

Effect of high hydrostatic pressure on bovine α -lactalbumin functional properties

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Abstract

The combined effects of high hydrostatic pressure (200, 400 and 600 MPa) and temperature (25, 40 and 55 °C) on selected functional properties of α -lactalbumin were evaluated in the pH range of 3.0–9.0. Processing at 600 MPa and 55 °C for 10 min had a positive effect on solubility, foaming capacity, foam stability, emulsion activity index and emulsion stability of this protein at pH 7. The foaming and emulsifying properties of the protein generally improved by treating its solution at high pressures at all pH values tested, this being in contrast to the reported behavior of pressure treated β -lactoglobulin. Most of the changes in the observed functional properties of pressure treated α -lactalbumin solutions were attributed to the modifications brought about in its solubility.

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1. Introduction

High hydrostatic pressure (HHP) technologies are based on exposing products to pressures ranging from 200 to 900 MPa in a specially designed vessel. The process is generally run batchwise, and applied to a number of liquid and semisolid pre-packed foods. There are only few instances where this process has been run either continuously or in a semicontinuous manner (Ludikhuyze et al., 2002). In practice, the batch time consists of an initial time required to reach the treatment pressure (also known as the come-up time), the holding time at the desired pressure, and finally, a short time necessary for releasing the pressure (also

known as the release time) (Hogan et al., 2005). HHP processes are very effective for inactivating vegetative microorganisms, but they are not known to be as effective for inactivating bacterial spores and certain enzymes (Rastogi et al., 2007).

With regard to the effect of HHP on proteins, it has been reported that covalent bonds are not affected by the high operating pressures employed. Thus, the primary structure of proteins remains intact during the process while secondary, tertiary and quaternary structures are affected to various degrees (Chapleau et al., 2004). High pressures (300 MPa or more) can induce irreversible denaturation due to the breaking down of hydrogen bonds. The tertiary structure is also affected at pressures over 200 MPa due to changes in hydrophobic and ionic interactions, while the quaternary structure – which depends on non-covalent unions – is affected at pressures of 150 MPa or less (Huppertz et al., 2004). These

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changes in molecular structure alter physical properties such as solubility and viscosity, and influence protein functionality, especially the role they play in processes such as gelation, emulsification, and foaming (Krešić et al., 2006). In general, HHP induced changes depend on factors such as temperature, pH, type of solvent used for processing and the applied pressure (Nabham et al., 2004).

Whey proteins are very important food ingredients due to their functional properties. HHP is known to induce changes which alter the functional properties for different food applications (López-Fandiño, 2006). One of the main components of whey proteins is α -lactalbumin (α -LA). The role of this protein in lactose production is well known. Its concentration in cow's milk varies from 1.2 to 1.5 g L⁻¹, and its concentration in the whey protein fraction at 20% is only second to β -lactoglobulin (β -LG). α -LA has good emulsifying and foaming properties, but its gelation ability is known to be poor (İbanoğlu and İbanoğlu, 1999; Rojas et al., 1997). At pressures below 400 MPa, no denaturation of α -LA has been observed. However, β -LG is known to suffer denaturation at pressures over 100 MPa. The degree of denaturation of both proteins, in general, increases with applied pressure, time duration over which the pressure is applied, temperature and pH (Huppertz et al., 2004). There are also reports on the formation of pressure-temperature-induced copolymers of whey proteins, as well as polymers of whey proteins with caseins (Nabham et al., 2004). There are studies on the effect of HHP on the functionality of β -LG (López-Fandiño, 2006), whey protein concentrate (WPC) and whey protein isolate (WPI) (Bouaouina et al., 2006; Krešić et al., 2006). However, little is known on the effect of HHP on the functional properties of α -LA, which is the subject matter of this paper.

2. Materials and methods

2.1. Protein sample

BioPURE – Alphasactalbumin™ (Production Batch JE 027-5-410) was kindly donated by Davisco Foods International, Inc. (Eden Prairie, MN, USA) and used directly. The composition of the sample, as per information of the suppliers was: moisture content 5.5% max, protein 95% min (91% min of which constituted α -LA), fat 0.5% max, carbohydrates 0.5% max. and ash 2.5% max.

2.2. Experimental design

A Box–Behnken three-level second-order experimental design for three factors was used to apply response surface methodology (RSM). The three independent variables were pressure, temperature and holding time with levels of 200, 400 and 600 MPa, 25, 40 and 55 °C, and 5, 10 and 15 min, respectively. The design had 18 runs which included five replicates of the central point and an untreated sample (α -LA in native state) which served as control; see Table 1. The response variables were selected functional proper-

Table 1
Box–Behnken three-level second-order experimental design for three factors^a

Run	Pressure (MPa)	Chamber temperature (°C)	Actual temperature ^b (°C)	Time (min)	pH after treatment
US	0.1	25	25	0	7.0
1	200	25	26.45	10	6.8
2	200	40	41.45	5	6.8
3	200	40	41.45	15	6.7
4	200	55	56.45	10	6.8
5	400	25	27.90	5	6.9
6	400	25	27.90	15	6.7
CP	400	40	42.90	10	6.7
12	400	55	57.90	5	6.8
13	400	55	57.90	15	6.8
14	600	25	29.35	10	6.9
15	600	40	44.35	5	6.6
16	600	40	44.35	15	6.7
17	600	55	59.35	10	6.9

^a Abbreviations: US = untreated sample, CP = central point (includes runs 7, 8, 9, 10 and 11, i.e. this run was performed in quintuplicate).

^b This value is the temperature reached after the pressurization and is due to adiabatic heat-up.

ties (solubility, emulsifying capacity, emulsion stability, foamability and foam stability) measured at four different pH values. All the analyses were performed in triplicate and the mean value and the standard deviations calculated. Differences among samples were evaluated by one way ANOVA. Modeling was performed with the aid of the Design Expert™ v.7 software (Stat-Ease, Inc., Minneapolis, MN, USA). One way ANOVA was used to determine the significance of the models and also to evaluate the significance of each of the terms in the RSM equations.

2.3. Methods

2.3.1. High pressure treatment

α -LA at a concentration of 5% (w/v) in distilled water was subjected to HHP in an isostatic press with yoke (Engineered Pressure Systems, Inc., Haverhill, MA, USA). The pressure chamber was cylindrical in shape (height = 0.255 m, diameter = 0.10 m). The pressurizing fluid was water. Samples were equilibrated in the chamber for 5 min so that they attained the desired process temperature. The maximum come-up time was 4.5 min and this was required to reach 600 MPa. The time taken to release the pressure was only 30 s. After exposure to high pressure, the pH values of the α -LA solutions were measured using a pH meter (Model 920A, ATI Orion Laboratory Products Group, Boston, MA, USA) and then the samples were freeze-dried and packed in sealed plastic bags. Freeze-drying was selected as the method to preserve samples because it has been shown to leave the protein structure intact (Alvarez et al., 2007).

2.3.2. Solubility

The solubility of the α -LA samples at room temperature (20 °C) and various pH values (3, 5, 7 and 9) was determined

by the method of Bera and Mukherjee (1989). One hundred milligrams of protein was dispersed in 10 mL of 0.1 M phosphate buffer. The suspension was stirred for 30 min, avoiding foam formation, and centrifuged at $10000 \times g$ for 30 min at 21 °C. The protein content of the supernatants was determined by the Lowry method (Boyer, 2000). The protein solubility (PS) in each sample was calculated as follows:

$$\text{PS (\%)} = \frac{\text{Amount of protein in the supernatant}}{\text{Amount of protein in the sample}} \times 100 \quad (1)$$

2.3.3. Foaming activity

The foaming capacity (FC) and foam stability (FS) of the α -LA samples were determined by the method of Kato et al. (1983) with modifications. Air ($90 \text{ cm}^3 \text{ min}^{-1}$) was introduced into a glass tube ($2.4 \times 30 \text{ cm}$) containing 10 mL of 0.1% protein in 0.1 M phosphate buffer (pH 3, 5, 7 and 9) for 15 s. The volume of foam in cm^3 was measured immediately after switching the gas flow off by directly reading the dispersion height in the glass tube. The foam capacity (FC) was evaluated using the following formula (Wilde and Clark, 1996): $\text{FC (\%)} = (V_f/V_s) \times 100$, where V_f = volume of foam after the air was introduced and V_s = volume of the original sample. Since foams are made of a number of bubbles separated by liquid or solid films, it is feasible to estimate the foamability by measuring the conductivity of the films (Kato et al., 1983). The foam stability (FS) was determined by measuring the conductivity of the foams produced when air at a constant flow rate of $90 \text{ cm}^3 \text{ min}^{-1}$ was introduced into 20 mL of a 0.1% protein solution in 0.1 M phosphate buffer. The flow was maintained until the foam reached a volume of 38.42 cm^3 . Conductivity readings were taken every minute for 5 min and a plot of \log_{10} (conductivity) vs. time was constructed and a regression equation of the form $y = mx + b$ (where y is \log_{10} (conductivity) and x is time) was calculated. All the equations thus determined gave $R^2 \geq 0.98$. FS was calculated from the following equation:

$$\text{FS (min)} = -\frac{b}{m} \quad (2)$$

The parameter b is equal to $\log(C_i)$, where C_i is the initial conductivity or foaming power as defined by Kato et al. (1983). The method was tested with ovalbumin, bovine serum albumin and α -LA and the values of FS obtained were comparable with the results presented by Kato et al. (1983) with the same proteins.

2.3.4. Emulsifying properties

The emulsifying activity index (EAI) and emulsion stability (ES) were measured by the turbidimetric technique reported by Pearce and Kinsella (1978). A mixture of 30 mL of 0.5% protein solution in 0.1 M phosphate buffer (pH 3, 5, 7 and 9) and 10 mL of corn oil was homogenized for 1 min at 2000 rpm with a waring blender (Oste-

rizer 465-43M, Tlalnepantla, México). Fifty milliliters of emulsion were dispensed into 5 mL of 0.1% SDS immediately and 10 min after homogenization. The absorbance of the solution at 500 nm was measured with a Spectrophotometer (Jenway Model 6405, Dunmow, Essex, England). The emulsifying activity index (EAI) was calculated as follows:

$$\text{EAI} = \frac{2T_b}{\phi C} \quad (3)$$

where C is the weight of protein per unit volume of aqueous phase before the emulsion is formed; ϕ is the volume fraction of dispersed phase (0.667 in this experiment); and T_b is turbidity defined by Pearce and Kinsella (1978) as:

$$T_b = \frac{2.303A}{l} \quad (4)$$

where A is the emulsion absorbance and l is the path length of the cuvette (1 cm). ES was calculated as follows:

$$\text{ES} = \frac{T_{b0}\Delta t}{\Delta T_b} \quad (5)$$

where ΔT_b is the change in turbidity over the time interval Δt and T_{b0} is the initial turbidity of the sample.

3. Results and discussion

3.1. Changes in temperature and pH in α -LA solutions under HHP

The temperature increased around 0.72 °C per 100 MPa during pressurization due to adiabatic heating. This increase is reported as “actual temperature” in Table 1. However, all the calculations and modeling were performed using the equipment chamber temperature which is the parameter that can be programmed into the equipment, so the data can be compared with earlier work, which also report the chamber temperature. It has been reported that in some cases, the degree of HHP-induced denaturation of whey proteins decreases when the pH during the treatment is less than 7 (Arias et al., 2000). In this study, the experimental variation of pH, which is reported in Table 1, was between 7.0 (α -LA without treatment) and 6.6 (for the sample treated at 600 MPa, 40 °C for 5 min). Even the mildest treatment (200 MPa, 25 °C for 10 min) resulted in a drop in pH (6.8). It is important to notice that the pH values of the solutions remained constant for at least 5 min after the pressure was released. Orlien et al. (2007) reported that the HHP-induced loss of tertiary structure results in a pH-decrease due to the contraction of solvent in the case of β -lactoglobulin. Over the temperatures and times studied here, HHP treatment created favorable pH conditions (pH < 7) which lowered the extent of denaturation of α -LA under pressure.

3.2. Solubility

The values of solubility (Table 2) were high for the untreated samples, ranging from 73.6% (pH 5) to 91.4% (pH 9). No significant changes ($p > 0.05$) were detected in the samples maintained at pH 3. The average solubilities for the untreated sample and the HHP-treated samples were 85.9% and 86%, respectively. At pH 9, the solubility was observed to decrease drastically to 67.4% when the experimental parameters were 600 MPa, 25 °C, and 10 min treatment time. Something similar also occurred at pH 7, and the results were comparable to the data reported by Bouaouina et al. (2006) for WPI (85% protein) at pH 6.7 and 300 MPa. A significant reduction in solubility was also reported by Krešić et al. (2006) in the case of WPI (97.8% protein) at pH 7 and treatment pressures of 300 and 600 MPa. In the case of the HHP-treated samples at pH 5 (near the isoelectric point), the decrease in solubility with increase in treatment intensity was very pronounced and was consistent with the study reported by Krešić et al. (2006) for WPI at pH 4.6. Fig. 1 shows a three-dimensional graph which allows a better visualization of the effects of pressure and temperature on the solubility at pH 7.

3.3. Foaming activity

Processing pressure, temperature, pH and time are very important parameters affecting the foamability of food proteins. The results of this study are shown in Table 3.

Table 2
Solubility (% w/v) of α -LA for the different processing conditions at four different pH values^{a,b}

Run	pH 3	pH 5	pH 7	pH 9
US	85.9 ± 1.5	73.6 ± 5.4	85.2 ± 0.7	91.4 ± 3.5
1	86.1 ± 4.8	66.1 ± 4.5	84.3 ± 4.3	82.7 ± 3.9
2	89.8 ± 6.0	71.2 ± 6.6	84.6 ± 2.7	96.9 ± 3.7
3	80.6 ± 5.9	70.4 ± 3.8	84.9 ± 2.5	94.7 ± 7.2
4	88.4 ± 2.8	67.7 ± 6.5	81.7 ± 1.6	83.6 ± 3.6
5	82.4 ± 4.3	63.7 ± 3.5	92.4 ± 3.7	95.2 ± 4.7
6	86.6 ± 5.5	75.4 ± 5.2	82.1 ± 1.2	94.4 ± 4.9
CP	89.5 ± 5.0	65.0 ± 5.9	82.9 ± 4.6	86.8 ± 8.4
12	84.2 ± 7.4	53.3 ± 3.9	77.7 ± 5.8	77.1 ± 3.7
13	84.1 ± 6.2	43.5 ± 4.4	74.4 ± 3.3	81.2 ± 2.8
14	83.7 ± 5.9	58.6 ± 1.2	80.5 ± 1.1	67.4 ± 2.2
15	88.7 ± 5.9	66.9 ± 4.3	89.6 ± 6.0	93.2 ± 4.7
16	90.4 ± 2.0	58.8 ± 5.2	88.5 ± 1.3	87.0 ± 6.2
17	83.1 ± 5.0	46.3 ± 3.6	90.1 ± 3.3	76.0 ± 6.7
Model	Quadratic	Quadratic	Cubic	Quadratic
<i>p</i> -Value	0.3433 ^{NS}	0.0012*	0.0045*	0.3924 ^{NS}
<i>R</i> ²	0.602	0.6668	0.9509	0.5797
Lack of fit MS	12.80	35.99	2.25	73.95
Pure error MS	5.52	20.26	2.97	53.20

^{NS} Not significant with 95% confidence level.

* Significant with 99% confidence level.

^a Abbreviations: US = untreated sample, CP = central point, MS = mean square.

^b Values ± standard deviation.

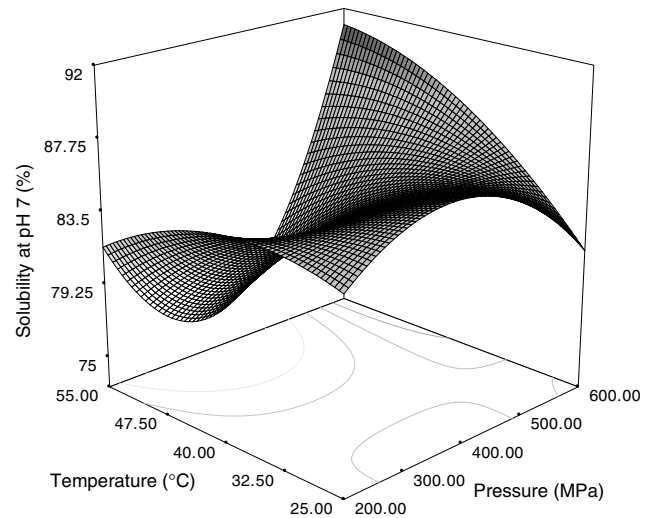


Fig. 1. Solubility of α -Lactalbumin at pH 7. Surface response plot of the equation: Solubility (%) = $97.0 + 0.23P - 0.25T - 6.48t - 0.004PT + 0.0058Pt + 0.212Tt$, with $t = 10$ min; $R^2 = 0.9509$.

3.3.1. Foaming capacity (FC)

All the untreated samples had the same FC regardless of the pH of the experiment. It can also be observed that all HHP treatments improved the FC of the α -LA solutions considerably. It is possible that the combined effect of HHP, temperature and pH can produce a wide range of molten globules. These globules are intermediates in the unfolding pathway of a globular protein, i.e. between the native and the fully unfolded protein there is a continuum of molecular intermediates known as molten globules (Aouzelleg et al., 2004). These partially unfolded structures have the right flexibility and secondary structure to form a stable film around the air–liquid interface (Cornec et al., 2001). At all pH values, the treatment at 400 MPa produced the highest FC. At 600 MPa, FC decreased marginally. This behavior was also observed by Van der Plancken et al. (2007) for HHP-treated egg white proteins at pH 7.6. İbanoğlu and Karataş (2001) reported that HHP treatment (150–450 MPa) of WPI (99% protein) at pH 6 and 5 reduced its FC. They concluded that the reduced overall foamability of WPI could result from its high β -LG content, since this protein significantly lost its solubility during the HHP treatments. The FC surface response at pH 3 is shown in Fig. 2. It was observed that pressure and temperature are significant ($p \leq 0.05$) for FC at all pH values, whereas process time is not significant at pH 3 and 5. İbanoğlu and Karataş (2001) found that process time is not significant for FC in the case of HHP-treated WPI in the pH range of 5–7.

3.3.2. Foam stability (FS)

The pH value had a definite influence on the FS of the untreated samples (see Table 3) with the highest stability at pH 9 (32.5 min). HHP treatment proved to be beneficial since all the treated samples had higher FS values than untreated proteins. The highest FS value was obtained at

Table 3
Foaming activity of α -LA for the different processing conditions at four different pH values^{a,b}

Run	pH 3		pH 5		pH 7		pH 9	
	FC (%)	FS (min)	FC (%)	FS (min)	FC (%)	FS (min)	FC (%)	FS (min)
US	286 ± 0	22.4 ± 0.4	286 ± 0	18.4 ± 0.6	286 ± 0	23.5 ± 0.8	286 ± 0	32.5 ± 0.3
1	381 ± 46	24.0 ± 0.2	373 ± 17	28.4 ± 0.7	380 ± 55	26.3 ± 0.3	375 ± 12	35.4 ± 0.1
2	414 ± 20	23.1 ± 0.2	397 ± 18	28.3 ± 0.9	394 ± 60	26.6 ± 0.3	380 ± 2	34.7 ± 0.3
3	426 ± 40	23.4 ± 0.3	407 ± 10	26.3 ± 0.3	417 ± 37	27.2 ± 0.3	429 ± 36	33.3 ± 0.2
4	464 ± 47	25.8 ± 0.2	426 ± 19	30.1 ± 0.8	421 ± 45	28.7 ± 0.4	434 ± 32	32.9 ± 0.3
5	479 ± 54	23.2 ± 0.3	418 ± 37	25.3 ± 0.8	427 ± 26	27.2 ± 0.5	417 ± 38	35.8 ± 0.3
6	479 ± 55	25.0 ± 0.6	439 ± 19	26.6 ± 0.6	422 ± 20	29.1 ± 0.4	438 ± 41	34.6 ± 0.2
CP	473 ± 49	22.3 ± 0.2	444 ± 42	26.9 ± 0.6	440 ± 15	31.7 ± 0.5	439 ± 30	37.0 ± 0.2
12	488 ± 58	24.3 ± 0.2	447 ± 20	26.8 ± 0.9	459 ± 25	30.9 ± 0.3	436 ± 38	36.6 ± 0.4
13	489 ± 57	23.2 ± 0.6	446 ± 46	26.8 ± 0.6	464 ± 18	29.2 ± 0.6	428 ± 38	36.7 ± 0.2
14	459 ± 52	26.7 ± 0.4	433 ± 38	24.2 ± 0.4	434 ± 32	32.6 ± 0.9	424 ± 32	35.2 ± 0.1
15	465 ± 37	23.6 ± 0.2	445 ± 43	23.0 ± 0.6	448 ± 30	31.0 ± 0.9	410 ± 37	39.5 ± 0.4
16	470 ± 42	24.7 ± 0.3	441 ± 47	23.7 ± 0.6	458 ± 10	31.4 ± 0.8	422 ± 22	38.2 ± 0.5
17	463 ± 48	22.6 ± 0.2	430 ± 54	27.2 ± 0.2	464 ± 4	31.4 ± 0.8	420 ± 24	38.1 ± 0.3
Model	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic	Linear	2FI	Quadratic
<i>p</i> -Value	<0.0001*	0.2090 ^{NS}	<0.0001*	0.0005*	<0.0001*	<0.0001*	0.0001*	<0.0001
<i>R</i> ²	0.9611	0.6700	0.9649	0.7625	0.9803	0.6309	0.8639	0.9106
Lack of fit MS	141.62	0.41	58.83	1.78	37.32	3.02	336.87	0.63
Pure error MS	97.80	3.08	105.30	3.37	64.70	2.06	226.30	0.30

^{NS} Not significant with 95% confidence level.

* Significant with 99% confidence level.

^a Abbreviations: US = untreated sample, CP = central point, FC = foaming capacity, FS = foaming stability, 2FI = two factor interaction.

^b Values ± standard deviation.

600 MPa, 40 °C, pH 9 and 5 min (39.5 min). This increase in FS with pressure was also observed by: Kresić et al. (2006) in WPC (61.3% protein) and WPI; İbanoğlu and Karataş (2001) in WPI; and Van der Plancken et al. (2007) in egg white proteins. The foams formed by α -LA have been considered to be weak and with low overrun (Luck et al., 2001), so the improvement in FC and FS by the HHP treatments could be very important for the development of proteins with specific foamabilities. The FS surface responses at pH 5 is shown in Fig. 3

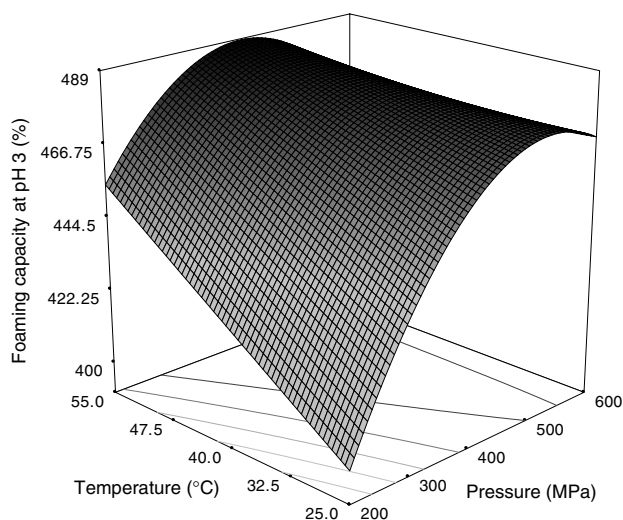


Fig. 2. Foaming capacity (FC) of α -Lactalbumin at pH 3. Surface response plot of the equation: $FC(pH 3) = 205.2 + 0.889P + 2.872T - 0.00515PT - 0.000733P^2$, $R^2 = 0.9611$.

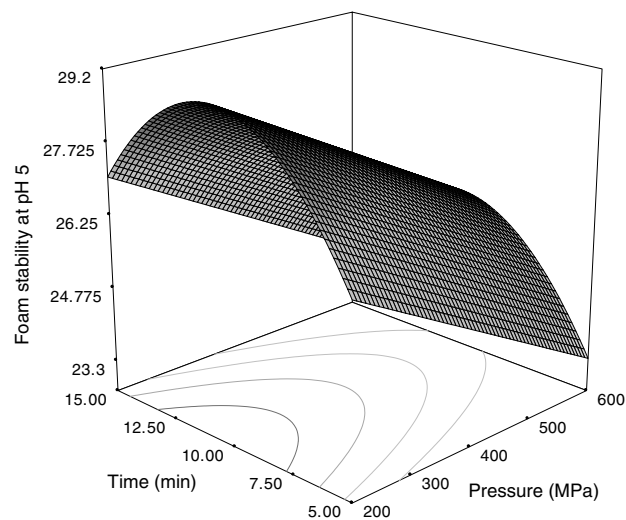


Fig. 3. Foam stability (FS) of α -lactalbumin at pH 5. Surface response plot of the equation: $FE(pH 5) = 18.07 - 0.0078P + 0.063T + 1.946t - 0.094t^2$, with $T = 40$ °C; $R^2 = 0.7625$.

3.4. Emulsifying properties

The results for the emulsifying activity index (EAI) and emulsion stability (ES) for the untreated and HHP-treated α -LA are summarized in Table 4. With the exception of the samples at pH 5, HHP treatment improves the emulsifying properties of the α -LA solutions. In general, proteins play two main roles in food emulsions: first, they lower the interfacial tension between the liquid phases, and second, they form a molecular film around the dispersed particles

Table 4
Emulsifying properties of α -LA for the different processing conditions at four different pH values^{a,b}

Run	pH 3		pH 5		pH 7		pH 9	
	EAI (m ² g ⁻¹)	ES (min)	EAI (m ² g ⁻¹)	ES (min)	EAI (m ² g ⁻¹)	ES (min)	EAI (m ² g ⁻¹)	ES (min)
US	116 ± 1.9	37.7 ± 1.8	101 ± 5.4	11.3 ± 1.1	111 ± 6.6	14.6 ± 1.4	105 ± 5.1	15.6 ± 1.1
1	121 ± 6.9	31.8 ± 2.7	106 ± 7.0	11.7 ± 1.8	111 ± 8.3	16.0 ± 0.9	105 ± 7.4	17.0 ± 1.3
2	126 ± 9.1	34.4 ± 2.1	109 ± 4.7	11.0 ± 1.1	117 ± 4.5	17.7 ± 0.9	106 ± 6.3	17.5 ± 1.7
3	127 ± 7.3	32.4 ± 1.4	110 ± 5.1	11.4 ± 1.2	113 ± 3.7	19.5 ± 0.6	103 ± 10.4	21.5 ± 2.1
4	128 ± 2.6	48.2 ± 2.1	104 ± 6.8	10.7 ± 2.0	114 ± 9.1	21.7 ± 2.1	109 ± 9.0	21.9 ± 1.3
5	133 ± 2.6	45.8 ± 2.3	109 ± 6.4	11.1 ± 1.9	112 ± 10.5	18.6 ± 1.7	109 ± 7.8	17.4 ± 1.2
6	127 ± 9.1	42.4 ± 3.0	102 ± 6.5	11.1 ± 1.9	109 ± 7.5	20.2 ± 2.0	112 ± 2.9	18.7 ± 1.1
CP	132 ± 7.4	44.6 ± 2.3	105 ± 4.4	10.8 ± 1.6	113 ± 10.1	20.3 ± 1.7	110 ± 4.7	20.3 ± 1.4
12	129 ± 9.9	42.4 ± 3.6	97 ± 6.6	11.3 ± 2.1	109 ± 9.7	22.9 ± 2.3	107 ± 5.9	26.7 ± 1.7
13	120 ± 8.0	58.9 ± 3.5	92 ± 7.0	10.6 ± 1.7	116 ± 4.1	22.8 ± 1.2	107 ± 3.3	24.1 ± 1.3
14	131 ± 9.6	31.3 ± 2.1	101 ± 7.1	10.9 ± 1.2	113 ± 8.6	19.7 ± 1.7	109 ± 6.4	22.1 ± 1.2
15	126 ± 9.2	44.9 ± 3.6	100 ± 6.9	10.9 ± 1.5	112 ± 7.8	22.6 ± 1.9	106 ± 3.7	24.4 ± 0.7
16	124 ± 6.1	49.7 ± 3.3	94 ± 7.6	10.9 ± 2.0	107 ± 9.3	30.8 ± 2.9	111 ± 5.2	28.4 ± 2.1
17	120 ± 5.2	61.4 ± 2.9	90 ± 5.7	10.9 ± 2.2	116 ± 9.5	38.0 ± 3.3	113 ± 2.6	24.3 ± 1.0
Model	Quadratic	Linear	Quadratic	2FI	Linear	2FI	Quadratic	Linear
p-Value	0.0014*	0.0704 ^{NS}	<0.0001	0.0307**	0.5558 ^{NS}	0.0013*	0.0072	0.0001
R ²	0.7744	0.3856	0.9347	0.6649	0.1338	0.8240	0.6357	0.7263
Lack of fit MS	11.01	54.96	4.45	0.065	8.80	11.72	4.89	4.86
Pure error MS	2.80	105.38	1.20	0.002	8.80	0.79	0.70	0.41

^{NS} Not significant with 95% confidence level.

* Significant with 99% confidence level.

** Significant with 95% confidence level.

^a Abbreviations: US = untreated sample, CP = central point, EAI = emulsifying activity index, ES = emulsion stability.

^b Values ± standard deviation.

which reduces coalescence, flocculation and oiling-off (Krešić et al., 2006). Again, in this case, it is possible that the process could have induced the formation of α -LA molten globules. These molecules appear to gain adequate flexibility in order to undergo conformational changes at the oil–water interface which is necessary to form a stable emulsion.

3.4.1. Emulsifying activity index (EAI)

At pH 5, EAI decreases at the highest values of pressure (400–600 MPa) and temperature (55 °C). Under these conditions, the solubility of α -LA is low (see Table 4). In the case of this protein and others, a high solubility is necessary for a good emulsifying activity and a possible increase in surface hydrophobicity (Liu et al., 2005) due to the HHP treatment is unable to compensate for the detrimental effect of solubility near the isoelectric point. For the other pH values, a slight increase in EAI is observed in the HHP-treated samples. The highest EAI values were obtained at pH 3 demonstrating that low-pH molten globules are good emulsifying agents (Lala et al., 1995). This is probably due to a high solubility shown at pH 3 and a possible increase in surface hydrophobicity. The EAI surface response at pH 3 is shown in Fig. 4.

3.4.2. Emulsion stability (ES)

No effect of HPP treatment could be observed at pH 5 where the lowest values of emulsion stability were obtained. This could be due to the aforementioned solubility loss possibly accompanied by some aggregation after

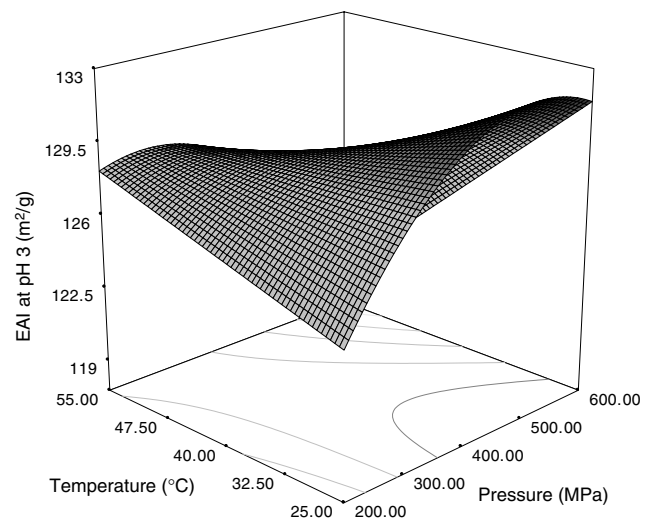


Fig. 4. Emulsifying activity index (EAI) of α -lactalbumin at pH 3. Surface response plot of the equation: $EAI (pH 3) = 105.02 + 0.119P + 0.397T - 0.451t - 0.0013PT - 0.000086P^2$, with $t = 10$ min; $R^2 = 0.7744$.

the HPP treatment. These factors could have acted together preventing the α -LA from forming a stable emulsion. At the other pH values, there was an increase in emulsion stability with increasing intensity of the HHP treatment. Bravo et al. (2007) also reported improvements in emulsifying properties by using HPP (300 MPa, 25 °C and 60 min) prior to lactosylation in α -LA. On the other hand, there are various studies on whey protein concentrates (WPC) containing 75% protein (Galazka et al.,

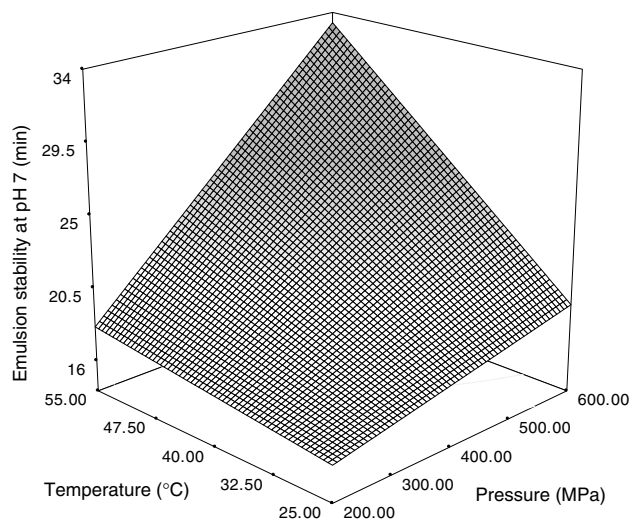


Fig. 5. Emulsion stability of α -lactalbumin at pH 7. Surface response plot of the equation: $ES(\text{pH } 7) = 16.21 - 0.028P - 0.048T + 0.166t + 0.00098PT + 0.0012Pt - 0.00838Tt$, with $t = 10$ min; $R^2 = 0.8240$.

1995) and on whey protein isolates (WPI) (Krešić et al., 2006) reporting a detrimental effect of the HHP treatment on the emulsifying properties of these protein solutions. The possible reason for this behavior is the presence of a high proportion of β -LG, a protein which shows lower emulsifying properties than the native form (López-Fandiño, 2006), when pressurized (300–900 MPa). The ES surface response at pH 7 can be observed in Fig. 5.

4. Conclusions

In general, the functional properties (with the exception of solubility) improved with the HHP treatments, which is a major difference in behavior between α -LA on the one hand and β -LG, WPC and WPI on the other. While HHP treatment adversely influences the foaming capacity and emulsifying properties of the latter three proteins, it works favorably in the case of α -LA. A process at 600 MPa and 55 °C for 10 min increased the solubility of α -LA by 5.75%, the foaming capacity by 62.23%, the foam stability by 19.39%, the emulsion activity index by 4.5% and the emulsion stability by 24.8% when the treated sample was reconstituted at pH 7. Further study must focus on the changes in the molecular properties induced in α -LA by HHP, in order to gain a more fundamental insight into the factors involved in bringing about changes in the functional properties of this protein.

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