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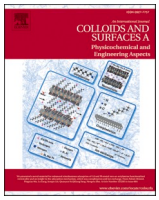

Improvement of the foaming properties of pea protein concentrate suspensions by physical or enzymatic treatments

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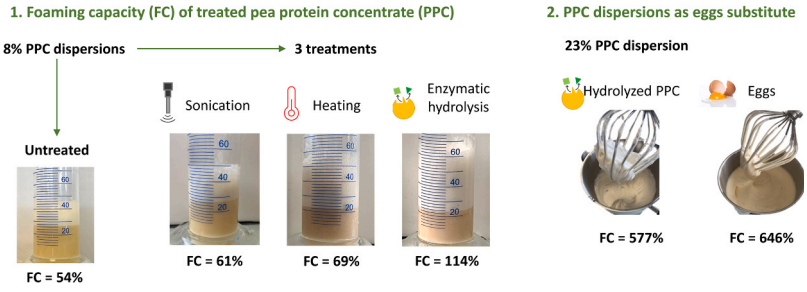
HIGHLIGHTS

- Enzymatic hydrolysis enhanced the foamability of pea proteins concentrates (PPC).
- Concentrated hydrolyzed PPC dispersion showed foamability akin to eggs.
- Hydrolyzed PPC adsorbed faster than egg amphiphiles at the air/water interface.
- Carbohydrates in PPC contributed to enhancing foam stability.

GRAPHICAL ABSTRACT



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ABSTRACT

The ability of proteins to foam is an important functionality in aerated food products. The functionalities of animal proteins over plant proteins are undeniable. However, plant-based ingredients have now became common substitutes. Among the available plant protein sources, yellow peas have been widely studied. This paper focuses on the functionalities of a pea protein concentrate (PPC) obtained after simpler and less damaging processes than those for isolates. Pea protein concentrate dispersions underwent ultrasounds, heating, or enzymatic hydrolysis. Physical treatments did not affect pea protein's structure, unlike enzymatic hydrolysis, which significantly altered the molecular size distribution. Enzymatic hydrolysis emerged as the most effective pathway for enhancing PPC foamability. Foams produced from concentrated hydrolyzed PPC (HYD PPC) dispersions were compared to whole egg foams. Interestingly, HYD PPC foams exhibited solid-like behavior, while whole egg foams displayed flowing behavior and lower stability over time. The solid fraction and the presence of polysaccharides in HYD PPC dispersions prevented drainage and coalescence in HYD PPC foams, ensuring excellent

Abbreviations: DH, Degree of hydrolysis; FC, Foaming Capacity; FS, Foaming Stability; HYD PPC, Hydrolyzed Pea Protein Concentrate; HYD PPI, Hydrolyzed Pea Protein Isolate; PPC, Pea Protein Concentrate; PPI, Pea Protein Isolate; TH PPC, Heated PPC; US PPC, Sonicated PPC.

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stability. Interfacial behavior differed in terms of kinetics of adsorption at the air/water interface between HYD PPC and whole egg proteins, yet both formed interfacial films with high viscoelastic properties.

1. Introduction

Dietary proteins, primarily derived from animals and plants, exhibit significant variations in molecular structure, amino acid composition, digestibility, and technical functionalities in foods, such as gelation, emulsification, foaming, and water binding [1].

A growing interest in plant-based ingredients as substitutes for animal constituents is undeniable, driven by sustainability considerations to meet the rising global population demand. Plant-based protein production boasts a lower carbon footprint than animal proteins [2,3]. Increasing health awareness and ethical concerns have further propelled consumer demand for plant proteins, prompting food companies to introduce plant-based alternatives.

Despite the advantageous functionalities of animal proteins such as egg proteins that exhibit excellent foaming, gelling or thickening properties [4], the shift towards plant-based proteins, particularly from sources like yellow peas (*Pisum sativum L.*), has gained traction. More than twelve million tons were produced worldwide in 2021 [5]. Yellow pea seeds contain approximately 20–30 % proteins and 65 % carbohydrates, mainly starch [6]. Pea proteins, available as flour (20–30 % proteins), protein concentrate (50 % minimum), or protein isolate (80 % minimum), are increasingly incorporated into plant-based substitutes for meat or yogurt [3].

Plant proteins can be categorized into four primary classes [6]: albumins, globulins, prolamins, and glutelins, based on their solubility and extractability in various solvents. Regarding pea proteins, globulins are the primary component (60 – 80 %). They exhibit poor water solubility contrary to albumins that account for the second major protein fraction (15 – 25 %) [7]. In contrast to animal proteins, which generally exhibit low molecular weight and a relatively simple globular structure [8], plant proteins display intricate quaternary structures with monomers of high molecular weight.

Although pea proteins exhibit functional properties such as emulsification and foaming, these functionalities fall short compared to animal proteins [9–11]. The limited physicochemical functionalities of plant proteins often constrain their utilization in food products; for instance, low solubility hinders their incorporation into aqueous-based products, and their tendency to be compact, aggregated, and inflexible restricts their capacity to adsorb at interfaces. Unlike animal proteins that are directly available, plant proteins need to be extracted from the seeds or the grains. The processes of extraction that are used comprise several steps such as milling followed by air fractionation or alkaline extraction/acid precipitation depending on the desired level of purity. For a wet extraction, a final drying step is added. These various extraction treatments tend to leave the proteins in a denatured and aggregated state with the majority of the functional groups buried inside the structure [8,12]. In order to improve their functionality, proteins modification can be implemented. The fundamental principles of protein modification approaches, based on physical and biochemical treatments, have been extensively reviewed elsewhere [9,13]

Physical treatments encompass heating [14,15], static high-pressure treatment [16], ultrasonication [17,18], pulsed electric fields [19], or other shear treatments [20]. These physical treatments denature proteins, inducing aggregation or unfolding by exposing hydrophobic residues previously buried within the protein's interior. Denatured albumins and globulins exhibit enhanced adsorption at interfaces compared to their native state, forming gels *via* disulfide bonds and hydrophobic interactions.

Among the deliberate biochemical modifications applied to proteins, enzymatic crosslinking or hydrolysis is common [21–23]. Proteases used for enzymatic protein hydrolysis are commonly obtained from either traditional or genetically modified microorganisms, and certain proteases are extracted from fruit sources. Hydrolysis has demonstrated its ability to improve solubility, gelling, and emulsifying characteristics of plant proteins [24].

Even though previous studies have already demonstrated the enhancement of pea protein foamability through various physicochemical treatments [17,25–27], they relied on protein isolates with high degree of protein purity. A specific nomenclature is used depending on the purity of the protein sources. Protein concentrates, typically contain 50–70 % protein, with the remainder consisting of carbohydrates, fats, and other nutrients. They are produced by removing some of the non-protein components (e.g. fats, fibers and carbohydrates) through basic processing methods like defatting, dispersion, filtration or centrifugation. Protein Isolates usually contain 80 % or more protein, making them highly pure. They undergo more extensive processing to remove almost all non-protein components. Techniques include isoelectric precipitation, ultrafiltration, ion exchange. In this study, we examine how different treatments – namely sonication, heat treatment, or enzymatic proteolysis – affect the foaming properties of yellow pea protein concentrates. Parameters such as solubility, foam overrun, stability, and dynamic tension at the water-air interface were analyzed. The results obtained for pea proteins were compared to a reference system based on whole eggs, which are commonly used for the production of aerated structures in foods.

2. Materials and methods

2.1. Materials

Pea protein concentrate (PPC, Pea Protein 55 – Deflavored) was obtained from AGT Foods Ingredients (Canada), and pea protein isolate (PPI, Vitessence Pulse 1803) from Ingredion (USA).

Whole eggs with a dry matter content of 23 % (MetroChef) were procured from Metro (France). Sodium hydroxide (NaOH 32 % w/v solution) and Endoprotease Sumizyme BNP were acquired from Sigma-Aldrich (France) and Takabio (France), respectively. Deionized water was employed in all experiments.

2.2. Compositional analysis

PPC and PPI powders were characterized according to AOAC methods to determine moisture content (14.004, 1999), fat content (2003.06, 2006) and ashes (942.05, 1942). The total nitrogen content of the PPC and PPI powders was determined using Kjeldahl method (AOAC method 991.20, 1995). A nitrogen-to-protein conversion factor of 5.4 was adopted [28]. The carbohydrates content was estimated as the remainder to 100 %.

2.3. Preparation of PPC and PPI dispersions

The protein powder was dispersed in demineralized water at ambient temperature by using a magnetic stirrer for around 30 min to allow powder hydration. Dispersions were prepared at 8 wt% or 23 wt% for PPC and 16 wt% for PPI. The weight percentages are given in relation to the total mass of the phase under consideration.

2.4. Physical treatments of PPC dispersions

2.4.1. Ultrasounds treatment

Sonication was applied to 8 wt% PPC dispersions using a SFX 550 (Branson-Emerson) sonicator. Ultrasound (US) treatment was delivered

with cycles (pulse duration of on-time 5 s and off-time 3 s) for a total of 20 min at 10 % full power output (550 W, 20 kHz). To control the temperature, an iced bath was used during US process and the temperature was monitored.

2.4.2. Thermal treatment

A two-step heat treatment was applied to the 8 wt% PPC dispersions: 90 min at 55°C, followed by 20 min at 80°C using water baths and under magnetic stirring. The second phase of the heat treatment at 80°C was adopted to replicate the enzyme inactivation step described in the next paragraph, and thus to compare systems identically processed. Immediately after the whole thermal treatment, PPC dispersions were chilled in an iced bath.

2.5. Enzymatic hydrolysis

2.5.1. Enzymatic proteolysis of 8 wt% PPC dispersions

Enzymatic proteolysis of PPC dispersions was performed for 90 min at 55°C using the Sumizyme BNP neutral protease extracted from *Bacilus subtilis* ($2.25 * 10^4$ AU/g, enzymatic activity determined with Anson method [29], equivalent to $3.75 * 10^{-4}$ kat) with an enzyme-to-substrate ratio (E/S) of 1000 AU/g.

A volume of 57.6 mL of demineralized water was used to disperse 5.6 g of PPC powder and heated. Once the dispersion attained 55°C, the enzyme solution (10 g/L) was added to reach a total volume of 70 mL and a dispersion concentration of 8 wt%.

Immediately after the hydrolysis step, the enzyme was inactivated through a thermal treatment of the hydrolysate at 80°C, for 20 min, and finally cooled down with an iced bath.

2.5.2. Scale up of enzymatic proteolysis of concentrated PPC or PPI dispersions

Enzymatic proteolyses of concentrated dispersions of PPC (23 wt%) and PPI (16 wt%) were performed under the same conditions as for 8 wt % PPC dispersions, at higher volume (500 mL) using 100 g/L enzyme solution. Stirring was achieved by a mechanical stirrer set at 400 rpm (RW 20 digital, IKA) equipped with a helix (5 cm diameter).

In the following sections, hydrolyzed PPC and PPI dispersions will be denoted as HYD PPC and HYD PPI, respectively.

2.6. Dispersions and hydrolysates characterizations

2.6.1. Soluble protein content

The soluble protein content of supernatants from PPC and PPI dispersions, obtained after centrifugation (4600 g, 15 min), was determined using bicinchoninic acid (BCA) spectrophotometric method with bovine serum albumin (BSA) as standard (Pierce rapid gold protein assay, Thermo Fisher Scientific, Massachusetts, USA). The absorption wavelength was 480 nm.

2.6.2. Degree of hydrolysis (DH)

The degree of hydrolysis allows to quantify the enzymatic proteolysis extent. It represents the percentage of peptides bonds cleaved compared to the initial number of peptide bonds of the protein. DH was calculated according to pH-Stat method [30] using Eq. [1]:

$$DH(\%) = \frac{B \times N_b}{M_p \times h_{tot} \times \alpha} \times 100 \quad (1)$$

B: base volume (mL), N_b: concentration of the base (NaOH, 0.5 mol/L), α: average dissociation degree, M_p: mass (g) of proteins (5.4 × N: nitrogen protein powder content), h_{tot}: theoretical total number of peptide bonds in the protein substrate (meqv.g^{-1} of protein). h_{tot} was calculated from the theoretical amino acid composition of the pea protein substrate by summing the concentrations of each amino acid, and was equal to 7.63 meqv.g^{-1} of protein.

α was calculated via Eq. [2] and found equal to 0.168, within the experimental conditions at pH = 6.37, and by adopting a pK value of 7.06.

$$\alpha = \frac{10^{(pH-pK)}}{1 + 10^{(pH-pK)}} \quad (2)$$

2.6.3. Size-exclusion chromatography (SEC)

The molecular weight distribution of the native proteins and of the hydrolysates was determined by size-exclusion chromatography on a Superdex Peptide 10/300 gl column (GE Healthcare, USA) using a Nexera Series HPLC apparatus (Shimadzu, Japan). The sample was filtered (0.2 μm) and 10 μL were injected. The column was equilibrated and eluted with water/acetonitrile/trifluoroacetic acid (60/40/0.1, v/v/v), at a flow rate of 0.6 mL/min, at room temperature. The peptides were detected at 214 nm. Proteins and PEG (polyethylene glycol) standards ranging from 160 Da to 38 kDa (Sigma-Aldrich) were used for calibration. The relative size distribution of the peptides and the proteins was estimated by integration of the area of the relevant peaks. SEC elution profiles were divided into 7 fractions determined to properly report the differences between the molecular weights of unhydrolyzed and hydrolyzed soluble pea proteins.

2.7. Foams preparation

Two devices were employed to prepare protein-based foams. The shear device was adapted to the sample volume. Indeed, the stirring system must preferably be of a size comparable to the container to ensure that the entire volume is continuously mixed. The initial series of experiments were conducted on limited volumes. The shear module used was a turbulent agitator, with the stirring rod being of a size comparable to the container's diameter. In the second series of trials, we aimed to scale up and compare the foaming capacity of protein suspensions with that of whole eggs, at comparable dry extract levels. In this case, a planetary mixer was used.

2.7.1. Preparation of foams from 8 wt% PPC dispersions

Foams were made using a high-speed shearing method. Briefly, 30 mL of dispersion were placed in a 100 mL graduated cylinder immersed in an iced bath. The dispersion was processed at 12,000 rpm for 1 min using a homogenizer (T25 digital ULTRA-TURRAX, IKA).

2.7.2. Preparation of foams from whole eggs or 16 wt% and 23 wt% pea protein dispersions

Foams were prepared using a planetary mixer equipped with a whisk (Cooking chef KCL95, Kenwood). Plant protein dispersions or eggs (approximately 300 mL) were placed in the mixer bowl and whipped for 15 min at the maximum speed (190 rpm).

2.8. Foams characterizations

2.8.1. Foaming capacity, FC, and foam stability, FS

FC corresponds to the air-to-liquid volume ratio (Eq. [3]) measured right after whipping. FS is defined as the ratio of the total foam volumes (Eq. [4]) measured after a time t equal to 1 or 2 hours (V_t) and after 5 min storage (V_5, the delay necessary to establish a discernable interface between the foam and the underlying liquid phase).

$$FC(\%) = \frac{V_{air}}{V_{liq}} \times 100 = \frac{V_{foam} - V_{liq}}{V_{liq}} \times 100 \quad (3)$$

$$FS(\%) = \frac{V_t}{V_5} \times 100 \quad (4)$$

2.8.2. Visco-elastic properties

Visco-elastic properties in the linear domain were measured with a controlled strain rheometer (AR G2, TA Instruments, USA) equipped with a parallel plate geometry (40 mm diameter) and 1 mm gap at 20°C.

Around 2 g of foam were carefully placed in the bottom plate and the gap was slowly narrowed. A cap was used to cover the geometry in order to prevent sample drying during the test.

The viscoelastic linear domain was determined by performing a strain sweep from 0.01 % to 1000 %, at a frequency of 1 Hz. The storage modulus G' (Pa) and loss modulus G'' (Pa) were measured in the linear domain, at a strain of 0.5 % after a 5 min equilibration step allowing sample relaxation after the gap was narrowed.

2.8.3. Microscopic images of foams

Foam samples were placed onto a hollow microscope slide under a phase contrast microscope (Olympus BX53) to observe the foam microstructure directly after whipping.

2.9. Air-water interfacial properties

Since foaming properties are, to a large extent, determined by protein adsorption, two techniques were implemented to characterize the kinetics of adsorption and the rheological properties of the protein layer at the water-air interface.

2.9.1. Dynamic surface pressure

Surface tension at the air/solution interface was measured as a function of time by the pendant drop technique (Tracker drop tensiometer, Teclis, France). The supernatants from HYD PPC as well as from eggs (4600 g, 15 min) were diluted with distilled water to adjust the soluble proteins concentration to 0.1 g/L. A 10-μL drop of sample was then generated and the evolution of the surface tension, σ(t), was recorded over time for a 1200–5400 s period. Data were converted to a time-dependent surface pressure Π (t) = σ_0 – σ(t), where σ_0 (72 mN/m) is the surface tension at the air/ pure water interface.

2.9.2. Dilatational interfacial elasticity

Interfacial dilatational properties of soluble egg proteins and soluble HYD PPC were characterized by the oscillating drop technique using the drop tensiometer (Tracker drop tensiometer, Teclis, France). A sinusoidal volume deformation of 5 % amplitude was applied to a 10 μL drop with a period of 1 s during 1200 s. The interfacial viscoelastic modulus, E (mN/m), is a complex number, comprising an in-phase component (real part), and an out-of-phase component (imaginary part). The two components were deduced from a Fourier transform using the apparatus software (Eq. [5]),

$$E = \frac{d\sigma}{dA/A} = \frac{-d\Pi}{dlnA} = E_d + iE_v \quad (5)$$

where E_d is the surface dilatational elastic modulus (mN/m), and E_v is the surface dilatational viscous modulus (mN/m).

2.10. Statistical analysis

All the results are presented as mean ± standard deviation from at least 3 replicates (n = 3). A one-way analysis of variance (ANOVA) was performed to identify significant differences ($p < 0.05$). Least significant difference (LSD) multi comparison tests were used to identify sample means that were significantly different. All the statistical analyses were performed with R software version 4.2.1 [31] using *agricolae* package for LSD test.

3. Results and discussion

3.1. Biochemical characterization of the protein powders

The composition of the studied commercial plant protein samples is presented in Table 1. The main component is protein, accounting for 50 % in PPC and for 71 % in PPI. PPC displays a significantly elevated carbohydrate content, nearly 36 % of its composition, in accordance with reported starch content of approximately 40 % [7]. The lipids content is lower for PPI than for PPC, 0.4 versus 2.1 % respectively.

Table 1
Biochemical composition of pea protein concentrate (PPC) and pea protein isolate (PPI) (Values are presented as mean ± standard deviation).

Composition (g/100 g)	PPC	PPI
Protein	49.97 ± 0.01	71.2 ± 0.3
Lipids	2.1 ± 0.4	0.44 ± 0.02
Carbohydrates*	36.2 *	16.0 *
Moisture	5.3 ± 1.2	7.1 ± 0.1
Ash	6.4 ± 0.2	5.27 ± 0.01

*Assumed to be equal to the complement to 100 % relative to all other measured components

3.2. Effects of physical treatments and enzymatic hydrolysis on dilute PPC dispersions

3.2.1. Impacts on the soluble protein fraction

Dilute PPC dispersions at 8 wt% were first examined. The soluble protein content was quantified following physical and enzymatic treatments to evaluate their impact on protein solubilization (Fig. 1 (A)). Regarding the enzymatic treatment, only one enzyme was used in this study. It was selected for its efficiency in terms of enzymatic activity across a wide range of conditions and its non-GMO nature. The operating conditions were optimized by evaluating the degree of hydrolysis, solubility, foaming capacity, and molecular weight distribution measured by size-exclusion chromatography (see Section 2.6.3). This optimization was conducted through an experimental design in which the selected control factors were temperature (35°C to 55°C), enzyme/substrate ratio (100–1000), dispersion dry matter content (2–8 %), and duration (15–90 minutes).

Sonication resulted in a slight improvement of the soluble protein concentration compared to the untreated PPC dispersion (25.6 ± 1.8 g/L *versus* 21.9 ± 1.5 g/L, respectively). This increase can be attributed to structural changes in the proteins induced by ultrasound, exposing hydrophilic groups and thereby enhancing protein water solubility. However, the solubility increase is less remarkable than reported in the literature [17], likely due to the lower intensity of the ultrasound treatment used here (55 W, 20 min compared to 200 W, 5 min). Indeed, we chose to use a low power level to ensure that the samples did not experience heating.

Despite the high temperature adopted (80°C for 20 minutes), the thermal treatment did not significantly alter solubility. This finding aligns with the work of Chao et al. [32] reporting that heating did not modify pea protein solubility when thermal treatments between 55°C and 90°C were applied to PPI dispersions.

In the conditions of enzymatic hydrolysis studied (55°C for 90 min, E/S = 1000 AU/g), the degree of hydrolysis (DH) of pea proteins was determined to be 13.7 ± 1.9 %. The soluble protein content of HYD PPC was unexpectedly the lowest among all the conditions studied (18.9 ± 1.5 g/L). This outcome was surprising, considering that hydrolysis is generally known to enhance protein solubility. For instance, Shahbal et al. [26] previously reported that enzymatic hydrolysis of various plant proteins using Alcalase improved solubility for all sources. Additionally, in the study by Shuai et al. [33], hydrolysis of PPI by different proteases increased protein solubility by at least 5 %, even at low degrees of hydrolysis (DH = 2 %). The increase in solubility was attributed to the reduction in the molecular size of proteins.

The unexpected result obtained in our case may be due to the limitation of the soluble protein assay (BCA assay), which may not accurately quantify very small peptides. Cortés-Rios et al. [34] indicated that the BCA assay could quantify tri-peptides as the minimum length. To verify this hypothesis, the dispersions were characterized for their soluble protein size profile using size exclusion chromatography (SEC). The

A

	PPC	US PPC	TH PPC	HYD PPC
Total protein concentration (g/L)	43.5			
Soluble protein concentration (g/L)	21.9 ± 1.5 *b*	25.6 ± 1.8 *a*	20.8 ± 0.3 *bc*	18.9 ± 1.5 *c*

B

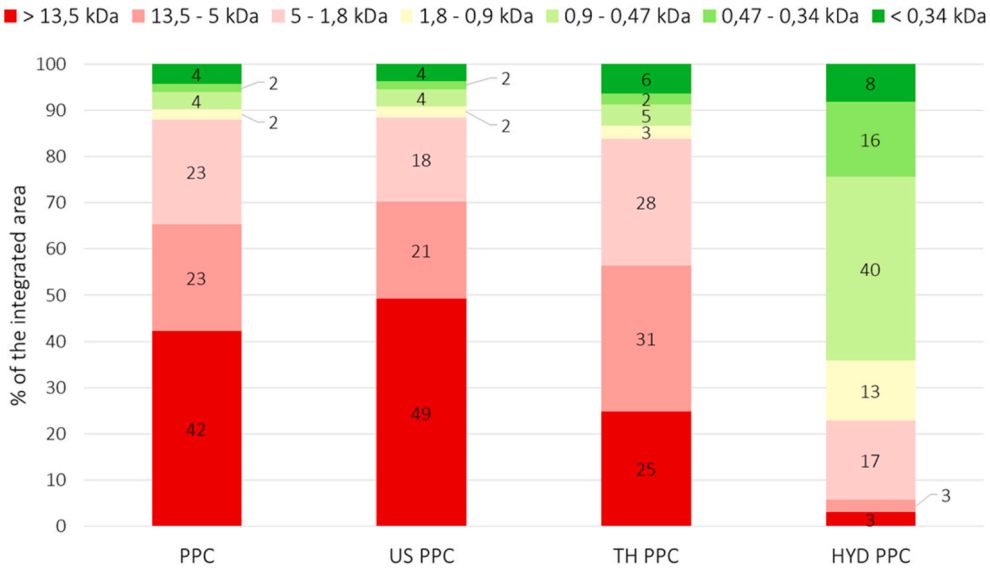

Fig. 1. (A) Total and soluble protein contents and (B) Size distribution from SEC-HPLC for native (PPC), sonicated (US PPC), heated (TH PPC) and enzymatically hydrolyzed (HYD PPC) pea protein concentrate dispersions at 8 wt%. (Values are presented as mean ± standard deviation; means with the same letter are not significantly different ($p < 0.05$)).

SEC elution profiles of both initial and treated PPC dispersions were segmented into seven size fractions (Fig. 1 (B)). The results concerning native PPC align with the expected composition, as the dispersion comprised more than 40 % of proteins over 13.5 kDa. Notably, pea albumins, the water-soluble protein fraction, are larger than 10 kDa [7].

Non-hydrolyzed dispersions, including those treated with ultrasound (US PPC) and heat (TH PPC), exhibited a protein molecular weight distribution very similar to that of native PPC, with over 84 % of the protein sizes exceeding 1.8 kDa. Sonication slightly raised the proportion of proteins over 13.5 kDa (49 % *versus* 42 %). It is likely that ultrasounds facilitate the solubilization of higher molecular weight proteins and form soluble protein complexes simultaneously [17]. TH PPC exhibited a lower proportion of proteins with a molecular weight greater than 13.5 kDa and an increased proportion of the 13.5–5 kDa size fraction. This change could be attributed to the aggregation of high molecular weight soluble proteins that were retained during sample filtration before SEC analysis. This hypothesis is supported by the lower signal intensity observed for TH PPC compared to native PPC (Fig. SI 1, Supplementary information). However, these proteins remained soluble and were quantified by spectrophotometric soluble protein assays, leading to similar soluble concentrations in TH PPC and native PPC (20.8 and 21.9 g/L, respectively). Elution profiles of native PPC, US PPC, and TH PPC presented the same pattern, suggesting that these physical treatments did not damage the ternary or quaternary structures of proteins.

As expected, enzymatic hydrolysis resulted in a clear shift in the molecular weight distribution of pea proteins: 64 % of the peptides had molecular weights lower than 0.9 kDa for HYD PPC, compared to only 10 % for native PPC. This aligns with our hypothesis regarding the BCA assay limitations to quantify small peptides, as the SEC profile shows that HYD PPC primarily comprised peptides lower than tripeptides that were not detected by the assay. Previous studies on the effects of the commonly used Alcalase endoprotease showed a similar trend, with the production of peptides with low molecular weights below 10 kDa. SDS-PAGE profiles demonstrated the disappearance of bands with molecular sizes between 17 and 100 kDa after proteolysis, leaving a thick area with indistinguishable bands below 10 kDa [26].

3.2.2. *Impact on the foaming properties*

The 8 wt% dispersions submitted to the different treatments were evaluated for their foaming capacity (FC) and foam stability (FS) after 1 h storage. The high-speed shearing method described in Section 2.7.1 was employed to generate foams from the diluted PPC dispersions. In Fig. 2, the results are compared to those obtained from the untreated dispersion (PPC).

In the realm of physical treatments, heat treatment demonstrated an enhancement of the foaming capacity (FC) when compared to the native PPC dispersion (69 ± 4 % *versus* 54 ± 2 %). Conversely, sonication did not exhibit any significant improvement (61 ± 10 %). The impact of enzymatic proteolysis was markedly more pronounced than that of the physical treatments, resulting in an FC as high as 114 ± 7 %, which is more than twice the FC value of the native PPC dispersion. Foaming stability (FS) surpassed 57 % for all examined conditions, indicating that more than half of the foam volume was retained one hour after foam production. Enzymatic hydrolysis emerged as the sole treatment that significantly enhanced foam stability compared to the native PPC dispersion, with values of 84 ± 3 % and 57 ± 2 %, respectively. A control test was performed with PPC and the enzyme thermally inactivated, which did not result in any improvement in FC nor FS parameter. Therefore, the improvement in foaming properties is clearly attributable to the use of the enzyme.

In the present study, sonication did not alter significantly the

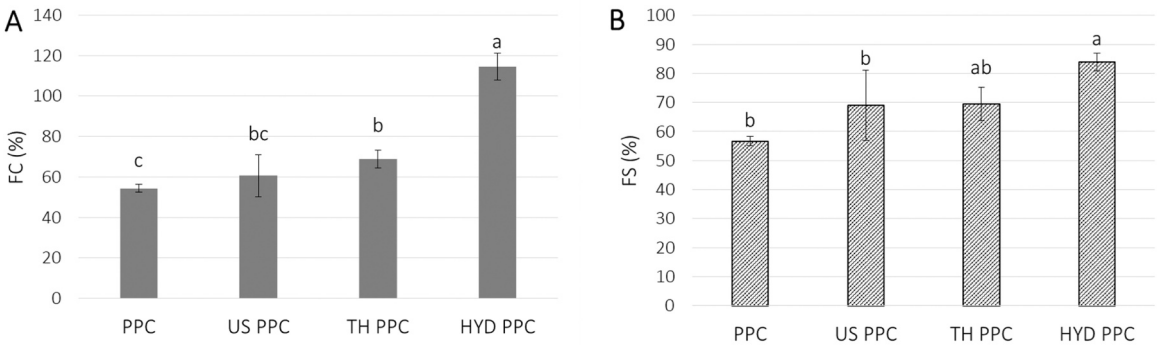

Fig. 2. (A) Foaming capacity (FC) and (B) foam stability (FS) of 8 wt% pea protein concentrate (PPC) dispersions after sonication (US PPC), thermal treatment (TH PPC) and enzymatic hydrolysis (HYD PPC) (significant differences between conditions are indicated by different letters, $p < 0.05$).

foaming properties of pea proteins, even though previous research has shown that this treatment can have an influence on it [17,27]. The enhancement of pea proteins' foaming properties after sonication is attributed to structural modifications of the proteins. Ultrasound induces partial unfolding of the proteins, leading to a quicker adsorption of unfolded proteins at the air/water interface, thereby improving foam formation.

The marginal improvement in foaming capacity observed for the TH PPC in this study is in line with observations made by Chao et al. [32]. These researchers noted an enhancement in foam stability but observed varying effects on the foaming capacity of PPI dispersions based on the applied thermal treatment: the foaming capacity remained unchanged after 30 min at 80°C, while it increased following 30 min at 50°C, suggesting that heating at high temperature reduces proteins foamability. In our study, results revealed that a treatment of 55°C for 1h30, followed by 20 min at 80°C, led to a 15 % increase in both foaming capacity and foam stability. Here, the time/temperature pattern used for the thermal treatment did not affect proteins foamability. Similarly to sonication, it can be hypothesized that protein unfolding and enhanced polypeptide chain flexibility induced by the thermal treatment facilitated adsorption at the air/water interface, thereby promoting foam formation.

Improvements in pea protein foaming capacity through enzymatic hydrolysis have been observed in various studies on PPI. For instance, there were increases from 100 % to 243 % with pepsin hydrolysis (76 g/L protein solution, DH = 9–12 %) as reported by Tang at al. [35], and from 45 % to 168 % with trypsin hydrolysis (67 g/L protein solution, DH = 4 %) as reported by Shuai et al. [33]. However, in those studies, foam stability did not show significant differences between the untreated and the hydrolyzed pea proteins.

In contrast, our results demonstrated a positive effect of enzymatic proteolysis on both foaming capacity and foam stability. The enhanced foaming capacity may be attributed to the reduction in molecular weight of the proteins, facilitating a faster migration rate at the air-water interface [35]. It is likely that due to their enhanced flexibility, these peptides develop stronger lateral interactions, creating a rigid layer that contributes to foam stability, as suggested by Wouters et al. [24].

Our findings unequivocally demonstrate that enzymatic proteolysis, involving peptide bond cleavage and molecular weight reduction, effectively functionalized pea proteins. In comparison to the two physical treatments, proteolysis resulted in a significant enhancement of foaming properties. This investigation, conducted on dilute PPC dispersions, led to the conclusion that enzymatic hydrolysis stands out as the optimal approach for augmenting their foaming properties.

3.3. Scale-up and functionalities of hydrolyzed concentrated dispersions

With the goal of using PPC dispersions as a food ingredient that could substitute whole eggs, they were formulated with the same dry extract (23 wt%). Their foaming characteristics were examined and compared to those of eggs. It is noteworthy that the foaming attributes of PPC dispersions can be affected by the presence of endogenous starch revealed by Maltese crosses in polarized micrographs (Fig. SI 2 (A), Supplementary Information). However, these crosses were no longer visible after thermal treatment (20 minutes at 80°C), indicating that starch underwent gelatinization during enzyme inactivation (Fig. SI 2 (C), Supplementary Information). To assess the role of gelatinized starch, PPI dispersions containing around 70 % protein (Table 1) and no starch were also examined. Their dry extract was fixed at 16 % to achieve the same protein content as whole eggs [36] and HYD PPC 23 wt% dispersions, which is 11.5 %. Foams were produced from the above-mentioned hydrolyzed concentrated protein dispersions and from whole eggs.

3.3.1. Foam characteristics

The foaming capacity (FC) and the foam viscoelastic moduli G' and G'' were measured and the values are reported in Table 2.

Whole eggs and the hydrolyzed dispersions exhibited the capability to trap substantial volumes of air, although with notable differences. HYD PPI 16 wt% showed the highest FC value (1111 %), followed by whole eggs (646 %), and finally HYD PPC 23 wt% (485 %). The foaming capacity of whole eggs is higher than the value (350 %) reported by Li et al. [37], possibly due to a difference in whipping duration (5 min *versus* 15 min in our study).

The viscoelastic properties of all studied foams exhibited a consistent trend, with the storage modulus (G') surpassing the loss modulus (G''), indicating a dominance of elastic behavior in the foams. For eggs and HYD PPI 16 wt% foams, the G' and G'' values were relatively low ($G' = 15$ Pa, $G'' = 10$ Pa for eggs; $G' = 99$ Pa, $G'' = 15$ Pa for HYD PPI 16 wt%) compared to HYD PPC 23 wt% foams ($G' = 459$ Pa, $G'' = 132$ Pa). In comparison to eggs and HYD PPI 16 wt%, HYD PPC 23 wt% foams exhibited a solid-like behavior: they did not show flowing behavior and supported their own weight. Microscope observations indicated that HYD PPC 23 wt% foams had smaller and more uniform bubble sizes compared to egg foams (Fig. 3 (A) and (B)). The bubbles in egg foams exceeded 200 μm in diameter, whereas most bubbles in HYD PPC 23 wt % foams measured around 100 μm.

Table 2
Foaming properties of whole eggs, hydrolyzed pea protein concentrate dispersions at 23 wt% (HYD PPC 23 wt%) and hydrolyzed pea protein isolate dispersion at 16 wt% (HYD PPI 16 wt%) (Means with different letters within the same column are significantly different, $p < 0.05$).

	FC (%)	Foam viscoelasticity (0.5 % strain)	
		G' (Pa)	G'' (Pa)
Eggs	646 ± 7^b	15.3 ± 1.3^c	9.5 ± 0.3^c
HYD PPC 23 wt%	485 ± 36^c	458.6 ± 20.1^a	132.3 ± 4.6^a
HYD PPI 16 wt%	1111 ± 37^a	98.9 ± 8.1^b	15.3 ± 2.2^b

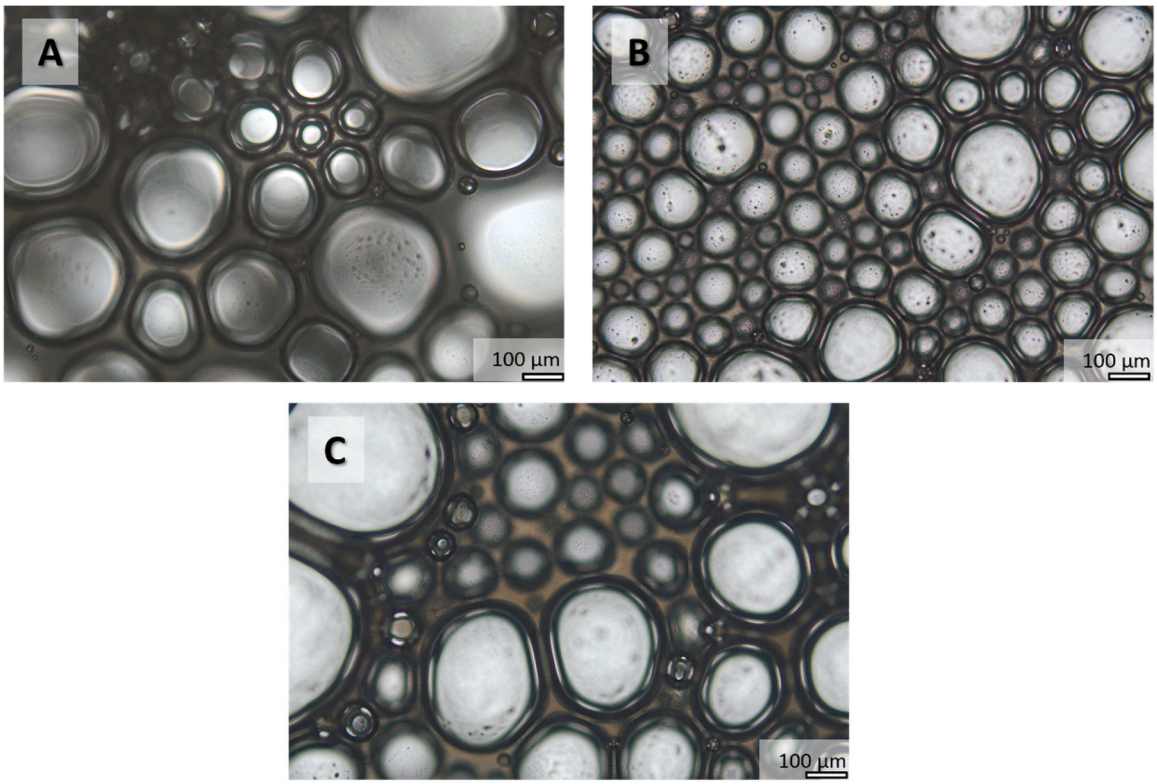

Fig. 3. Micrographs of whole eggs (A), HYD PPC 23 wt% (B) and HYD PPI 16 wt% (C) foams.

The observed differences can be attributed to the presence of gelatinized starch in HYD PPC dispersions. A similar correlation was noted in the study by Martin Torrejon et al. [38], which involved foams made from gelatin suspensions with and without pregelatinized tapioca starch. Substantial differences were observed in terms of the incorporated air volume: in presence of gelatinized starch, the highly viscous suspension could not incorporate as much air as the suspension without. A further comparison between foams obtained from HYD PPC 23 wt% and HYD PPI 16 wt% highlights the disparities between a pea protein concentrate and a pea protein isolate. Despite nearly identical total protein contents (11.5 %), foam characteristics differed significantly in terms of air volume fraction, primarily due to the elevated carbohydrate content of PPC dispersions. Concomitantly, the higher viscosity of the HYD PPC 23 % dispersions generated higher viscous stresses during the whipping process and thus smaller bubbles than the HYD PPI 16 % dispersions, as evidenced by microscope observations (Fig. 3 (B) and (C)).

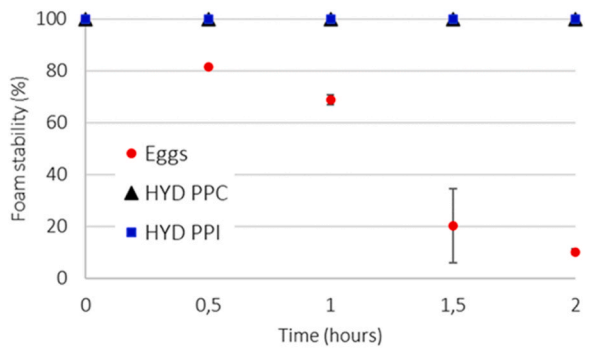

Fig. 4. Foam stability (FS) over time of whipped wholes eggs, hydrolyzed pea protein concentrate dispersion (HYD PPC 23 wt%) and hydrolyzed pea protein isolate dispersion (HYD PPI 16 wt%). Results are presented as means; standard deviations are shown as error bars.

Foam stability was monitored for up to 2 hours (Fig. 4). During the initial hour, the egg foam remained relatively stable, with a volume loss close to 30 % (FS = 69 ± 2 %). However, a significant decline in egg foam stability occurred after 1.5 hours, resulting in almost complete collapse. In contrast, foams generated from HYD PPC and HYD PPI exhibited consistent stability throughout the entire observation period.

The destabilization of aqueous foams is attributed to three primary mechanisms: drainage, disproportionation (Ostwald ripening), and coalescence [39]. Coalescence, the merging of air bubbles, typically occurs as a result of liquid drainage. As films between bubbles and Plateau borders (interstitial zones in between 3 adjacent bubbles) thin out, the interfaces approach to each other, leading to eventual film disruption. In our investigation, we noted both drainage and coalescence contributing to the destabilization of whole eggs foam. Indeed, over the observation time, we could observe an increase of the drained liquid as well as a swifter raise of the air bubbles sizes than the average, revealing the occurrence of coalescence phenomena [40] (Fig. SI 3, Supplementary Information). In their study involving microscopic observation of whole egg foam, Spencer et al. [41] revealed a destabilization mechanism via disproportionation. Over time, bubble size increased as gas diffused from smaller bubbles to larger ones. Previous research has explored the destabilizing impact of egg yolk on egg white foams. Li et al. [37] found that adding 30 % egg yolk to egg white increased foam drainage by 45 % within 30 minutes. Wang et al. [42] determined that even a low concentration of 0.5 % egg yolk decreased egg white foam stability by 10 % and increased drainage by 40 % within the same timeframe. Therefore, given the use of whole eggs in our study, the observed limited foam stability is in agreement with these authors' findings. Egg white has a favorable effect on both foaming and stability, while egg yolk predominantly exerts a destabilizing effect.

For HYD PPC and HYD PPI foams, no destabilization similar to that observed in whole eggs was identified and the total foam volume remained unaffected. Indeed, no drainage was observed over the 2-hour observation period, even though HYD PPC and HYD PPI foams evidenced significantly bigger bubbles sizes as the experiment was going on

(Fig. SI 3, Supplementary Information).

The lifespan of foams is generally influenced by drainage through the liquid films separating air bubbles and the Plateau borders [39]. In HYD PPC 23 wt% foams, the continuous phase is thickened due to the dissolved polysaccharides and the gelatinization of starch, resulting in a measured viscosity of 65 mPa.s for HYD PPC 23 wt% *versus* 10 mPa.s for whole eggs at 500 s^{-1}. Furthermore, aggregates from the insoluble fraction form an interconnected three-dimensional network which slows down drainage. Interestingly, in Fig. 3 (B), dark spots seem to indicate the presence of particles at the air-water interfaces. It is likely that particles or clusters from the insoluble fraction adsorb at the air-water interfaces or intercalate into the films, thus preventing them from thinning.

3.3.2. *Interfacial properties of hydrolyzed pea proteins soluble fraction and whole egg proteins*

A critical factor influencing the formation and stability of protein foams is the ability of proteins to function as surface-active species, thereby reducing the interfacial tension between air and water and generating interfacial stiffness.

Drop tensiometry measurements were conducted to compare the performance of egg proteins and hydrolyzed pea proteins at the air-water interface. For HYD PPC 23 wt%, the measurements were conducted using only the water-soluble fraction.

The surface pressure (Π) was monitored over time for eggs and HYD PPC at 0.1 g/L (Fig. 5 (A)). The observations were continued until the surface pressure value approached a nearly asymptotic value. The process of protein adsorption typically involves three distinct steps: diffusion, adsorption, and rearrangement. The diffusion phase is characterized by a lag period in the evolution of surface pressure as proteins move towards the interface. Subsequently, the surface pressure increases rapidly as proteins adsorb at the interface. Finally, during the rearrangement phase, only minimal alterations in surface pressure are observed as proteins undergo rearrangements [8].

For HYD PPC proteins, no lag phase was observed. The surface pressure exhibited a rapid increase within the initial 100 seconds, rising from 1 to 8 mN/m, indicating a swift diffusion of peptides to the interface. Similar immediate surface pressure increases have been reported in studies on the interfacial properties of pea or legume proteins. In a study by Kornet *et al.* [43], unhydrolyzed soluble pea proteins (1 g/L) raised the surface pressure by 10 mN/m in 100 s. However, our achieved surface pressure value after 1000 s was slightly lower (17 mN/m compared to 21 mN/m).

The surface pressure curve for eggs exhibited a prolonged induction time of approximately 500 seconds before a sharp increase. Between 300 and 2000 s, the surface pressure increased by 10 mN/m and reached a value of 15 mN/m after 3000 seconds. In a study by Jin et al. [44], the surface pressure in binary systems of egg white proteins (ovalbumin/ovomucoid 5:1) reached a value exceeding 20 mN/m, which is higher than the measurement obtained here for whole eggs. In the case of whole eggs, the three steps described for protein adsorption are clearly observable, contrary to the observations of Jin et al. [44] on egg white proteins. The extended lag phase at the beginning may indicate a slower diffusion of proteins to the interface because of the presence of proteins of higher molecular weight (e.g., ovomucin, ovotransferrin). Li et al. [45] demonstrated that contamination of egg white with egg yolk reduces its ability to adsorb at the air-water interface and, consequently, its foaming properties.

For both HYD PPC and eggs, the surface pressure did not truly reach a plateau regime at the end of the observation, but its evolution slowed down. The continuous increase of surface pressure over time suggests that protein adsorption is a dynamic phenomenon with constant reorganizations at the interface.

Dilatational interfacial properties may contribute to understanding the phenomena involved in foam formation and the differences in foaming properties between whole eggs and HYD PPC. Foam formation relies on the ability of proteins to quickly adsorb at the interface when the system undergoes perturbations that introduce air or stretch the interfaces.

The viscoelastic modulus (E) curves exhibited different shapes (Fig. 5 (B1)). For eggs, an induction period occurred within the first 200 s, after which the viscoelastic modulus increased continuously before stabilizing at around 55 mN/m after 1000 s. In contrast, for HYD PPC, there was no induction phase. The viscoelastic modulus increased rapidly between the beginning of the observation and 200 s, reaching a plateau value of 40 mN/m.

The viscoelastic moduli curves closely resembled those obtained for the elastic modulus, E_d (Fig. SI 4, Supplementary Information), with E_d being higher than the viscosity modulus, E_v, indicating the dominance of elastic behavior at the interface. The absence of an induction period for HYD PPC suggests rapid protein adsorption at the interface. In a study by Shen et al. [46], the viscoelastic modulus of native PPIs also exhibited an early increase. However, the value reached upon protein adsorption at the interface was much lower than that observed for our pea protein hydrolysate (10 mN/m *versus* 40 mN/m). The higher viscoelastic modulus values for HYD PPC suggest that hydrolyzed pea proteins establish stronger interactions at the interface compared to native pea proteins.

We observed significant variability in the duration of the induction phase for eggs. This is illustrated in Fig. 5 (B2), which presents two

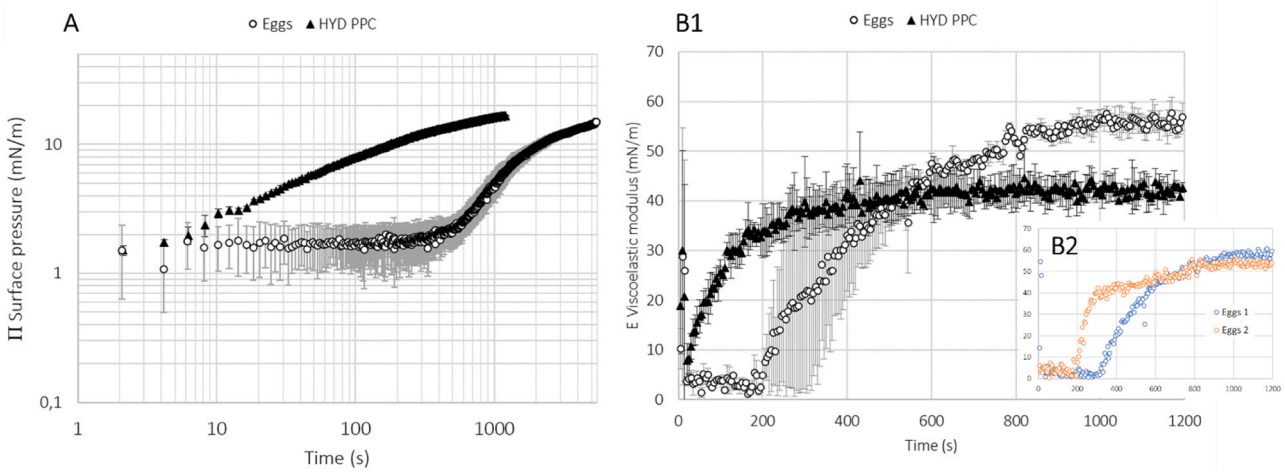

Fig. 5. Time-dependent changes in (A) surface pressure (Π) and (B1) viscoelastic modulus, E, at the air-water interface of proteins (0.1 g/L) from whole eggs and hydrolyzed pea protein concentrate (HYD PPC, soluble fraction). (B2) Detail of the two curves for whole eggs represented as a mean in (B1).

separate measurements. As depicted in Fig. 5 (B1) representing the mean of the replicates, the large error bars in the viscoelastic modulus mainly result from the difference in lag time that could extend up to 300 seconds. Nevertheless, the trend in the evolution of interfacial viscoelasticity remains consistent across both replicates. A competition for adsorption at the interface may occur between proteins from egg white and egg yolk, resulting in an induction phase of variable duration at the beginning of the observation. This is in contrast to the study by Jin et al. [44], where the viscoelastic modulus of egg white proteins exhibited a sharp increase without any induction phase. Despite this difference, our results align with those presented in the literature regarding the reached viscoelastic modulus values: at long times of observation, they were comparable for egg white proteins (around 50 mN/m in the study of Jin et al. [44]) and whole eggs (55 mN/m). This suggests that the long term- interfacial properties of whole eggs are primarily influenced by egg white proteins.

The trend in the evolution of viscoelastic moduli for HYD PPC and whole eggs shown in Fig. 5 hints at a potentially faster strengthening of the interfaces covered by HYD PPC proteins. This phenomenon can be attributed to the lower molecular weight resulting in higher flexibility of the hydrolyzed pea proteins compared to globular and rigid egg proteins. In both systems, high values of viscoelastic modulus (> 40 mN/m) were obtained, indicating the solid-like nature of the interfacial films formed.

It is worth noting that the apparatus employed for investigating the air/water interface may have limitations in elucidating further distinctions between the two systems. Particularly in the context of surface viscoelasticity measurement, the applied deformations may not mirror the real conditions of foam formation and evolution [47]. Furthermore, these findings regarding interfacial behavior solely reflect the influence of the soluble fractions.

In summary, under the examined conditions, both systems demonstrated similar surface pressure and viscoelastic modulus values, indicating their capability to foam through the formation of rigid films with in-plane (lateral) protein interactions. Contrary to systems where enhanced stability against coalescence was associated to an increase in surface viscoelasticity [48], our observations did not support this correlation. This suggests that, in our systems, other factors are more influential. Again, it can be argued that in foams based on HYD PPC, the presence of both polysaccharides dissolved in the aqueous phase and aggregates formed by the insoluble fraction lead to increased viscosity and even gelation, which slows down the draining process and thus extends the lifespan of the foams.

4. Conclusion

In this study, our primary objective was to identify protein modification methods that could enhance the foamability of pea protein concentrates. We explored both physical and enzymatic treatments on low dry matter pea protein dispersions, with a focus on characterizing the soluble fraction before conducting foaming experiments. Sonication increased the soluble protein content and resulted in the formation of soluble protein complexes. Thermal treatment did not modify the soluble protein concentration. Analysis of protein size distribution revealed significant alterations in protein structures with enzymatic proteolysis, leading to the formation of low molecular weight peptides.

Size exclusion chromatography did not show any modification of the primary protein structure with physical treatments. However, the enhancement of foaming capacity indicated that physical treatments induced structural changes in the polypeptide chains, promoting protein unfolding and exposure of hydrophobic regions that facilitated the formation of aqueous foams and stabilized them. Following enzymatic modification, the foaming properties of proteins were significantly higher compared to the native protein dispersion. The comparison of different systems highlighted the synergy of soluble and insoluble components in hydrolyzed protein concentrates. The reduced molecular weight of the hydrolyzed species facilitated their diffusion towards the interface and resulted in the formation of firm interfaces. Our study was focused on protein modification, but other components may contribute to the improvements in foaming properties through sonication, heat treatment, or enzymatic proteolysis. For instance, the presence of insoluble particles and polysaccharides contributed to bolstering physical stability.

Using plant protein concentrates in place of isolates could be a way to develop stable foams by taking advantage of the texturing properties of the carbohydrate content without resorting to food additives.

CRediT authorship contribution statement

Fernando LEAL-CALDERON: Writing – review & editing, Methodology, Conceptualization. **Martine CREPIN:** Project administration. **Cécile LEBRUN:** Project administration, Funding acquisition. **Mathieu DELAMPLE:** Project administration. **Mathilde ROZE:** Writing – review & editing. **Raphaëlle SAVOIRE:** Writing – review & editing, Methodology, Conceptualization. **Christelle HARSCOAT-SCHIAVO:** Writing – review & editing, Methodology, Conceptualization. **Lucie Périé:** Writing – original draft, Visualization, Methodology, Investigation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: PERIE reports financial support was provided by ASSOCIATION NATIONALE RECHERCHE TECHNOLOGIE. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.colsurfa.2024.136076.

Data Availability

Data will be made available on request.

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