Structure and Stability of Ion Induced Whey Protein Aerated Gels

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Abstract

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The microstructure and stability of aerated whey protein gels were determined. Foamed whey protein gels were obtained using a novel method applying a simultaneous gelation and aeration process. Whey protein gels were produced at different protein concentrations and pH by calcium ion induction at ambient temperature. Two concentrations of calcium ions were used: 20 and 30mM to produce foamed gels with different microstructure. Foamed gels obtained at 30mM Ca^{2+} were composed of thick strands and irregular, large air bubbles. For these gels, larger synaeresis and bubble size reduction were observed. Fine-stranded, small bubble size aerated gels obtained at 20mM Ca^{2+} were very stable during storage. Decreased protein concentration and increased pH of the gels resulted in an increased bubble size.

Keywords: foam; globular protein; microstructure; syneresis; Turbiscan

Innovation in the milk industry strongly depends on the ability to implement new ideas and new ingredients. The cost-effective manufacture of whey proteins allowed to use these ingredients in many functional food applications (Heino *et al*. 2007). The biological value of whey protein exceeds that of egg protein, the functional properties of which are also intensively investigated (Houška *et al*. 2004). Advanced processing techniques have facilitated the manufacture of specialised second-generation whey protein products: high protein/peptide isolates, and fractionated/purified bioactive proteins for specific high-value applications (Warner *et al*. 2001; Smithers 2008). A previous paper (Tomczyńska-Mleko 2012) presented the rheological properties of aerated whey protein gels. A novel method was used to induce simultaneous gelation and aeration of pre-heated whey protein dispersions. Gelation of whey protein was induced by calcium ions at room temperature. Under suitable conditions of pH, protein concentration, and concentrations of ions it was possible to obtain globular protein gels able to retain air bubbles. Aerated whey protein gels can be used to produce new food products with unique textural and nutritional properties. However, further studies are required to determine their structure, average air bubble diameter and stability under storage. Aerated gels represent a group of fragile systems with possible syneresis and changes in air bubble diameter.

In recent years, laboratory analysers have emerged, with a possibility of scanning the sample in a vertical glass container. Currently, only two companies in the world offer similar equipment: Beckman Coulter in the USA and Formulaction in France, which produces a Turbiscan apparatus. They are used especially for colloidal systems such as emulsions or suspensions. Using this analyser, the particle size and its changes, and their migration can be observed. Therefore, this device allows to describe quantitatively such phenomena as synaeresis, coalescence, flocculation, sedimentation or

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aggregation. The advantage of this device is that it is a non-invasive method and the measurement is performed on the actual sample without dilution. The direction of migration can be determined, which allows the distinction between such phenomena as sedimentation and coalescence (Xu *et al*. 2011). An expanded version called Turbiscan LabCooler allows to perform measurements in the temperature range from 4°C to 60°C. There are few studies in which this device was used to determine the stability of food foams, especially drainage (Sceni & Wagner 2007). Rouimi *et al*. (2005) used Turbiscan for determining the average size of air bubbles in foams produced from milk protein solutions. There is no data in the literature on the stability of aerated gels with a vertical scan analyser.

The objective was to determine the microstructure of aerated whey protein gels and changes occurring in them under the influence of storage.

MATERIAL AND METHODS

Whey protein isolate (88.0% protein) was purchased from Arla Foods Ingredients (Viby, Denmark). The protein content was determined by analysing for nitrogen using the macro Kjeldahl method and calculating protein as N × 6.38. The result is the mean of three replications.

Foamed gel formation. 6.0, 7.0 or 8.0% (w/w) protein dispersion was made by hydrating in distilled water at 22°C for 30 minutes. The pH of the native protein solution was 6.68. The pH of the dispersions was adjusted to 7.34 (average value between 6.68 and 8) or 8.0. The above protein concentrations and pH were selected based on preliminary research on the sensorically observed ability of WPI gels to form foams. Dispersions were heated in water bath at 80°C for 30 min and after heating they were immediately cooled down. Calcium chloride was added in concentrations of 20mM or 30mM. Immediately after adding the salt, solutions were foamed for 30 s or 60 s at 2000 rpm using a Compact Digital Lab Mixer (Cole-Parmer, Montreal, Canada). Foamed semisolid gels were carefully transferred to a flat-bottomed glass cylindrical Turbiscan sample cells and stored at a temperature of 7°C for 20 hours.

Scanning electron microscopy *(SEM).* Samples of foamed gels were fixed by immersion in 2.5% glutaraldehyde solution in 0.1M sodium cacodylate buffer. The samples were dehydrated in serial dilutions of ethanol and acetone and dried at the critical point in liquid carbon dioxide. Preparations were coated with gold using an EMITECH K550× vacuum evaporator (Emitech, Ashford, UK). Preparations were viewed and photographed using a VEGA II LMU scanning electron microscope (Tescan, Canberra, USA).

Foamed gel average bubble size and destabilisation analysis using Turbiscan apparatus. Foamed gel samples were stored at 7°C for 41 days (and scanned every 7 days) or stored and scanned continuously in Turbiscan at 7°C for 72 hours. The average bubble size and destabilisation of the gels were investigated by Turbiscan apparatus (Formulaction, L'Union, France). The samples were scanned by a pulsed near infrared light source (wavelength 880 nm) and two synchronised detectors were used to collect the transmitted and backscattering lights. The obtained data was expressed as percentage intensity of the transmission or backscattering. During the scan, a temperature of 7°C was maintained, which was the same as that of the storing conditions. The average size of the air bubbles dispersed in the gel was calculated using Turbiscan Lab expert software (Formulaction, L'Union, France). All measurements were done in triplicate and the scans present the averaged curves.

RESULTS AND DISCUSSION

Figure 1 shows the structure of aerated whey protein gel obtained at native pH 6.68 and induced by calcium ion addition at the concentration of 30mM. Thick strands of the gel network are observed. The air is entrapped in irregular empty spaces between the elements forming the network (Figure 1a). Such a structure could influence the stability of air bubbles entrapped in the gel matrix, as the air migration is possible. Air bubbles could move and transform, and finally a release of the air from the volume of the gel could be observed. This structure does not exclude the possibility of multiple-phase air bubble entrapment. Large air bubbles are contained in the areas of the loose, porous microstructure of the gel, but small air bubbles are probably present in the thick strands forming the gel matrix. In Figure 1b air cavities of several micrometres in size are observed. Stability of the aerated gel was measured with a Turbiscan device. The gel was obtained under the above-mentioned conditions, aerated for 30 s, stored at

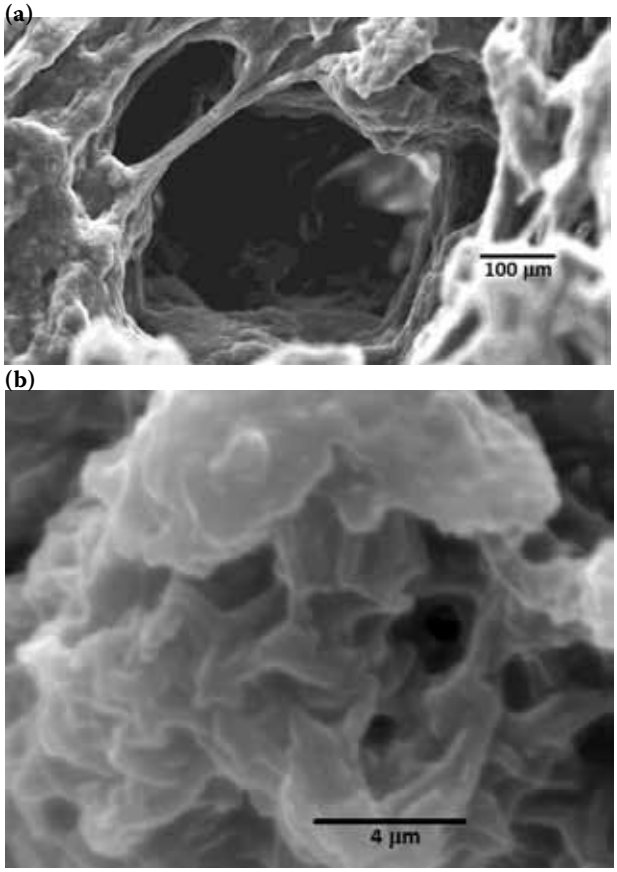

Figure 1. Scanning electron microscopy view of foamed gel obtained from 8% protein dispersion at pH 6.68 and induced by 30mM calcium ions

7°C for 41 days and tested every day. Turbiscan transmission and backscattering profiles are presented in Figure 2. All obtained foamed gels were turbid, so only backscattering profiles were used for analysis. The initial average size of air bubbles was 393 µm. This volume was dramatically reduced to 33.3 µm within 7 days of storage at 7°C. Longer storage time did not induce any further changes in the average air bubble size. Foamed gels behave in a different way than foams, as the migration of air bubbles through the liquid phase is different from the solid one. In foams bubbles collapse due to a loss of large bubbles on the foam top (Rouimi *et al.* 2005; Sceni & Wagner 2007). In gels, in a porous matrix the air can migrate through pores and then the gel shrinks and a syneresis is observed. Syneresis occurred in the sample after 24 h of storage, as greater transmission was measured in the sample. A 3.08 mm layer of the solution appeared on the bottom of the cuvette. After two weeks, this layer expanded to 5.25 mm. The final syneresis after 41 days of storage was measured as a 3.97 mm thick layer. At the same time of 41 days, the storage of aerated gel caused the formation of an opaque layer of the solution on the top of the cuvette reaching 1.09 mm in size. It appears that aerated gels induced by calcium ions are characterised by rather quickly occurring syneresis. This probably results from their microstructure. The particulate, heterogeneous gel structure contains a lot of free space which enables water migration (Foegeding *et al.* 1990). Similar changes were observed in gels obtained with aeration for 60 s (the profiles are not shown). The initial average size of air bubbles in aerated

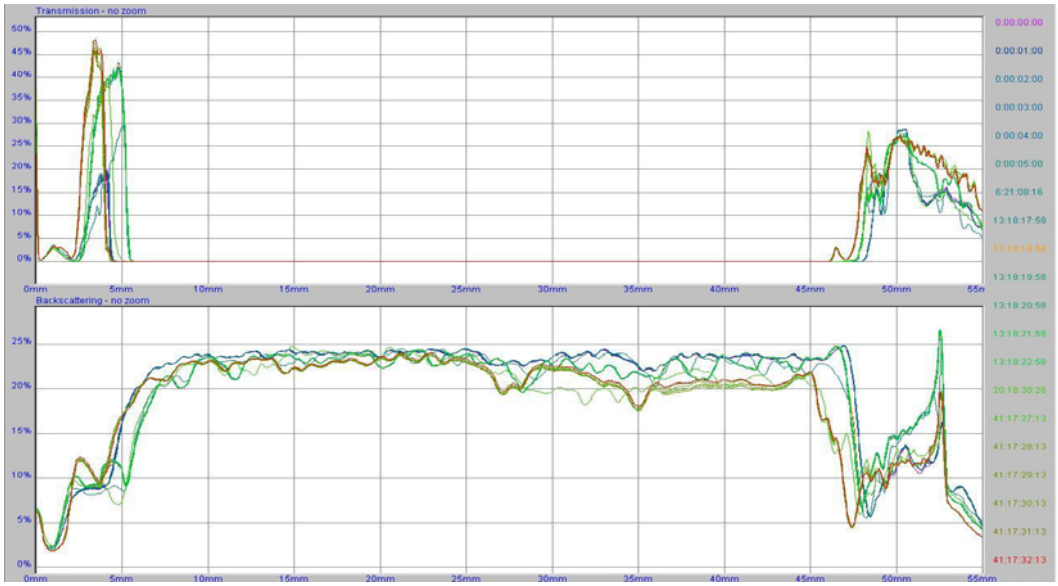

Figure 2. Turbiscan transmission and backscattering profiles of foamed gel obtained from 8% protein dispersion at pH 6.68 and 30mM calcium ions by mixing for 30 s at 60 rpm and stored at 7°C for 41 days

Table 1. Influence of calcium concentration, protein concentration, and pH of the gel on average air bubble size

Calcium (mM)	Protein (%)	pH of aerated gel	Average bubble size (mm)
	6	6.68	2360
	7	6.68	560
20	8	6.68	71.9
	8	7.34	169
	8	8.00	251
	6	6.68	3156
	7	6.68	1326
30	8	6.68	393
	8	7.34	670
	8	8.00	812

gel determined by Turbiscan was 208 μm. After three weeks there was a dramatic decrease in the diameter of air bubbles to 5.5 mm. After 6 days of storage, a 5.1 mm syneresis layer appeared at the bottom of the sample. At the same time a semi-translucent layer appeared on the surface of the sample. These studies showed that the aeration time had no significant effect on the stability of aerated gels. In both cases, syneresis and bubble size reduction were observed. Studies by other investigators showed that the variable whipping time affects foaming capacity, but does not affect stability (Ibanoglu & Ibanoglu 2000). In foams obtained from whey protein solutions it has been observed that the longer whipping time caused an increase in foaming capacity and the yield stress of obtained foam (Nastaj 2009).

Table 1 presents the average diameter of air bubbles in gels induced by 20mM or 30mM calcium ions and obtained at different protein concentrations and pH. The backscattering decreased at higher pH. Increasing pH produced more transparent samples than at higher pH, the more fine-stranded gel structure is formed. Increased pH resulted in an increased bubble size, as the average air bubble diameter in 20mM calcium gels was 72, 169, and 251 mm for pH 6.68, 7.34, and 8.0, respectively. The same regularity was observed for foamed gels induced by 30mM calcium ions (Table 1). The more fine-stranded gel matrix was capable to hold larger bubbles (Tomczyńska-Mleko 2010). It takes a longer time to form ion-induced gel at a higher pH value and air bubbles could migrate to join together into larger forms. An opposite effect was observed for the influence

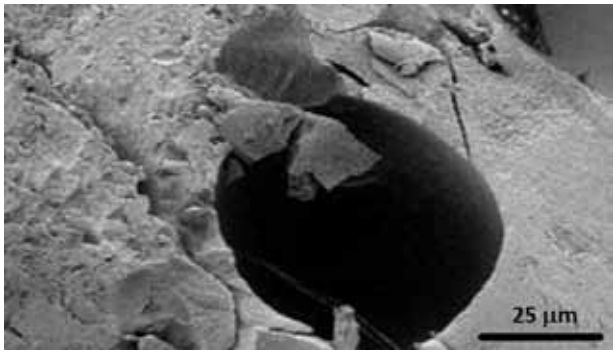

Figure 3. Scanning electron microscopy view of foamed gel obtained from 8% protein dispersion at pH 6.68 and induced by 20mM calcium ions

of the protein concentration (Table 1). Increased protein content in the gels resulted in a sudden decrease in the diameter of air bubbles, both for 20 and 30mM calcium induced gels. The increased protein concentration resulted in gels with increased hardness (Tomczyńska-Mleko 2012). Probably the stronger protein matrix was capable to hold smaller bubbles with higher pressure.

A different microstructure of aerated gel was observed at calcium ion concentration of 20mM (Figure 3). A regular round bubble in the fine-stranded gel matrix is observed. The stability of aerated gel produced at the 8% protein concentration, pH 6.68 and by addition of calcium ions at a concentration of 20mM is presented in Figure 4. Aerated gel was continuously scanned at 7°C for 72 h using a Turbiscan LabCooler. The average diameter of air

Table 2. Influence of calcium concentration, protein concentration and pH of the gel on the summary upper and lower layer synaeresis after 41 days of storage at 7°C

Calcium (mM)	Protein (%)	pH of aerated gel	Summary syneresis (mm)
	6	6.68	0.89
	7	6.68	0.62
20	8	6.68	0.43
	8	7.34	0.79
	8	8.00	0.35
	6	6.68	8.24
	7	6.68	6.01
30	8	6.68	5.06
	8	7.34	3.12
	8	8.00	1.88

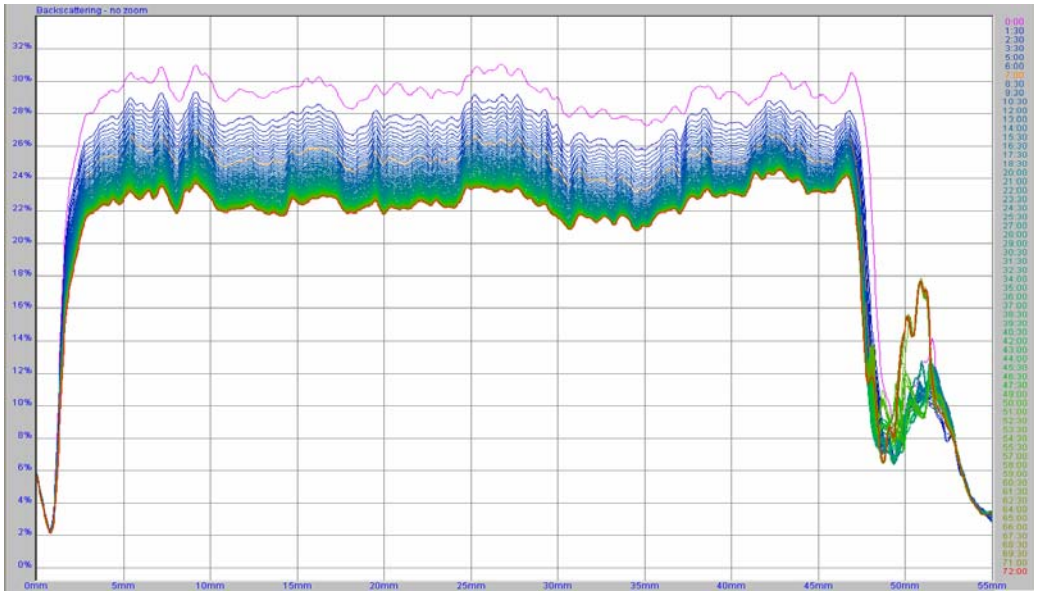

Figure 4. Turbiscan backscattering profiles of foamed gel obtained from 8% protein dispersion at pH 6.68 and 20 mM calcium ions (constant measurements at 7°C for 72 h)

bubbles counted in the first two hours of storage was 76.5 μm. After 72 h of storage, the average diameter of air bubbles measured 69.2 mm. Small changes in the average bubble size were observed for the gels stored at 7°C for 41 days (Turbiscan profiles not shown). Aerated gel induced by 20mM calcium ions was very stable during storage as there was only a small decrease in the average bubble size. Table 2 summarises the results of aerated gel syneresis measurements obtained by Turbiscan. Results are presented as the sum of the lengths of the lower and upper layer of translucent liquid. For all foamed gels obtained using 20mM calcium ions, only small layers (below 1 mm) were observed after 41 days of storage at 7°C. Higher calcium concentration produced higher syneresis, which was probably caused by the more porous structure of particulate whey protein gels observed by scanning electron microscopy (Figure 1). An increase in protein concentration resulted in gels with lower syneresis as the denser gel structure was obtained. An increase in pH also produced gels with lower syneresis as higher repulsive forces between protein molecules cause the formation of smaller gel aggregates with lower porosity (Foegeding *et al*. 1990).

CONCLUSION

Aerated whey protein gels composed of air bubbles in fine-stranded gel are stable under storage at 7°C. Manipulating with protein concentration and pH enables to obtain products with different bubble size. Aerated whey protein gels are a new possibility to extend the application of whey protein preparations. Their unique organoleptic and nutrition properties could be used for novel foods for sportsmen and physically active people.

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