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Foaming properties of egg white proteins affected by heat or high pressure treatment

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Abstract

This paper deals with the effect of heat (50–85 °C) and high pressure treatment (400–700 MPa at 10–60 °C) on the foaming properties of egg white solutions (10% v/v or 9.64 mg protein/mL). These physical treatments (20 min) were performed at two pH levels: pH 7.6 corresponding to the pH of fresh egg white and pH 8.8, corresponding to that of older egg white. Both heat and pressure treatment affected the foaming properties of egg white proteins. While foams from untreated egg white solutions were crispy and subject to collapse of the foam column after a long standing period, foams from heat and pressure-treated egg white solutions were moist and creamy, showing smaller bubble size and little or no sensitivity to foam collapse. The effect of physical treatments was strongly dependent on the pH during treatment. The most voluminous foams were obtained at pH 8.8, while the most stable, dense foams were obtained at pH 7.6, for both heat and pressure treatment. Based on previous data on the effect of heat and pressure treatment on the physicochemical properties of egg white proteins, the relationship between the processing-induced changes in these physicochemical properties and the foaming properties of egg white was investigated. Treatments resulting in a high level of protein unfolding, yet accompanied by a certain degree of residual protein solubility (primarily at pH 8.8), resulted in egg white solutions with improved foaming ability. A high level of unfolding combined with extensive protein solubility loss (primarily at pH 7.6) was associated with increased foam stability and density. Foams with high volume and average stability and density were obtained by pressure treatment at pH 8.8 (above 500 MPa). The processing-induced changes in the foaming properties could not be attributed to the changes in a single physicochemical property. The foaming ability was in part determined by the sulfhydryl content and protein flexibility. Improved protein–protein interactions (solubility and exposed SH groups) contributed to increased foam stability of treated egg white solutions. Other properties, not measured in this study, probably also contribute to the foaming properties of processed egg white solutions, especially after pressure treatment.
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1. Introduction

It is almost impossible to ignore the significance of eggs in the world of food. Chicken egg is a multi-purpose ingredient, meeting the requirements of a variety of food formulations. It combines not only excellent foaming, gelling and emulsifying properties, but also a high nutritional quality of its proteins (Hatta, Hagi, & Hirano, 1997; Mine, 1995). This study focuses on the foaming properties of egg white proteins. The excellent foaming capacity of the egg white protein and the stability of the resulting foams (even when subjected to heating) are applied in food industry in the preparation of, among others, meringues and cakes.

For most applications in food industry, pasteurization of egg white is a prerequisite, because of possible contamination with pathogen *Salmonella* sp. (Cunningham, 1986). It is known that heat-treatment can induce protein denaturation, which can, depending on the severity of the process, result in changes in the functional properties of the treated proteins. The correlation between the structural

properties of egg white proteins and their functional properties has been the subject of many studies, with an emphasis on the contribution of the major egg white protein, ovalbumin (Donovan, Mapes, Davis, & Garibaldi, 1975; Kato, Fujimoto, Matsudomi, & Kobayashi, 1986; Mine, Noutomi, & Haga, 1990; Van der Plancken, Delattre, Indrawati, Van Loey, & Hendrickx, 2004; Van der Plancken, Van Remoortere, Indrawati, Van Loey, & Hendrickx, 2003; Van der Plancken, Van Loey, & Hendrickx, 2005b, 2006). In these studies much attention was paid to the changes in structural properties of ovalbumin or the other egg white proteins induced by heating, leading to changes in the functional properties. Heating of egg white solutions in the temperature range of 50–85 °C results in significant unfolding of the proteins, as evidenced by an exposure of hydrophobic groups and sulfhydryl (SH) groups, priorly buried in the protein core, and a higher sensitivity towards proteases. The decrease in denaturation enthalpy indicates loss of secondary protein structure (Ma & Harwalkar, 1991; Privalov & Khechinashvili, 1974). Depending on the pH during heat-treatment, these changes result in massive loss of protein solubility and the formation of turbid protein suspensions after prolonged heating at elevated temperature, due to hydrophobic interactions and the formation of disulfide (SS) bonds through SH/SS exchange reactions and SH oxidation (Van der Plancken et al., 2005b). These changes in physicochemical properties due to heat-treatment are expected to result in changes in the foaming characteristics of the treated egg white solutions.

High pressure processing is being more and more suggested as an interesting alternative for heat-treatment as this non-thermal processing technique often results in a better balance between food safety on the one hand and food quality on the other. High hydrostatic pressure can, however, also induce protein denaturation, depending on the protein concentration, pressure, temperature and pH (Balny & Masson, 1993; Barbarosa-Cánovas, Pathakamury, Palou, & Swanson, 1997). Similar to heat-treatment, pressure treatment of egg white proteins above 450 MPa results in a loss of secondary structure (Hayakawa, Linko, & Linko, 1996). These pressure-induced structural changes in egg white proteins can also be demonstrated by the exposure of previously buried hydrophobic and SH groups and increased flexibility (measured as susceptibility to enzymatic hydrolysis) (Iametti et al., 1998, 1999; Van der Plancken et al., 2004, 2005b; Van der Plancken, Van Loey, & Hendrickx, 2005a, Van der Plancken, Van Loey, & Hendrickx, in press). However, the increase in surface hydrophobicity is less pronounced at elevated pressure, compared to elevated temperature at ambient pressure. On the other hand, the decrease of exposed SH groups due to SH oxidation is enhanced by pressure. Both observations might explain why pressure treatment induced protein solubility loss to a lesser extent compared to heat-treatment. Due to high pressure, smaller aggregates stabilized by disulfide bonds (arising from SH oxidation) are formed, while heat-treatment induces the formation of large, insoluble aggregates that are only partly stabilized by disulfide bonds (resulting from SH–SS exchange mechanisms), but more importantly by hydrophobic interactions. The larger size of the aggregates formed at elevated temperature at ambient pressure might explain why the resulting suspensions are more turbid (Van der Plancken et al., 2005b). Pressure-treated egg white proteins are more flexible, as evidenced by the higher susceptibility to enzymatic hydrolysis compared to the untreated protein (Van der Plancken et al., 2004). An antagonistic effect between pressure and temperature is observed, since at elevated pressure the changes in physicochemical properties are faster at lower temperature, while at atmospheric pressure this occurs at elevated temperature (Suzuki, 1960; Van der Plancken et al., 2004, in press). These pressure-induced changes in protein structure and physicochemical properties will affect the foaming properties of the pressurized egg white solutions. As the mechanism of protein denaturation appeared to be different for heat and pressure treatment, it can be expected that this difference is reflected in the foaming properties of the treated egg white proteins. Thus, similar to heat-treatment, it will affect the functional properties of the treated egg white. Although several studies have been conducted on the pressure-induced gelation of egg white (Bridgman, 1914; Doi, Shimizu, Oe, & Kitabatake, 1991; Hayashi, Kawamura, Nakasa, & Okinaka, 1989; Richwin, Raasch, Teichgraber, & Knorr, 1992), few studies are available on the effect of pressure on the foaming properties of treated egg white. The existing literature is chiefly focused on the lower pressure range, up to 400 MPa (Richwin et al., 1992; Strohalm et al., 2000).

Therefore, the objective of this work was to compare the foaming properties of egg white solutions treated either by heat or by high pressure and to relate possible differences in these properties to differences in the denaturation mechanisms of the two preservation techniques. As the pH of egg white increases during storage up to 9.5 (Gu, Matsuda, & Nakamura, 1986), the processing-induced changes in all parameters were studied both at the pH of fresh egg white (pH 7.6) and at pH 8.8, corresponding to the pH of older egg white.

In our previous work (Van der Plancken et al., 2004, 2005a, 2005b; Van der Plancken, Van Loey, & Hendrickx, 2006, in press), the effect of the heat and pressure treatments on the physicochemical properties of egg white solutions was discussed. Based on these data, the relationship between the processing-induced changes in these physicochemical properties and the foaming properties of egg white was investigated.

2. Materials and methods

2.1. Materials

Fresh eggs (68) were obtained from the Zootechnological Centre (K.U. Leuven, Belgium). The egg white (approximately 2.6 L) was separated from the egg yolk and the

chalazae were removed. The albumen was gently mixed and stored at −40 °C, without any conversion to S-ovalbumin, as demonstrated by differential scanning calorimetry measurement (data not shown) (Donovan & Mapes, 1976). The protein content of the egg white was determined to be $9.64 \pm 0.78\%$ (w/v). A 10-fold dilution of the egg white (10% v/v or 9.64 g protein/L) was prepared in 0.2 M TrisHCl at pH 7.6 and 8.8. Under these conditions, no precipitation was observed, but at pH 8.8, a hazy solution was obtained.

2.2. Physical treatments

Thermal treatment of 10 mL egg white solution (10% v/v) was performed in 14 mL polypropylene tubes with screw cap (15 mm in diameter, Greiner Bio-One, Frickenhausen, Germany). The tubes were heated for 20 min in a temperature-controlled water bath (Thermo Haake, Karlsruhe, Germany) set at a constant temperature, ranging from 50 to 85 °C. After heating, samples were taken out of the water bath and immediately transferred to an ice bath to stop further denaturation. Under the conditions applied, no gel was obtained. However, a turbid suspension of protein aggregates was observed.

High pressure experiments were performed in a laboratory scale multivessel high-pressure equipment (HPIU-10.000, serial no. 95/1994, Resato, Roden, The Netherlands), consisting of six individual vessels (volume = 40 mL) surrounded by a thermostatic mantel, connected to a cryostat. The protein solutions were filled in 14 mL polypropylene tubes with flexible silicone stopper (15 mm in diameter, Greiner Bio-One, Frickenhausen, Germany), packed in double polyethylene plastic packs, to avoid any direct contact with the pressure medium, and vacuum sealed (down to 30 mbar, Multivac A300/16, Wolferteschwenden, Germany). Next, the tubes were enclosed in the pressure vessels already equilibrated at the desired temperature. Introduction of the samples in the pressure equipment took exactly 3 min, after which pressure was built up slowly (100 MPa/min) to minimize adiabatic heating. After attaining the desired pressure, all individual vessels were isolated and the central circuit was decompressed. The vessels were decompressed after 20 min of high pressure treatment. In these experiments, a pressure range of 400–700 MPa and temperature range of 10–60 °C was used. Exactly 1 min after pressure release, the samples were taken out of the pressure equipment and cooled in ice water to stop any further heat-denaturation. Under the conditions applied, no gel was obtained. However, a turbid suspension of protein aggregates was observed.

Heated or pressurized solutions from different tubes were mixed to render the necessary volume for determination of foaming properties. Foaming characteristics were assessed after 24 h storage at 4 °C. In this way, only the irreversible changes in egg white proteins were taken into account.

2.3. Determination of foaming properties

Heat-treated or pressurized egg white solutions (10% v/v) were diluted to 8% v/v with demineralized water to prepare foams. A volume of 50 mL (20 °C) foaming solution was placed in a glass beaker of 400 mL (diameter = 7.7 cm) and whipped for 4 min with a rotating anchor (45 mm diameter) at 1300 rpm and ambient temperature, using a laboratory stirrer with microprocessor controlled constant speed (OST basic, IKA, Germany). In Fig. 1, a schematic presentation is shown of the experimental set-up. The foam produced and any remaining liquid were gently transferred into a graduated glass cylinder (3.5 cm diameter, 31 cm height, graded up to 0.250 L) using a small size plastic spatula and plastic, broad neck funnel, within 5 min. Any air pockets were removed by holding up the base of the cylinder with one hand and the top of the cylinder with the other hand. With the cylinder held upright, two quick downward shakes were given (Patel, Stripp, & Fry, 1988).

At regular time intervals, close-up photographs of the foam column were taken using the super macro modus of a high-end digital camera (Canon Powershot Pro 1) from a distance of 5 cm. Arbitrary sections (25 mm^2) of these photographs were analyzed using imaging analysis software (analySIS pro 3.1 and analySIS 5, Soft imaging System, Bensheim). The number and cross section area (mm^2) of the air bubbles surrounded by protein film were determined.

The foam-forming ability (FA) was defined as the foam volume (L) 5 min after the end of whipping. The liquid in foam (LF) was expressed as the volume of liquid retained in a fixed volume of foam, 5 min after whipping had

Fig. 1. Schematic presentation of the experimental set-up of the whipping device (dimensions in mm). Fifty milliliters of foaming solution (8% v/v) was whipped in a glass beaker of 400 ml with a rotating anchor.

stopped. Drainage of liquid was recorded during 1 h. The foam stability (FS) was defined as the percentage of liquid still present in the foam after 1 h compared to the situation at 5 min after whipping. Samples were determined in triplicate.

2.4. Determination of physicochemical properties

The procedures for and the results of the determination of the physicochemical properties of treated egg white solutions were described in previous studies (Van der Plancken et al., 2004, 2006). Residual denaturation enthalpy was determined using differential scanning calorimetry (DSC). Solubility and turbidity were determined spectrophotometrically. Samples (1:10 dilutions of the 10% v/v samples in distilled water) were centrifuged for 15 min at 19,900g and 4 °C. Protein content of the supernatant was determined using Sigma Procedure No. TRPO-562 at 562 nm. Solubility was expressed as the percentage of protein remaining in the supernatant as compared to the untreated sample. The transmittance of a 1/20 dilution of the (un)treated egg white solution (10% v/v) in demineralized water was measured at 650 nm. A turbidity of 0% corresponds to a totally clear solution (transmittance in comparison to the blank is zero). (Aromatic) surface hydrophobicity (S_0) was determined using the fluorescent probe ANS (anilino-naphthalenesulfonic acid) as described by Alizadeh-Pasdar and Li-Chan (2000). The total, buried and exposed sulfhydryl (SH) content was determined spectrophotometrically using Ellman's reagent (5′,5-dithiobis (2-nitrobenzoic acid) or DTNB) (Kalab, 1970). The susceptibility to enzymatic hydrolysis (DH10), a measure for protein's flexibility, was determined using a pH-stat method (Adler-Nissen, 1986; Pedersen & Eggum, 1981).

2.5. Data analysis

The foaming properties of egg white solutions treated under different conditions of temperature and pressure were analyzed by Tukey's Studentized Range Test using the ANOVA procedure of the SAS Software (SAS Institute, 1999). Significant differences between means were decided based on a significance level (α) of 0.05.

The data on the physicochemical and foaming properties of treated egg white solutions were subjected to multiple regression analysis using least squares methodology and the REG procedure of the SAS software (SAS Institute, 1999). Stepwise multiple regression analysis was performed on the individual foaming properties as dependent variables and the physicochemical properties as independent variables. Logarithmic transformations of the variables and interaction terms between two variables were also taken into account. The best combination of independent variables was found by eliminating variables of no significance to simultaneously improve the standard error of the estimate (S), the adjusted r^2 (r^2_{adj}) and the sum of squares of the residuals.

3. Results and discussion

3.1. Foaming properties of untreated egg white solutions

Egg white solutions (8% v/v) were whipped using the experimental set-up as described above. Increasing both whipping time and rotation speed of the anchor resulted in the formation of higher volume foams with lower density (data not shown). Foam making conditions of 4 min at 1300 rpm and ambient temperature were chosen.

A significant difference ($\alpha = 0.05$) was observed in foam volume of foams prepared from untreated egg white solutions at pH 7.6 and pH 8.8 (0.1837 ± 0.0033 and 0.2170 ± 0.0054 L, respectively). Furthermore, it was observed that foams prepared at the lower pH contained more liquid (0.103 ± 0.007 and 0.064 ± 0.006 L liquid/L foam at pH 7.6 and pH 8.8, respectively). Once formed, foams of untreated egg white solutions were only marginally stable, as the liquid retained in the foam drained as a function of standing time. From Fig. 2, it is clear that drainage occurs in two stages. Initially, liquid flows freely as a result of gravitational force, however, flow is counteracted as function of the interface tension gradient. In a later stage, the slower liquid drainage is due to the collapse of foam bubbles (German, O'Neill, & Kinsella, 1985). The stability of the pH 8.8 foams was significantly lower than at pH 7.6, as at the former pH only 26.0 ± 0.8% of the liquid initially present in the foam was retained after 1 h, while at pH 7.6, this was 28.8 ± 1.0%. The foam volume could not be determined as a function of time, as the foams of untreated samples collapsed internally. Consequently, foam stability had to be determined based on the amount of liquid retained in the foam after a fixed delay time.

Hammershoj, Prins, and Qvist (1999) studied the surface and foaming properties of diluted (dried) egg white solutions (0.01% protein w/v) at different pH values. They used two methods to prepare the foams: 25 s shaking and 60 s
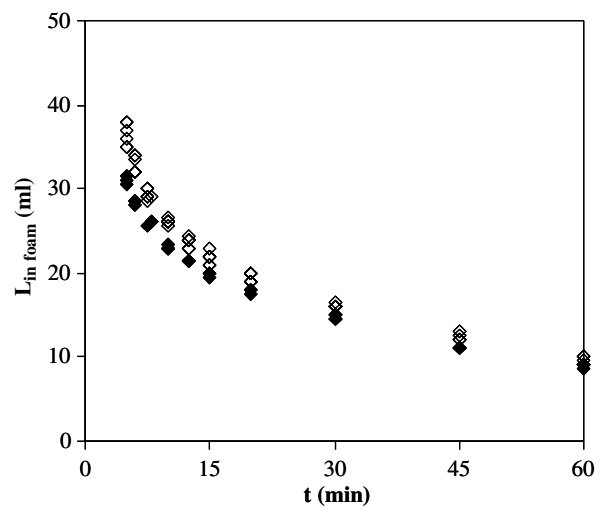

Fig. 2. Liquid drainage from foams of untreated egg white solutions at pH 7.6 (♦) and pH 8.8 (◊).

stirring with a fan-shaped whisk at 2500 rpm. The latter resembled better the method used in our work. For that method, a somewhat lower overrun was observed at pH 7.0 compared to 9.2, while for the overrun of foams prepared using the shaking method, the opposite was observed. For both methods, the foam stability (based on the residual foam volume), was the lowest at pH 9.2.

Brittle foams were formed upon whipping of untreated egg white solutions. After foam transfer and 5 min after the end of whipping, bubble cross section area ranged from $0.05\,mm^2$ to $0.55\,mm^2$ with an average of $0.077 \pm 0.010\,mm^2$ at pH 7.6. At the higher pH, bubbles were somewhat larger, ranging from 0.011 to $0.70\,mm^2$ with an average of $0.090 \pm 0.009\,mm^2$. Bubble-size distribution measurements through a glass wall can be erroneous, as a relatively higher number of larger bubbles is observed (Bisperink, Ronteltap, & Prins, 1992). Furthermore, as the boundaries of these air bubbles were selected manually (because of the low contrast between air bubble and surrounding medium, although this boundary was clearly distinguishable by eye), and only a small section of a single sample was examined, these values are approximate and therefore merely informational. Keeping in mind the determination conditions used, the difference in average bubble cross section area between the two pH values cannot be regarded significant.

In course of time, egg white solution drained from the foam, resulting in drying-up of the foam and distortion of the bubbles. While the bubbles were more or less spherical at first, they became more polyhedrical after time. The amount of bubbles decreased from 12.2 to 3 bubbles/mm^2 at pH 7.6 and from 9.8 to 4 bubbles/mm^2 at pH 8.8, within 45 min after whipping. A clear shift to larger bubbles and a broad range of bubble cross section areas was observed as after a delay of 45 min, the average bubbles cross section area was increased to $0.323\,mm^2$, with a maximum of $4.47\,mm^2$ at pH 7.6 and $0.231\,mm^2$, with a maximum of $3.13\,mm^2$ at pH 8.8. It has to be stressed that at the glass interface, bubbles can become distorted and disproportionation and coalescence rates may be affected. Therefore, these values are not an adequate representation of the overall changes in bubble size distribution due to aging of the foam. The foam column collapsed 45 min after whipping, leaving a dry, fragile matrix of egg white proteins.

These changes in bubble cross section area and number can be accounted for by two possible mechanisms. Firstly, coalescence of two bubbles due to the rupture of the interfacial film results in larger, though less numerous bubbles. Secondly, disproportionation due to internal gas pressure differences between large and small bubbles, results in the shrinking and even disappearing of small bubbles in favour of the larger bubbles. Distinguishing between these two phenomena is often difficult (Bisperink et al., 1992). Under the present conditions of bubble cross section area determination, no significant shrinking of bubbles, typical for bubble disproportionation, could be observed. The drainage of liquid due to gravitational force leads to thinning and finally breaking of the interfacial film. Therefore, it was concluded that besides liquid drainage, due to gravitational force, bubble coalescence was the main process leading to foam instability.

3.2. Effect of heat-treatment on foaming properties of egg white proteins

Temperature- and pH-dependent changes could be observed in the foaming properties of heat-treated egg white solutions, as shown in Fig. 3. Increasing temperature resulted in a decrease of the volume of the foams formed, until a pH-dependent minimum was reached (Fig. 3A). Further increasing the temperature, again improved the foaming ability, at pH 8.8 even to a higher level than of foams made out of untreated egg white solutions. At pH 8.8, this decrease in FA coincided with an increase in turbidity of heat-treated egg white solutions and the first phase in protein solubility loss, shown in an earlier study (Van der Plancken et al., 2006). With increasing treatment temperature, foams with lower foam volume were formed, until the temperature at which maximum turbidity occurs, was reached. From this temperature on, the FA of heat-treated egg white solutions increased again until the second phase of protein solubility loss commenced.

At the lower pH, however, FA initially did not decrease when the turbidity started to increase (above 55 °C). Only when the temperature of the second phase in the turbidity increase was reached (above 65 °C), FA of heat-treated egg white solutions decreased until a minimum was reached at 75 °C, the temperature above which no further changes in turbidity could be observed. Above this temperature, heat-treated egg white solutions showed a further loss in protein solubility and improved foam volume (Van der Plancken et al., 2006).

Thus, extensive protein solubility loss at pH 7.6 (maximal at 85 °C) did not impair the ability of the egg white proteins to form voluminous foam (83% of the untreated foam volume). This would indicate that the loss of soluble protein able to unfold readily at the interface was counterbalanced by the other structural changes induced by heat-treatment. At pH 8.8, changes in protein structure were not accompanied by extensive protein insolubilization (Van der Plancken et al., 2006). This can explain the improved foaming ability of egg white proteins treated at temperatures above 70 °C. The proteins can readily unfold at the interface by heat-induced increased flexibility (Van der Plancken et al., 2004), without being hindered by large aggregate sizes. Hagolle, Relkin, Popineau, and Bertrand (2000) also observed an increase in foaming ability when ovalbumin and lysozyme solutions (pH 7.0) were preheated. For ovalbumin, the solution had to be heated above 70 °C to obtain improved foaming power. Lysozyme showed no foaming ability, unless it was heated above 80 °C. The heat-induced conformational changes in this rigid protein were indicated as the cause for this phenomenon.
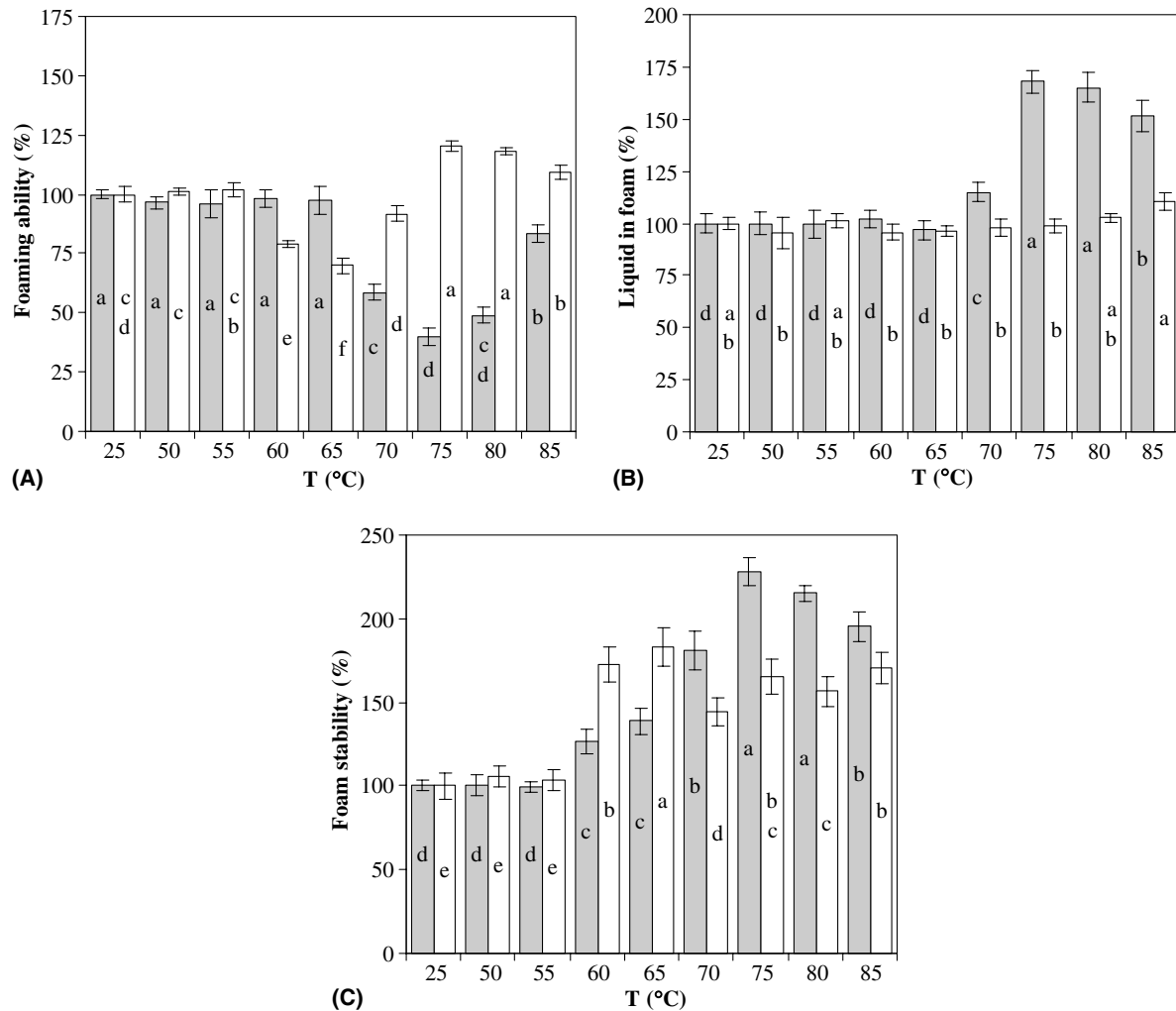
Fig. 3. Effect of 20 min heat-treatment of egg white solutions (10% v/v) at pH 7.6 (grey bars) and pH 8.8 (white bars) on foaming ability (A), foam density (B), and foam stability (C) as compared to the untreated egg white solution of each individual pH (%). Error bars represent the standard deviation of triplicate measurement. For each individual pH, means with the same letter are not significantly different ($\alpha = 0.05$).

At the neutral pH, maximum foam density was obtained in the same temperature range wherein a minimum in foam volume occurred (Fig. 3B). Egg white solutions treated at pH 7.6 and 75 °C, formed low volume, highly dense foams, that were however relatively stable (Fig. 3C). At pH 8.8, however, the FD was only mildly affected by heat-treatment. A heat-induced change in foam volume was thus paralleled by a proportional change in liquid retained in the foam. An increase in foam density was also observed for pre-heated ovalbumin solutions (pH 7) above 70 °C. The foams of heated lysozyme showed also increased initial density when the temperature of the heat-treatment was increased (Hagolle et al., 2000).

For both pH values, foam stability was improved by a 20 min heat-treatment at temperatures above 55 °C (Fig. 3C). Maximal FS was observed for treatments resulting in minimal FA. The FS is dependent on the ability of the proteins at the interface to form a cohesive network by both covalent and non-covalent interactions. In stable foams, this film can resist physical perturbations and the

approach of adjacent films. Heat-treatment at high temperatures (above 55 °C) seemed to enhance these protein–protein interactions at the interface, possibly by interactions between heat-exposed hydrophobic groups or sulfhydryl groups (Van der Plancken et al., 2005b, 2006).

The higher density of foams prepared from egg white solutions treated at temperatures above 65 °C, resulted in a moist and creamy appearance, contrasting with the crispy and dry appearance of the foams prepared from untreated egg white solutions. These foams were sticky and resembled clotted cream. No changes in color (white) of the foams could be observed with the naked eye.

Foams prepared from heat-treated samples showed more and smaller bubbles compared to those from untreated egg white solutions. For example, egg white solutions treated for 20 min at 75 °C and pH 7.6 had an initial average bubble cross section area of 0.043 mm^2. Foams treated under the same conditions but at pH 8.8 showed similar cross section areas. The higher foam density observed after heat-treatment at pH 7.6, thus indicates that

at the lower pH, the amount of interlamellar liquid was higher, as no significant difference in bubble size was observed for the different pH levels. This difference in foam structure compared to the untreated egg white solutions was also observed for ovalbumin and lysozyme foams obtained by sparging of heat-treated protein solutions (Hagolle et al., 2000).

The collapse of the foam column observed for foams prepared from untreated egg white solutions was reduced or even prevented by heat-treatment. Foams prepared from egg white solutions treated at 50 or 55 °C, showed some collapse of the foam column, but for egg white solutions treated at temperatures above 60 °C this was not observed upon aging. For these treatments, the bubble size did increase as a function of time, but not as pronounced as for foams from untreated egg white solutions (Fig. 4).

For instance, egg white heat-treated at 75 °C for 20 min had an average cross section area of 0.090 mm^2 at pH 7.6 and 0.18 mm^2 at pH 8.8 1 h after whipping. At pH 7.6, the heat-induced protein–protein interactions seem to be involved in limiting the drainage and subsequently coalescence of bubbles, resulting in a very stable structure, with smaller bubbles.

The relationship between heat-induced changes in the physicochemical properties of egg white solutions reported in earlier studies (Van der Plancken et al., 2004, 2005b, 2006) and the properties of the foams prepared from heat-treated egg white solutions were examined. When correlating the foaming properties of heat-treated egg white solutions with the observed changes in their physicochemical properties, it is important to keep in mind that during foam formation, the proteins undergo additional denaturation at the interface (Clarkson, Cui, & Darton, 1999). For instance, it has been shown that conformational changes in ovalbumin at the air–water interface lead to the formation of new intermolecular disulfide bonds due to oxidation of exposed sulfhydryl groups (Kitabatake & Doi, 1987). Heat-induced changes in the physicochemical properties of the egg white proteins can facilitate or counteract these changes, and thus enhance or diminish their foaming properties. Nevertheless, the physicochemical properties of the treated egg white solutions do not necessarily correspond to those of the proteins at the interface.

Pair wise correlation analysis of all foaming properties of the heat-treated egg white solutions showed that, however significant, no strong linear correlation existed between the individual properties, especially at pH 8.8 (Table 1). Pair wise correlation analysis between the individual foaming properties on the one hand and the corresponding physicochemical properties of these egg white
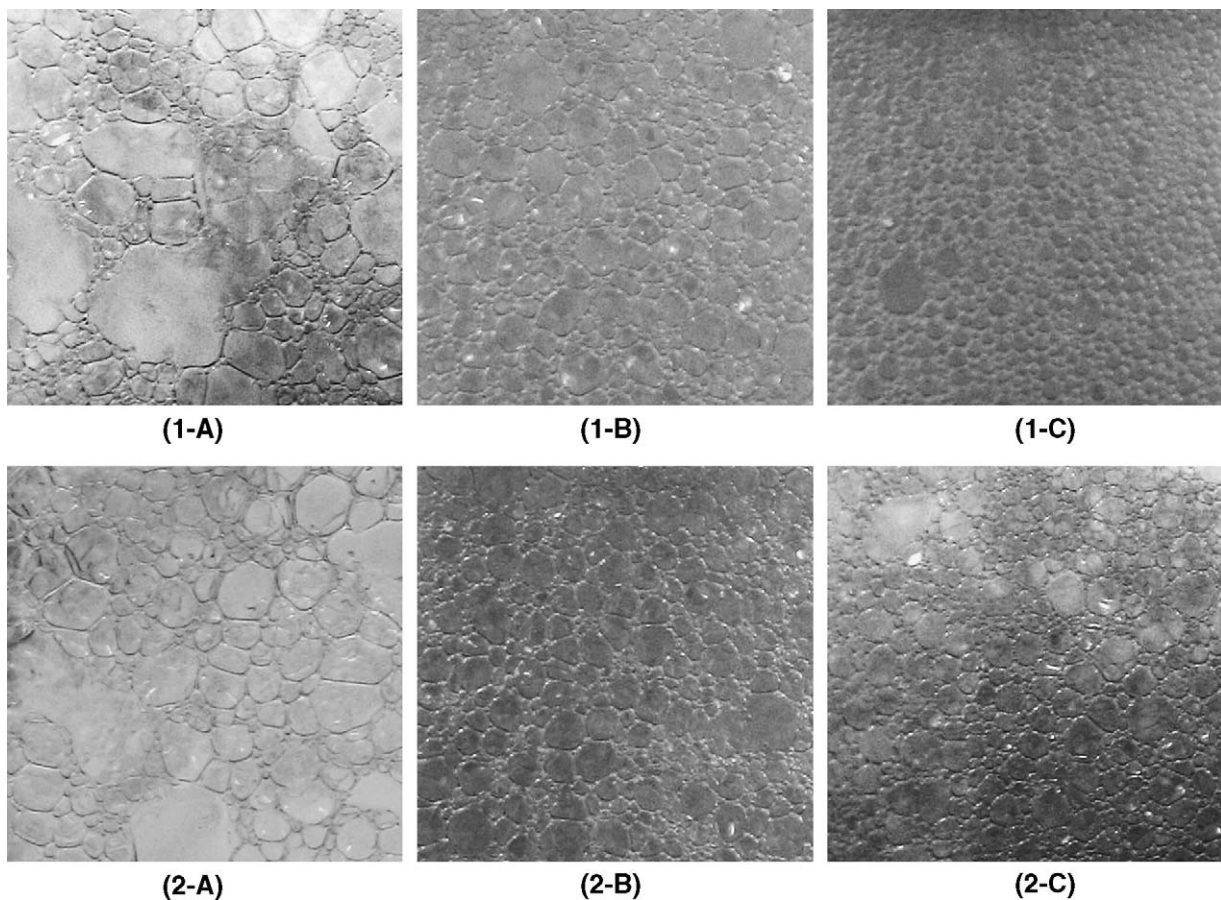

Fig. 4. Appearance of foams prepared from untreated egg white solutions (A) and egg white solutions heat-treated at 65 °C (B) and 75 °C (C), at pH 7.6 (1) or pH 8.8 (2), 60 min after whipping. The width of the photograph corresponds to 9 mm.

Table 1
Pearson correlation coefficients among foaming properties of egg white solutions (8% v/v) heat-treated at a concentration of 9.64 g protein/L, and their physicochemical properties, presented in earlier studies (Van der Plancken et al., 2003, 2005b, 2006)

Property	pH 7.6			pH 8.8		
	FA	LF	FS	FA	LF	FS
FA	–	−0.816[a]	−0.883[a]	–	0.402	−0.252
LF	−0.816[a]	–	0.901[a]	0.402	–	0.090
FS	−0.883[a]	0.901[a]	–	−0.252	0.090	–
Enthalpy	0.742[a]	**−0.955[a]**	−0.943[a]	−0.641	−0.593	−0.526
Solubility	0.747[a]	**−0.956[a]**	−0.938[a]	−0.464	−0.567	−0.668
Turbidity	−0.775[a]	0.898[a]	**0.970[a]**	−0.008	0.171	0.898[a]
A_{650}	−0.717[a]	0.942[a]	0.925[a]	0.011	0.178	0.888[a]
S_0	−0.716[a]	0.917[a]	0.920[a]	0.449	0.525	0.663
Total SH	0.790[a]	**−0.953[a]**	−0.932[a]	−0.647[a]	−0.616	−0.497
Exposed SH	−0.713[a]	**0.961[a]**	0.895[a]	0.719[a]	0.503	0.469
Buried SH	0.733[a]	**−0.963[a]**	−0.907[a]	−0.694	−0.557	−0.485
DH10	−0.778[a]	0.945[a]	**0.964[a]**	0.620	0.537	0.570

Correlation coefficients higher than 0.95 are highlighted.
FA foaming ability (L) of 8% v/v egg white solution.
LF liquid in foam (L/L) of 8% v/v egg white solution.
FS foam stability (%) of 8% v/v egg white solution.
A_{650} absorbance of 1/20 dilution of the treated egg white solution (10% v/v).
S_0 surface hydrophobicity (a.u.).
SH sulfhydryl content (% of total SH content of untreated egg white solution).
DH10 degree of hydrolysis after 10 min of enzymatic hydrolysis with trypsin and α-chymotrypsin.
[a] Significant at $P < 0.0001$.

solutions on the other hand revealed that although some significant correlation existed (and this primarily at pH 7.6), the heat-induced changes in none of the foaming properties could be accounted for by the heat-induced changes in one single physicochemical property measured (Table 1). This could be expected as both protein-stabilized foam formation and heat-induced protein denaturation are complex phenomena involving several different types of protein interactions.

Therefore, stepwise multiple regression analysis was performed on the individual foaming properties as dependent variables and the physicochemical properties (transformed if necessary) as independent variables. The optimal combination of independent variables was selected based on the lowest standard error of the estimate (S), the highest adjusted r^2 (r^2_{adj}) and the lowest sum of squares of the residuals. Only models consisting of maximum three physicochemical properties and maximum four parameters (including intercept) were considered. The denaturation enthalpy was not considered as the determination of this property was performed on egg white solutions treated at a much higher concentration (75% v/v) than the other properties.

For each individual pH, a specific set of physicochemical properties could be found to predict accurately the foaming ability of heat-treated egg white solutions (more than 95% of the variation in the property was explained by the prediction equation), as shown in Table 2. Several factors contribute to the formation of protein-stabilized foam: among others, protein flexibility, hydrophobic–hydrophilic ratio and electrostatic forces. Furthermore, heat-induced egg white protein denaturation was shown to proceed through

different mechanisms, depending on the pH. Therefore, it is not surprising that at the different pH values, different physicochemical properties are determinant for the foaming ability of heat-treated egg white solutions.

At both pH values, the sulfhydryl groups seemed to be involved in the mechanism of foam formation. This was also observed for foams prepared from ovalbumin. During foam formation, new intermolecular disulfide bonds are formed due to oxidation of exposed sulfhydryl groups (Kitabatake & Doi, 1987). These covalent bonds contribute to the formation of a protein film around the bubble. Heat-induced changes in these groups thus affect the foaming ability of the egg white proteins.

The foaming ability of a protein strongly depends on its flexibility at the interface. Susceptibility to enzymatic hydrolysis was demonstrated to be a measure for protein flexibility (Kato, Komatsu, Fujimoto, & Kobayashi, 1985). At pH 7.6, an increase in DH10 had a positive effect on the foaming ability of the heat-treated egg white proteins (Table 2). This was also observed for ovalbumin and lysozyme solutions by Kato et al. (1986). Although the exposure of hydrophobic groups at the interface reduces the surface tension, no positive effect of S_0 was observed. At pH 8.8, even a negative contribution of the heat-induced exposure of hydrophobic groups to the foaming ability was observed, when the other physicochemical properties were also taken into account (Table 5).

No significant relationship could be found between foaming ability and surface hydrophobicity of different untreated proteins (Townsend & Nakai, 1983). This observation was ascribed to the extensive exposure of hydrophobic groups upon unfolding a protein at the interface. As a

Table 2
Multiple regression models for prediction of the foaming properties of egg white solutions (8% v/v) heat-treated at a concentration of 9.64 g protein/L, based on their physicochemical properties, presented in earlier studies (Van der Plancken et al., 2003, 2005b, 2006)

Property	pH	Predictor variable	Parameter estimate	t-Value	$P > t$	S	r^2_{adj}
FA (mL)	7.6[a]	Intercept	3263.6 ± 201.4[b]	16.2	<0.001	8.71	0.962
		Exposed SH	-17.52 ± 1.15[b]	-15.3	<0.001		
		ln(buried SH)	-665.1 ± 43.6[b]	-15.3	<0.001		
		ln(DH10)	28.54 ± 9.19[b]	3.1	0.0052		
	8.8[a]	Intercept	-7402.7 ± 638.5[b]	-11.6	<0.001	6.30	0.968
		Total SH	-34.75 ± 2.49[b]	-14.0	<0.001		
		ln(total SH)	2450.3 ± 193.8[b]	12.6	<0.001		
		ln(S_0)	-34.58 ± 1.67[b]	-20.7	<0.001		
LF (L/L)	7.6[a]	Intercept	-1.359 ± 0.163[b]	-8.35	<0.001	0.0062	0.984
		Exposed SH	0.0331 ± 0.0039[b]	8.33	<0.001		
		Total SH $\times$ exposed SH	$-280.6\ 10^{-6} \pm 40.0\ 10^{-6}$[b]	-7.01	<0.001		
		ln(buried SH)	0.3305 ± 0.0353[b]	9.36	<0.001		
	8.8	No model established					
FS (%)	7.6[a]	Intercept	-169.88 ± 16.99[b]	-10.00	<0.001	1.916	0.983
		ln(solubility)	43.40 ± 3.64[b]	11.92	<0.001		
		Turbidity $\times$ DH10	0.3145 ± 0.0184[b]	17.10	<0.001		
	8.8[a]	Intercept	60.22 ± 1.09[b]	55.38	<0.001	1.293	0.977
		Exposed SH	-0.121 ± 0.018[b]	-6.84	<0.001		
		ln($A_{650\,nm}$)	8.077 ± 0.299[b]	26.95	<0.001		

FA foaming ability (L) of 8% v/v egg white solution.
LF liquid in foam (L/L) of 8% v/v egg white solution.
FS foam stability (%) of 8% v/v egg white solution.
A_{650} absorbance of 1/20 dilution of the treated egg white solution (10% v/v).
S_0 surface hydrophobicity (a.u.).
SH sulfhydryl content (% of total SH content of untreated egg white solution).
DH10 degree of hydrolysis after 10 min of enzymatic hydrolysis with trypsin and α-chymotrypsin.
[a] Significant at $P < 0.001$.
[b] Standard error.

result, the S_0 measured in solution may be quite different to the actual exposed hydrophobicity of a protein unfolded at the interface. In that study, a strong contribution of total hydrophobicity, viscosity and dispersibility to the foaming capacity of untreated proteins was demonstrated. Surface hydrophobicity of heat-treated ovalbumin or lysozyme was not related to their foaming ability. However, a strong correlation with emulsifying activity, another surface activity, was demonstrated (Kato et al., 1986).

Significant correlations existed between the physicochemical properties, especially at pH 7.6 (data not shown). Therefore, the contribution of the other structural properties to foaming properties of heat-treated egg white solutions cannot be excluded based on the models obtained. Furthermore, other physicochemical properties that were not considered in this study (e.g. charge density and aggregate size) can also contribute to the foaming ability of heat-treated egg white proteins.

No model meeting the prerequisites could be found to predict the foam density of egg white solutions treated at pH 8.8. This could be expected as little significant change ($\alpha = 0.05$) in this property occurred at the alkaline pH. Probably some other physicochemical properties are responsible for the constant density by counterbalancing

the heat-induced changes in the physicochemical properties. The foam density of egg white solutions heat-treated at pH 7.6 could be adequately predicted based on their sulfhydryl content (Table 2).

Although proteins need to be sufficiently flexible to unfold at the interface, they also need to retain a considerable secondary and even tertiary structure at the interface in order to form a stable film around the foam air bubbles (German et al., 1985). Therefore, it can be assumed that physicochemical properties facilitating the formation of a protein–protein network will contribute to the foam stability. In Table 2, the best prediction equations for the foam stability of heat-treated egg white proteins based on their physicochemical properties, are listed.

Although a relatively strong correlation was observed between the foam stability and the sulfhydryl content of egg white solutions heat-treated at pH 7.6, the former did not form part of the optimal model predicting the foam stability at this pH. At pH 8.8, the amount of exposed SH groups contributed negatively to the foam stability (Table 2). The heat-induced exposure of the buried SH groups of ovalbumin can facilitate the formation of disulfide bonds observed at the interface. However, the formation of these disulfide bonds proved not to be essential

for a stable foam, as the addition of DTNB to ovalbumin prior to foam formation does not impair the formation of a stable foam (Kitabatake & Doi, 1987).

For each individual pH, the optimal model predicting the foam stability consisted of a term corresponding to the degree of aggregation of the egg white proteins (either turbidity or solubility). Strong protein–protein interactions are necessary to form a stable foam. Increased aggregation had a positive influence on the foam stability.

3.3. Effect of pressure treatment on foaming properties of egg white proteins

Similar to heat-treatment, pressurization of egg white solutions resulted in irreversible changes in the foaming properties of the treated solutions. These changes were either beneficial or detrimental, depending on the pH and the temperature–pressure combination applied (Figs. 5–7).

The effect of pressure on the foaming ability of pressure-treated egg white solutions was strongly dependent on the pH of the egg white solutions. At pH 8.8, a beneficial effect could be observed at all levels of temperature studied (Fig. 5B). With increasing pressure, foams of higher volume could be obtained. At the highest pressures, the improvement of the FA was even more pronounced than obtainable by heat-treatment at this pH. The effect of temperature was not clear below 60 °C at any pressure. For the pressure levels studied, no significant lower pressure was needed to change the FA at lower pressure, as would be expected based on the antagonistic effect between pressure and temperature observed for the physicochemical properties of pressure-treated egg white proteins. At 60 °C however, pressure had a significantly stronger, enhancing effect on the FA of egg white solutions.

At pH 7.6, the effect of pressure on the foaming ability was less straightforward than at the higher pH (Fig. 5A). Below 60 °C, an initial increase in FA due to pressure treatment at pressures below 500 MPa, was followed by a decrease, resulting in egg white solutions with lower foaming capacity compared to the untreated proteins. This detrimental effect at elevated pressure was stronger at lower temperature. At 60 °C, no significant effect of pressure on the FA of the treated egg white solutions could be observed ($\alpha = 0.05$).

Except for 60 °C, pressure treatment resulting in excessive loss of protein solubility (600–700 MPa at pH 7.6) reduced the foaming ability of the treated egg white solutions. At pH 8.8 little insolubilization of protein occurred due to pressure treatment. The combination of high residual protein solubility and pressure-induced changes in the protein structure can explain the improved foaming ability of egg white proteins pressure-treated at this pH.

Richwin et al. (1992) observed that pressure treatment up to 400 MPa results in decreasing foam expansion of the pressure-treated egg white, almost independent of temperature (20–50 °C), while in our study the FA was improved by pressure treatment at 400 MPa below 60 °C.
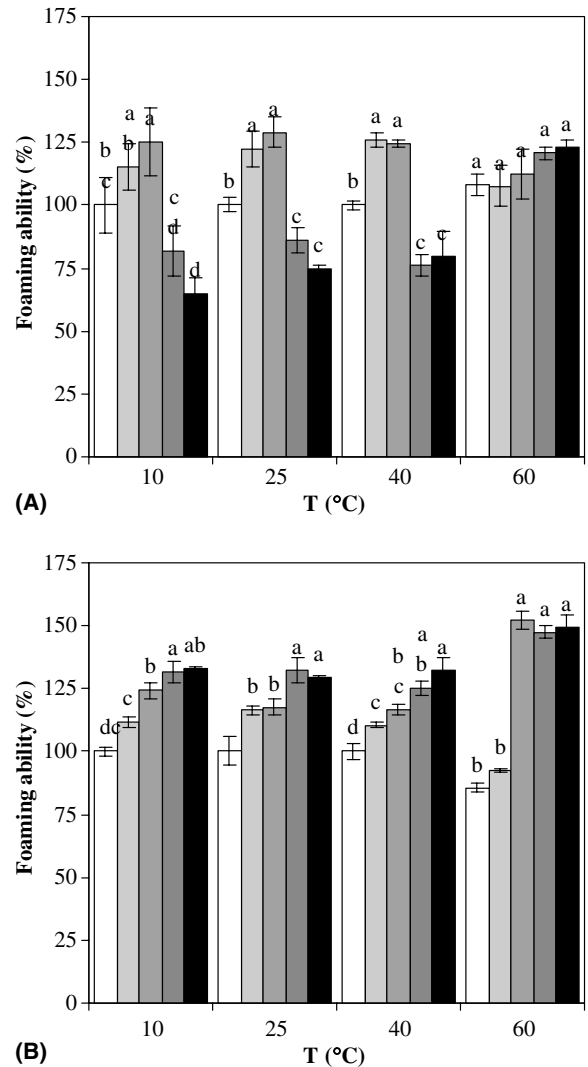

Fig. 5. Effect of 20 min pressure treatment of egg white solutions (10% v/v) at pH 7.6 (A) or pH 8.8 (B) and at 0.1 (☐), 400 (☐), 500 (☐), 600 (☐), and 700 MPa (■) on foaming ability as compared to the untreated egg white solution of each individual pH (%). Error bars represent the standard deviation of triplicate measurement. For each individual pH and temperature, means with the same letter are not significantly different ($\alpha = 0.05$).

In the study by Strohalm et al. (2000), however, no significant change in foam volume could be observed for egg white pressurized at 25 °C and pressures up to 400 MPa. This difference can be due to the fact that our study was conducted on diluted egg white solutions, while in the other studies both pressure treatment and foam formation where performed with pure egg white. In the first study, foams were prepared by stirring, while in the second the pressurized egg white was whipped.

As was observed for heat-treatment, high pressure treatment exerted little effect on the density of the foams prepared from egg white solutions pressure-treated at pH 8.8 (Fig. 6B). Little or no effect of temperature during pressure treatment was observed either. Since the volume of the foams formed was indeed affected by pressure treatment
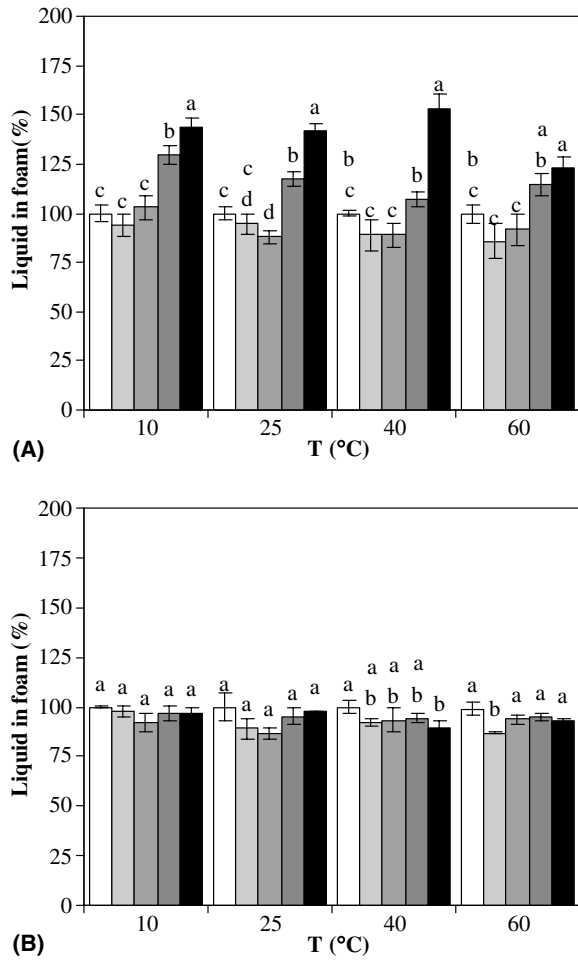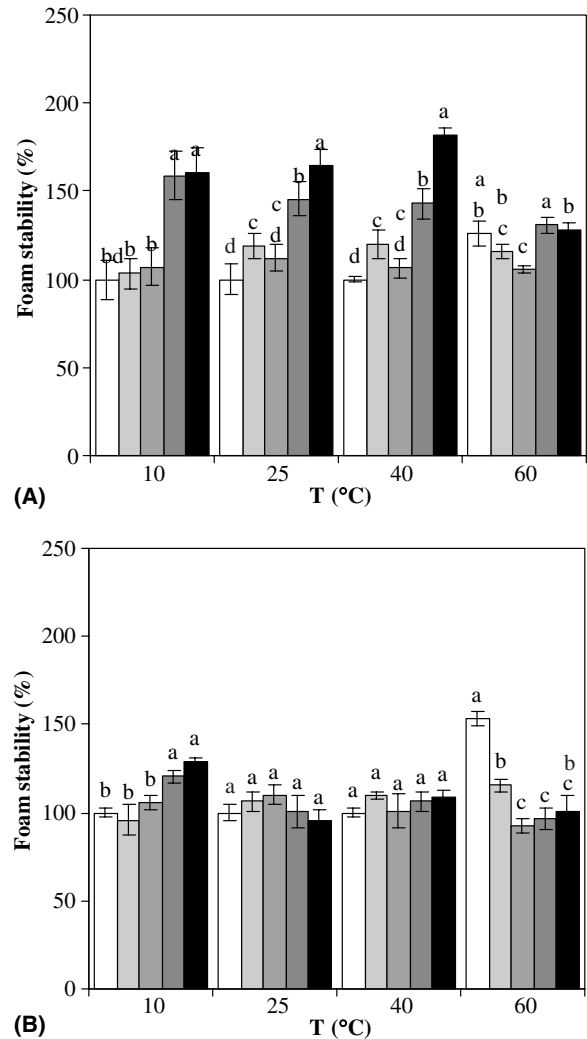
Fig. 6. Effect of 20 min pressure treatment of egg white solutions (10% v/v) at pH 7.6 (A) or pH 8.8 (B) and at 0.1 (▯), 400 (▯), 500 (▯), 600 (▯), and 700 MPa (▮) on foam density as compared to the untreated egg white solution of each individual pH (%). Error bars represent the standard deviation of triplicate measurement. For each individual pH and temperature, means with the same letter are not significantly different ($\alpha = 0.05$).

Fig. 7. Effect of 20 min pressure treatment of egg white solutions (10% v/v) at pH 7.6 (A) or pH 8.8 (B) and at 0.1 (▯), 400 (▯), 500 (▯), 600 (▯), and 700 MPa (▮) on foam stability as compared to the untreated egg white solution of each individual pH (%). Error bars represent the standard deviation of triplicate measurement. For each individual pH and temperature, means with the same letter are not significantly different ($\alpha = 0.05$).

(Fig. 5B), at pH 8.8 a pressure-induced change in foam volume was paralleled by a proportional change in liquid retained in the foam.

At the lower pH, pressure-treated egg white solutions (above 500 MPa) gave rise to denser foams (Fig. 6A). As for heat-treated egg white solutions, a higher density coincided with a lower foam volume (Fig. 5A). Under the conditions applied, the foams prepared from pressure-treated egg white solutions were less dense compared to those from heat-treated solutions (Fig. 3B). Iametti et al. (1999) showed that pressure treatment (5 min at 600 MPa and 25 °C) enhanced the foam density of the treated egg white; the addition of 10% sucrose prior to pressure treatment enhanced this effect, while the presence of 10% NaCl resulted in less dense foams.

At both pH values, it could be observed that pressure treatment had no detrimental effect on the stability of the foams prepared from the resulting egg white solutions

(Fig. 7), as was the case for heat-treated egg white solutions (Fig. 3C). The positive effect on this foaming property of the non-thermal treatment was however less pronounced. The low volume, dense foams produced from egg white solutions pressure-treated above 500 MPa (10–40 °C) were significantly more stable than those prepared from egg white solutions with pressure-induced improved foaming ability. Probably the formation of aggregates in the former solutions did not allow the incorporation of large volumes of air, but the strong intermolecular interactions stabilized the small bubbles formed to a higher extent.

Literature data present contradictory observations regarding the stability of foams prepared from egg white pressurized up to 400 MPa. Richwin et al. (1992) observed that increasing pressure results in decreasing foam volume of the pressure-treated egg white, almost independent of

temperature (30–50 °C). In the higher temperature regions (40–50 °C), foam stability decreased most likely due to lower soluble protein content. When the foam stability was defined based on the amount of liquid drained from the foam (like in our study), it was observed that increasing pressure up to 400 MPa resulted in more stable foams (Strohalm et al., 2000).

Foams prepared from pressure-treated egg white solutions had a moist and creamy appearance, similar to foams from egg white solutions heat-treated above 65 °C. Severe pressure treatments (700 MPa) led to dense foams that were sticky and resembling clotted cream. During the whipping of egg white solutions processed under these conditions, splashing of the solution occurred and the resulting foam adhered to the glass wall and the rotating anchor.

The collapse of the foam column observed for foams prepared from untreated egg white solutions was prevented by pressure treatment (400–700 MPa). No coarsening was observed upon aging of foams from pressure-treated egg white solutions. Foams prepared from pressure-treated egg white solutions with low foaming ability (600–700 MPa at 10–40 °C and pH 7.6) showed small bubble size. For instance, foams of egg white solutions pressurized for 20 min at 700 MPa and 10 °C had an average bubble cross section area of 0.035 mm². This small bubble size was more or less maintained during standing of the foam (Fig. 8). This is not surprising as these foams showed high stability. Under conditions of improved foaming ability, a bubble size comparable to that of untreated egg white foams was observed at pH 7.6, while at pH 8.8 smaller bubble sizes were observed compared to untreated foams, both at low and high temperature. For instance after 20 min treatment at 700 MPa and 10 °C, the average initial bubble cross section area was 0.064 mm².

At pH 8.8, the bubble size after 60 min standing was similar to those of foams of heat-treated egg white solutions, both for egg white solutions pressurized at low temperatures and at 60 °C. Foams prepared from egg white solutions pressurized at 60 °C at the lower pH showed bubble sizes after 60 min of standing comparable to those of untreated egg white solutions (Fig. 8).

The relationship between the pressure-induced changes in the physicochemical properties of egg white solutions reported in earlier studies (Van der Plancken et al., 2004, 2005a, in press) and the properties of the foams prepared from these pressure-treated egg white solutions were examined. It was observed that the pressure-induced changes at 60 °C in both physicochemical properties and foaming properties strongly deviated from those of egg white solutions pressurized at 10–40 °C (Van der Plancken et al., in press). Therefore, pair wise correlation analysis of all foaming properties of the pressure-treated egg white solutions was performed for the two temperature domains separately. No strong, significant linear correlation existed between the individual properties for neither of the two temperature domains, as was also observed for heat-treated egg white solutions (Tables 3 and 4).

Some significant correlation existed between the individual foaming properties on the one hand and the corresponding physicochemical properties of these egg white solutions on the other hand in the lower temperature range (and this primarily at pH 7.6) (Table 3). At 60 °C however, little linear correlation was observed between foaming properties and physicochemical properties (Table 4). Pressure treatment at 60 °C indeed had a different effect on foaming properties than pressure treatment in the lower temperature range. For instance, at 60 °C, pressure treatment resulting in low protein solubility did not impair the foaming ability of the treated egg white proteins, while in the lower temperature range a low residual protein solubility of the pressurized egg white solutions was accompanied by poor foaming ability. These opposite relationships for different temperature ranges were also observed between the other physicochemical properties and foaming properties. This clearly indicates that pressure-induced denaturation at elevated temperatures occurs through a different mechanism compared to moderate temperatures.

None of the foaming property changes were accounted for by pressure-induced changes in a single physicochemical property. Therefore, stepwise multiple regression analysis was performed on the individual foaming properties as dependent variables and the physicochemical properties (transformed if necessary) as independent variables. Again, this analysis was performed separately for the two temperature ranges.

An acceptable model (80% of the variation in the property was explained by the prediction equation) was found to describe the increase in foaming ability at moderate pressure (400 and 500 MPa) and the decrease in this property at high pressure (600 and 700 MPa) at pH 7.6, based on the susceptibility to enzymatic hydrolysis, surface hydrophobicity and buried SH content of the pressure-treated egg white solutions (Table 5). At pH 8.8, the changes in this property could be described based on the egg white protein's susceptibility to enzymatic hydrolysis and surface hydrophobicity alone (Table 5). The lower r^2_{adj} in comparison to the models describing this property for heat-treated egg white solutions can be due to the limited amount of data points or to the contribution of unmeasured physicochemical properties to the FA of pressure-treated egg white solutions. Contrary to what was observed for foams from heat-treated egg white solutions, surface hydrophobicity had a positive effect on the FA of egg white proteins pressure-treated in the temperature range of 10–40 °C.

No model meeting the prerequisites could be found to describe the changes observed in foaming ability of egg white solutions pressure-treated at 60 °C based on their physicochemical properties. Under the restrictions applied, no higher r^2_{adj} than 0.460 could be obtained. This can be due to the insignificant changes ($\alpha = 0.05$) in this property. Furthermore, other properties than the ones measured can be responsible for the foaming activity at this temperature. As shown in Table 5, the foaming ability of proteins pressurized
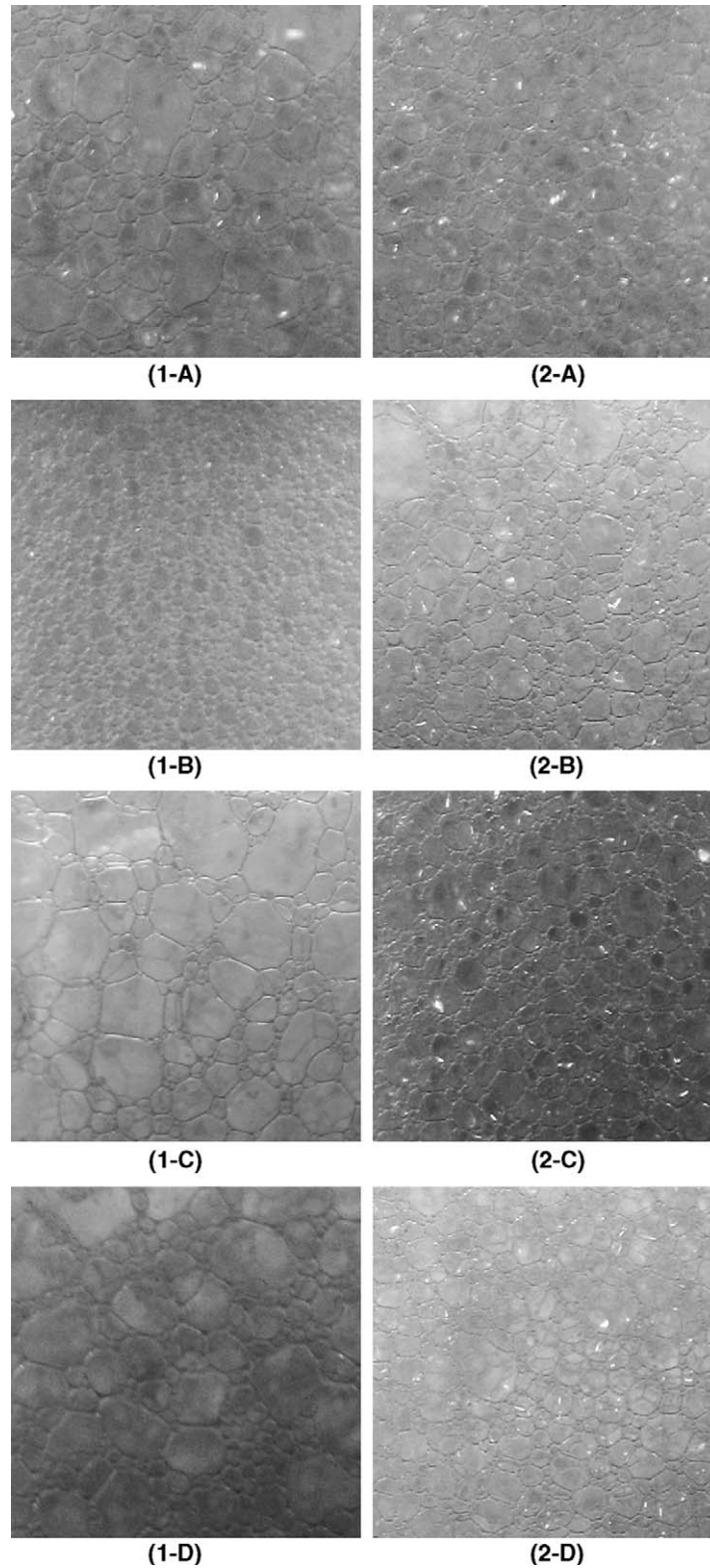
Fig. 8. Appearance of foams prepared from egg white solutions pressure-treated at 10 °C and 500 MPa (A) or 700 MPa (B) and at 60 °C and 500 MPa (C) or 700 MPa (D), at pH 7.6 (1) or pH 8.8 (2), 60 min after whipping. The width of the photograph corresponds to 9 mm.

at 60 °C and pH 8.8 could be accurately described based on their solubility and surface hydrophobicity.

Similar to heat-treated egg white solutions, the insignificant changes in the foam density at pH 8.8, made it impossible to construct a predictive model for this property based on the physicochemical properties measured (Table 5). Probably other physicochemical properties are responsible for this invariable density at all temperature levels.

Table 3

Pearson correlation coefficients among foaming properties of egg white solutions (8% v/v) pressure-treated at 10–40 °C at a concentration of 9.64 g protein/L, and their physicochemical properties, presented in earlier studies (Van der Plancken et al., 2004, 2005a, in press)

Property	pH 7.6			pH 8.8		
	FA	FD	FS	FA	FD	FS
FA	–	−0.832[a]	−0.742[a]	–	−0.585[a]	0.032
FD	−0.832[a]	–	0.876[a]	−0.585[a]	–	0.603[a]
FS	−0.742[a]	0.876[a]	–	0.032	0.603[a]	–
Enthalpy	0.689[a]	−0.868[a]	−0.906[a]	−0.860[a]	0.518[b]	−0.045
Solubility	0.752[a]	−0.911[a]	−0.936[a]	−0.423	0.510	0.648
Turbidity	−0.564[a]	0.637[a]	0.773[a]	0.681[a]	−0.296	0.029
A at 650 nm	−0.593[a]	0.642[a]	0.785[a]	0.680[a]	−0.298	0.028
S_0	−0.674[a]	0.856[a]	0.904[a]	0.915[a]	−0.557[b]	0.041
Total SH	0.577[a]	−0.815[a]	−0.868[a]	−0.837[a]	0.519[b]	0.039
Exposed SH	−0.708[a]	0.848[a]	0.805[a]	0.756[a]	−0.289	0.288
Buried SH	0.726[a]	−0.928[a]	−0.926[a]	−0.829[a]	0.438	−0.097
DH10	−0.771[a]	0.920[a]	0.925[a]	0.837[a]	−0.515[b]	0.109

FA foaming ability (L) of 8% v/v egg white solution.
LF liquid in foam (L/L) of 8% v/v egg white solution.
FS foam stability (%) of 8% v/v egg white solution.
A_{650} absorbance of 1/20 dilution of the treated egg white solution (10% v/v).
S_0 surface hydrophobicity (a.u.).
SH sulfhydryl content (% of total SH content of untreated egg white solution).
DH10 degree of hydrolysis after 10 min of enzymatic hydrolysis with trypsin and α-chymotrypsin.
[a] Significant at $P < 0.0001$.
[b] Significant at $P < 0.001$.

Table 4

Pearson correlation coefficients among foaming properties of egg white solutions (8% v/v) pressure-treated at 60 °C at a concentration of 9.64 g protein/L, and their physicochemical properties, presented in earlier studies (Van der Plancken et al., 2004, 2005a, in press)

Property	pH 7.6			pH 8.8		
	FA	FD	FS	FA	FD	FS
FA	–	0.581	0.405	–	0.046	−0.847
FD	0.581	–	0.669	0.046	–	0.337
FS	0.405	0.669[b]	–	−0.847[a]	0.337	–
Enthalpy	−0.778	−0.828[b]	−0.407	−0.796[b]	0.205	0.757
Solubility	−0.686	−0.746	−0.328	−0.423	−0.715	−0.043
Turbidity	0.756	0.651	0.266	0.526	0.811[b]	−0.067
A at 650 nm	0.800[b]	0.762	0.415	0.523	0.812[b]	−0.065
S_0	0.796[b]	0.754	0.388	0.773[b]	−0.129	−0.699
Total SH	−0.628	−0.709	−0.290	−0.506	0.033	0.349
Exposed SH	0.706	0.660	0.445	0.636	−0.158	−0.546
Buried SH	−0.710	−0.584	−0.218	−0.522	0.047	0.371
DH10	0.805[b]	0.842[b]	0.440	0.745	−0.165	−0.787

FA foaming ability (L) of 8% v/v egg white solution.
LF liquid in foam (L/L) of 8% v/v egg white solution.
FS foam stability (%) of 8% v/v egg white solution.
A_{650} absorbance of 1/20 dilution of the treated egg white solution (10% v/v).
S_0 surface hydrophobicity (a.u.).
SH sulfhydryl content (% of total SH content of untreated egg white solution).
DH10 degree of hydrolysis after 10 min of enzymatic hydrolysis with trypsin and α-chymotrypsin.
[a] Significant at $P < 0.0001$.
[b] Significant at $P < 0.001$.

The surface hydrophobicity seemed to contribute to the density of foams of heat-treated egg white solutions.

Only for egg white solutions pressurized at pH 7.6 in the temperature range of 10–40 °C, a model could be found to predict the foam stability (Table 5). This is not surprising, as pressure treatment did not affect significantly the foam stability at the other level of pH, or in the higher tempera-

ture range. With increasing degree of protein precipitation, the foam stability improved. However, other physicochemical properties can attribute to this property as less than 90% of the variation in the FS was explained by the prediction equation. This again shows that protein–protein interactions are important to form stable foams. The current data are however insufficient in number to fully grasp the

Table 5
Multiple regression models for prediction of the foaming properties of egg white solutions (8% v/v) pressure-treated at a concentration of 9.64 g protein/L, based on their physicochemical properties, presented in earlier studies (Van der Plancken et al., 2004, 2005a, in press)

Property	T-Range	pH	Predictor variable	Parameter estimate	t-Value	P > t	S	r^2_{adj}
FA (%)	10–40 °C	7.6[a]	Intercept	71.1 ± 16.71[b]	4.25	0.0001	9.95	0.802
			DH10	-15.9 ± 1.7[b]	−9.25	<0.0001		
			$\ln(S_0)$	15.7 ± 2.4[b]	6.65	0.0023		
			ln(buried SH)	-8.5 ± 2.6[b]	−3.27	<0.0001		
		8.8[a]	Intercept	61.8 ± 4.7[b]	13.11	<0.0001	3.63	0.905
			DH10	1.139 ± 0.296[b]	3.85	0.0004		
			$\ln(S_0)$	6.908 ± 0.720[b]	9.60	<0.0001		
	60 °C	7.6	No model established					
		8.8[a]	Intercept	1479 ± 161[b]	9.18	<0.0001	5.81	0.972
			S_0	-0.0465 ± 0.00465[b]	−10.00	<0.0001		
			$\ln(S_0)$	176.5 ± 14.5[b]	12.21	<0.0001		
			ln(solubility)	-580.2 ± 52.7[b]	−11.01	<0.0001		
LF (%)	10–40 °C	7.6[a]	Intercept	153.5 ± 9.3[b]	16.45	<0.0001	5.55	0.934
			DH10	7.57 ± 0.96[b]	7.91	<0.0001		
			$\ln(S_0)$	-6.80 ± 1.3[b]	−5.17	<0.0001		
			ln(buried SH)	-5.32 ± 1.45[b]	−3.67	0.0007		
		8.8[a]	No model established					
	60 °C	7.6[a]	Intercept	260.5 ± 25.5	10.22	<0.0001	3.75	0.923
			S_0	0.0095 ± 0.0010	9.47	<0.0001		
			$\ln(S_0)$	-25.0 ± 3.7	−6.76	<0.0001		
		8.8	No model established					
FS (%)	10–40 °C	7.6[a]	Intercept	371.3 ± 13.5[b]	27.41	<0.0001	9.24	0.888
			ln(solubility)	-57.6 ± 3.2[b]	−18.02	<0.0001		
		8.8	No model established					
	60 °C	7.6	No model established					
		8.8	No model established					

FA foaming ability (L) of 8% v/v egg white solution.
LF liquid in foam (L/L) of 8% v/v egg white solution.
FS foam stability (%) of 8% v/v egg white solution.
A_{650} absorbance of 1/20 dilution of the treated egg white solution (10% v/v).
S_0 surface hydrophobicity (a.u.).
SH sulfhydryl content (% of total SH content of untreated egg white solution).
DH10 degree of hydrolysis after 10 min of enzymatic hydrolysis with trypsin and α-chymotrypsin.
[a] Significant at $P < 0.001$.
[b] Standard error.

effect of pressure-induced denaturation on the foaming properties of egg white solutions.

4. Conclusions

Both heat and pressure treatment affected the foaming properties of egg white proteins. While foams from untreated egg white solutions were crispy and subject to collapse of the foam column after a long standing period, foams from heat and pressure-treated egg white solutions were moist and creamy, showing smaller bubble size and lower sensitivity to bubble coalescence as evidenced by the reduced tendency of collapse of these foams.

The effect of physical treatments was strongly dependent on the pH during treatment. The most voluminous foams were obtained at pH 8.8, while the most stable, dense foams were obtained at pH 7.6, for both treatments. Treatments resulting in a high level of protein unfolding however combined with a certain degree of residual protein solubility (primarily at pH 8.8) resulted in egg white solutions with improved foaming ability. A high level of unfolding combined with extensive protein solubility loss (primarily at pH 7.6) was associated with increased foam stability and density.

A pH-dependent minimum could be observed in the foaming ability of heat-treated egg white proteins coinciding with maximal foam stability. The heat-induced changes in the foaming properties could not be attributed to the changes in one single physicochemical property. The foaming ability of heat-treated egg white solutions was in part determined by the sulfhydryl content of ovalbumin, the major egg white protein. Increased protein flexibility

(measured as the susceptibility to enzymatic hydrolysis) due to heating also contributed to the volume of foams prepared from heat-treated egg white solutions. Surface hydrophobicity showed to have no or a negative effect on the foam volume. This was also observed in literature (Kato et al., 1986; Townsend & Nakai, 1983). The foaming ability of pressure-treated egg white solutions was improved at all levels of temperature and pressure at pH 8.8, while at pH 7.6 in the lower temperature range a negative effect of pressure was observed for pressure treatments resulting in pronounced protein denaturation. This difference in foaming ability of egg white solutions pressure-treated at different pH values can be attributed to the marked difference in aggregation behaviour under pressure. Furthermore, an effect of pH during foam formation (for instance electrostatic interactions) cannot be excluded. This observation does not necessarily imply that it is advisory to use older eggs before physical treatment. Firstly, aging of eggs can reduce the microbial quality of eggs before pasteurization, compelling more severe treatments. Secondly, this study was performed on egg white in a Tris–HCl buffer system ($pK_a = 8.1$). A contribution of the buffer to the different aggregation behaviour at different levels of pH, through differences in shielding of negatively charged egg white proteins, cannot be excluded.

While little or no effect of physical treatment was observed on the foam density at pH 8.8, a clear increase in density of foams prepared from egg white solutions heat- or pressure-treated at pH 7.6 under conditions of pronounced protein aggregation (above 65 °C at ambient pressure and above 500 MPa at 10–40 °C, respectively) was observed. The high density of foams prepared from egg white solutions treated under these conditions was also shown by a small bubble size. The stability of dense foams was in general high. Protein–protein interactions (solubility, SH groups) showed to be determinant for this property.

The best foams (high volume and average density) were obtained by pressure treatment at pH 8.8. For practical applications in meringues and angel cakes, the heat-stability of the foams prepared from treated egg white should be investigated. Also, microbiological studies are required to explore the effect of both heat and high pressure treatment on *Salmonella*, so that processes can be designed that are both safe and result in excellent foaming properties.

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