



Aerated whey protein gels as new food matrices: Effect of thermal treatment over microstructure and textural properties



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ABSTRACT

Aerated gels (AG) contain both bubbles and entrapped water in their structure, thus offering ample versatility in product development. The objective of this study was to evaluate the effect of thermal treatment of whey protein isolate (WPI) dispersions over the microstructure and textural properties of AG fabricated with these dispersions. WPI dispersions (9% w/w) with NaCl (0.4% w/w) at three pH's (6.50, 6.75 and 7.00) were prepared. Dispersions were subjected to thermal treatment at three temperatures (70, 75 and 80 °C) at different times depending on the pH. After thermal treatment, dispersions were aerated by mechanical agitation at 2000 rpm for 3 min. Finally, aerated dispersions were cooled at 10 °C for 24 h to set the AG structure. AG were characterized in terms of their gas hold-up capacity, microstructure (bubble sizes) and textural properties. Thermal treatment temperature influenced the gas hold-up and mean diameter (D_M) of air bubbles in the AG, both decreasing with an increase in temperature. Maximum gas hold-up was about 70% and D_M were in the range of 530–700 μm . The increase in apparent viscosity of thermally treated dispersions produced lower air incorporation in AG structure and smaller bubble sizes. Compression stress at break decreased with an increase in the thermal treatment temperature. Control of thermal treatment conditions for WPI dispersions allows to fabricate AG with different microstructural and textural properties which can be used as innovative food matrices for obesity control or delivery of bioactives.

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1. Introduction

The foods in a modern shopping basket increasingly feature a novel, but often overlooked ingredient: bubbles. Aerated foods gain importance as manufacturers seek to exploit the novelty and versatility of bubbles as food ingredients. However, bubbles in food have been present for centuries in products such as beer, bread and ice cream. Bubbles are desirable elements in gastronomy creations. Mousses and soufflés, recognized as emblematic forms in culinary art, are classic examples in which the incorporation and retention of bubbles is a critical factor in the success of the dish. Besides, modern innovative cooks exploit bubbles in their creations (Zúñiga and Aguilera, 2008).

Air bubbles are structural elements in solids, semi-solids and liquid foods. The mere incorporation of air in a food product change their physical and sensory properties, which depend on the structure formed during processing, in this regard a scientific understanding of structure formation in aerated food is needed

with the aim of designing products with tailored physical and sensory properties (Zúñiga and Aguilera, 2008). The final objective of structuring foods is to obtain structure–property relationships, in other words, the underlying connection between the structure and how the product behaves (Aguilera and Lillford, 2007).

The positive benefits of aerated foods primarily relate to texture. Fluid products such as whipped cream and mousses obtain smoothness and novelty, while solid products such as breakfast cereals and snacks become light and crispy. An aerated structure may facilitate mastication, enzyme accessibility to substrates and enhance flavor delivery (Zúñiga and Aguilera, 2008). Introducing a gas phase into a food matrix not only affects its texture and firmness making the product softer, but also changes the appearance, color and mouth-feel (Campbell and Mougeot, 1999). Thus, the presence of bubbles in gel-based food products may result in unique properties conferred by the additional gaseous phase and the increased internal surface area (Nussinovitch et al., 1992).

In recent years, new aerated foods appearing in the market are perceived as lighter in terms of calories, thus satisfying urgent consumer needs. An important benefit of air inclusion, or other gases, in foods is the reduction in the “caloric density”, understanding

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caloric density as the amount of calories per unit of volume. Dispersed air in foods reduces the energy consumed per unit of volume making portions less caloric-dense, in line with the suggestions of food psychologists who advocate for constant volume but fewer calories in rations (Osterholt et al., 2007; Rolls et al., 2000). These studies showed that volume, independent of weight, can have an effect on food and energy intake. In fact, Osterholt et al. (2007) demonstrated that, within limits, people eat by volume. In this study, subjects consumed a greater volume but less weight and energy of the more-aerated snack compared to the less-aerated snack; however, no differences were found in terms of fullness after consumption. Rolls et al. (2000) showed that increasing the volume of a food by incorporating air can affect satiety, thus decreasing the energy intake at the subsequent meal. In agreement with these results, higher ratings of fullness (satiety) were found after consumption of a high-volume food compared with the same food at lower volume (Bell et al., 2003). Thus, aerated foods may provide a sense of fullness higher or comparable to the non-aerated food, but giving fewer calories. In addition, it was demonstrated that the inclusion of air bubbles may be an alternative for the reduction of sodium chloride or sucrose in food products (Goh et al., 2010). In this work, the reduction of total sugar or salt (by volume) was proportional to the incorporation of bubbles, thus the concentration of sugar or salt in the continuous gel phase was the same for non-aerated or aerated gel, not affecting the perception of sweetness or saltiness. In this way, air incorporation would decrease caloric density in solid foods making people feel as satisfied as they eat a normal food. A strategy in food design may then be to maintain the taste perception of demanded energy-dense foods while imperceptibly adding air as small bubbles and/or immobilizing water in the food matrix, thus lowering their caloric content per portion.

There is increased evidence that the quantity, composition and microstructure of the food ingested affect health (Norton et al., 2007; Zúñiga and Troncoso, 2012). Besides, the physical form of the food may profoundly alter the sensation of fullness and satiety. In solid foods, the microstructure has a major influence on the sensation of satiety by slowing down the rate of breakdown in the gastrointestinal tract. It is believed that a slower breakdown in the stomach can lead to a more lasting sensation of satiety (Marciani et al., 2001; Norton et al., 2006). Enhancing satiation may restrict the daily food intake and the desire of overeating, therefore, contributing to control of body weight. In this sense, tailoring the continuous phase of AG (by changing polymer concentration) could increase the initial sense of fullness, but the effect of an aerated structure on the rate of breakdown in the gastrointestinal tract needs to be determined. Designed AG with tailored texture and low caloric density may help in developing new dietetic foods for the treatment of obesity.

Most foods are structurally complex and their structure determines its physical, sensory and nutritional properties. Entrapping abundant amounts air in gel matrices may be one alternative to design products that promote satiety with reduced caloric density (Zúñiga and Aguilera, 2008, 2009; Zúñiga et al., 2011). In this sense, protein gelation could be a mechanism to “entrap” bubbles in a solid matrix that contain high amounts of water. Tomczyńska-Mleko (2013a) developed a novel method to induce simultaneous gelation and aeration of pre-heated WPI dispersions, gelation was induced by ions at room temperature (similar to cold-gelation) and the structure was able to retain air bubbles. The same author used “reversibility” of the gelation process to production of AG. Weak non-covalent interactions can be broken up and reformed after removing the shearing force used at the aeration (Tomczyńska-Mleko, 2013b). Recently, the same group employed whey protein AG as floating matrices for the controlled release of minerals in simulated gastric conditions (Tomczyńska-Mleko and

Mleko, 2014a). From the above discussion, the objective of this work was to study the effect of thermal treatment and pH of WPI dispersions on the structural and textural characteristics of AG made from WPI.

2. Materials and methods

2.1. Raw materials

Whey protein isolate (WPI) (BiPro, Davisco Foods International, USA) was used as gelling material. WPI is mainly composed of three proteins: β -lactoglobulin, α -lactalbumin and bovine serum albumin, but their functional properties are result of their β -lactoglobulin content. Proximate analysis of WPI was provided by manufacturer: 4.6 ± 0.3 of moisture content (% wb), 97.6 ± 0.3 of protein content (% db), <0.5 of fat content (% wb), 2.0 ± 0.2 of ash content (% wb), and 0.4 ± 0.2 of lactose content (% wb). The pH of the native WPI dispersion was about 6.8.

2.2. Methods

2.2.1. Formation of WPI dispersions

Dispersions of WPI (9% w/w) and NaCl (0.4% w/w) in distilled water were prepared by slow stirring (200 rpm) at 25 °C for 2 h, avoiding foam formation. The pH of the dispersions (6.50, 6.75 and 7.00) was carefully adjusted with 1 N HCl or NaOH solutions (Sigma–Aldrich Corp., St. Louis, Mo, U.S.A.). In previous papers (Zúñiga et al., 2010, 2011), it was demonstrated that thermally-induced β -lactoglobulin aggregates produced at this narrow pH range had very different physicochemical properties, which could influence the foaming properties of the protein. The WPI dispersions were left at 5 °C for at least 12 h to allow complete hydration of the protein.

2.2.2. Thermal treatment of WPI dispersions

Samples (7 mL) were heated in test tubes (inner diameter: 10 mm; length: 120 mm) in order to induce denaturation of the WPI dispersions. The tubes were immersed in a water bath (Mettler, model Basic WNB, Germany) at a constant temperature of 70, 75 or 80 °C (the time to reach constant temperature inside the tubes was about 45 s). The time of thermal treatments (Table 1) was chosen based on previous experiments following the methodology of Kerstens et al. (2006). Briefly, the tubes were taken out for observation at regular intervals, and the gel point was defined empirically as the time at which a tube could be turned upside-down without observable downwards flow of its contents, indicating the formation of a self-supporting gel. Times before dispersions became a gel were chosen for each experimental condition. After the heat treatment, samples were cooled in ice

Table 1
Combination of pH and thermal treatment conditions for the denaturation of WPI dispersions.

Experimental run	pH of dispersion	Thermal treatment temperature (°C)	Thermal treatment time (min)
1	6.50	70	31
2	6.75	70	24
3	7.00	70	18
4	6.50	75	10
5	6.75	75	9
6	7.00	75	8
7	6.50	80	3.5
8	6.75	80	3.5
9	7.00	80	3.5

water (4 °C) to ambient temperature (25 °C) in order to form a weak gel, able to be aerated.

2.2.3. Formation of aerated WPI gels

After cooling, 20 mL of the sample was put into a 120 mL beaker (inner diameter: 5 cm). Aeration was done with an overhead stirrer (Nahita, model 686/2, China) with a paddle impeller (4 cm width × 3 cm height) at 2000 rpm for 3 min. After aeration, WPI foams were stored for 24 h at 10 °C to set the AG structure. Control WPI gels (non-aerated gels) were done by heating WPI dispersions at 80 °C for 30 min and cooled in water at 4 °C for 20 min. Finally, samples were equilibrated to room temperature for 1 h before analyses. All experiments were done at least in triplicate.

2.3. Measurements

2.3.1. Apparent viscosity of WPI dispersions

Apparent viscosity was measured directly in the test tubes used for the thermal treatment. Dispersions were equilibrated at room temperature for 1 h and a rotational viscometer (Fungilab, model Visco Basic-R, Spain) with spindle 7R (diameter: 3.5 mm) was employed for the measurements at a rotational velocity of 50 rpm.

Effect of thermal treatment temperature on apparent viscosity of WPI dispersions was followed by the linearized Arrhenius type equation as shown below:

$$\ln(\mu_{\text{app}}) = \ln(\mu_0) - \frac{E_A}{RT} \quad (1)$$

where μ_{app} is the apparent viscosity of WPI dispersion (cP), μ_0 is the pre-exponential factor, E_A is the activation energy for viscosity (kJ/mol), R is the ideal gas constant (kJ/mol K), and T is the absolute temperature (K).

2.3.2. Density and gas hold-up of aerated WPI gels

Density of AG was determined by the flotation method (Zúñiga and Aguilera, 2009; Zúñiga et al., 2011). AG were placed in a glass container filled with water at 25 °C and corked. The density of AG (ρ_{ag}) was calculated as:

$$\rho_{\text{ag}} = \frac{\rho_w \times m_{\text{ag}}}{m_{\text{ag}} + m_w - m_{w+\text{ag}}} \quad (2)$$

where ρ_w is the density of water at 25 °C (997.05 kg/m³), m_{ag} , m_w and $m_{w+\text{ag}}$ are the mass of the AG (kg), the mass of the container filled with water alone (kg) and the mass of the container with water and the AG (kg), respectively. After measurement AG were blotted with paper towel and weighed after the assay to assure that no water was absorbed. The method actually determines the apparent density of the AG, although this property will be referred to as density. Density of control gels (ρ_{cg}) was evaluated in the same way as AG.

Gas hold-up (ϕ) was calculated by comparing the density of the AG (ρ_{ag}) with the density of control gels (ρ_{cg}).

$$\phi = \left(1 - \frac{\rho_{\text{ag}}}{\rho_{\text{cg}}}\right) \times 100\% \quad (3)$$

All measurements were done at least in triplicate and the results are expressed together with standard deviation values.

2.3.3. Microstructure of aerated WPI gels

Microstructure of AG was evaluated by means of quantification of gel bubble sizes (Zúñiga and Aguilera, 2009; Zúñiga et al., 2011). Bubble sizes were determined from images of selected slices (thickness of 3 mm) of the AG. Slices were cut with a new razor blade each time. Images were obtained with an inverted light microscope (Olympus CK40, Optical Co. Ltd., Tokyo, Japan). In this

way, light passed through the sample and the outlines of whole bubbles could be visualized. Images were recorded with a digital CCD camera (Toupcam, model UCMOS08000KPA, Touptek, China) connected to the microscope and each image (3264 pixels × 2448 pixels) was saved as a 24 bits TIFF image file of approximately 15 MB, without compression. Image processing for bubble size analysis was carried out semi-automatically with the camera imaging software (ToupView, version ×86, Touptek, China). The boundaries of bubbles were manually traced and the image analysis software measured and calculated the size of the traced objects.

A substantial number of bubbles ($N > 100$) were counted to obtain statistical estimates of the bubble size distribution in each sample. A bubble size distribution was generated by grouping the bubbles into classes. The frequency distribution of bubble sizes was computed using MS-Excel (Microsoft® Excel 2010). The relative frequency of any class interval was calculated as the number of bubbles in that class (class frequency) divided by the total number of bubbles, and expressed as a percentage. The mean bubble diameter (D_M) and the standard deviation of the class belonging to D_M are presented.

2.3.4. Textural properties of aerated WPI gels

Textural properties of gels were measured by uniaxial compression tests. Compression measurements were carried out between lubricated plates at a constant deformation speed of 0.1 mm/s until breaking of the structure (Zúñiga and Aguilera, 2009; Zúñiga et al., 2011). Aerated gel cylinders (50 mm diameter; 30 mm height) were compressed at 25 °C using a texture analysis machine (Zwick/Roell, model Z0.5, Zwick GmbH & CO, Germany) with a 100 mm diameter plate. Four replications of each treatment were performed.

The absolute deformation of gels was expressed as the Hencky's or true strain (ε_H), which is preferred for calculating strains resulting from large deformations (Hamann et al., 2006; Steffe, 1996):

$$\varepsilon_H = -\ln\left(\frac{H}{H_0}\right) \quad (4)$$

where H and H_0 (m) are the final and initial height after deformation, respectively. The overall stress acting on the sample during compression was expressed as the so-called true normal stress, which is the force normal to the cylinder cross section divided by the area of the sample (Hamann et al., 2006):

$$\sigma = \frac{F}{A} \quad (5)$$

where σ is the true normal stress (Pa), F is the normal force acting over the gel and A is the cross-sectional area of the gel (m²). Stress (σ_B) and strain at break (ε_B) values are reported.

2.4. Statistical analysis of data

Analysis of variance (ANOVA) tests were used to analyze the data at a confidence level of 95%, using Statgraphics Plus 5.1 software (Manugistics Inc., Statistical Graphics Corporation, Rockville, USA). Differences between samples were evaluated using Multiple Range Test, by means of Tukey's HSD multiple comparison method.

3. Results and discussion

3.1. Apparent viscosity of WPI dispersions

Apparent viscosity of WPI dispersions increased with thermal treatment temperature (Fig. 1). It is known that the thermal treatment of whey protein dispersions produce the denaturation and

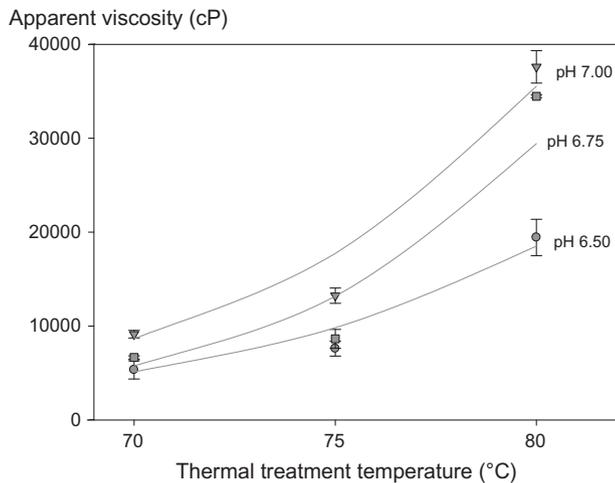


Fig. 1. Effect of the thermal treatment on apparent viscosity (measured at 50 rpm) of whey protein dispersions at different pH. Lines represent values obtained by the viscosity model of Eq. (1).

aggregation of proteins (Kulozik, 2007; Zúñiga et al., 2010). Also, has been reported that a higher temperature of thermal treatment increase the denaturation degree of proteins (Kulozik, 2007), during denaturation molecules unfold to generate greater interactions between them and the solvent, with the concomitant increase in apparent viscosity of dispersions. Changing the pH toward the isoelectric point (approximately 5.2 for β -lactoglobulin), aggregation is promoted in a higher degree due to the decreased intermolecular charge repulsion of the protein (Zúñiga et al., 2010), leading to a decrease in the interactions protein–solvent producing lower apparent viscosity of dispersions.

When the logarithm of the apparent viscosity was adjusted versus the reciprocal of absolute temperature ($1/T$) (Eq. (1), data not shown), a straight line was obtained. Arrhenius's model fitted reasonably well with the experimental data and gave high correlation coefficients (0.97–0.98) at a 95% confidence level. The estimated values of μ_0 and E_A for each pH of WPI dispersions are shown in Table 2, where it is possible to appreciate that the highest μ_0 and E_A values were exhibited by the WPI dispersions at pH 6.75. E_A represents the activation energy of the flow and it indicates a large effect of temperature on viscosity (Steffe, 1996). The explanation is that there was an effect of pH contributing in the change of apparent viscosity. It is known that the rheological behavior of proteins depend not only on the specific protein and the amount present, but also on environmental conditions like temperature, pH, ionic strength and other constituents present, conditions that induce conformational changes of protein structure (Luyten et al., 2004). For example, it has been reported that the heating of whey protein isolate solutions at 80 °C results in an increase of unordered structure. Nevertheless, the difference between the contents of unordered structure in the unheated and heated samples increased with increasing pH of the solution. However, when pH of whey protein isolate solutions was varied between 3.0 and 10.0, no tendency for the percentage of ordered (i.e., α -helices, β -

sheets, and β -turns structures) and unordered structures was found (Tomczyńska-Mleko et al., 2014b). In turn, Law and Leaver (2000) studied the effect of pH on thermal denaturation of β -lactoglobulin by heating skim milk at 80 °C for 0.5–20.0 min over the pH range 5.2–8.8. The authors found that the rate constants for denaturation of β -lactoglobulin increased between pH 5.2 and 6.2, followed by a decrease to about pH 7.0, and thereafter a rapid increase to pH 8.8. These results indicate that there is not a clear relationship between pH and structural changes of proteins when they are also subjected to thermal treatment. Similar results have been also reported by Bazinet et al. (2004) for the study of the rheological behavior of WPI dispersion as a function of pH and protein concentration.

3.2. Formation of aerated WPI gels

AG were successfully formed under the experimental conditions (Fig. 2). At the protein concentration used in this study, all AG maintained their physical integrity and could be easily handled. No bubbles were detected by the naked eye and no visual differences were noticed between AG produced at different thermal treatments or at different pH's.

3.3. Gas hold-up and bubble sizes of aerated WPI gels

Because density of control gels did not presented statistical differences between treatments ($p > 0.05$), its average value ($1586 \pm 131 \text{ kg/m}^3$) was used to calculate the values of gas hold-up (ϕ) of AG. Thermal treatment temperature affected significantly ($p > 0.05$) ϕ values of AG, decreasing linearly with an increase in temperature (Fig. 3). A maximum value of $\sim 70\%$ was found for AG fabricated at 70 °C of thermal treatment and pH 7.0. The lowest values of ϕ were found for the dispersion made at pH 6.75. Tomczyńska-Mleko and Mleko (2014a) obtained ϕ values in the range of 38–47% when fabricated aerated WPI gels with the addition of calcium, magnesium and iron, probably the difference in aeration time (30 s versus 3 min in this work) gave these results.

Previous studies have shown that two physical properties, surface tension and viscosity, strongly impact the aeration process of protein dispersions (Zúñiga and Aguilera, 2009; Zúñiga et al., 2011). Due to their amphiphilic nature, proteins behave as macromolecular surfactants lowering the surface tension of dispersions and acting as foaming agents, creating and stabilizing a greater



Fig. 2. Image of an aerated whey protein isolate gel.

Table 2
Arrhenius's model parameters for the effect of thermal treatment temperature on apparent viscosity of WPI dispersions.

pH of dispersion	μ_0 (cP)	E_A (kJ/mol)	Correlation coefficient, R^2
6.50	2.83×10^{23}	30997.2	0.979
6.75	6.22×10^{28}	39302.9	0.969
7.00	3.80×10^{25}	33977.7	0.979

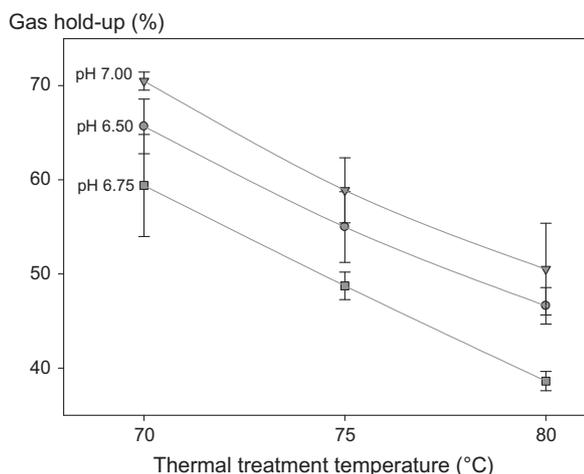


Fig. 3. Effect of thermal treatment on gas hold-up of aerated whey protein gels at different pH.

interfacial area (i.e. higher number of tiny bubbles). The ability to create foams has been correlated with the surface tension of protein dispersions and especially to the rate of decrease in surface tension, because the lower the surface tension, the greater the extent to which bubbles can be broken up during intense shearing or turbulent flow (Davis and Foegeding, 2004; Dickinson, 2003). Thermal treatments are able to change the interfacial properties of protein dispersions, and this change is affected by the pH of the dispersion. Thermal treatment changes the conformation of proteins exposing hydrophobic groups thus increasing surface hydrophobicity (Zhu and Damodaran, 1994). However, an extensive denaturation of proteins reduces their interfacial properties, decreasing their capacity to form and stabilize foams (Bals and Kulozik, 2003; Kim et al., 2005; Zúñiga et al., 2011). WPI dispersions treated at 70 °C were able to hold more air into the AG structure, probably because the thermal treatment was less severe and a lower number of aggregates with a lower average size were formed.

On the other hand, higher viscosities produce lower degrees of aeration (Walstra, 2003). In fact, for the generation of foams it is necessary to deliver energy to the system to incorporate air and create new interfaces (i.e. in the form of bubbles). For the same level of energy delivered from the overhead stirrer (same value of rpm), there was a lower incorporation of air (Fig. 3), due to the higher resistance to flow (higher apparent viscosity) of dispersions (Fig. 1). This is because a greater amount of energy is employed in the movement of the fluid and less energy is used for the creation of new interfaces.

On the other hand, there is no clear effect of pH on the gas hold-up of the AG, being only possible to note that a change in the natural pH of dispersions (~6.8) led to a higher incorporation of air in the AG; however, no conclusions can be drawn regarding this variable.

Selected images from optical microscopy of AG at different processing conditions are shown in Fig. 4. From these images, bubble sizes were obtained, and the bubble size distributions were generated (data not shown). D_M values were in the range of 535–708 μm (Table 3) and no significant differences ($p > 0.05$) were observed between D_M obtained at pH 6.50 and 6.75, but at pH 7.00 D_M values were statistically higher. Thermal treatment temperature had a significant effect ($p > 0.05$) on the D_M values of AG, with higher temperatures decreasing their value. Values of average bubble size in the range of 72–3156 μm were found by Tomczyńska-Mleko (2013a) for AG fabricated at WPI concentration between 6% and

8% (w/w), pH's between 6.68 and 8.00 and calcium content of 20 or 30 mM. In line with our results, increasing pH resulted in an increased bubble size. Using reversibility of the gelation process, Tomczyńska-Mleko (2013b) fabricated AG at 9% (w/w) of WPI, where bubble diameters increased from 167 to 221 μm when pH augmented from 3.0 to 6.7; but diameters decreased to 84 μm with a further increase from pH 6.7 to 10.0.

Destabilization processes (i.e. disproportionation and coalescence of bubbles) are produced during setting of aerated food gels, which affects the food structure. These phenomena are affected by the viscosity of the continuous phase of the aerated dispersion (Zúñiga and Aguilera, 2009). An increase in the viscosity of the continuous phase may delay the gas transfer (disproportionation) between small bubbles with higher internal pressure, toward big bubbles and, on the other hand, decrease the drainage of the continuous phase thereby retarding the break of the lamella between bubbles, and avoiding the merge of two or more bubbles into a one big bubble (coalescence). Bubbles with smaller sizes and lower dispersion of sizes could minimize the effect of buoyance and vertical stratification of bubbles, improving the appearance of aerated products. Monodisperse bubbles could reduce disproportionation due to a reduction in the difference of pressure because of their similar size (Zúñiga and Aguilera, 2008). Therefore, it is important to control the size and dispersion of bubbles in order to produce homogeneous products. Mean bubble sizes (535–708 μm) and size distributions (range from 37 to 1430 μm) obtained in this work were not small and narrow enough to ensure the homogeneity of AG, hence a better device (e.g. rotor/stator dispersing system or membrane foaming apparatus) and/or processing conditions (e.g. dynamic heating) are needed to properly design AG with controlled properties.

3.4. Mechanical properties of aerated WPI gels

Stress–strain relationships for AG presented a non-linear behavior until break of the structure (results not shown) and did not show the typical sigmoid stress–strain behavior found in most cellular foams (Corradini and Peleg, 2007). These authors stated that cellular solids with thick walls and very small air bubbles can exhibit an almost linear compressibility pattern with absence of a “prominent shoulder” in the stress–strain relationship, discussion in agreement with the results presented in this study and the results of previous works (Zúñiga and Aguilera, 2009; Zúñiga et al., 2011).

Higher thermal treatment temperatures produced a decrease in σ_B and ε_B of AG (Table 4). The structure of globular protein gels (i.e. the molecular network of the gel) strongly affects their mechanical properties. This structure is highly dependent on the thermal treatment employed to set the gel and the pH of the dispersion used to fabricate the gel (Aguilera and Rademacher, 2004). Lower temperatures of the thermal treatment produced higher ϕ values, but AG fabricated under these conditions showed higher σ_B and ε_B values (Table 4), probably because in a more aerated structure larger compression can be possible. It is known for cold gelation of globular proteins that the size of the protein aggregates formed during the denaturation step affects the mechanical properties of the gels (Alting, 2003). In addition, recently it has been reported that WPI gels containing protein particles (aggregated WPI) were reported to be weaker (fractures at much lower strain values) than gels without particles at the same total protein concentration. Increasing the volume fraction of the WPI particles led to a further decrease in the gel strength (Sağlam et al., 2014).

Although all gels obtained under the pH conditions employed in this study can be referred as “fine-stranded”, there could be differences in their microstructure. It is very probable that at higher temperatures bigger aggregates have been formed producing a

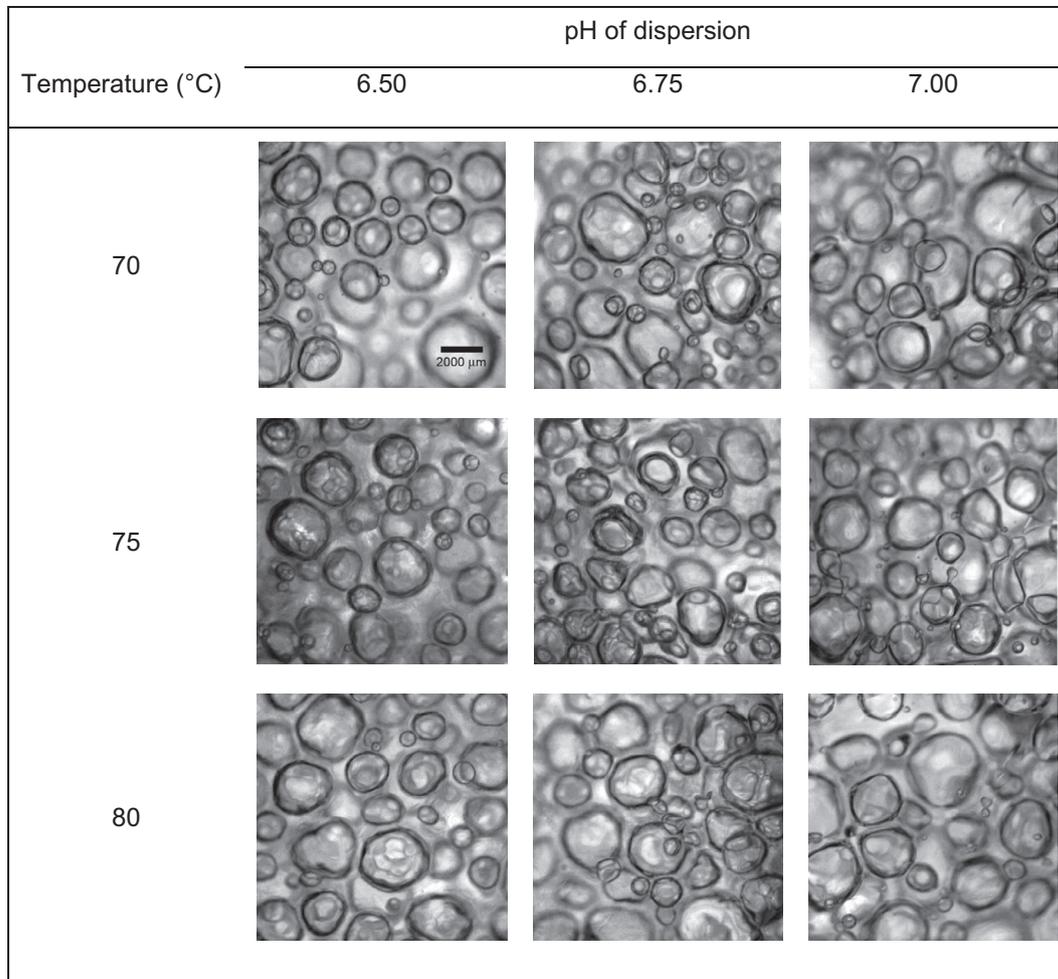


Fig. 4. Gallery of images from optical microscopy of aerated whey protein gels under different conditions of fabrication.

Table 3
Mean bubble diameters (D_M in μm) of aerated whey protein isolate gels.*

Thermal treatment temperature ($^{\circ}\text{C}$)	pH of dispersion		
	6.50	6.75	7.00
70	$653 \pm 44^{\text{aA}}$	$647 \pm 42^{\text{aA}}$	$708 \pm 40^{\text{bA}}$
75	$627 \pm 39^{\text{aB}}$	$616 \pm 49^{\text{aB}}$	$685 \pm 44^{\text{bB}}$
80	$537 \pm 37^{\text{aC}}$	$535 \pm 37^{\text{aC}}$	$619 \pm 48^{\text{bC}}$

* Different lower case letters indicate significant differences ($p < 0.05$) between pH of dispersions and different capital letters indicate significant differences ($p < 0.05$) between thermal treatment temperatures.

Table 4
Textural properties of aerated whey protein isolate gels.*

Thermal treatment temperature ($^{\circ}\text{C}$)	pH of dispersion	Stress at break (kPa)	Strain at break (-)
80	6.50	$0.92 \pm 0.22^{\text{aA}}$	$0.34 \pm 0.16^{\text{aA}}$
	6.75	$1.70 \pm 1.24^{\text{aBA}}$	$0.38 \pm 0.15^{\text{bA}}$
	7.00	$2.85 \pm 0.88^{\text{bA}}$	$0.53 \pm 0.04^{\text{bA}}$
75	6.50	$0.98 \pm 0.24^{\text{aA}}$	$0.43 \pm 0.09^{\text{aB}}$
	6.75	$1.68 \pm 0.40^{\text{aBA}}$	$0.57 \pm 0.10^{\text{bB}}$
	7.00	$2.94 \pm 1.87^{\text{bA}}$	$0.57 \pm 0.11^{\text{bB}}$
70	6.50	$1.30 \pm 0.50^{\text{aA}}$	$0.45 \pm 0.14^{\text{aB}}$
	6.75	$2.63 \pm 1.80^{\text{aBA}}$	$0.60 \pm 0.10^{\text{bB}}$
	7.00	$3.40 \pm 3.06^{\text{bA}}$	$0.67 \pm 0.04^{\text{bB}}$

* Different lower case letters indicate significant differences ($p < 0.05$) between pH of dispersions and different capital letters indicate significant differences ($p < 0.05$) between thermal treatment temperatures.

lower amount of “junction zones” in the three-dimensional gel network, generating weaker AG. On the other hand, at pH values toward the isoelectric point (~ 5.2 for whey proteins) aggregates of bigger size are produced by thermal treatments (Zúñiga et al., 2010), explaining the data presented in Table 4, where at lower pH's AG were less resistant to break.

It seems that at the ϕ levels obtained in this work, the microstructure of the gels walls of the AG had a strong influence over the break properties of the gel. For example, for a thermal treatment at 70°C a difference in ϕ values of $\sim 5\%$ was found for pH 6.50 and 6.75 (Fig. 3); but the σ_B increased twofold when pH was increased (Table 4).

3.5. Structure–property relationships

As seen from Fig. 3 and Table 4 different structures of AG were formed depending on the thermal treatment and pH of WPI dispersions. In order to analyze how the microstructure affected macro-properties of AG, the relationship between D_M and ϕ was plotted (Fig. 5). A linear relationship with a correlation coefficient (R^2) equals to 0.82 was found. If we assume that all the bubbles in the structure of AG have the same diameter (the D_M value), the incorporation of air in the structure will be a function of the bubble diameter and the number of bubbles incorporated into the AG structure. As a next step we plot the ϕ values against σ_B obtaining relationships that were function of the pH of the dispersion (Fig. 6). This pH dependence could be indicative that the microstructure of

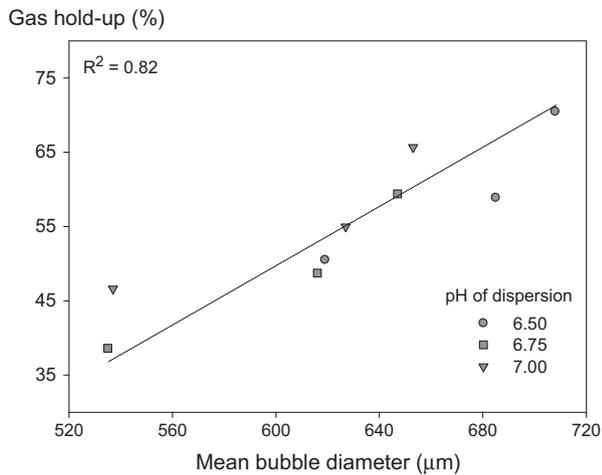


Fig. 5. Correlation between mean bubble diameters and gas hold-up for aerated whey protein gels.

the gel walls had more influence over the textural properties than the ϕ in the range of values obtained. Zúñiga and Aguilera (2009) found a power law relationship between density and fracture stress for gas-filled gelatin gels, in that case aerated gelatin gels with different concentration of gelatin and gas hold-up were formed. Probably, a master curve was found because the aerated gel walls had the same microstructure, which is not the case presented here.

4. Conclusions

Aerated gels with different structures and mechanical properties were fabricated changing the pH of whey protein isolate dispersions and the time–temperature relationship of the thermal treatment used to denature the proteins. The increase in the temperature of the thermal treatment produced a decrease in gas hold-up and the average bubble size because of the higher viscosity of the protein dispersions. The change in the pH of the dispersions only had a marked effect on the mechanical properties of the aerated gels because possibly it generated protein aggregates of different size, which influenced the formation of stronger three-dimensional network at pH values far from the isoelectric point.

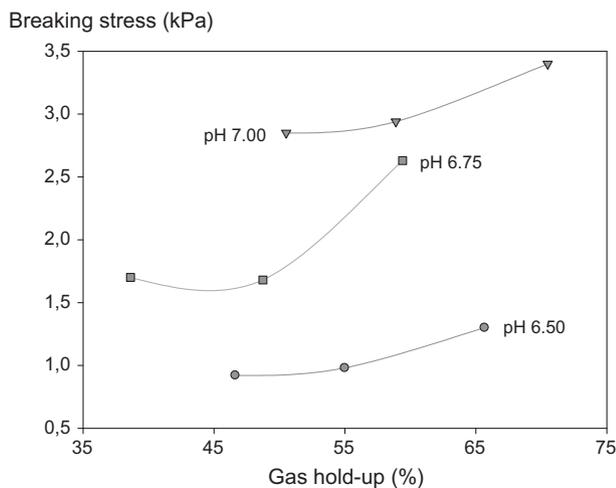


Fig. 6. Relationship between gas hold-up and breaking stress for aerated whey protein gels. Error bars were not included in the figure to improve visual clarity; however standard deviations for breaking stress are shown in Table 4.

Control of thermal treatment of protein dispersions allows the fabrication of aerated gels with tailored structural and textural properties which can be used as new food matrices.

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