Structure and rheology of aerated whey protein isolate gels obtained at different pH

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Summary
Aerated whey protein isolate gels were obtained at different pH using reversibility of heat-induced gels. The aerated gels were characterized by different air bubble size and different microstructure. Aeration produced gels with 30–70% lower puncture test force, but 2–3 times higher storage modulus than for non-aerated gels. Native pH 6.7 was optimal for obtaining aerated gels, as the hardest gels were produced. At pH 9, strong gels were formed but these were less susceptible to rehealing after aeration. Aerated gels were stable at storage for 41 days at 7 °C and no statistically significant changes in the average bubble size were noted. The aerated gels can be used as matrices for active ingredients release or as a new product with lower caloric value and a unique textural and sensorial properties.

Keywords
whey; protein; gel; aeration; foam; microstructure; rheology

Whey protein concentrates or isolates are commonly used as functional ingredients in foods. They enrich food products with valuable amino acids and shape their rheological properties. The use of whey protein allows for the manufacturing of the products with excellent texture, stability and nutritional value [1]. Whey proteins have very good stabilizing effect on emulsions and stirred acid gels [2]. Gelation is one of the most important functional properties of whey protein. At the process of heating above denaturation temperature, proteins unfold and form aggregates. At some conditions of protein concentration, ionic strength and pH, a continuous gel matrix is formed. The gelling occurs via covalent intermolecular disulfide bonds and non-covalent interactions, such as van der Waals, ionic and hydrophobic interactions [3]. Gelation is a key reaction used to generate food texture. Heat-induced gelation of globular proteins produces two general types of gel microstructure designated as fine-stranded and particulate. Fine-stranded gels are formed at a pH value distant from isoelectric point of the proteins and/or at low ionic strength [4].

In the last few years, some research was conducted on aerated gels [5–8]. Introducing a gas phase into a gel changes its texture, appearance, colour and mouth-feel [9]. In previous research, the influence of locust bean gum concentration on reversibility of whey protein gel was investigated [10]. Mixed gels of whey protein/locust bean gum were obtained and their structure was destroyed by shear forces. The texture of the destroyed gels was partially reversible and the process depended on locust bean gum concentration. Reversibility of the gelation process after shearing can be applied to production of aerated gels [6]. The associations between attractive groups of the protein lead to the formation of physical bonds, which are, in contrast to chemical bonds, reversible [11]. Weak physical bonds (hydrogen bonds, ionic interactions) can be broken and reformed after removing the shearing force used at the aeration. Aerated foods have a lower caloric value and aeration is cost effective process since it increases the volume of the product with a lower ingredients content. Aerated gels can be also used as matrices for release of active ingredients [12].

Stability of the aerated gels is crucial in all their applications. Turbiscan vertical scanning analyzer (Formulaction, L'Union, France) was used to determine foam stability [13]. Rouimi et al. [14] used

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Turbiscan to measure average size of air bubbles in foams derived from milk protein solutions. There are no data in the literature regarding the stability of aerated gels using Turbiscan scanning analyzer.

In this study, the effect of pH on whey protein gels aeration process was investigated. It was presumed that different pH will produce aerated gels with different microstructure, bubble size, stability and rheological properties.

MATERIAL AND METHODS

Whey protein isolate Lacprodan produced by Arla Foods Ingredients (Viby, Denmark) was used. Protein concentration (88.0%) was determined by the Kjeldahl procedure [15]. Sodium chloride, sodium hydroxide, hydrochloric acid, ethanol and acetone were purchased from Polskie Odczynniki Chemiczne (Gliwice, Poland). Glutaraldehyde was obtained from Chempur (Katowice, Poland) and sodium cacodylate from Merck (Warsaw, Poland). All chemicals were high purity analytical grade reagents.

Whey protein isolate dispersions (9% w/w) were made in 0.1 mol·l^{-1} NaCl by mixing for 60 min with a magnetic stirrer. pH was adjusted to 3.0, 9.0 or 10.0 using 2.0 mol·l^{-1} HCl or NaOH. pH of native 9% protein dispersion was 6.7.

Thermal properties of whey protein isolate dispersions

Thermal properties of whey protein isolate dispersions were investigated by differential scanning calorimetry (DSC) using the instrument DSC 204 F1 (Netzsch, Selb, Germany). A dispersion of 10% (w/w) whey protein isolate in 0.1 mol·l^{-1} NaCl was used in all samples. The dispersions were heated in the temperature range 20–100 °C with a rate of 10 °C·min^{-1}. The measurements were monitored by Measurement Netzsch software v. 4.8.2 (Netzsch). The temperature of endothermic peak and the enthalpy were determined from the thermograms. All tests were performed in triplicate.

Dispersions (200 ml) were heated in 50 mm diameter beakers in water bath at 80 °C for 30 min and, after heating, immediately cooled down at cold tap water for 5 min. Obtained gels (50 g) were aerated in 100 ml beakers for 30 s at 33 Hz speed using an RW 11B mixer (IKA, Staufen, Germany) equipped with an R 1001 paddle stirrer (IKA). Non-aerated (control) gels were immediately after cooling put into the refrigerator. All the samples after storing at 7 °C for 20 h were equilibrated at 21 °C for 2 h and subjected to evaluation of their physicochemical properties and microstructure.

Rheology of gels

Hardness of aerated and non-aerated gels was measured with a TA-XT2i Texture Analyser (Stable Micro Systems, Godalming, United Kingdom). The samples were analysed inside the beakers where they were formed by puncture test using 10 mm in diameter ebonite cylinder probe. The probe was plunged into 25 mm high gels at a speed of 1.0 mm·s^{-1} for 10 s. Gels were obtained in 50 mm diameter beakers to avoid the edge effect. Hardness of the samples was measured as the maximal force of penetration. Six measurements were carried out for each test.

Dynamic oscillatory measurements were performed using RS300 rheometer (ThermoHaake, Karlsruhe, Germany) with a serrated parallel steel plate geometry (35 mm diameter) to limit the potential sliding effects. Gel samples (35 mm in diameter and 3 mm thick) were cut using a scalpel and were analysed by strain and frequency sweep. For strain sweeps, a frequency of 0.1 Hz was used.

Structure of gels

Scanning electron microscopy (SEM) was used to examine the structure of the gels. Samples of the aerated and non-aerated gels were fixed by immersion in 2.5% glutaraldehyde solution in 0.1 mol·l^{-1} sodium cacodylate buffer. The gels 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 a vacuum evaporator K550x (Emitech, Ashford, United Kingdom). Preparations were viewed and photographed using a scanning electron microscope VEGA II LMU (Tescan, Canberra, Pennsylvania, USA).

The microstructure of the samples was investigated using also confocal laser scanning microscopy (CLSM). Gel samples were cut using a scalpel and viewed under confocal laser scanning microscope LSM 510 (Carl Zeiss, Jena, Germany). Images were analysed using LSM 5 Image Examiner software (Carl Zeiss).

The average bubble size of the aerated gels was evaluated using Turbiscan apparatus equipped with a LabCooler (Formulaction). The semiliquid aerated gels were carefully transferred (poured) to a flat-bottomed glass cylindrical 20 ml disposable glass cell (external diameter 27.5 mm, height 70 mm). The samples were stored at 7 °C for 41 days, being scanned every 7 days. During the scan, temperature of 7 °C was maintained with a labCooler (Formulaction). The TurbiScan Lab reading head consists from a pulsed near infrared light source (880 nm) and two synchronous detectors. The transmission detector receives the light

flux transmitted through the product, the backscattering detector measures the light backscattered by the product at angle 135°. The reading head acquired transmission and backscattering data at every 40 μm while moving along the 55 mm cell height. The average size of the air bubbles dispersed in the gel was calculated by Turbiscan Lab expert software (Formulaction) using the backscattering data. The backscattering level is directly linked to the photon transport mean free path through the foamed gel. Knowing the refractive index of the continuous phase and the one of the dispersed phase, and also the gas volume fraction, the Turbiscan software calculates the diameters as a function of time [14]. All measurements were made in triplicate and the scans represent the averaged curves.

Statistical analysis

Statistical analysis of results (standard deviation and analysis of variance) was done using the statistical program Statistica 5.0 PL (StatSoft Polska, Warsaw, Poland). The significance of differences between means was determined using the Tukey's test at a confidence level of $p \leq 0.05$ based on the least significant difference.

RESULTS AND DISCUSSION

Tab. 1 presents the temperature at the endothermic peak and enthalpy for whey protein isolate dispersion obtained at 10% protein concentration at different pH. A decrease in the temperature in the endothermic peak temperature (minimum) was observed with increasing pH. BOYE et al. [16] studied whey protein concentrate dispersions using DSC. At pH 7, they observed an endothermic peak at 76.8 °C, which was attributed to changes taking place in β-lactoglobulin. This value is similar to that observed in the present study (77.5 °C). FITZSIMONS et al. [17] studying the whey protein isolate with a protein content of 91% observed an endothermic peak at pH 7.0 at 75 °C, while NICORESCU et al. [18] at 77 °C. Denaturation of β-lactoglobulin occurs at lower temperatures (between 59 °C and 63 °C) [18]. Tests carried using Fourier transformation infrared spectroscopy revealed that probably the DSC measurement performed at high concentrations of protein in solution (10%) shows not only the process of protein denaturation, but also their aggregation. Because of this, the endothermic peak is not to be regarded as the temperature of denaturation but a result of the endothermic and exothermic denatured protein aggregation [16]. In the present study, the highest temperature peak was found at pH 3.0 – 82.2 °C, and the lowest at pH 10.0 – 54.2 °C. BOYE et al. [16] observed, for a solution of whey protein concentrate at pH 3.0 and 9.0, endothermic peaks at 82.7 °C and 72.3 °C, respectively. Denaturation of whey protein takes place at high pH much faster, but these proteins are more stable at low pH values. At pH 9.0, partial denaturation of the protein takes place already at room temperature, followed by deprotonation of sulfhydryl groups. At this pH, there is a possibility of formation of disulfide bridges already at room temperature without prior heating of the protein solution, which leads to the formation of the gel [16, 19]. At low pH, strong protonation of sulfhydryl groups takes place and, therefore, formation of disulfide bridges is strongly inhibited [20]. The temperature of the endothermic peak observed at pH 3.0 is higher than at pH 6.7. Heating of β-lactoglobulin allows hydrophobic interactions to contribute to the gel formation. At pH close to neutral, the majority of hydrophobic groups of β-lactoglobulin molecules are at the surface, while at pH 3.0, a significant amount of hydrophobic groups is in the interior [21]. Enthalpy values indicate the energy absorbed at the process of heating and reflect the energy of electrostatic and hydrophobic interactions, as well as forming and breaking of hydrogen and disulfide bonds. The highest value of enthalpy was observed at pH 9 and pH 10, at which partial denaturation at room temperature allows for intensive interactions at heating [22]. DSC measurements reveal that, at different pH, there is different intensity of bonds formation, which probably leads to different gel structures.

The microstructure of aerated gels was investigated using confocal laser scanning microscopy and scanning electron microscopy. Fig. 1 shows the microstructure of the aerated gels obtained from 9% whey protein isolate dispersion at differ-

Tab. 1. Temperature at the endothermic peak and enthalpy for whey protein isolate dispersion obtained at 10% protein concentration at different pH.

pH	Temperature at the endothermic peak [°C]	Enthalpy [J·g^{-1}]
3.0	82.2 ± 0.1^{a}	0.56 ± 0.07^{b}
6.7	77.5 ± 0.3^{b}	0.34 ± 0.02^{c}
9.0	58.5 ± 0.8^{c}	1.54 ± 0.27^{a}
10.0	54.2 ± 0.3^{d}	1.24 ± 0.13^{a}

Values represent mean ± standard deviation. Values followed by the same letter in the same column are not significantly different at 95% confidence level.

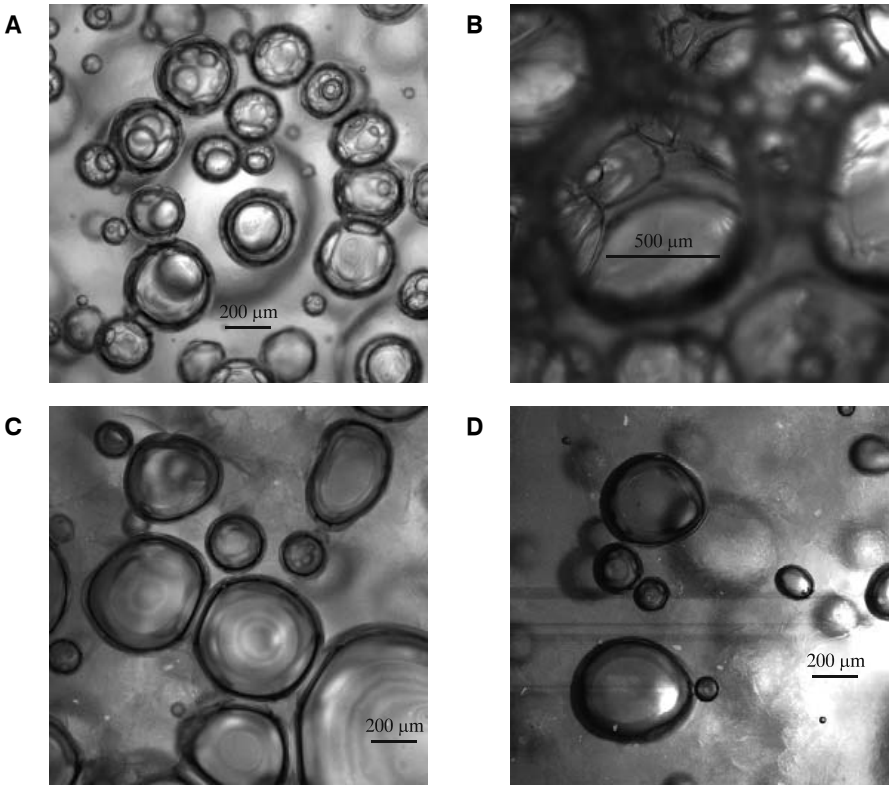

Fig. 1. Confocal laser scanning microscopy view of foamed gel obtained from 9% whey protein isolate at different pH.

A – pH 3.0, B – pH 6.7, C – pH 9.0, D – pH 10.0.

ent pH. At pH 3.0, a very interesting structure of air bubbles was observed (Fig. 1A). Larger bubbles contained smaller ones and these contained other smaller bubbles. This microstructure resembled a fractal structure. IKEDA et al. [23] reported a fractal nature of whey protein gels. Similarly, the fractal nature of the whey protein gels surface was investigated by CHEN and DICKINSON and the fractal dimension of the particulate gels was calculated as 1.15 [24, 25]. The formation of air bubbles in the gel structure is a phenomenon at the interface gel/air. Perhaps the fractal nature of the gel causes the air bubbles to be arranged in a fractal structure. This requires further study. In Fig. 2A, the fractal structure of the air bubble is shown. The proposed fractal model (Fig. 2B) was made

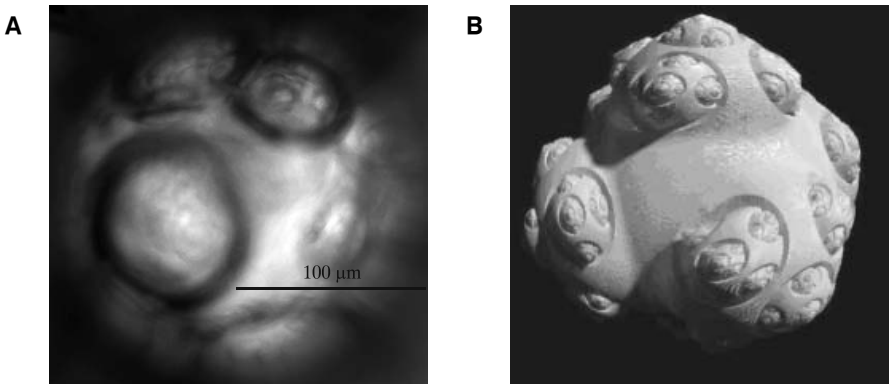

Fig. 2. Fractal structure of the air bubble obtained from whey protein isolate dispersion at pH 3.0.

A – fractal structure, B – the proposed fractal model made in Adobe Photoshop (Adobe Systems).

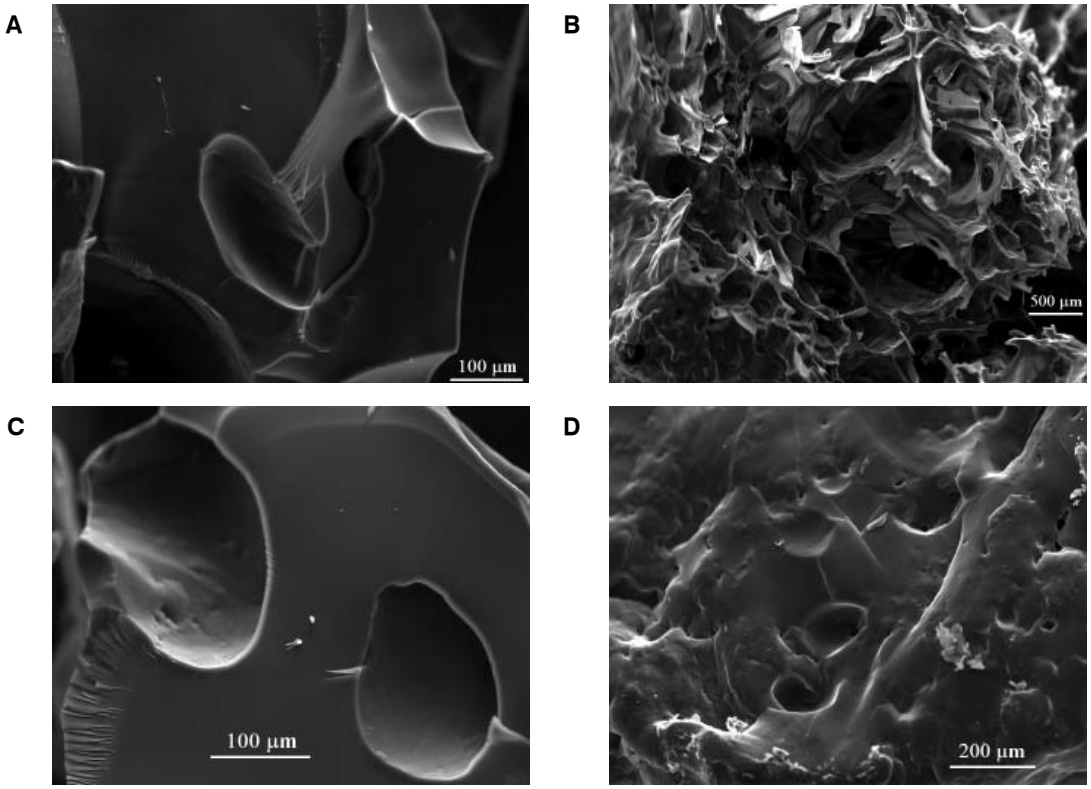

Fig. 3. Scanning electron microscopy view of foamed gel obtained from 9% whey protein isolate at different pH.
A – pH 3.0, B – pH 6.7, C – pH 9.0, D – pH 10.0.

in Adobe Photoshop CS6 13.0.4. (Adobe Systems, San Jose, California, USA). At native pH 6.7, a different structure of air bubbles was observed (Fig. 1B). Air bubbles had a comparatively larger size, and smaller bubbles inside the larger ones were not arranged in self-similar fractals. At pH 9 and pH 10 (Fig. 1C and 1D), gel was aerated by singular air bubbles and no tendency to bubble-in-bubble structure was observed. The gel obtained at pH 10.0 was transparent, indicating small size of the matrix-forming aggregates [26].

The microstructure of the aerated gels was examined also by scanning electron microscopy. At pH 3.0; pH 9.0 and pH 10.0, a smooth gel microstructure was observed (Fig. 3A, 3C, 3D). At these pH values, repulsive electrostatic force between

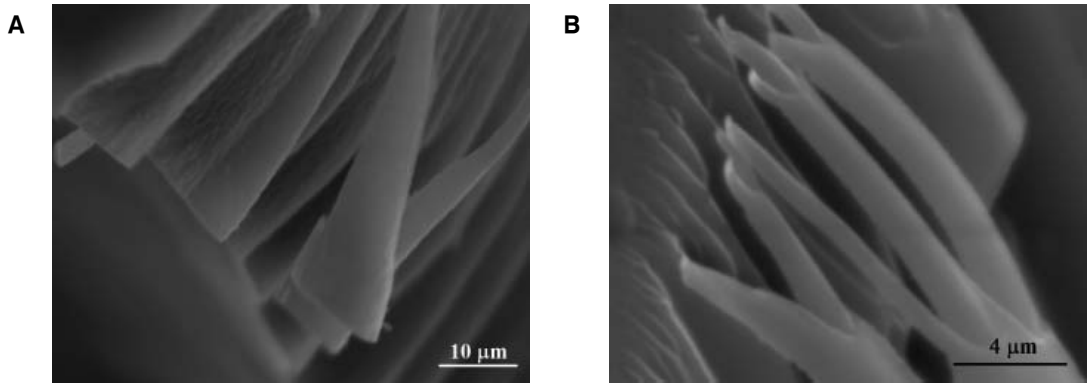

Fig. 4. Scanning electron microscopy view of foamed gel obtained from 9% wheat protein isolate at different pH.
A – pH 3.0, B – pH 9.0.

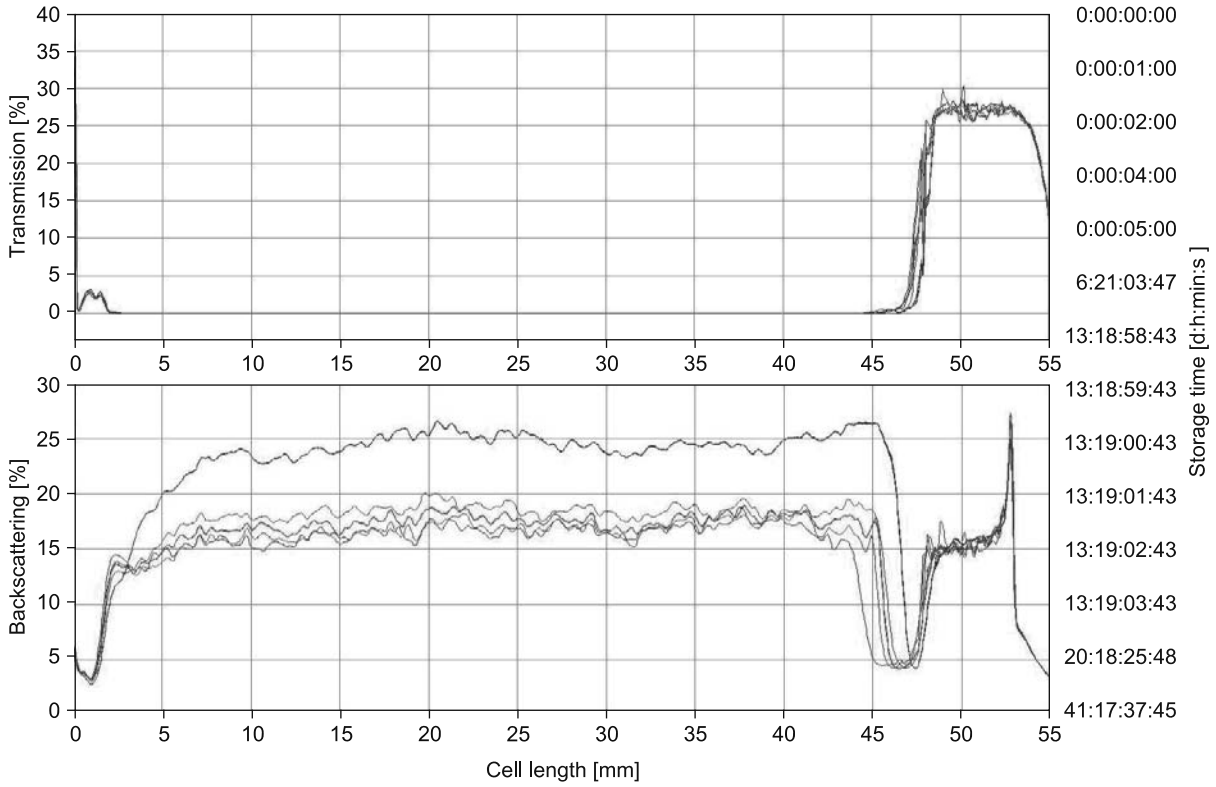

Fig. 5. Turbiscan backscattering and transmission profiles of aerated gel obtained from 9% whey protein isolate dispersion at pH 6.7 and stored for 41 days at 7 °C.

charged protein chains are effective [26]. The picture shows the smooth structure of the gel, since in these pH conditions, the gel network is formed by fibres of the size of a few nanometers. Visible cavities illustrate the air bubbles space. At higher magnification of the gel formed at pH 9.0, a characteristic microstructure is observed (Fig. 4B). We called this "fingers structure". It is formed at the process of peeling off the gel from its surface. The same "fingers structure" was not seen at pH 3.0 (Fig. 4A). This structure suggests that the gel was composed of concentric structures, which were located one in another. This is similar to the structure of a coaxial cable. The observed microstructure allows to offer a new theory of the formation of the gel structure at high pH values. Perhaps at pH 9.0, the gel is formed in a way that the protein chains are initially connected in "strings of beads" by strong disulfide bonds [27]. Next, these strings are stacked one on top of another and attached forming concentric layers. The apparent diameter of the smallest structure of "the finger" is about 200 nm, and thus is similar to the size of the diameter of a single "string of beads" observed by other investigators [26]. A different microstructure of aerated gels was observed at native pH 6.7 and was similar to a sponge (Fig. 3B). The size of the air bubble was much larger than at other investigated pH values. This microstructure allows for release of air from the air bubbles. It was observed using Turbiscan measurements (Fig. 5). During the storage, the backscattering slightly decreased, which suggested formation of more translucent foamed gels with larger bubbles. An average bubble size increased, for aerated gels obtained at pH 6.7 and stored for 41 days at 7 °C, from 221 μm

Tab. 2. Average bubble size before and after storage of aerated gel obtained from 9% whey protein isolate dispersion at different pH.

pH	Average bubble size [μm]	Average bubble size after 41 days at 7 °C [μm]
3.0	167 ± 8^{c}	154 ± 7^{c}
6.7	221 ± 11^{a}	234 ± 8^{a}
9.0	186 ± 4^{b}	173 ± 9^{bc}
10.0	84 ± 3^{d}	80 ± 10^{d}

Values represent mean ± standard deviation. Values followed by the same letter are not significantly different at 95% confidence level.

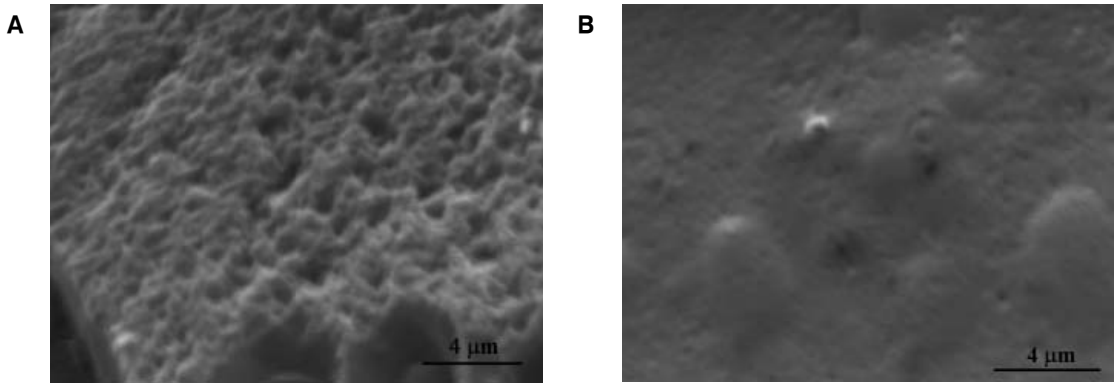

Fig. 6. Scanning electron microscopy view of the surface of foamed gel obtained from 9% whey protein isolate at pH 3.0 and pH 10.0.

A – pH 3.0, B – pH 10.0.

to 234 μm, but this change was not statistically significant. Also for other stored aerated gels, no significant changes in the average bubble size were found (Tab. 2). Scanning samples of aerated gels by Turbiscan scan analyzer was found to be a valuable test method by which the average diameter of the air bubbles can be determined. Studies carried out over a long period of time (41 days) demonstrated that aerated whey protein gels had very good stability and can be successfully used as matrix for the release of active substances.

Although both gels obtained at pH distant from the isoelectric point of whey protein are referred to as "fine-stranded", there are differences in their microstructure. Fig. 6 compares the appearance of the surface of the gels at pH 3 and pH 10. The gel produced at pH 10 had a smoother and more consistent texture than the gel obtained at pH 3.0. The more porous and less consistent structure of the gel prepared at pH 3 is probably responsible for its weaker texture. This microstructure results from the absence of disulphide bonds in the gel formed at pH 3.0 [28]. The texture of the gels was evaluated using a puncture test (Fig. 7). Aeration caused a decrease in puncture test force, as air bubbles weakened the gel structure by making crack propagation regions. The highest puncture test force of the aerated gel was noted at pH 6.7 (Fig. 7). In these conditions, there is a balance between repulsive and attractive forces between protein molecules, and the gel disrupted at the aeration process can be rehealed [29]. The largest puncture test

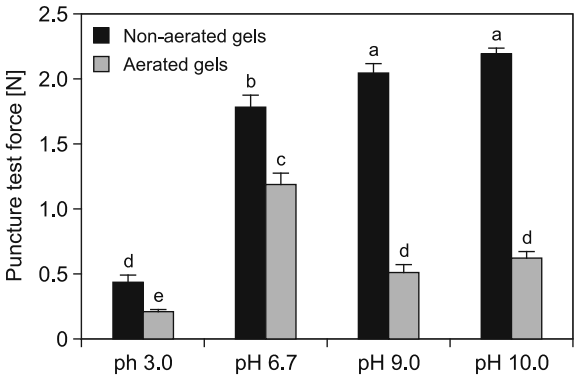

Fig. 7. Puncture test force for aerated and non-aerated gels obtained from 9% whey protein isolate dispersion at different pH.

Values marked by the same letter are not significantly different at 95% confidence level.

Tab. 3. Storage modulus *G*', loss modulus *G*" and tan delta values observed at frequency of 1 Hz for non-aerated and aerated gels obtained from 9% whey protein isolate dispersion at different pH.

pH	*G*' [Pa]		*G*" [Pa]		Tan delta	
	Non-aerated gel	Aerated gel	Non-aerated gel	Aerated gel	Non-aerated gel	Aerated gel
3.0	937	186	213	33	0.23	0.18
6.7	2617	5769	386	912	0.15	0.16
9.0	1416	4615	119	481	0.08	0.10
10.0	569	517	38	42	0.07	0.08

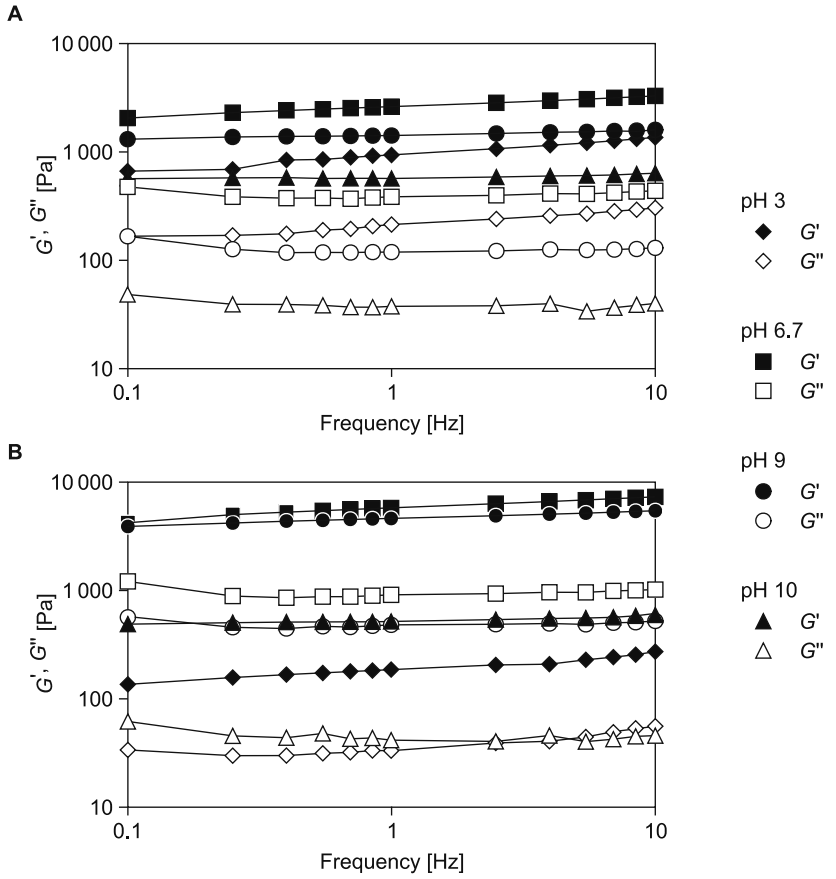

Fig. 8. Frequency sweep for aerated and non-aerated gels obtained from 9% whey protein isolate dispersion at different pH.

A – non-aerated gels, B – aerated gels.

forces for the gels formed at pH 9.0 and pH 10.0 corresponded to weak aerated gels obtained in this conditions. It is well documented in the literature that at this pH strong, transparent whey protein gels can be formed as a result of strong disulfide bonds. Too strong gel structure is less susceptible for rehealing [29]. Non-aerated and aerated gels behaved as strong gels with storage moduli values several times higher than loss moduli (Tab. 3). For most samples, moduli values increased with increased frequency, which reflected the increase in elasticity of the gels with decreased time of deformation (Fig. 8). The highest values of storage modulus was observed at pH 6.7, at which there is a balance between repulsive and attractive forces between protein molecules [26]. In most cases, aerated gels have higher tangent delta value than non-aerated gels (Tab. 3), which supports large strain rheological data, as the higher tangent delta reflects larger share of viscous properties in comparison to the elastic properties. The lowest values of storage moduli were noted for non-aerated and aerated gels with fine-stranded structure at the lowest pH (pH 3.0) and the highest pH (pH 10.0). The changes in elasticity with pH are caused by the change in chemical nature of the gel junction zones (from physical junctions driven by H bond and hydrophobicity at pH 3.0 to chemical S-S bonds at pH 9.0). Strong chemical disulfide bonds can be formed also at pH 6.7 [30]. Low values of storage moduli observed for the non-aerated and aerated gels at pH 10.0 were probably caused by alkaline hydrolysis of the proteins at heating at this pH for 30 min at 80 °C [31].

CONCLUSIONS

Aeration of whey protein isolate gels obtained by heat-induced gelation produced rehealed aerated gels. The aerated gels were characterized by different air bubble size and different microstructure of the bubbles. Aeration produced weaker but still elastic structure capable to hold air bubbles

during storage for 41 days at 7 °C. The aerated gels can be used as matrices for active ingredients release or as a new product with a lower caloric value and a unique textural and sensorial properties. High nutritional value of whey proteins can be used for production of novel foods for athletes and physically active people.

Acknowledgements

This work was supported by the National Science Centre, Krakow, Poland, Grant No. 2011/01/N/NZ9/04563.

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Received 8 January 2013; revised 9 February 2013; 2nd revised 18 February 2013; accepted 22 February 2013.