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Mild high hydrostatic pressure processing: Effects on techno-functional properties and allergenicity of ovalbumin

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ABSTRACT

The effects of mild (250–350 MPa) high hydrostatic pressures (HHP) on the technological properties of ovalbumin were studied. Thermal gels were prepared using HHP-treated ovalbumin. Their characteristics and the efficacy of HHP processing to inhibit allergenicity were evaluated. The samples treated at 250 MPa/15 min, 350 MPa/10 min and 350 MPa/15 min showed the best results for solubility and water and oil absorption capacities, respectively. Regardless of treatment duration, foaming capacity increased with pressure. The foam stability only increased significantly in the samples subjected to 350 MPa for 10 and 15 min. On the contrary, the mildest treatment yielded the highest emulsifying activity index and emulsion stability. Improved gel strength and water holding capacity were observed, particularly under 300 MPa, resulting in a maximum inhibition of allergenicity (46.75%).

1. Introduction

Ovalbumin constitutes 54% of the protein content by weight and is the primary protein in egg whites (Mine, 1995). This globular protein has a molecular weight of 45 kDa, an isoelectric point of 4.5, one disulfide bond, and four free sulfhydryl groups that are buried in the protein core (Gharbi and Labbafi, 2019). Additionally, it comprises a carbohydrate chain that enhances the protein's foaming and gelling capabilities (Lv et al., 2015).

Ovalbumin is recognized as one of the main food allergens, causing various forms of food intolerance in humans (Zhang et al., 2020). Hovewer, due to its nutritional, gelling, emulsifying and foaming properties, ovalbumin is a widely used food ingredient.

High hydrostatic pressure (HHP) processing induces modifications in macromolecules like proteins (Queirós et al., 2018). Therefore, utilizing this technology can enhance the functional properties of such macromolecules in line with industry requirements.

Several studies have documented the effects of HHP on the functional properties of ovalbumin. Protein concentrations below 5% and pressures above 400 MPa were the most frequently used conditions in these experiments. Under these HHP-conditions, changes in protein structures could occur that would affect even the secondary structure (He et al., 2014), which in turn favors a non-reversible denaturation of proteins through unfolding and aggregation processes (Gharbi et al., 2022).

The effect of moderate pressures (200–400 MPa) would be limited to tertiary and quaternary structures, supported mainly by hydrogen bonds, and inter- and intramolecular hydrophobic interactions (Queirós et al., 2018). As the tertiary structure provides protein functionalities, any change in this sense could result in changes in the proteins' technological properties (Hoppe, 2010; Kavuşan and Serdaroğlu, 2019) such as solubility, foaming, emulsification, and gelation. Furthermore, the disruption of intramolecular disulfide bonds and the subsequent unfolding of proteins generally reduce allergenicity (Naderi et al., 2017).

In addition, ovalbumin treated with moderate pressures could be used to elaborate/produce gels, an application that has received much attention in the food industry in recent years (Munialo et al., 2018). In particular, gels with a protein content greater than 15% may be of interest for certain groups such as the elderly and people suffering from dysphagia. These patients are limited in the type and amount of food they can eat and therefore, are more prone to suffer malnutrition. In order to manage dysphagia, nutritional management must ensure both, an adequate food texture and an appropriate intake of energy and proteins (Jukic Peladic et al., 2023).

Despite the advantages of the HHP technology, the related investment and operational costs may limit a broader industrial use (Liu et al., 2019). Regarding the former, the investment cost of the equipments

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increases as the working pressure get higher (Velazquez et al., 2005). What is more, as protein powders are preferred for industrial applications the use of highly concentrated solutions allows maximizing protein recovery after pressurization.

Thus, the two objectives of this study were (1) to evaluate the effects of mild HHP (250–350 MPa) on the technological properties and the allergenicity of ovalbumin, and (2) to study the physical characteristics of thermal gels prepared from HHP-treated ovalbumin.

2. Materials and methods

2.1. Ovalbumin dispersions

About 1.5 kg of protein dispersion (12 %, w/w, pH 6.50 ± 0.02) were prepared by dispersing egg ovalbumin powder (protein content 82 %, water <8 % ashes <6 % salt 3.1 %, citric acid 2 %, carbohydrates <0.2 %, lipids <0.1 % HeLa SL, Spain) in distilled water. The dispersion was homogenized by stirring for 30 min at room temperature (20 ± 2 °C) with a magnetic stirrer. The dispersion (300 ± 10 g) was introduced into polyethylene bags, avoiding the introduction of air bubbles. The bags were packed in double polyethylene bags to prevent direct contact with the pressure medium and vacuum sealed. For allergenicity assays, the dispersions were prepared at a concentration of 0.1% (w/w).

2.2. High-pressure treatment

A high-pressure unit (IDUS 50 L, maximum pressure 600 MPa, HHP Systems, Metronics Technologies, Spain) with an inner diameter and capacity of 210 mm and 50 L, respectively, was used in this study. The samples were introduced at room temperature (20 ± 2 °C) and were subjected to 250, 300 and 350 MPa for 5, 10 and 15 min. The treatment times did not include come-up and depressurization times. The pressure-transmitting medium was water and reached a maximum temperature of 29 ± 2 °C due to adiabatic heating. Pressurization rate was about 350 MPa min^{-1} and pressure was released almost immediately after completion of the pressurization cycle. After the HHP treatment, the ovalbumin dispersions were freeze-dried in a LyoBeta freeze drier (Telstar®, Spain), vacuum-packed and stored at 20 ± 2 °C until use. Unpressurized samples were also freeze-dried and used as controls. The dispersions for the allergenicity assays were stored at 4 °C until use.

2.3. Technological properties

2.3.1. Protein solubility

Protein solubility was determined according to the method of Yin et al. (2008), with slight modifications. HHP-treated and untreated freeze-dried ovalbumin (0.02 g) were dispersed in 20 mL of distilled water and stirred at 25 °C for 30 min in a thermostatic bath with agitation (Unitronic P, JP Selecta, Spain). Then, the dispersions were centrifuged at RT (2627×*g*; 20 min) in a Sigma 3K30 centrifuge (Sigma Laborzentrifugen, Germany). Protein content of the supernatants was determined by Lowry's method using bovine serum albumin as the standard. Protein solubility was calculated as follows (Eq (1)):

$$PS\ (\%) = \frac{Protein\ content\ in\ the\ supernatant\ (g)}{Total\ protein\ content\ (g)} * 100 \quad \text{(Eq 1)}$$

2.3.2. Water and oil absorption capacities

Water (WAC) and oil (OAC) absorption capacities were determined according to Beuchat (1977). A mixture of 1 g of dried protein and 10 g of distilled water or sunflower oil was prepared in a centrifuge tube, vortexed for 30 s and left to stand at RT (20 ± 2 °C) for 30 min. Afterwards, the sample was centrifuged at RT (30 min, 950×*g*) using a Sigma 3K30 centrifuge (Sigma Laborzentrifugen, Germany) and the supernatant was weighed. Water and oil absorption capacity were calculated as follows (Eq (2)):

$$WAC \Big/ OAC\ \left(\frac{g\ water/oil}{g\ protein}\right) = \frac{Water/oil\ weight\ (g) - Supernatant\ weight\ (g)}{g\ protein} \quad \text{(Eq 2)}$$

2.3.3. Foaming capacity (FC) and foam stability (FS)

Foaming capacity and foaming stability were determined following the method of Paredes-López et al. (1991). For this purpose, 10 mL of solution (1 %, w/v) were whipped with an Ultra Turrax T-25 homogenizer (IKA, Germany) at 11000 rpm for 2 min and volumes were registered before and after whipping. After 30 min at room temperature (RT), the remaining volume was also registered. Foaming capacity was calculated by equation (3):

$$FC\ (\%) = \frac{V_2 - V_1}{V_1}\ x\ 100 \quad \text{(Eq 3)}$$

where V2 and V1 are the solution volumes after and before whipping, respectively. Foaming stability was determined as the remaining foam volume after 30 min, expressed as percentage of the initial volume.

2.3.4. Emulsifying activity index (EAI) and emulsion stability index (ESI)

The method of Brishti et al. (2020) was followed for the determination of the EAI and the ESI, with some modifications. Briefly, 5 mL of a 3 % (w/v) dispersion of ovalbumin in water and 2.5 mL of sunflower oil were homogenized with an Ultra Turrax T25 basic (IKA, Germany) at 11000 rpm for 1.5 min. An aliquot of 1 mL of the emulsion was diluted in 250 mL distilled water and the absorbance was immediately read at 500 nm. For ESI, the absorbance of the samples was read again after 24 h at 4 °C.

Equation (4) was used for calculating EAI ($m^2\ g^{-1}$):

$$EAI = \frac{4.606 \times A_0 \times F}{\theta \times C \times l \times 1000000} \quad \text{(Eq 4)}$$

where A_0 is the absorbance read at 500 nm at time 0, F is the dilution factor (250), θ is the oil fraction used to form the emulsion (0.33), C is the protein weight/volume unit (0.03 g cm^{-3}) before emulsion formation, and l is the optical path (0.01 m).

ESI (h) was calculated as follows (Eq (5)):

$$ESI = \frac{A_0}{A_0 - A_{24}} \times t \quad \text{(Eq 5)}$$

where A_0 and A_{24} are the absorbances read at time 0 and after 24 h, respectively and t is the time interval (24 h).

2.3.5. Gelation

The least gelation concentration (LGC) was determined following the method described by Sathe et al. (1982). A series of dispersions with increasing protein concentration (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 %, w/w) was prepared. The dispersions were heated in a water bath at 70 °C for 15 min followed by cooling at RT (20 ± 2 °C) for 30 min and further cooling at 4 °C for 24 h. The LGC concentration was defined as the concentration at which a stable gel was formed, and the sample did not fall or slip upon inversion of the tubes.

2.4. Characterization of the heat-induced gels

2.4.1. Preparation of the heat-induced ovalbumin gels

Untreated and HHP-treated freeze-dried ovalbumin was used to prepare 25 % (w/w) protein dispersions. Heat-induced ovalbumin gels were obtained following the method described above for gelation. Once prepared, the gels were stored under refrigeration (8 ± 1 °C) until use.

2.4.2. pH and color

The pH of the gels was measured with a CRISON pH 25 (Hach, USA) pH-meter, using a penetration probe at different sites of the sample.

Color was measured on three points of each sample, using a Minolta CM-2500 d spectrophotometer (Minolta CO, Japan) calibrated to a standard white tile (y −93.5, x −0.3155, y −0.3320) and using the CIE*Lab* color space (CIE, 1976), the illuminant D_{65} and the 10° observer. The coordinates L*, a* and b* were determined and the chroma ($\sqrt{(a^*)^2 + (b^*)^2}$) and hue angle ($H^\circ = \tan^{-1}$ (b*/a*)) were calculated.

2.4.3. Gel strength

Gel strength (N) was defined as the maximum force used in a uniaxial compression test performed with a stainless steel, P/25 probe. A TA-XT. plus texture analyzer and the Exponent, v. 6.1.16.0 software (Stable Micro Systems, England) were used for the assays. To avoid manipulation of the samples, the tests were carried out on gels formed in plastic bottles (34 mm diameter, 68 mm height; 20 mL gel/bottle) without removing them from the containers (Hu et al., 2013). The test conditions were as follows: constant speed of 2 mm s^{-1} and target distance of 5 mm (25 % of the sample initial height).

2.4.4. Water holding capacity (WHC)

The WHC of the heat-induced ovalbumin gels was measured according to the method of Kocher and Foegeding (1993). Gel samples (0.5 g) were placed in micro-centrifuge tubes (Spin-X, Centrifuge Tube Filter, ThermoFisher Scientific, USA) equipped with a filter insert (cellulose acetate, 0.22 μm pore size). After centrifugation (VWR Micro Star 17 Avantor, USA) at RT (400×*g*, 10 min), the water released by the sample was weighed and the WHC was calculated using the following equation (Eq 6):

$$WHC\ (\%) = \frac{(total\ g\ of\ water\ in\ the\ sample - g\ of\ released\ water)}{g\ of\ sample}\ x\ 100 \quad \text{(Eq 6)}$$

2.4.5. Scanning electron microscopy (SEM)

The microscopic network of heat-induced HHP-treated ovalbumin gels was observed according to the method described by Wang et al. (2019). Freeze-dried gel samples were mounted onto a scanning electron microscopy (SEM) plate using conductive carbon tabs, and Pt-coated by sputtering of 7 nm. The three-dimensional network microstructure of the gels was then observed and photographed using a JSM-5610-LV scanning electron microscope (JEOL, Ltd. Tokyo, Japan) at a 20-kV acceleration voltage. The magnification used for the gel observation was ×500 and the images were analyzed with the Image J 1.52a image analysis software. A circularity index was determined, which varies from 0 (non-circular shapes) to 1 (perfect circles).

2.5. Allergenicity

A Proteon EGG® (ref. ZE/PR/OVS (ZEULAB 40 S.L., Spain) commercial enzyme-linked immunosorbent assay (ELISA) kit was used to quantify the allergen present in the control and the HHP-treated ovalbumin samples following the manufacturer's instructions. Samples were prepared using the provided extraction buffer, pipetted (100 μL) in duplicate into the wells and incubated for 30 min at 20 ± 2 °C. The plates were emptied and washed three times with washing buffer (300 μL) removing afterwards the residual liquid. Then conjugate was added (100 μL) into each well and incubated 30 min at 20 ± 2 °C. The washing step was repeated as before and the substrate (100 μL) was added and leaved for 30 min at 20 ± 2 °C. The reaction was developed (blue color) and the enzyme reaction was stopped by adding stop solution (50 μL) into each well. The absorbance was measured using a microplate scanning spectrophotometer at 450 nm.

The results were expressed as allergen inhibition (%).

3. Statistical analyses

The experiments were conducted in quadruplicate, except for gelation capacity that was determined in duplicate. Data were subjected to a one-way analysis of variance (α = 0.05) using the IBM SPSS Statistics Version 25 software (IBM Corp. USA). When statistically significant differences were found, mean treatments were compared using Tukey's test.

4. Results and discussion

4.1. Technological properties

4.1.1. Protein solubility (PS)

A fundamental goal in protein product manufacturing is the preservation of protein solubility as it constitutes a pre-requisite for other functional properties (Baier and Knorr, 2015). The solubility of the untreated samples was of 79.50 ± 0.84 %. Both, pressure intensity and treatment time significantly ($p < 0.05$) affected the PS of the ovalbumin protein (Fig. 1).

In effect, all the samples pressurized at 250 MPa showed higher solubility than the control with a gradual increase in this property with increasing treatment time. On the contrary, under 300 and 350 MPa, the solubility of the protein only increased in the 5-min treatment whilst the formation of insoluble aggregates could be responsible for the decrease in this property in the 10- and 15-min treatments. Indeed, the lowest solubility (76.31 ± 0.56 %) was observed under the more severe conditions (350 MPa/15 min).

Similar results were observed in egg white (Andrássy et al., 2006) and rice bran protein (Zhu et al., 2017) after HHP treatments. These authors reported that protein solubility increased at low pressures up to 200 MPa but decreased with further increases in pressure intensities. High pressure treatments at low pressure levels (~200 MPa) may provoke the dissociation of oligomeric proteins, a progressive unfolding of the protein structures, and consequently, an enhancement of protein-solvent interactions resulting in an improved solubility (Liu et al., 2019; Silva et al., 2014). On the contrary, high pressure intensities (>300–400 MPa), or prolonged treatment times may result in complete protein unfolding, the exposure of hydrophobic amino acid residues and subsequently, the formation of insoluble aggregates and the loss of protein solubility (Wang et al., 2022).

4.1.2. Water and oil absorption capacities

Ovalbumin water (WAC) and oil (OAC) absorption capacities were significantly affected by both, pressure intensity and treatment time (Table 1). The WAC tended to increase with pressure. In effect, the highest water absorption value of 2.90 ± 0.70 g g^{-1} protein, which was almost 3 times that of the control, was achieved after 10 min of

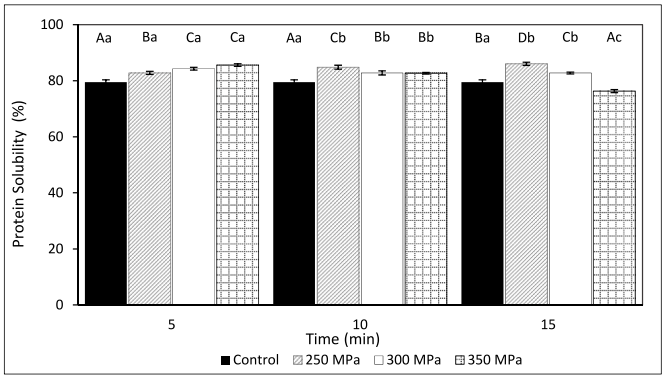

Fig. 1. Protein solubility index (%) of untreated and HHP-treated (250, 300 and 350 MPa; 5, 10 and 15 min) ovalbumin samples. Values represent the mean of 4 determinations. Error bars represent the confidence interval (95%). Different capital letters indicate, for each treatment time, significant differences among the pressures applied ($p < 0.05$). For each pressure, different lower case letters indicate significant differences among treatment duration ($p < 0.05$).

Table 1
Water (WAC) and oil (OAC) absorption capacity (g g^{-1} protein) of control (0.1 MPa) and HHP-treated samples of ovalbumin.

	Time (min)	Pressure (MPa)			
		0.1	250	300	350
WAC	**5**	1.02 ± 0.32 BAa	2.21 ± 0.58 Bbb	1.61 ± 0.31 ABa	2.18 ± 0.20 Bab
	10	1.02 ± 0.32 BAa	1.09 ± 0.32 Aab	1.48 ± 0.31 BAa	2.90 ± 0.70 Bba
	15	1.02 ± 0.32 ABa	0.74 ± 0.12 Aab	1.36 ± 0.16 BBa	1.89 ± 0.09 Caa
OAC	**5**	1.74 ± 0.04 BAa	3.94 ± 0.14 Bab	4.10 ± 0.14 BBa	4.40 ± 0.19 Caa
	10	1.74 ± 0,04 BAa	4.13 ± 0.08 Bab	4.04 ± 0.01 BBa	4.69 ± 0.23 Cab
	15	1.74 ± 0.04 BAa	4.15 ± 0.06 Bbb	4.29 ± 0.20 ABa	4.82 ± 0.06 Cba

Values are the mean ± standard deviation (n = 4). Different capital letters indicate, for each treatment time, significant differences among the pressures applied ($p < 0.05$). For each pressure, different lower-case letters indicate significant differences among treatment duration ($p < 0.05$).

treatment at 350 MPa. However, extending the treatments to 15 min, did not exert any positive effects as WAC was reduced, with the lowest value (0.74 ± 0.12 g g^{-1} protein) observed in the 250 MPa/15 min combination.

All the HHP-treated samples exhibited higher OAC than the control, with the maximum (4.82 ± 0.06 g g^{-1} protein) reached with pressurization at 350 MPa for 15 min. OAC increased with pressure intensity and with more extended treatments, except for the 300 MPa treatment. Under these conditions, the OAC only showed slight, still no significant increases with longer pressurization time.

Different factors, such as the hydrophilic-hydrophobic balance of amino acids and the conformational changes in the protein molecules after the HHP treatments can influence both properties. In effect, after pressurization previously enclosed amino acid side chains and polar side chains contained in the carbohydrates, become available to interact with water (Naderi et al., 2017; Paredes-López et al., 1991), whilst the exposition of non-polar, hydrophobic groups, originally buried in the interior of the protein, are able to bind more oil (Zhang et al., 2015).

4.1.3. Foaming capacity and foam stability

Fig. 2a shows the FC of the control and the HHP-treated ovalbumin samples. Foaming capacity of the control samples was of 188.49 ± 0.31 %. Regardless of treatments' time, the FC gradually increased when pressures of 250 and 300 MPa were used. At the highest pressure of 350 MPa, and even when no further increases were found, the FC was still higher than in the untreated and the 250 MPa-treated samples.

HHP treatments can improve the FC of ovalbumin by the exposure of the naturally buried sulfhydryl groups (Wang et al., 2016) and the enhancement of hydrophobic interaction between protein molecules (Ding et al., 2022). In this sense, Yang et al. (2009) found HHP treatments of 350 MPa/10 min as the best combination in enhancing the FC and FS of egg whites. These authors associated the improvement in foaming properties with the exposure of hidden hydrophobic groups after pressurizing and a consequent increase in surface hydrophobicity.

On the contrary, Strohalm et al. (2000) and Ding et al. (2022) reported no significant changes in the foam volume of egg white pressurized up to 400 MPa, for 5, 10 and 15 min at 25 °C whilst Richwin et al. (1992) observed, regardless of temperature, less foam volume in pressure-treated egg white as pressure increased up to 400 MPa.

Foam stability only increased after the more intense treatments (350 MPa, 10 and 15 min) (Fig. 2b). In contrast, when lower pressures or times were used, no significant differences (300 MPa) or even lower FS (250 MPa) than in the control samples were found. Similar results were observed in walnut protein isolates by Qin et al. (2013). According to these authors, the aggregation that follows the pressurization enables a thicker, more cohesive, and viscoelastic film to be formed around the gas bubbles, resulting in an improved FS.

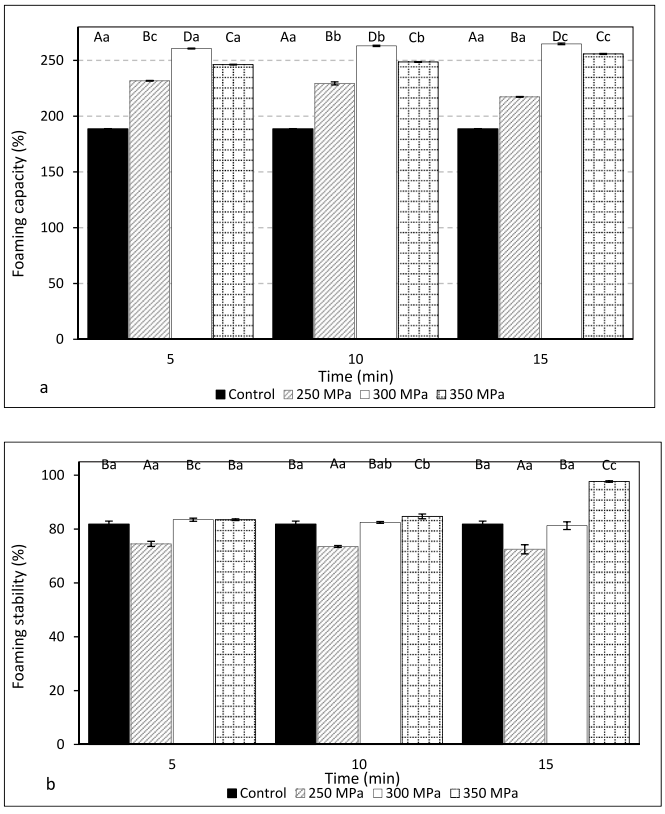

Fig. 2. Foaming capacity (%) (a) and foaming stability (%) after 30 min standing (b) of untreated and HHP-treated (250, 300 and 350 MPa; 5, 10 and 15 min) ovalbumin samples. Values represent the mean of 4 determinations. Error bars represent the confidence interval (95%). Different capital letters indicate, for each treatment time, significant differences among the pressures applied ($p < 0.05$). For each pressure, different lower case letters indicate significant differences among treatment duration ($p < 0.05$).

4.1.4. Emulsifying activity index (EAI) and emulsion stability index (ESI)

The emulsifying activity **(a)** and emulsion stability **(b)** indexes are shown in Fig. 3. The highest EAI (11.27 ± 0.08 m^2g^{-1}) was obtained under the 250 MPa/5 min combination and both, higher pressure intensities and more extended treatments, resulted in a decrease of the EAI. Nonetheless, all the HHP-treated samples presented higher EAI in comparison with the control.

Low-intermediate (200–400 MPa) pressures were reported to enhance the emulsifying activity of walnut (Qin et al., 2013), soy (Li et al., 2011; Wang et al., 2008), red kidney beans (Ahmed et al., 2018; Yin et al., 2008) and rice bran (Zhu et al., 2017) proteins. The positive effect of HHP in increasing the emulsifying activity of proteins has been attributed to an increased interfacial activity because of the partial unfolding and exposure of hydrophobic groups occurring after pressurization (Queirós et al., 2018) and to changes in molecular flexibility (Zhou et al., 2016).

On the contrary, the use of higher pressures (500–600 MPa) resulted in an impairment of the emulsifying activity. According to Qin et al. (2013) the formation of aggregates that occurs under these pressures leads to protein loss at the interface, which in turn causes a decrease in EAI.

HHP treatments also resulted in a significant increase in ESI, mainly in those samples pressurized at 250 MPa with the highest ESI value (33.82 ± 0.40 min) observed in the 250 MPa/15 min treatment. As with the EAI, further increasing the pressure to 300 and 350 MPa, provoked a

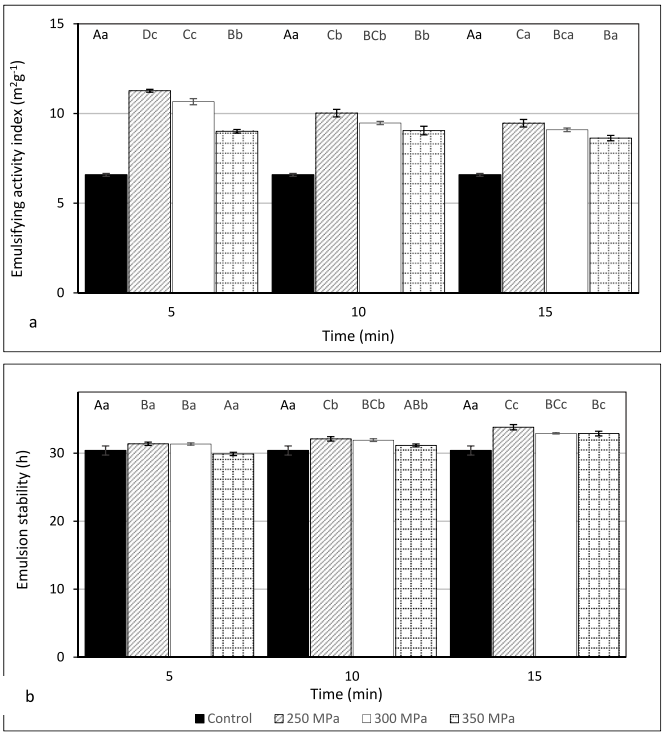

Fig. 3. Emulsifying activity (a) and emulsion stability (b) indexes of untreated and HHP-treated (250, 300 and 350 MPa; 5, 10 and 15 min) ovalbumin samples. Values represent the mean of 4 determinations. Error bars represent the confidence interval (95%). Different capital letters indicate, for each treatment time, significant differences among the pressures applied ($p < 0.05$). For each pressure, different lower case letters indicate significant differences among treatment duration ($p < 0.05$).

decrease in the stability of the emulsions, noticeably in the shortest treatments.

Our results are in accordance with those reported for red kidney beans (Ahmed et al., 2018; Yin et al., 2008) and rice bran proteins (Zhu et al., 2017). These authors showed that pressures in the low-intermediate range (200–400 MPa) increased ESI whilst an increase in either pressure or holding time did not exert any additional effect or it could even cause a decrease in the EAI and/or the ESI.

4.1.5. Gelation

All the samples presented the same least gelation concentration (LGC) of 8%, except for those subjected to the 250 MPa/5 min and 300 MPa/15 min combinations, with a LGC of 6 and 10%, respectively. Similar results were reported by He et al. (2014) in rapeseed protein isolates pressurized at 200–600 MPa. These authors stated that protein unfolding after HHP treatment enhanced interactions through hydrophobic bonding and thus, an increased strength of the gel networks obtained, and a lower amount of protein required to form the gel. Additionally, the relatively high protein content of the HHP-treated dispersions, could favor the protein-protein interaction, with a direct effect of gel formation (Wang et al., 2022; Yan et al., 2010).

4.2. Characterization of the heat-induced gels

4.2.1. pH and color

The HHP treatments did not affect the pH of either the dispersions or the thermal gels obtained thereafter, with values in the ranges of 6.50–6.52 for the former and 6.57–6.60 for the latter. Accordingly, Strohalm et al. (2000) also found that the pH of egg white pressurized at 200–400 MPa for 5–15 min remained unchanged when compared to untreated samples.

No significant differences were observed in the color parameters due to pressure intensity or treatment duration. The luminosity, chroma and hue values of the gels obtained from the control samples were 61.44 ± 2.57, 11.75 ± 0.94 and 96.30 ± 2.27, respectively. Even when a decrease in the luminosity (51.82–54.07) and hue (89.40–91.18) was observed in the gels obtained from the HHP-treated ovalbumin, these differences were not visually detected.

4.2.2. Gel strength and water holding capacity (WHC)

The gel strength and WHC of the thermally-formed gels are shown in Table 2. Gel strength increased progressively with pressure and time, reaching the maximum value (174.18 ± 1.93 N) in the 300 MPa/15 min treatment. Further increases to 350 MPa lead to significant decreases, with values even lower than those of the control, especially in the longest treatment.

The maximum WHC (80.97 ± 0.75 g g-1) was observed in the 300 MPa/5 min combination. As with gel strength, increasing the pressure to 350 MPa, or more extended treatments resulted in lower WHC values.

HHP-treatments up to 300 MPa enhanced the WHC and gel strength of soy (Li et al., 2011) and wheat gluten (Wang et al., 2019) gels obtained from HHP-treated protein. According to these authors, the partial unfolding of the protein molecules together with the exposure of hydrophobic groups after the HHP treatment, allow the formation of a flexible network capable of entrapping water, which in turn increases gel strength and WHC. In the same way, at pressures ≥400 MPa and treatment time longer than 15 min, both, gel strength and WHC decreased, probably due to the aggregation and re-folding of proteins with the consequent embedding of free sulfhydryl and hydrophobic groups.

4.2.3. Scanning electron microscopy (SEM)

The three-dimensional network is an important factor determining the rheological properties, gel strength, and WHC of a gel, which can also affect the sensorial attributes of gel-based foods (Wang et al., 2019). In the SEM images analysis, the samples treated at 250 MPa for 5 min exhibited the highest circularity index (0.58 ± 0.20) and a more compact structure in comparison with the untreated samples (circularity index of 0.47 ± 0.19), what in turn is consistent with the higher strength and WHC of these gels (Fig. 4, a, and b). However, after the more intense treatment (350 MPa/15 min), the gel showed a disordered network with larger cavities and the lowest circularity index (0.40 ± 0.17) (Fig. 4c) resulting in softer gels with reduced WHC. The application of HHP to ovalbumin resulted in a loss of the α-helix structure (Hayakawa et al., 1996) together with the formation of intermolecular hydrogen bonds due to rearrangements in the secondary structure of the protein (Mine, 1995). These phenomena could be responsible for the ordered network with small cavities observed in the gels formed from the 250 MPa pretreated ovalbumin. However, when the time and pressure increase (>300–400 MPa) a complete protein unfolding, the exposure of hydrophobic amino acid residues and subsequently, the formation of insoluble aggregates and the loss of protein solubility were produced (Wang et al., 2022). Consequently, a less compact network with larger cavities in the thermal gels was observed.

4.3. Allergenicity

The HHP conditions applied in this study were not able to completely reduce the allergenicity of the ovalbumin samples. The maximum (46.75%) and minimum (15%) inhibitions were obtained at 300 MPa/5 min and 250 and 300 MPa/15 min, respectively, without a clear tendency for pressure intensity or treatment extent. Contradictory results were found in the bibliography with positive results in walnut (Yang et al., 2017) and peanuts (Huang et al., 2014) and no effects in egg ovalbumin (Ma et al., 2015) and walnuts (Cabanillas et al., 2014). In general, positive results (inhibition values up to 86 %) were associated with pressures higher (>600 MPa for 10 min) than those used in our

Table 2
Gel strength (N) and water holding capacity (g g^{-1} gel) of thermal gels obtained from control (0.1 MPa) and HHP-treated (250, 300 and 350 MPa/5, 10 and 15 min) samples of ovalbumin.

	Time (min)	Pressure (MPa)			
		0.1	250	300	350
Gel strengh	**5**	103.17 ± 0.40 AAa	127.03 ± 3.01 Ca	153.09 ± 0.70 Da	123.51 ± 0.89 BCE
	10	103.17 ± 0.40 ABa	132.02 ± 1.16 Cb	173.49 ± 1.19 Db	170.03 ± 1.02 Ab
	15	103.17 ± 0.40 ABa	170.42 ± 0.57 *Cc*	174.18 ± 1.93 Db	163.12 ± 0.83 Aa
WHC	**5**	176.27 ± 0.60 BBa	179.59 ± 0.30 Cb	180.97 ± 0.75 Db	174.26 ± 0.59 Aa
	10	176.27 ± 0.60 ABa	177.25 ± 0.67 Ba	179.84 ± 0.75 Cb	175.56 ± 0.26 Ab
	15	176.27 ± 0.60 BBa	176.87 ± 0.57 Ba	178.15 ± 0.55 Ca	173.33 ± 0.63 Aa

Values are the mean ± standard deviation (n = 4). Different capital letters indicate, for each treatment time, significant differences among the pressures applied ($p < 0.05$). For each pressure, different lower-case letters indicate significant differences among treatment duration ($p < 0.05$).

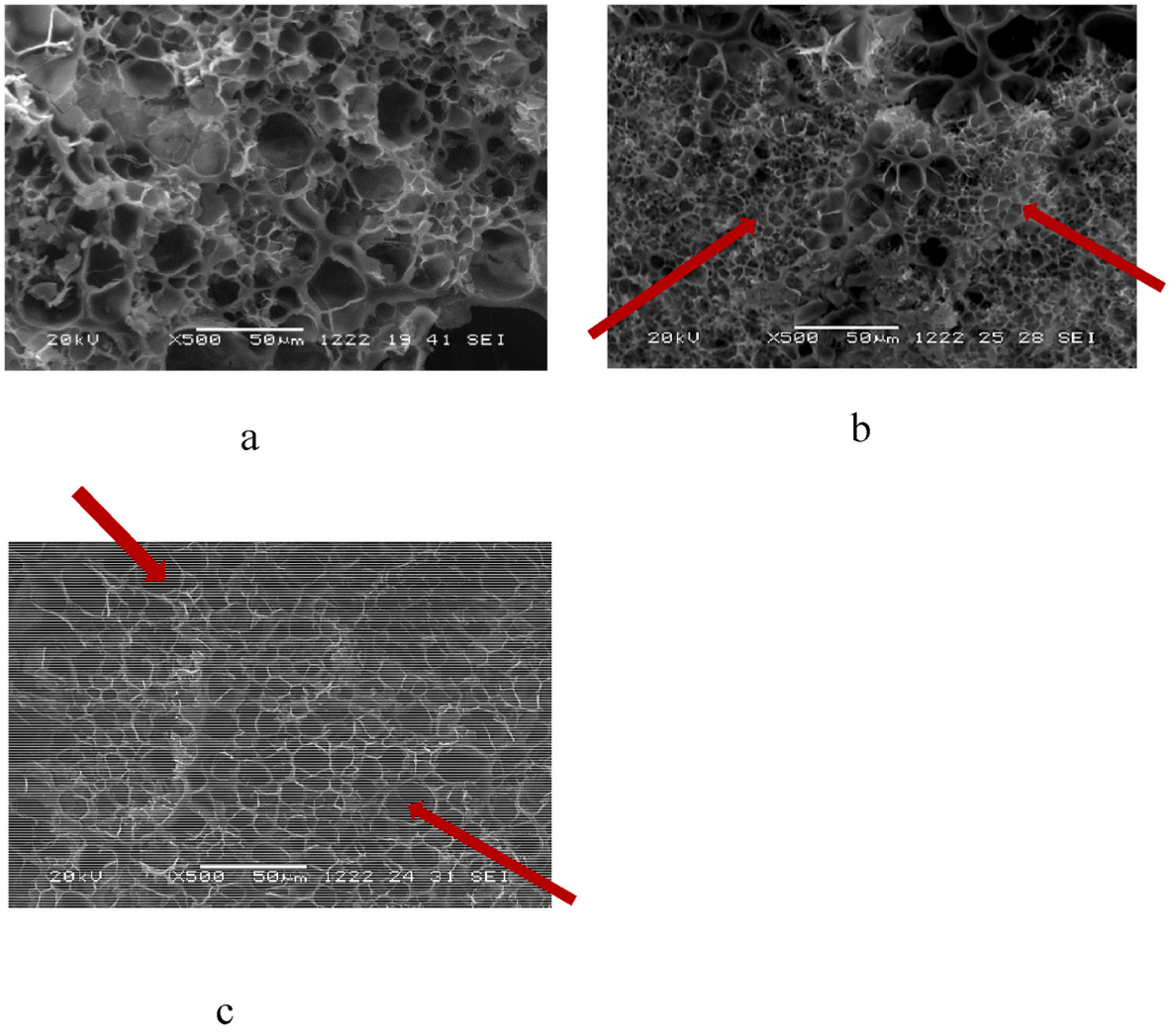

Fig. 4. SEM images of unprocessed (a) and HHP-treated ovalbumin samples under 250 MPa/5 min (b) and 350 MPa/15 min (c).

study and were related to changes in the protein structure. In addition, these allergenicity reductions were observed in plant proteins, which present a different conformation and consequently, also different modifications after HHP treatments.

5. Conclusions

HHP treatments in the range of 250–350 MPa were found to be a valuable technique in improving ovalbumin technological properties which are of interest in food products' development. In effect, the greater solubility, and water and oil absorption capacities together with the enhanced foaming and emulsifying properties observed in the HHP-treated samples, could be exploited for the formulation of new food products with a wide range of textures, which could be targeted to different populations, mainly those with special needs. However, as not all the proteins show the same behaviour after pressurization, specific studies for each one must be carried out before their use.

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CRediT authorship contribution statement

Sandra Horvitz: Investigation, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Cristina Arroqui:** Investigation, Methodology, Resources, Writing – review & editing. **Paloma Vírseda:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

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.

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