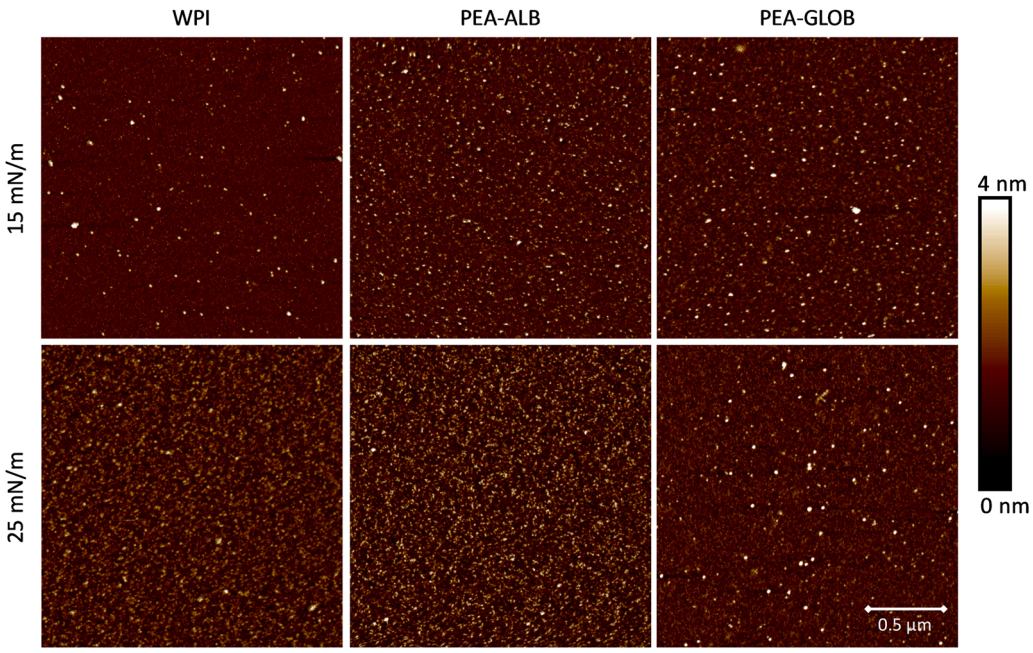


Fig. 5. AFM images of Langmuir-Blodgett films made from whey proteins, pea albumin and globulin extract, visualizing the interfacial microstructures. Abbreviations: WPI (whey protein isolate), PEA-ALB (pea albumin), and PEA-GLOB (pea globulin). The surface pressures during film sampling are indicated on the vertical axis. The scale bar is indicated in the bottom-right corner, and is the same for all images.

3.6. Interfacial films explained by molecular properties

The ability of albumins to form stiff and solid-like interfacial films could be related to the smaller size compared to globulins. A smaller protein size allows for more proteins to adsorb on the surface and create denser layers. Also, the lower net-electrostatic charge of albumins might lead to a more compact/dense interface. In comparison, globulins are larger and often more aggregated (Kornet et al., 2020) with a higher net charge, which, upon adsorption, leads to a less dense and more heterogeneous interfacial microstructure. Therefore, albumins cover the interface more effectively than globulins, resulting in the formation of stiff and cohesive interfacial layers. One point of attention is the pH-dependency of the net-electrostatic charge of proteins. As a result, the protein charges vary at different pHs, thus affecting the interactions at the interface.

Based on the Osborne classification, albumins are also more water-soluble than globulins, and increased soluble protein content positively affects foaming properties. Several properties of albumins are comparable to whey proteins, as the major whey proteins β-lactoglobulin and α-lactalbumin also have low molecular weights (14–18 kDa) (Butré et al., 2012) and are highly water-soluble (Mavropoulou & Kosikowski, 1973). Such similarities in physico-chemical properties between whey proteins and albumins could have resulted in a comparable performance at the interface and in foams. In addition, albumins formed foams that were up to three times more stable than foams made with egg white proteins, probably due to the faster adsorption of albumins towards the air-water interface.

Another major difference between albumins and globulins is their surface-exposed hydrophobicity, which was demonstrated for rapeseed proteins (Ntone et al., 2021). In rapeseed globulins (named cruciferins), the hydrophobic and hydrophilic regions are broadly distributed over the protein's surface. In contrast, the rapeseed albumin (napin) has a single large hydrophilic domain and a single, somewhat smaller, hydrophobic domain. Thus, the rapeseed albumin has a structure that has similarities with a Janus particle with two distinct regions with different wettability (Ntone et al., 2021). Albumins from various sources show similarities in their secondary and tertiary structures, and species-related changes in primary protein structure (amino acid sequence) barely affect the three-dimensional structure of these proteins (Souza, 2020). Therefore, the albumins in this work could also have characteristics similar to a Janus particle (distinct hydrophilic/hydrophobic regions), similar to the structure of rapeseed albumin. Such a distinct distribution of hydrophobic and hydrophilic regions could promote fast adsorption of the albumins at the interface, and stronger intermolecular interactions once adsorbed.

Albumins possess substantially better foaming properties compared to globulins, and albumins even have comparable foaming properties as WPI and EPI. The albumin proteins could be valorized as a side-stream from globulin extraction. We could also consider the production of a protein mixture, containing both albumins and globulins, which we will discuss in the following section.

3.7. Including albumins by extracting albumin – globulin mixtures

Albumins and globulins can also be co-extracted as a mixture, by omitting the isoelectric point precipitation step of the globulins. Other non-proteinaceous solutes can be removed using dialysis or filtration. We extracted a BGN-protein mixture (BGN-PM) with a protein purity of 62.1% (w/w) using a co-extraction method. As a result, the BGN-PM showed the presence of albumins, which were absent in the BGN-GLOB extract (Fig. 6a). The presence of the albumins in the BGN-PM resulted in a significant difference with a 9-fold higher foam overrun and an 8-fold higher foam stability compared to BGN-GLOB with only globulins (Fig. 6b & c). We also included a BGN-ALB sample with a 0.18% (w/w) protein, equal to the amount of albumins in the BGN-PM sample. The BGN-PM had a lower foam overrun, but higher foam stability compared to the BGN-ALB sample. The globulins in BGN-GLOB created foams with low overrun and appear to negatively influence the foaming properties of the protein mixture, where globulins and albumins are both present. On the other hand, globulins in the protein mixture also seemed to induce an additional increase in foam stability on top of the contribution of the albumins. Excluding the defatting step results in the presence of lipids, as the BGN-PM had an oil content of 10.8%. These lipids did not seem to affect the foaming properties, which was also demonstrated by previous work on rapeseed protein extracts, produced with a similar method (Yang et al., 2020). Here, the lipids are

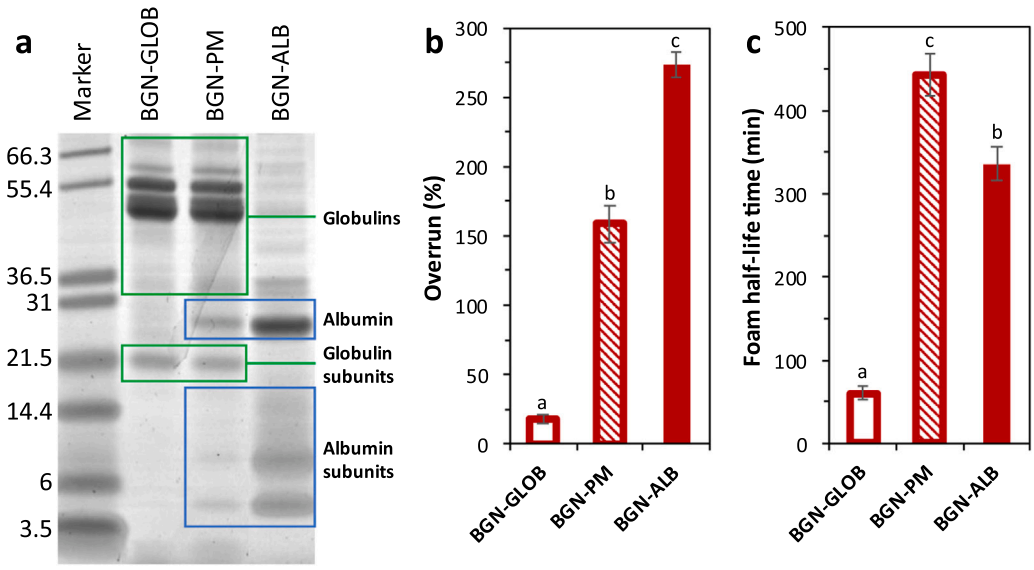


Fig. 6. (a) SDS-PAGE profile under reducing conditions of BGN-GLOB (Bambara groundnut globulin), BGN-PM (Bambara groundnut – protein mixture) and BGN-ALB (Bambara groundnut albumin). A molecular weight marker is included, and the corresponding molecular weights (kDa) are indicated on the left. The foam overrun (b) and foam half-life time (c) of these samples are also shown. The BGN-GLOB and BGN-PM-stabilized foams were produced from 1.0% (w/w) protein solutions. The BGN-ALB-stabilized foams were produced from 0.18% protein solutions, which was similar to the amount of albumin in a 1.0% (w/w) BGN-PM protein solution. Samples were dissolved in a 20 mM phosphate buffer, pH 7.0. The averages and standard deviation were calculated from three replicates.

able to affect the interfacial properties, but foams with high stability (half-life times above 5 hrs) can be obtained. The proteins seems to be able to outcompete the lipids for the interface, as the lipids are trapped in their original colloidal structures, called oleosomes or oil bodies (Yang et al., 2021).

Interestingly, the protein mixture led to higher foam stability than a foam stabilized by albumins, revealing a contribution of globulins to the foam stability. This could be related to the processing of the concentrates, as the isoelectric point precipitation step of globulins was excluded in the production of the protein mixture. The avoidance of the isoelectric point precipitation was previously found to prevent the formation of more, larger and insoluble globulin aggregates (Kornet et al., 2020), which suggests the presence of less aggregated globulins in the protein mixture, leading to increased foaming properties. Also, for emulsions, a synergy between albumin and globulins from rapeseed was observed, as the albumins were more effective in stabilizing the oil-water interface, whereas the globulins formed a secondary layer on top of the albumin layer that protected the oil droplets against flocculation (Ntone et al., 2021).

4. Conclusion

The current focus on plant protein functionality, with respect to interface and foam stabilizing properties, is focused on globulin-dominated extracts. In the extraction of plant globulins, albumins is generated as a side-stream, thus generally underutilized in food systems. In this work, we show the high potential of albumins in interface and foam stabilization, as albumins are able to form dense and stiff interfacial layers. Albumins are able to form such layers due to their small molecular size, low protein charge, and probably their distribution in hydrophobicity. This leads to the stable foams, comparable to whey protein stabilized foams, and even more stable than egg white protein stabilized ones. On the other hand, the globulin-dominated extracts are poor foam stabilizers, probably due to their largely aggregated structure. The foam stabilizing potential of plant albumins can also be utilized by co-extracting them with globulins using a mild extraction method, yielding a protein mixture. The protein mixture showed a significantly higher foam overrun compared to globulins, and even a higher foam stability compared to albumins.

Albumins are a promising food ingredient with the ability to substitute dairy- and egg-derived foaming agents. A drawback of some albumins, such as those extracted from pea and mustard seeds, is that they can trigger allergenic responses (Souza, 2020). This attribute should be carefully addressed before specific albumins can be applied as food ingredients, but also in general, as traces of albumin can remain in plant globulin-rich extracts. We should increase the attention paid to obtain plants albumins and unravel their functionalities in industry and academia. The sustainability aspect of plant-derived ingredients will increase immensely by upgrading plant albumins from a side stream into a valuable key ingredient in our food products.

Conflict of interest

The authors have declared that no competing interest exist. This manuscript has not been published and is not under consideration for publication in any other journal. All authors approve this manuscript and its submission to Food Structure.

Acknowledgements

The project is funded by TiFN, a public-private partnership on pre-competitive research in food and nutrition. The public partners are responsible for the study design, data collection and analysis, decision to publish, and preparation of the manuscript. The private partners have contributed to the project through regular discussion. This research was performed with additional funding from the Netherlands Organisation for Scientific Research (NWO), and the Top Consortia for Knowledge and Innovation of the Dutch Ministry of Economic Affairs (TKI). NWO project number: ALWTF.2016.001.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foostr.2022.100254.

References

Aiking, H., & de Boer, J. (2018). The next protein transition. *Trends in Food Science and Technology, 105*(2018), 515–522. https://doi.org/10.1016/j.tifs.2018.07.008

Amagliani, L., & Schmitt, C. (2017). Globular plant protein aggregates for stabilization of food foams and emulsions. *Trends in Food Science and Technology, 67*, 248–259. https://doi.org/10.1016/j.tifs.2017.07.013

Barać, M. B., Pešić, M. B., Stanojević, S. P., Kostić, A. Z., & Čabrilo, S. B. (2015). Techno-functional properties of pea (Pisum sativum) protein isolates-a review. *Acta Periodica Technologica, 46*, 1–18. https://doi.org/10.2298/APT1546001B

Butré, C. I., Wierenga, P. a, & Gruppen, H. (2012). Effects of ionic strength on the enzymatic hydrolysis of diluted and concentrated whey protein isolate. *Journal of Agricultural and Food Chemistry, 60*(22), 5644–5651. https://doi.org/10.1021/jf301409n

Campbell, G. M., & Mougeot, E. (1999). Creation and characterisation of aerated food products. *Trends in Food Science and Technology, 10*(9), 283–296. https://doi.org/10.1016/S0924-2244(00)00008-X

Chéreau, D., Videcoq, P., Ruffieux, C., Pichon, L., Motte, J. C., Belaid, S., & Lopez, M. (2016). Combination of existing and alternative technologies to promote oilseeds and pulses proteins in food applications. *OCL - Oilseeds and Fats, Crops and Lipids, 41*(1). https://doi.org/10.1051/ocl/2016020

Cheung, L., Wanasundara, J., & Nickerson, M. T. (2014). Effect of pH and NaCl on the emulsifying properties of a napin protein isolate. *Food Biophysics, 10*(1), 30–38. https://doi.org/10.1007/s11483-014-9350-7

Chua, J. Y., & Liu, S. Q. (2019). Soy whey: More than just wastewater from tofu and soy protein isolate industry. *Trends in Food Science and Technology, 91*, 24–32. https://doi.org/10.1016/j.tifs.2019.06.016

Davis, J. P., & Foegeding, E. A. (2007). Comparisons of the foaming and interfacial properties of whey protein isolate and egg white proteins. *Colloids and Surfaces B: Biointerfaces, 54*(2), 200–210. https://doi.org/10.1016/j.colsurfb.2006.10.017

Dickinson, E. (1999). Adsorbed protein layers at fluid interfaces: Interactions, structure and surface rheology. *Colloids and Surfaces B: Biointerfaces, 15*(2), 161–176. https://doi.org/10.1016/S0927-7765(99)00042-9

Diedericks, C. F., de Koning, L., Jideani, V. A., Venema, P., & van der Linden, E. (2019). Extraction, gelation and microstructure of Bambara groundnut vicilins. *Food Hydrocolloids, 97*, Article 105226. https://doi.org/10.1016/j.foodhyd.2019.105226

Djemaoune, Y., Cases, E., & Saurel, R. (2019). The effect of high-pressure microfluidization treatment on the foaming properties of pea albumin aggregates. *Journal of Food Science, 84*(8), 2242–2249. https://doi.org/10.1111/1750-3841.14734

Ercili-Cura, D., Miyamoto, A., Paananen, A., Yoshii, H., Poutanen, K., & Partanen, R. (2015). Adsorption of oat proteins to air-water interface in relation to their colloidal state. *Food Hydrocolloids, 44*, 183–190. https://doi.org/10.1016/j.foodhyd.2014.09.017

Fetzer, A., Herfellner, T., & Eisner, P. (2019). Rapeseed protein concentrates for non-food applications prepared from pre-pressed and cold-pressed press cake via acidic precipitation and ultrafiltration. *Industrial Crops and Products, 132*, 396–406. https://doi.org/10.1016/j.indcrop.2019.02.039

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., & Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Science, 327*(5967), 812–818. https://doi.org/10.1126/science.1185383

Hinderink, E. B. A., Sagis, L., Schroën, K., & Berton-Carabin, C. C. (2020). Behavior of plant-dairy protein blends at air-water and oil-water interfaces. *Colloids and Surfaces B: Biointerfaces, 192*, Article 111015. https://doi.org/10.1016/j.colsurfb.2020.111015

Kapp, G. R., & Bamforth, C. W. (2002). The foaming properties of proteins isolated from barley. *Journal of the Science of Food and Agriculture, 82*(11), 1276–1281. https://doi.org/10.1002/jsfa.1177

Karaca, A. C., Low, N., & Nickerson, M. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International, 44*(9), 2742–2750. https://doi.org/10.1016/j.foodres.2011.06.012

Keppler, J. K., Heyse, A., Scheidler, E., Uttinger, M. J., Fitzner, L., Jandt, U., & Biedendieck, R. (2021). Towards recombinantly produced milk proteins: Physicochemical and emulsifying properties of engineered whey protein beta-lactoglobulin variants. *Food Hydrocolloids, 110*(July 2020). https://doi.org/10.1016/j.foodhyd.2020.106132

Kim, W., Wang, Y., & Selomulya, C. (2020). Dairy and plant proteins as natural food emulsifiers. *Trends in Food Science and Technology, 105*(September), 261–272. https://doi.org/10.1016/j.tifs.2020.09.012

Kornet, C., Venema, P., Nijsse, J., van der Linden, E., van der Goot, A. J., & Meinders, M. (2020). Yellow pea aqueous fractionation increases the specific volume fraction and viscosity of its dispersions. *Food Hydrocolloids, 99*, Article 105332. https://doi.org/10.1016/j.foodhyd.2019.105332

Kornet, R., Yang, J., Venema, P., van der Linden, E., & Sagis, L. (2021). Optimizing pea protein fractionation to yield protein fractions with a high foaming and emulsifying capacity. *Food Hydrocolloids, 126*(December 2021), Article 107456. https://doi.org/10.1016/j.foodhyd.2021.107456

Lam, A. C. Y., Can Karaca, A., Tyler, R. T., & Nickerson, M. T. (2018). Pea protein isolates: Structure, extraction, and functionality. *Food Reviews International, 34*(2), 126–147. https://doi.org/10.1080/87559129.2016.1242135

Loveday, S. M. (2020). Plant protein ingredients with food functionality potential. *Nutrition Bulletin, 45*(3), 321–327. https://doi.org/10.1111/nbu.12450

Lu, B. Y., Quillien, L., & Popineau, Y. (2000). Foaming and emulsifying properties of pea albumin fractions and partial characterisation of surface-active components. *Journal of the Science of Food and Agriculture, 80*(13), 1964–1972. https://doi.org/10.1002/1097-0010(200010)80:13<1964::AID-JSFA737>3.0.CO;2-J

Malabat, C., nchez-Vioque, R. I. S., Rabiller, C., & Gu guen, J. (2001). Emulsifying and foaming properties of native and chemically modified peptides from the 2S and 12S proteins of rapeseed (Brassica napus L. *Journal of the American Oil Chemists' Society, 78*(3), 235–242. https://doi.org/10.1007/s11746-001-0251-x

Martinez, K. D., Carrera Sanchez, C., Pizones Ruiz-Henestrosa, V., Rodríguez Patino, J. M., & Pilosof, A. M. R. (2007). Soy protein-polysaccharides interactions at the air-water interface. *Food Hydrocolloids, 21*(5–6), 804–812. https://doi.org/10.1016/j.foodhyd.2006.11.005

Mavropoulou, I. P., & Kosikowski, F. V. (1973). Composition, solubility, and stability of whey powders. *Journal of Dairy Science, 56*(9), 1128–1134. https://doi.org/10.3168/jds.S0022-0302(73)85321-4

Narsimhan, G., & Xiang, N. (2018). Role of proteins on formation, drainage, and stability of liquid food foams. *Annual Review of Food Science and Technology, 9*, 45–63. https://doi.org/10.1146/annurev-food-030216-030009

Ntone, E., Wesel, T., Van, Sagis, L. M. C., Meinders, M., Bitter, J. H., & Nikiforidis, C. V. (2021). Adsorption of rapeseed proteins at oil / water interfaces. Janus-like napins dominate the interface. *Journal of Colloid and Interface Science, 583*, 459–469. https://doi.org/10.1016/j.jcis.2020.09.039

Osborne, T. B. (1924), *Longmans green and coThe vegetable proteins*.

Peng, D., Jin, W., Arts, M., Yang, J., Li, B., & Sagis, L. M. C. (2020). Effect of CMC degree of substitution and gliadin/CMC ratio on surface rheology and foaming behavior of gliadin/CMC nanoparticles. *Food Hydrocolloids, 107*, Article 105955. https://doi.org/10.1016/j.foodhyd.2020.105955

Poirier, A., Stocco, A., Kapel, R., In, M., & Ramos, L. (2021). Sunflower proteins at air − water and oil − water interfaces. *Langmuir, 37*, 2714–2727. https://doi.org/10.1021/acs.langmuir.0c03441

Rodríguez Patino, J. M., Carrera Sánchez, C., & Rodríguez Niño, M. R. (2008). Implications of interfacial characteristics of food foaming agents in foam formulations. *Advances in Colloid and Interface Science, 140*(2), 95–113. https://doi.org/10.1016/j.cis.2007.12.007

Sagis, L. M. C., Humblet-Hua, K. N. P., & van Kempen, S. E. H. J. (2014). Nonlinear stress deformation behavior of interfaces stabilized by food-based ingredients. *Journal of Physics-Condensed Matter, 26*(46), 9. https://doi.org/10.1088/0953-8984/26/46/464105

Sagis, L. M. C., Liu, B., Li, Y., Essers, J., Yang, J., Moghimikheirabadi, A., & Schroen, K. (2019). Dynamic heterogeneity in complex interfaces of soft interface-dominated materials. *Scientific Reports, 9*(1), 1–12. https://doi.org/10.1038/s41598-019-39761-7

Samtiya, M., Aluko, R. E., & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Production, Processing and Nutrition, 2*(1), 1–14. https://doi.org/10.1186/s43014-020-0020-5

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

Shevkani, K., Singh, N., Kaur, A., & Rana, J. C. (2015). Structural and functional characterization of kidney bean and field pea protein isolates: A comparative study. *Food Hydrocolloids, 43*, 679–689. https://doi.org/10.1016/j.foodhyd.2014.07.024

Souza, P. F. N. (2020). The forgotten 2S albumin proteins: Importance, structure, and biotechnological application in agriculture and human health. *International Journal of Biological Macromolecules, 164*, 4638–4649. https://doi.org/10.1016/j.ijbiomac.2020.09.049

Tripathi, A. D., Mishra, R., Maurya, K. K., Singh, R. B., & Wilson, D. W. (2018). Estimates for world population and global food availability for global health. *The role of functional food security in global health*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-813148-0.00001-3

Wang, L., Wu, Z., Zhao, B., Liu, W., & Gao, Y. (2013). Enhancing the adsorption of the proteins in the soy whey wastewater using foam separation column fitted with internal baffles. *Journal of Food Engineering, 119*(2), 377–384. https://doi.org/10.1016/j.jfoodeng.2013.06.004

Wierenga, P. A., Meinders, M. B. J., Egmond, M. R., Voragen, F. A. G. J., & Jongh, H. H. J. de (2003). Protein exposed hydrophobicity reduces the kinetic barrier for adsorption of ovalbumin to the air-water interface. *Langmuir, 19*, 8964–8970.

Wong, A., Pitts, K., Jayasena, V., & Johnson, S. (2013). Isolation and foaming functionality of acid-soluble protein from lupin (Lupinus angustifolius) kernels. *Journal of the Science of Food and Agriculture, 93*(15), 3755–3762. https://doi.org/10.1002/jsfa.6249

Yang, J., Berton-Carabin, C. C., Nikiforidis, C. V., van der Linden, E., & Sagis, L. M. C. (2021). Competition of rapeseed proteins and oleosomes for the air-water interface and its effect on the foaming properties of protein-oleosome mixtures. *Food Hydrocolloids, 122*, Article 107078. https://doi.org/10.1016/j.foodhyd.2021.107078

Yang, J., Faber, I., Berton-Carabin, C. C., Nikiforidis, C. V., van der Linden, E., & Sagis, L. M. C. (2020). Foams and air-water interfaces stabilised by mildly purified rapeseed proteins after defatting. *Food Hydrocolloids, 112*, Article 106270. https://doi.org/10.1016/j.foodhyd.2020.106270

Yang, J., Lamochi Roozalipour, S. P., Berton-Carabin, C. C., Nikiforidis, C. V., van der Linden, E., & Sagis, L. M. C. (2021). Air-water interfacial and foaming properties of whey protein - sinapic acid mixtures. *Food Hydrocolloids, 112*, Article 106467. https://doi.org/10.1016/j.foodhyd.2020.106467

Yang, J., & Sagis, L. M. C. (2021). Interfacial behavior of plant proteins – novel sources and extraction methods. *Current Opinion in Colloid & Interface Science, 56*, Article 101499. https://doi.org/10.1016/j.cocis.2021.101499

Yang, J., de Wit, A., Diedericks, C. F., Venema, P., van der Linden, E., & Sagis, L. M. C. (2022). Foaming and emulsifying properties of extensively and mildly extracted Bambara groundnut proteins: A comparison of legumin, vicilin and albumin protein. *Food Hydrocolloids, 123*, Article 107190. https://doi.org/10.1016/j.foodhyd.2021.107190

Yi-Shen, Z., Shuai, S., & Fitzgerald, R. (2018). Mung bean proteins and peptides: Nutritional, functional and bioactive properties. *Food and Nutrition Research, 62*, 1–11. https://doi.org/10.29219/fnr.v62.1290

Yong, S., Sim, J., Srv, A., & Chiang, J. H. (2021). Plant proteins for future foods: A roadmap. *Foods*, 1–31.