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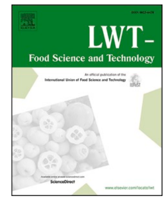

Foaming properties of plant protein blends prepared using commercial faba bean and hemp protein concentrates at different faba bean/hemp protein ratios

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ABSTRACT

Faba bean and hemp protein blends offer potential for the formulation of plant-based products due to their complementary amino acid compositions. This study evaluated the chemical composition, particle size, protein solubility and foaming properties of four commercial protein concentrates (two faba bean-based: FBC1 and FBC2, and two hemp-based: HPC1 and HPC2) and their blends at different faba bean/hemp protein ratios. The concentrates consisted mainly of albumins and globulins, some of which could have suffered alterations during processing, especially in HPC2 and FBC2. Both FBCs and HPCs made proportional contributions to the protein solubility observed in the blends. However, FBC1:HPC2 blends consistently demonstrated smaller particle sizes across all investigated ratios than those predicted by linear interpolation. Foaming properties of the four concentrates were comparable, except for HPC2, which did not foam. FBC1:HPC2 blends formed stable foams, whereas FBC2:HPC2 blends resulted in unstable foams. These results suggested that foam-destabilising factors were dominant in HPC2, which were counteracted by foam-stabilising factors in FBC1 and/or dilution effects. FBC1:HPC2 blends exhibited early indications of synergistic interactions, enhancing foam properties. This study demonstrates the potential of faba bean and hemp protein blends for foam-based food applications and the importance of considering their extraction processing history.

1. Introduction

New perspectives in the sustainable food sector have led to an increased demand for innovative plant-based products with a high protein content, which also deliver balanced amino acid profiles (Estell et al., 2021). The nature and characteristics of plant and animal proteins differ. While the fractionation and functionalisation of animal proteins is overall well established, extraction of plant proteins still represents a challenge. Several approaches are currently being used in the food industry to extract protein from plants using both, dry and wet strategies. Dry extraction results in techno-functional concentrates with rather low protein content (40–70%), whereas wet extraction can lead to concentrates with higher protein content (60–95%) but often with reduced techno-functionality (Assatory et al., 2019; Kumar et al., 2021). Furthermore, the digestibility and nutritional quality of plant proteins may be lower than those of animal proteins (Day et al., 2022). Protein quality is commonly measured using methods such as the protein digestibility-corrected amino acid score (PDCAAS) or the digestible indispensable amino acid score (DIAAS). For instance, whey protein isolate has PDCAAS and DIAAS of 97 and 100%, respectively, whereas brown rice protein isolate has PDCAAS and DIAAS scores of 46 and 64%, respectively (Day et al., 2022; Jiménez-Munoz et al., 2021).

Recently, blending animal and plant proteins has been considered as an approach to improve the techno-functional and nutritional properties of proteins for the formulation of new food products. However, this may not be an option for consumers who exclusively follow a plant-based diet (Alves & Tavares, 2019; Hinderink et al., 2020). Therefore, blending different plant protein sources has been proposed as a promising alternative, not only to adjust the amino acid profile, but also to improve techno-functional properties. When different plant proteins with

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complementary amino acid profiles are combined, e.g., pulses-cereals or pulses-oilseeds, their PDCAAS and DIAAS scores can be close or equal to those of animal proteins, reaching optimal levels for human nutrition (Herreman et al., 2020; Jiménez-Munoz et al., 2021). Moreover, it has been previously reported that blending plant proteins could lead to protein-protein interactions during processing, e.g., high-shear mixing, thermal treatment, hydration, and/or pH shift, resulting in an improvement in the overall techno-functionality of the blends (He et al., 2020). However, studies focusing on the techno-functional properties of plant protein blends are limited (Jiménez-Munoz et al., 2021).

Foaming is one of the most important techno-functional properties in the food industry, and animal proteins present in milk and egg whites are the most widely used for foam production (Amagliani et al., 2021). Foams are commonly employed in the food industry to manufacture products such as creamers or ice cream. During foaming, proteins are adsorbed at the air/water interface, reducing the surface tension between the air bubbles, thus stabilising the foam (Narsimhan & Xiang, 2018). Protein attributes such as structure, hydrophobicity, surface charge and flexibility at the air/water interface are key parameters for foam stabilisation (Sagis & Yang, 2022). Some plant-based foaming agents, such as those derived from soy, peas, and other plant sources, are commonly used in the formulation of plant-based food and beverage products. They play a crucial role in achieving texture, structure, and mouthfeel similar to their animal-based counterparts. For example, plant based globulins have been reported to be effective foam stabilisers, exhibiting high foaming capacity and foam stability (Amagliani et al., 2021; Sagis & Yang, 2022).

Plant proteins derived from hemp (*Cannabis sativa*) and faba bean (*Vicia faba*) are of current interest in the food industry. Hemp is an oleaginous seed, and its protein is often recovered as a side-stream of oil extraction (Nasrollahzadeh et al., 2022). Faba bean is a pulse and, in some countries, is regarded as surplus production (Bangar & Kajla, 2022). Consequently, the valorisation and incorporation of these plant proteins into the food industry are attractive from a sustainability perspective. While hemp proteins have been reported to exhibit poor techno-functional properties, especially due to the presence of insoluble edestin (Liu et al., 2023), faba bean proteins have been reported to be excellent foam stabilisers (Alavi, Chen, Wang, & Emam-Djomeh, 2021; Martínez-Velasco et al., 2018; Mattila et al., 2018). In terms of their nutritional properties, faba bean and hemp proteins have complementary amino acid profiles. Faba bean protein is rich in lysine, whereas hemp protein contains only small amounts thereof. Conversely, faba bean protein is low in sulfur-containing amino acids (methionine and cysteine), which are abundant in hemp protein. Therefore, the blend of these two plant proteins could be beneficial from both nutritional and techno-functional perspectives and could serve as an alternative to animal protein-based foams.

This study aimed to investigate the foaming properties of commercial faba bean and hemp protein concentrates, both individually and in combination, using different faba bean to hemp protein ratios (100:0, 80:20, 60:40, 50:50, 40:60, 20:80, and 0:100) at 3% w/w protein and pH 7.0. The investigation involved characterising the protein composition, analysing particle sizes, assessing protein solubility, and investigating foaming properties such as foam capacity and foam stability for blends of faba bean and hemp protein concentrates.

2. Materials and methods

2.1. Materials

Four commercial plant protein concentrates, two faba bean-based and two hemp-based, were used to generate protein blends. They all had a protein content below 90% (w/w), and were therefore named protein concentrates in the current study, in accordance with Schutyser and van der Goot (2011). Faba bean protein concentrates FFBP-60 (FBC1) and FFBP-90 (FBC2) were supplied by AGT Foods (Regina, SK, Canada). FBC1 was obtained via air classification (dry fractionation), whereas FBC2 was obtained via alkaline extraction followed by isoelectric precipitation. Hemp protein concentrate V-70 (HPC1) was supplied by Applied Foods (Austin, TX, US), while Pure 85% Hemp Protein concentrate (HPC2) was supplied by Good Hemp (Barnstaple, North Devon, UK). HPC1 and HPC2 were both obtained using wet processing. For HPC1, the extraction procedure included dehulling, heat treatment, hydrostatic pressure, solvent extraction, and milling treatments. For HPC2, no further information was provided by the supplier. All chemicals and reagents were purchased from Sigma-Merck (Buchs, Switzerland), unless otherwise stated.

2.2. Compositional analysis

The nitrogen content of the protein concentrates was analysed using a LECO system (FP828 P, Leco, Michigan, US) following the Dumas method. Combustion was performed at 950 °C under argon atmosphere, and EDTA was used as a reference for calibration. A nitrogen-to-protein-conversion factor of 6.25 was applied to estimate the protein content. Moisture content was determined using a halogen dryer (HC 103, Mettler Toledo, Switzerland). The concentrates (1 g) were placed in the middle of the furnace, heated to 137 °C and kept at this temperature until no weight changes were detected. The weight loss after water evaporation was used to determine the moisture content. The lipid content of the concentrates was determined by batch solvent extraction using n-heptane. Finally, the carbohydrate fraction was calculated as remainder after subtracting the protein, water and lipid contents.

2.3. Preparation of protein suspensions

The concentrates (100:0, 0:100) were mixed at different faba bean: hemp protein ratios (80:20, 60:40, 50:50, 40:60, 20:80) and suspended in water to obtain suspensions with a final protein content of 3% (w/w) following the method described in Silva et al. (2019) with some modifications. Briefly, the samples were stirred for 30 min at 25 °C and stored at 4 °C overnight (approximately 16 h) to ensure proper hydration. The following day, the samples were maintained at 25 °C for 1 h and their pH was adjusted to 7.0 using 1N NaOH or 1N HCl. The samples were then homogenised using a Polytron high-shear mixer (Kinetica AG, Malters, Switzerland) at 8000 rpm for 1 min.

2.4. Gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to characterise the protein profile of soluble and total protein suspensions. To prepare the soluble protein fraction, total protein suspensions were centrifuged at 13,000×*g* for 5 min. The supernatant was mixed with Laemmli loading buffer (Bio-Rad, Cressier, Switzerland). For total protein, no centrifugation step was performed before electrophoresis.

For SDS-PAGE under reducing conditions, samples were suspended in Laemmli loading buffer containing 5% β-mercaptoethanol. For non-reducing conditions, samples were resuspended in Laemmli loading buffer in the absence of β-mercaptoethanol. Then, 25 μL of each sample (diluted to a final protein content of 50 μg) was placed in a precast gel (Criterion Precast Gel, 12.0% TGX, Bio-Rad). After running the gel at 150 V for 45 min, it was incubated for 30 min in a solution of 40% (v/v) methanol, 10% (v/v) acetic acid, and 50% (v/v) water, followed by a staining solution (0.1% (w/v) Coomassie R-250 in 40% (v/v) methanol, 10% (v/v) acetic acid, and 50% (v/v) water) for another 30 min. After removal of the staining solution, the gel was washed with deionised water and destained with a solution containing 40% (v/v) methanol, 10% (v/v) acetic acid, and 50% (v/v) water for 30 min, followed by another solution of 10% (v/v) methanol, 10% (v/v) acetic acid, and 80% (v/v) water until it was fully destained.

For SDS-PAGE of the foam samples, foam (1 g) was collected and

freeze-dried using a LSCplus freeze dryer (Martin Christ, Osterode am Harz, Germany). The freeze-dried foam was then resuspended in water at 1% (w/v) and added to the SDS-PAGE gel following the protocol described above. For foam samples, the SDS-PAGE was performed only under reduced conditions.

2.5. Particle size determination

The particle sizes of the protein suspensions were analysed using an LS13320 Beckman Coulter (Nyon, Switzerland). The suspensions were added to the universal liquid module until an obscuration value between 9 and 12% was reached. The refractive index of water was set to 1.33. The D [4,3] values, i.e. the De Brouckere mean diameters, were automatically calculated using the equipment software.

2.6. Fluorescence microscopy

Images of the suspensions were obtained as previously described in Silva et al. (2019) with some modifications. An aliquot of the protein suspension (20 μL) was stained with 20 μL of Rhodamine B (final concentration = 2.5 ppm). The samples were then observed under a Leica DMI3000B fluorescence microscope (Leica, Wetzlar, Germany) using an N2.1 filter cube and a magnification of 20x. Five to ten micrographs were taken for each sample and the most representative was selected.

2.7. Protein solubility

An aliquot of each protein suspension (1 mL) was taken and centrifuged for 2 min at 20 °C and 5000×g using a MicroStar 17 centrifuge (VWR International, Dietikon, Switzerland). The supernatant was transferred into a tube and an aliquot of the sample was collected and tested for protein content, using a bicinchoninic acid (BCA) assay kit (ThermoFisher, Reinach, Switzerland) following the manufacturer's instructions. The supernatants were immediately diluted with water to a 1/10 (v/v) ratio, and 25 μL aliquots of the dilutions were pipetted into a clear 96-well plate along with 200 μL of BCA reagent. The plate was incubated at 37 °C for 30 min in the absence of light, and the absorbance was measured afterwards at 562 nm using an Epoch plate reader (BioTek, Vermont, US). A colour blank was added to each set of samples by substituting the BCA reagent with water. Bovine serum albumin was used as a standard. Protein solubility was calculated using the following formula:

$$Solubility\ (\%) = \frac{PC_{susp}\ (g)}{PC_{total}\ (g)}\ x\ 100$$

where PC_{susp} was the protein content in the supernatant and PC_{total} was the total protein content before centrifugation (3% w/w).

2.8. Foaming properties

Foaming properties of protein suspensions were determined in triplicate using a dynamic foam analyser (DFA-100, Kruss GmbH, Germany). Samples at a protein concentration of 3% (w/w) were foamed in a CY4571 column (40 mm prism with two electrodes for liquid content measurement) (Kruss) with a FL4551 filter paper insert (12–15 μm pore size). The foam was generated by air streaming into the column at a flow of 0.3 mL min^{-1} until the foam reached a height of 180 mm. Foam properties were recorded for 30 min or until its full collapse. ADVANCE software, Version 1.10 (Kruss) was used to monitor the foams decay and to take photographs. Foaming capacity and foam stability were calculated using the following equations:

$$Foaming\ capacity\ (\%) = \frac{V_{foam,\ t_0}\ (mL)}{V_{liquid,\ t_0}\ (mL)}\ x\ 100$$

$$Foam\ \ stability\ (\%) = \frac{V_{foam,\ t_{30}}\ (ml)}{V_{foam,\ t_0}\ (mL)}\ x\ 100$$

where V_{foam} and V_{liquid} at t_0 indicated the volume of the foam and liquid, respectively, measured when the foam reached its maximum height, i.e., 180 mm, and t_{30} after 30 min (Dachmann et al., 2020). Foaming of the soluble fraction of the FBC1:HPC2 50:50 blend was performed following the same methodology as described above but only using the supernatant obtained after centrifugation for 5 min at 20 °C and 5000×g in a Sigma 6-16 KS centrifuge (Sigma Laborzentrifugen, Osterode am Harz, Germany).

2.9. Statistical analysis and graph plotting

All experiments were performed at least in triplicate and the uncertainties were expressed as the standard deviation (SD). Statistical analyses were performed using one-way ANOVA with GraphPad Prism 9.00 (San Diego, CA, USA). Differences were considered statistically significant at $P < 0.05$. GraphPad Prism was used to plot graphs. Linearly interpolated values were calculated based on the sum of the experimental values obtained for the individual protein components (100% FBC1, FBC2, HPC1, and HPC2) proportional to the protein ratios used (80:20, 60:40, 50:50, 40:60, 20:80, faba bean protein:hemp protein).

3. Results and discussion

3.1. Compositional analysis of protein concentrates

In the present study, four commercial plant protein concentrates, two derived from faba bean (FBC1 and FBC2) and two from hemp seed (HPC1 and HPC2), were characterised. FBC1 was obtained by dry extraction, whereas FBC2, HPC1 and HPC2 were produced using wet extraction. The processes employed to obtain each concentrate are described in the materials section, however, only limited information was provided by the suppliers.

Results of the compositional analyses performed on the concentrates are shown in Table 1. FBC1 had the lowest protein content (58.7% w/w), which was consistent with the literature for faba bean protein concentrates obtained by dry fractionation (Felix et al., 2019; Hall & Moraru, 2021). In contrast, FBC2 had the highest protein content (88.5% w/w) of all concentrates evaluated in this study, which was also in agreement with protein contents reported for faba protein concentrates obtained via wet extraction (Nivala et al., 2017). HPC1 and HPC2 showed similar protein contents (approximately 79% w/w), comparable to the values reported in the literature (55–76% w/w) for hemp protein concentrates obtained via wet extraction (Nasrollahzadeh et al., 2022). All concentrates showed similar moisture contents (9–12% w/w). Their lipid contents were low (<1.5% w/w), probably because of the removal of fat prior to the protein extraction step (Eze et al., 2022). As previously stated, the use of dry fractionation to obtain protein concentrates leads to plant protein concentrates with lower protein and higher

Table 1
Compositional analysis of the faba bean and hemp protein concentrates.

Concentrate	Protein (%)	Moisture (%)	Lipid (%)	Carbohydrate[a] (%)
FBC1	58.7 ± 1.3^c	9.0 ± 0.1^b	1.03 ± 0.2^b	31.2
FBC2	88.5 ± 1.8^a	9.3 ± 0.1^b	$<0.1^c$	2.1
HPC1	78.8 ± 2.3^b	8.5 ± 0.2^c	$<0.1^c$	12.6
HPC2	79.1 ± 0.3^b	11.7 ± 0.4^a	1.30 ± 0.1^a	7.8

FBC: faba bean protein concentrate, HPC: hemp protein concentrate. Values represent the mean ± SD (n = 3). Superscript letters indicate significant differences between samples.

[a] Carbohydrate content was determined by difference, i.e., as the residual after subtracting the three other fractions.

carbohydrate content. In contrast to FBC1, the other concentrates (i.e., FBC2, HPC1 and HPC2) had much lower carbohydrate contents (ranging from 2 to 12%), indicating a more effective isolation of the protein fraction using wet extraction conditions compared to dry fractionation.

3.1.1. SDS-PAGE

Fig. 1 shows SDS-PAGE gels of soluble and total protein fractions of the protein suspensions under reducing (Fig. 1 A, B) and non-reducing conditions (Fig. 1 C, D). The gels corresponding to the soluble protein fractions (Fig. 1 A, C) revealed fewer and less intense bands than the gels corresponding to the total protein fractions (Fig. 1 B, D). This finding indicates that some protein fractions were partially or totally insoluble in water. For example, globulins such as edestin (bands 7a and 7b) and legumin (band 4), the main protein fractions in hemp and faba bean, respectively, are soluble in saline aqueous solutions or at alkaline pH (Yang & Sagis, 2021). Consequently, these proteins were no longer part of the protein phase in the supernatant after centrifugation. On the other hand, water-soluble albumins (bands 6 and 9 with subunits 9a and 9b) were present in the soluble phase. The SDS-gels containing HPC1 and HPC2 showed almost no bands after centrifugation, indicating a very small fraction of soluble protein.

FBC 1 and FBC2 showed differences in the lipoxygenase (LOX) band patterns between 75 and 100 kDa (bands 1a, b, c (Warsame et al., 2020)). Thereby, FBC1 revealed the three expected bands whereas FBC2 did not elute band 1b. Processing steps such as high pressure and heat treatment can cause disulfide bond-induced aggregation of LOX and therefore alter its conformation and solubility (Tangwongchai et al., 2000). Additionally, the lack of bands for subunits of legumin (bands 5b and 5c) in the FBC2 soluble protein gel, but not in the total protein gel, supports the assumption that the processing conditions of FBC2 favoured legumin denaturation (and aggregation). The high molecular weight material that remained on top of the stacking gels is an additional indication of the probable undesirable impact of thermal processing.

For hemp protein concentrates, distinct differences were observed between the soluble and total protein gels as well as between the HPC1 and HPC2 gels. While edestin (bands 7a, b) subunits appeared under reducing conditions for both concentrates, edestin (band 7) was nearly absent in the HPC2 gels under non-reducing conditions. This finding indicates that large, non-migrating, disulfide-bridge-dominated aggregates probably developed during processing, e.g., due to unfavourable temperature or pH conditions. A similar reduction in band intensity was also reported by Nasrollahzadeh et al. (2022), who compared the SDS-PAGE profiles of five commercial hemp protein concentrates under non-reducing conditions and reported that samples with higher protein denaturation levels showed less intense bands than those with low levels of denaturation. The authors indicated that highly denatured samples had higher amounts of disulfide bonds in the protein than those with low denaturation levels, a consequence of unfolding and aggregation resulting from alkaline solubilisation and isoelectric precipitation.

The unidentified band for HPC2 at 37 kDa in the non-reducing gel (Fig. 1 D) could be a degradation product of edestin. As for FBC2, high molecular weight material that remained on top of the stacking gels was also found in the HPC2 concentrate, indicating a severe extraction process.

In summary, HPC1 and FBC1 seem to have been subjected to processing conditions which caused little protein alteration, whereas indications of protein denaturing conditions were found for HPC2 and FBC2. No new bands were found while blending the concentrates, indicating that proteins were simply mixed and no modifications on a molecular level seemed to occur at the conditions tested (Supplementary

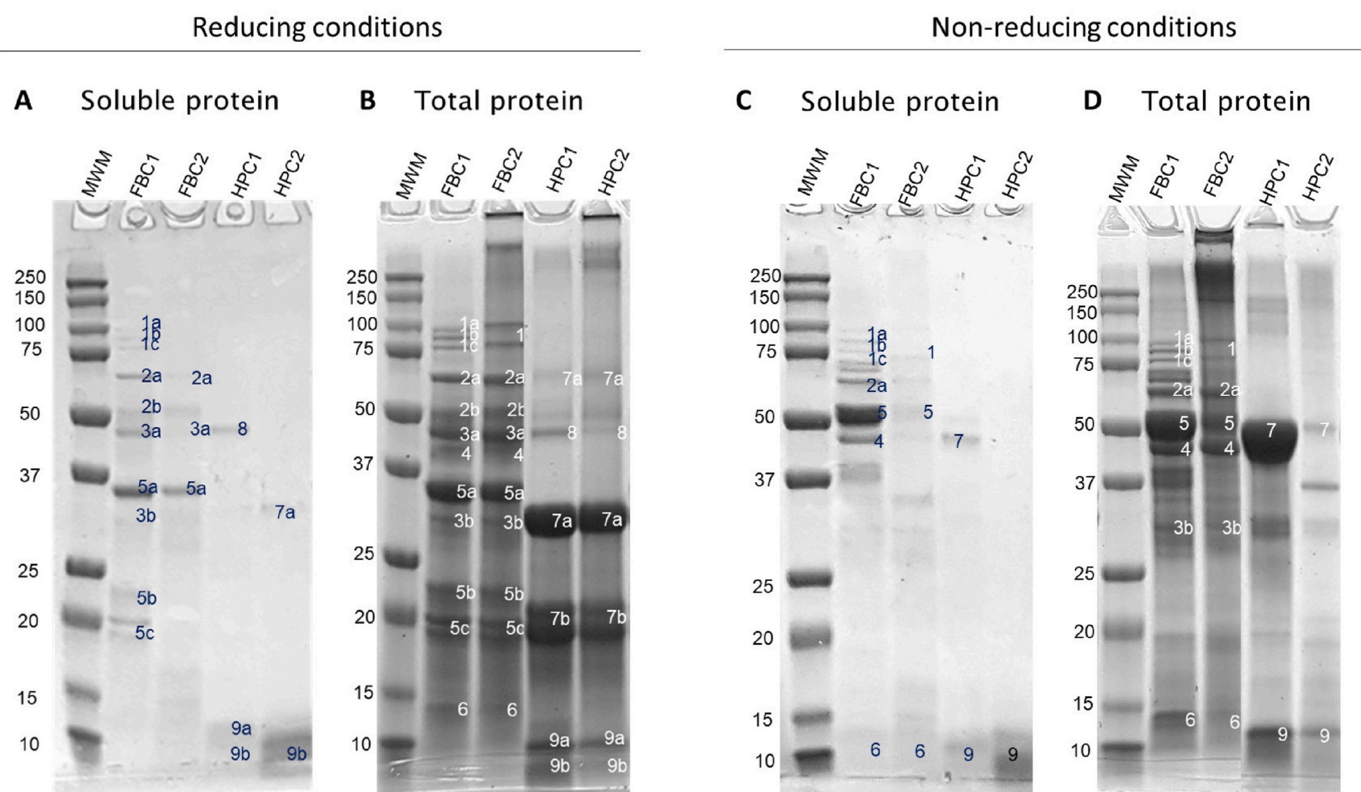

Fig. 1. Sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) of the faba bean and hemp protein suspensions. A) soluble protein under reducing conditions, B) total protein under reducing conditions, C) soluble protein under non-reducing conditions and D) total protein under non-reducing conditions. Soluble protein: residual protein in supernatant after centrifugation. MWM: molecular weight marker, FBC: faba bean protein concentrate, HPC: hemp protein concentrate. Legend: 1 a, b, c: lipoxygenases (LOX), 2 a, b: convicilin; 3 a, b: vicilin; 4: high molecular weight legumin; 5: legumin complex; 5 a, b, c: legumin α, β1 and β2,; 6 albumin; 7: edestin; 7a: acid edestin; 7b: basic edestin; 8: vicilin-like protein; 9: albumin complex; 9a: albumin subunit 1; 9b: albumin subunit 2.

Fig. 2).

3.2. Physicochemical properties

3.2.1. Particle size

Fig. 2 shows the values of the mean diameters D [4,3] of the protein suspensions. Additional particle size distributions and fluorescence microscopy images of the protein suspensions are shown in Supplementary Fig. 1. The D [4,3] values ranged from 24 ± 4 μm for FBC1 to 132 ± 11 μm for HPC2, with a monomodal distribution for FBC1, FBC2 and HPC2 and a multimodal distribution for HPC1 (Supplementary Fig. 1).

The particles of the FBC2 and HPC2 suspensions observed in the micrographs (Supplementary Fig. 1) and their D [4,3] values were significantly higher compared to those of FBC1 and HPC1, supporting the discussion regarding process-related protein alterations presented in section 3.1.1.

Blends containing HPC1 (i.e., FBC1:HPC1 and FBC2:HPC1) showed a linear correlation between the experimental and interpolated particle size values, possibly indicating that no particular interactions occurred between the different proteins (Fig. 2 A, C), confirming the previous SDS-PAGE results (Supplementary Fig. 2). However, blends containing HPC2 (i.e., FBC1:HPC2 and FBC2:HPC2, Fig. 2B–D, respectively) had smaller particle sizes than expected. In suspensions of FBC2:HPC2, these deviations of particle sizes were observed only at 60:40 and 80:20 ratios, but in the case of the suspensions FBC1:HPC2, they were observed at all ratios tested. This finding could suggest that an interaction occurred between these proteins, as partial replacement of HPC2 by FBC1 resulted in a reduction of HPC2 aggregates.

No information was found in the literature regarding particle size distributions in blends of protein isolates or concentrates deviating from the proportional contributions of the individual samples. Therefore, the reason for this behaviour remains unclear. In the FBC1:HPC2 blends (Fig. 2B), the reduced particle size could potentially be linked to an increase in solution viscosity, influenced by the higher carbohydrate content in FBC1 (Table 1). This elevated viscosity might facilitate transmission of shear forces, thereby reducing aggregates (Herceg et al., 2007). It is also conceivable that blending could have led to a dilution of critical components in HPC2, such as polyvalent ions, leading to aggregation, as well as highly aggregated and insoluble material, such as insoluble fibre (Zheng et al., 2019).

3.3. Techno-functional properties

3.3.1. Protein solubility at pH 7.0

The protein solubility of faba bean and hemp protein suspensions and their blends was studied at pH 7.0 and the results are shown in Fig. 3. The solubility varied between the protein concentrates; while HPC1, HPC2, and FBC2 were highly insoluble (<10% solubility), FBC1 exhibited a protein solubility of 60%. The protein solubility values of HPC1 and HPC2 were in agreement with those reported in the literature for hemp protein concentrates, with 15% solubility at pH 7.0, as reported by Liu et al. (2022); Malomo et al. (2014); Malomo and Aluko (2015). Regarding the solubility of faba bean protein concentrates, other studies have reported values between 30 and 90% at pH 7.0, depending on the extraction and processing conditions (Alavi, Chen, & Emam-Djomeh, 2021; Karaman et al., 2022). Considering the high D [4, 3] values of FBC2 (Fig. 2), it seems possible that the limited solubility (<10%) observed derives from protein denaturation or aggregation occurring during the extraction process applied by the manufacturer (Shanthakumar et al., 2022). Another potential factor contributing to the limited solubility could also be the extraction of predominantly low water-soluble proteins (e.g., globulins) as a result of alkaline

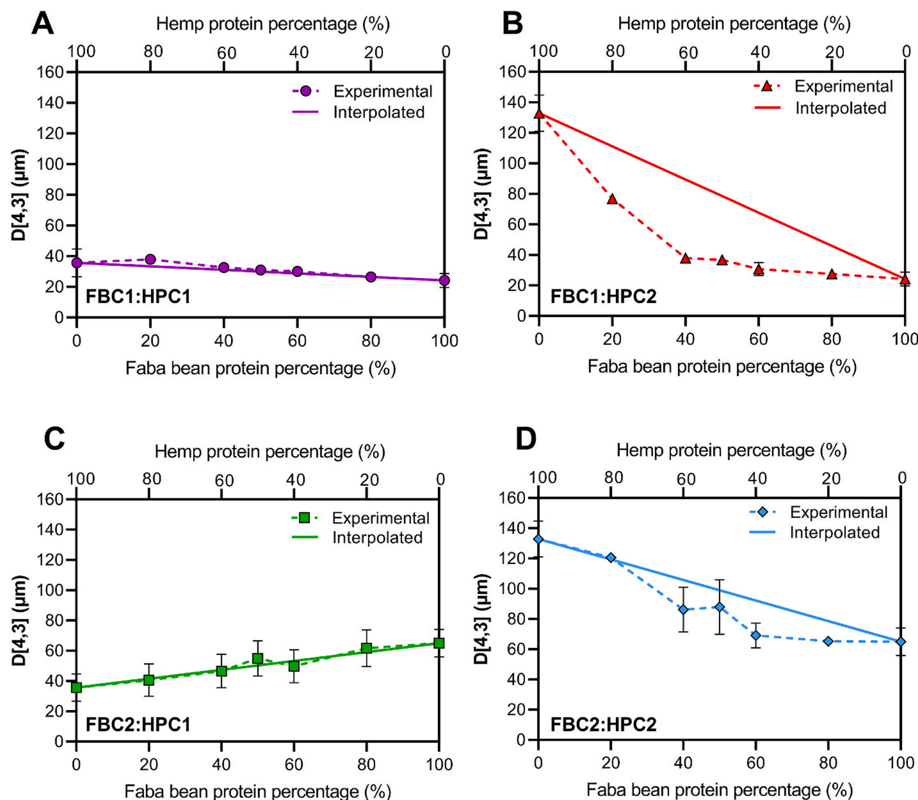

Fig. 2. Particle size values D [4,3] of the protein suspensions and their blends, at pH 7.0: (A) FBC1:HPC1, (B) FBC1:HPC2, (C) FBC2:HPC1 and (D) FBC2:HPC2. FBC: faba bean protein concentrate, HPC: hemp protein concentrate. Symbols represent the experimental values (mean ± SD, n = 3), with dotted lines added as a visual guide. Solid lines represent the linear interpolation of the D [4,3] values, calculated as the sum of the proportional contributions of the individual concentrates (i.e., 100:0 and 0:100 ratios) and the respective ratios used for each blend.

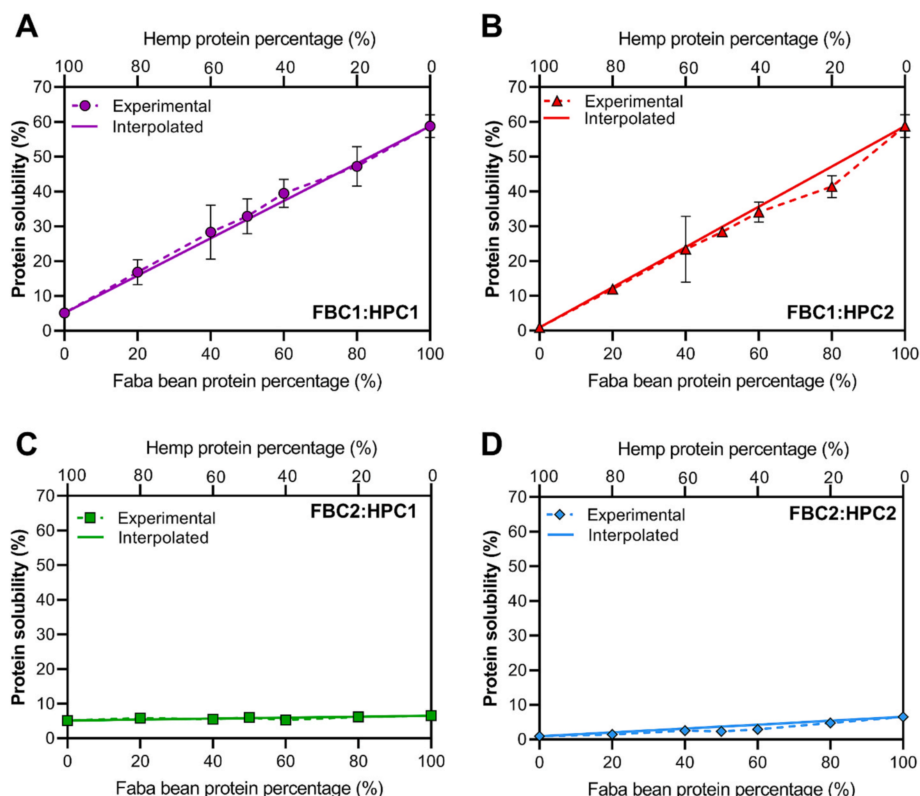

Fig. 3. Protein solubility of the protein suspensions and their blends. (A) FBC1:HPC1, (B) FBC1:HPC2, (C) FBC2:HPC1 and (D) FBC2:HPC2. FBC: faba bean protein concentrate, HPC: hemp protein concentrate. Symbols represent the experimental values (mean ± SD, n = 3), with dotted lines added as a visual guide. Solid lines represent the linear interpolation of the protein solubility values, calculated as the sum of the proportional contributions of the individual concentrates (i.e., 100:0 and 0:100 ratios) and the respective ratios used for each blend.

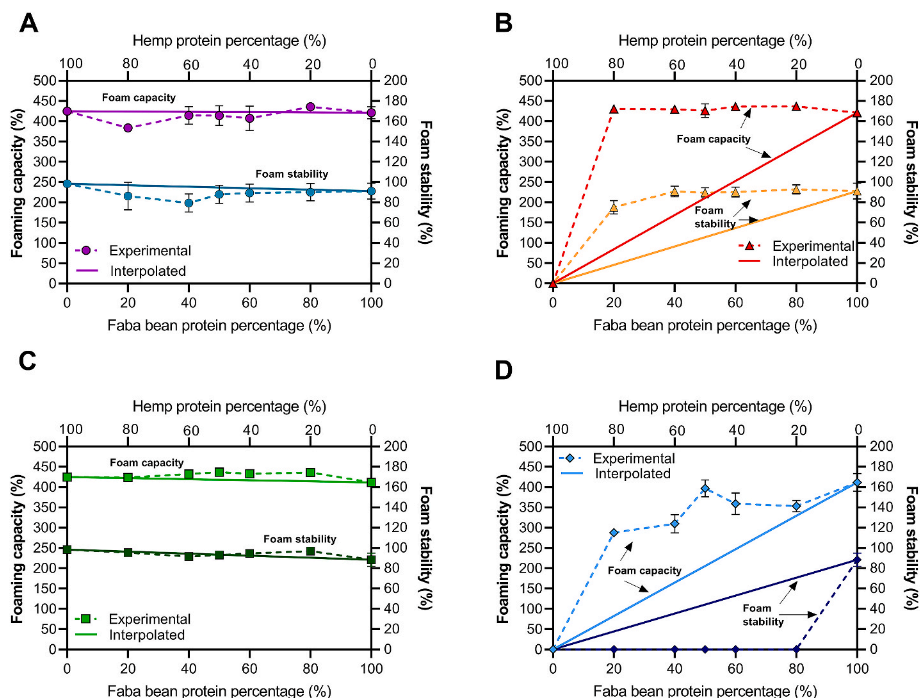

Fig. 4. Foaming capacity and foam stability of the samples and their blends at pH 7.0: A) FBC1:HPC1, B) FBC1:HPC2, C) FBC2:HPC1 and D) FBC2:HPC2. FBC: faba bean protein concentrate, HPC: hemp protein concentrate. Symbols represent the experimental values (mean ± SD, n = 3), with dotted lines added as a visual guide. Solid lines represent the linear interpolation of the foam capacity and stability values, calculated as the sum of the proportional contributions of the individual concentrates (i.e., 100:0 and 0:100 ratios) and the respective ratios used for each blend.

solubilisation and isoelectric precipitation.

Despite the reduction in the average particle size observed for FBC1:HPC2 and FBC2:HPC2 blends (Fig. 2), their protein solubility values matched with the expected values based on interpolation. This behaviour may seem contradictory in view of the changes in D [4,3] values reported in Fig. 2 B, D. However, regardless of the size of the particles, the solubility of these proteins did not change with blending. Similar results were reported by Zhou et al. (2021), who mixed pea (~60% solubility) and grass carp (~55% solubility) protein isolates at equal protein concentrations, resulting in a blend with a protein solubility of ~58%. They suggested that no protein-protein interactions occurred within the blends, which agrees with the findings presented herein.

3.3.2. Foaming capacity, foam stability and foam structure

The foaming capacity (421 ± 14, 411 ± 6, 424 ± 7%) and foam stability (91 ± 7, 88 ± 6 and 98 ± 1%) of FBC1, FBC2 and HPC1, respectively, were not significantly different (Fig. 4 A, B, C). Contrarily, HPC2 did not have the ability to foam (Fig. 4 B, D). Since the air/water interface is mainly stabilised by colloidal dissolved proteins, the lack of foaming properties in HPC2 could be related to its low contents of soluble protein in the suspension as well as its large particle size values (Fig. 2 B, D and 3 B, D). The lack of foamability of HPC2 seems unusual. Liu et al. (2023) found different foaming capacities and foam stabilities for different cultivars, but never a complete absence of foamability. However, the hemp isolates studied by these authors were all obtained using the same pH-shift extraction method, were freeze-dried rather than spray-dried, and foaming was performed using a different methodology than in the present study, making a direct comparison difficult.

The morphology of the foams (Fig. 5) showed that (i) the bubble size after 5 min was similar in all foams (HPC1, FBC1, and FBC2) and (ii) coalescence occurred in all foams after 30 min, although it was more pronounced in HPC1 than in FBC1 and FBC2. Nevertheless, the high foam stability of these samples (Fig. 4) indicates that, although bubble coalescence occurred, foams remained stable. After 30 min, FBC2 resulted in foams with more variety in structures than those formed by FBC1 and HPC1, characterised by the co-existence of both, small and large, bubbles, as well as a visually thicker air/water interface. In addition, the bubbles of the FBC2 foams appeared to be more spherical than in the FBC1 and HPC1 foams after both 5 and 30 min, suggesting a higher interfacial tension.

The variation in foam structures among different samples may have resulted from different conformations of relevant proteins such as large globulin-like proteins in FBC2 observed in the SDS-PAGE, or by an altered ratio of foam-stabilising and -destabilising factors due to different extraction procedures. Furthermore, the variations in the foaming properties could be caused by non-proteinaceous material within the different concentrates (Góral & Wojciechowski, 2020).

Blends from FBC1:HPC1 and FBC2:HPC1 exhibited similar foam capacities (approximately 425%) and stabilities (90–100%). Both foam capacities and foam stabilities coincided with a linear interpolation assuming proportional contributions from each component (Fig. 4 A, C). The similarity in foam capacity and stability within these blends indicates that the stabilisation mechanism was not protein-type specific, thus allowing interchange of the particles adsorbed at the interface. In contrast, for blends FBC1:HPC2 and FBC2:HPC2, the experimental values differed significantly from those expected. A proportion of 20% FBC1 protein in the blend (the smallest FBC1 protein proportion investigated) resulted in an improvement of the foam capacity and stability for FBC1:HPC2 with values that were comparable to those of FBC1:HPC1 and FBC2:HPC1.

The addition of FBC2 to HPC2 also led to a significant improvement in foam capacity, but none of these foams were stable and collapsed within seconds after foam formation (Fig. 4 D).

3.3.3. Protein profiles of foams

To gain a better understanding of which proteins were relevant for interfacial properties in the faba bean and hemp protein suspensions and their blends, the protein profile of each foam was analysed by SDS-PAGE. For the SDS-PAGE gels, the same quantity of freeze-dried material was used for each sample, i.e., it was not standardised for protein content. Supplementary Fig. 3 shows how at the end of foaming analysis the sensors located on top of the column (i.e., S7 and S6, from where the foam was taken for analysis), detected low residual fluid/serum. This finding could indicate that the proteins revealed in the SDS-PAGE (Fig. 6) were mainly adsorbed at the air/water interface. Many of the bands previously observed in the SDS-PAGE of the protein concentrates (Fig. 1 B) were also observed in these gels but at lower overall intensities due to lower protein contents in the foams compared to the corresponding suspensions (Fig. 6). The bands observed for the protein blends were similar to those of the foams of the individual concentrates. The main proteins identified in the foams were convicilin (2a), vicilin (3a) and legumin (5a) from faba bean protein and edestin (7a, 7b) and albumin (9a) from hemp protein (Fig. 6). The foam produced with HPC1 exhibited clear high-intensity bands corresponding to edestin and albumin. This result could suggest that these proteins, mainly edestins, vicilins, legumins and albumins may play a key role in foam formation since, and, regardless of their low solubility, they could also stabilise foams as particles (Han et al., 2023). However, this statement requires further investigation since some of these proteins could also be part of the residual foam serum and do not necessarily occupy the foam interface.

3.3.4. Contribution of FBC1 and HPC2 to the foaming properties of their blends

To understand the impact of the soluble protein fraction on foaming, the blend FBC1:HPC2 50:50 (with approximately 30% protein solubility, Fig. 3 B) was centrifuged, and the supernatant was subjected to foaming. The protein content of the supernatant and the non-centrifuged sample was not normalised; hence, the foamed supernatant fraction contained less protein (especially hemp protein due to its low solubility). The supernatant (soluble protein fraction) showed a foaming capacity and stability values of 434 ± 5 and 75 ± 2%, respectively, whereas the non-centrifuged sample (total protein) had values of 426 ± 17 and 89 ± 5%, respectively (Table 2). Although no differences in foam capacity were found between the centrifuged and non-centrifuged samples, foam stability was significantly reduced when the sample was centrifuged, indicating that part of the insoluble material from FBC1 and/or HPC2 contributed to increase the foam stability. Therefore, it can be assumed that in this blend, soluble proteins predominantly determine foaming capacity, whereas insoluble proteins are rather contributing to foam stability. Previous studies have indicated that insoluble proteins can significantly impact the techno-functional properties of protein concentrates (Nikbakht Nasrabadi et al., 2021; Stone et al., 2015). The conformation and flexibility of these proteins play a crucial role in their ability to be adsorbed at the interface, thereby stabilising the foam (Amagliani et al., 2021). Yang et al. (2018) reported that after the disruption of insoluble faba bean protein aggregates and exposure of their hydrophobic areas, the foam capacity increased, while stability remained unchanged.

For additional insights of which source of protein was dominant during foam formation and stabilisation of the FBC1:HPC2 blends, suspensions of FBC1 alone, but with the same mass fraction as in blends with HPC2, were subjected to foaming (Fig. 7). Therefore, these samples had different dry masses and total protein contents compared to the blends. A comparison of the foaming properties of FBC1 alone with those of FBC1:HPC2 showed that both foaming capacity and foam stability were approximately 10% higher in the blends (Fig. 7). This suggests that, when combined with FBC1, HPC2 can be integrated into foam structures, implying the existence of an interaction or synergy between hemp and faba bean proteins in the foam (Fig. 6).

The similarity of the trend of foam capacity and stability of FBC1 alone and FBC1:HPC2 across the ratios examined implies that FBC1

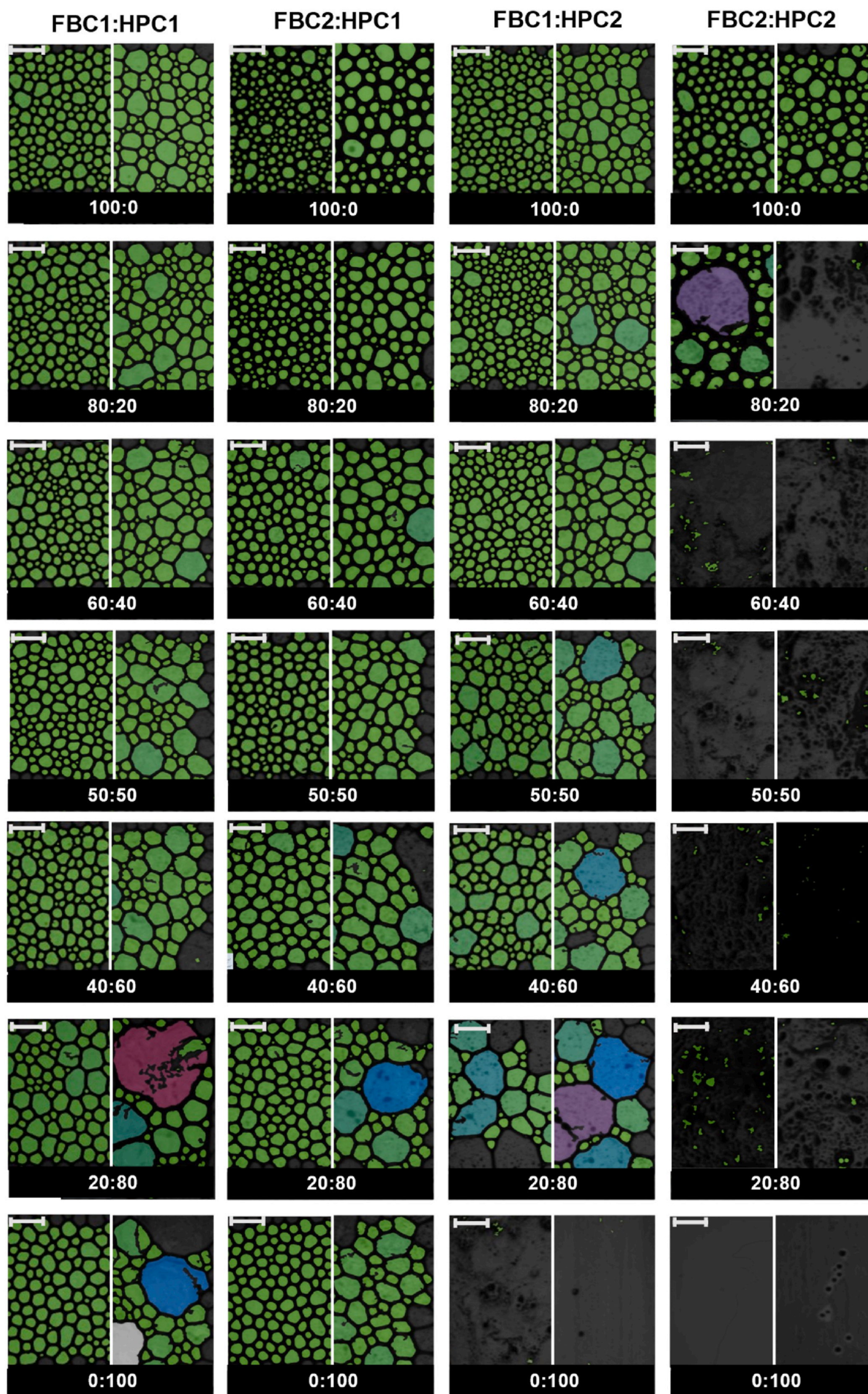

Fig. 5. Images of the foams at 5 (left) and 30 (right) min after foam formation using the protein suspensions and their blends. FBC: faba bean protein concentrate, HPC: hemp protein concentrate. Colours represent different bubble sizes. The scale bar on the top left indicates 2 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

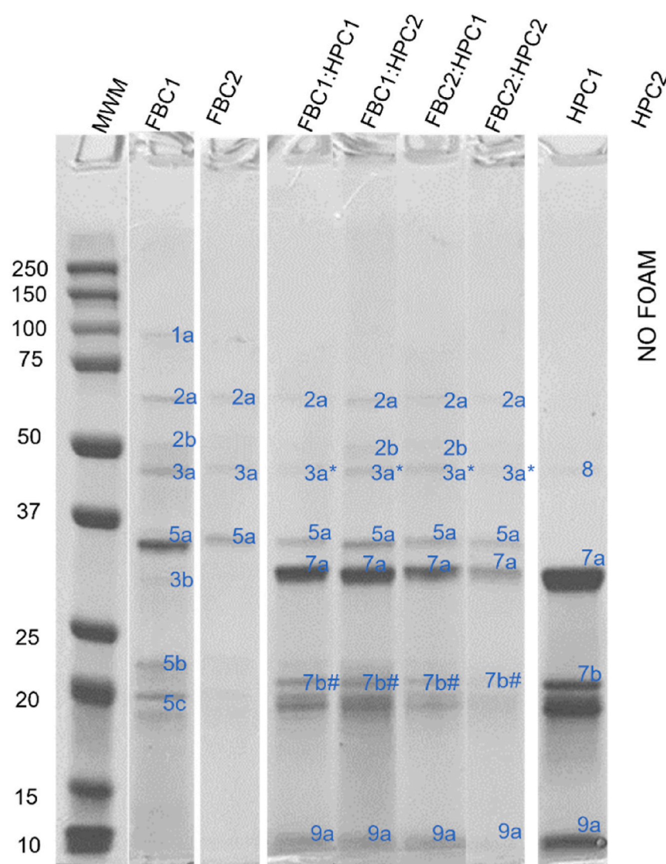

Fig. 6. Sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) under reducing conditions of the freeze-dried foams obtained from the protein suspensions. Blends correspond to a 50:50 faba bean-hemp protein ratio. MWM: molecular weight marker, FBC: faba bean protein concentrate, HPC: hemp protein concentrate. Legend: 1a,: lipoxygenase, 2 a,b: convicilin; 3 a,b: vicilin; 5 a,b,c: legumin α, β1 and β2; 7a: acid edestin; 7b: basic edestin; 8: vicilin-like protein; 9a: albumin subunit 1; 9b: albumin subunit 2.* Indicates that band could be 3a, 8 or contain both proteins, # indicates that band could be 7b, 5c or contain both proteins.

Table 2
Foaming capacity and stability of sample FBC1:HPC2 50:50 after collection of the supernatant following centrifugation (soluble protein) and without the centrifugation step (total protein).

Sample	Foaming capacity (%)	Foam stability (%)
FBC1:HPC2 50:50 soluble protein	434.0 ± 5.6 [a]	75.0 ± 1.7 [b]
FBC1:HPC2 50:50 total protein	426.1 ± 16.9 [a]	89.4 ± 5.0 [a]

Different letters denote statistical differences between samples for each column. FBC: faba bean protein concentrate, HPC: hemp protein concentrate.

dominated the interface and was at least not totally displaced by the addition of an increasing HPC2 fraction in the blend.

3.3.5. *Possible foam stabilising and destabilising factors*

Of the four protein concentrates investigated herein, three had the ability to form stable foams with high air-holding capacities, i.e., FBC1, FBC2 and HPC1. Since all three protein concentrates can form stable foams on their own, they can be used at various mixing ratios without any impact on foaming properties. The FBC1:HPC1 and FBC2:HPC1 blends (Fig. 4 A and C, respectively) exhibited foam capacity and stability values in the same range, regardless of the mixing ratio, suggesting similar mechanisms of foam stabilisation.

The combination of HPC2 and FBC2 resulted in unstable foams that collapsed within 30–60 s. However, the replacement of low quantities (20% protein) of HPC2 by FBC1 in the suspension was sufficient to result in foams with improved foaming capacity and stability comparable to those of FBC1:HPC1 and FBC2:HPC1 blends (Fig. 4). Since the addition of FBC1, but not FBC2, was able to improve the foam stability of HPC2 blends, it can be hypothesised that FBC1 predominantly contains foam-stabilising factors that favour foam formation, while HPC2 may be dominated by destabilising factors. It is assumed that these foam-destabilising factors are, to a certain extent, in balance with foam-promoting factors, e.g., soluble proteins and surface-active insoluble protein/aggregates/particles. Thus, the formation of stable foams arises when foam-promoting factors predominate or a potential synergistic effect among proteins occurs, although the available data do not allow for definitive conclusions. Some foam-stabilising and destabilising mechanisms that occur in these blends could be.

i) Protein solubility is considered an important factor in foam stabilisation (Amagliani et al., 2021). The term "protein solubility" refers to molecularly dissolved proteins and colloidal proteins in equilibrium dispersed homogeneously in a solution (Garidel, 2013). As shown in Fig. 3, the protein solubility of HPC1 (5%) was significantly higher than that of HPC2 (<1%), suggesting that HPC1 contained more interface-active proteins available for foam formation. As shown in Fig. 1 D, very low intensity bands, especially edestin, were eluted by HPC2 in the SDS-PAGE suggesting that this protein concentrate was obtained under extraction conditions which promoted protein denaturation, and changes in protein structure leading to low solubility. This argumentation would explain why at neutral pH, HPC2 contained higher amounts of insoluble proteins than HPC1.

ii) HPC1 and HPC2 differed substantially in terms of size (Fig. 2 C and D). The average particle size was significantly lower in HPC1 (40 μm) than in HPC2 (130 μm). In contrast, the particle diameters of the FBC1:HPC2 blends were found to be (a) significantly lower than those predicted by linear interpolation, (b) of the same order of magnitude as those of the FBC1:HPC1 blends, and (c) significantly lower than those of the FBC2:HPC2 blends. These findings indicate that there is a critical particle size above which a foam destabilising effect occurs, analogous to the mode of action of an antifoam. In the present study, foams produced from samples with a particle size of >80 μm were not stable (Figure 2 and 4).

iii) Cations such as Na^+ and Ca^{2+} which might be added into the concentrates during processing (salt-assisted extraction) could lead to a significant reduction in foam stability, even at relatively low concentrations. Previous studies have shown that the utilisation of salt-assisted extraction for pea proteins led to a notable reduction in foam stability (up to 10% lower) compared to pea proteins obtained through an alkaline extraction followed by isoelectric precipitation (Stone et al., 2015). This effect could be attributed to the direct complexation of proteins via ion bridges (Zheng et al., 2019). Additionally, the possible presence of phytic acid could have comparable effects, and although the mechanism is unclear, at pH 7.0 it is suspected that complexation between phytic acid and protein molecules via ion bridges involving Ca^{2+} or other minerals could cause relevant proteins to precipitate (Amat et al., 2022, 2024; Wang & Guo, 2021).

iv) Other compounds, such as free fatty acids or saponins, can actively compete for adsorption at the interface during foam formation, causing destabilisation or stabilisation, respectively. For example, free fatty acids are effective destabilisers in beer foams (Wilde et al., 2003). Free fatty acids can be released and solubilised during protein extraction when the material is subjected to high pH values or enzymatic hydrolysis, favouring lipid breakdown. In the present study, HPC2 had a significant higher lipid content than HPC1 (Table 1) which, together with the other

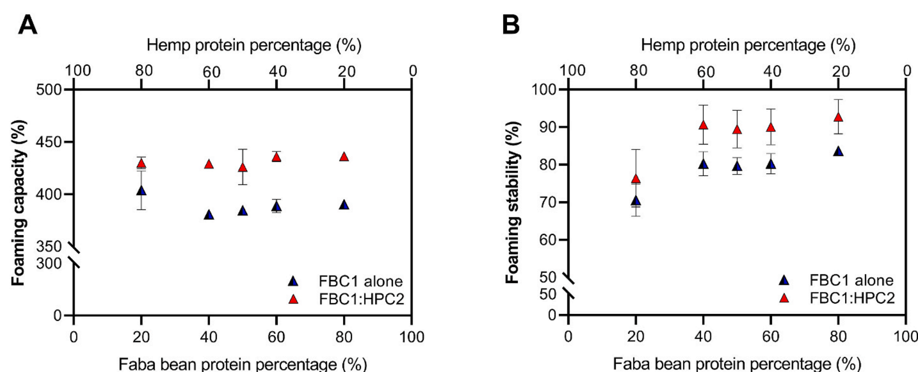

Fig. 7. Foaming capacity (A) and foam stability (B) of faba bean protein concentrate and hemp protein concentrate blends (FBC1:HPC2, full red triangles), along with the foaming capacity and stability of the same samples generated with the same mass of FBC1 but no HPC2 (FBC1 alone, half blue triangles). All samples were adjusted to pH 7.0. Data are represented as mean ± SD (n = 3).

factors mentioned above, could explain the low foam capacity of HPC2. The surface stabilising effect of saponins that could be present in the FBC concentrates could also have contributed to the improvement of the foaming capacity and stability of the FBC1:HPC2 blends (Góral & Wojciechowski, 2020; Saha et al., 2022).

4. Conclusion

This study focused on the investigation of foaming properties of concentrates and their blends, using commercial protein concentrates from faba beans (FBC1 and FBC2) and hemp seeds (HPC1 and HPC2). The findings showed that the protein solubility of the blends was influenced proportionally by both FBC and HPC. Interestingly, when HPC2 was blended with FBC1, smaller mean particle sizes were observed compared to the expected values calculated by linear interpolation. HPC2 had poor foaming properties, unlike the FBCs and HPC1. The observed differences in foaming properties were likely due to different extraction strategies and/or processing conditions applied by the manufacturers to produce the protein concentrates, although the specific methods and parameters remain unknown. However, when the non-foaming HPC2 was blended with FBC1, it demonstrated a foaming capacity comparable to that of the other samples (concentrates and blends). Furthermore, the comparison between FBC1 foams with FBC1:HPC2 foams revealed a relevant contribution of non-foaming HPC2 to foam capacity and stability, suggesting a synergistic interaction underlying joint foam structures. To draw further insights regarding blends with HPC2, additional investigations with different ranges of absolute protein concentrations and ratios would be required. Expanding these parameters could address the uncertainties present in this study. Moreover, probing the factors that could potentially account for the absence of foaming properties in HPC2, as discussed earlier, could provide valuable insights. Looking forward, the identification of components responsible for interfacial stabilisation and destabilisation of the concentrates and their impact on their foaming capacities is crucial. This understanding can guide targeted adaptations in extraction processes, leading to optimised protein blend formulations and facilitating product development for the food industry.

CRediT authorship contribution statement

Maria Cermeño: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Juliana V.C. Silva:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Mario Arcari:** Writing – review & editing, Formal analysis. **Christoph Denkel:** Writing – review & editing, Supervision, 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.115948.

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