Abbreviations
EWI; Egg white isolate, DEWA; dried egg white albumin

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crystallized egg white protein – ovalbumin is used or commercial egg white protein is desalinated (Weijers et al., 2002; Weijers et al., 2006). Arntfield (1996) used calcium and magnesium to obtain “cold set” ovalbumin gels. Increased salt concentration causes formation of stronger gels (Artfield 1996; Savoie et al., 1996). Savoie et al. (1996) reported that the fracture stress showed a maximum at a certain calcium concentration although the storage modulus increased with increasing calcium concentration.

Lately a new commercial egg white isolate was obtained by Kewpie Corporation (Tokyo, Japan). Using a patented process, calcium, magnesium, sodium and potassium concentration was reduced accordingly to 22.2; 29.7; 12.7 and 14.2% of egg white dried albumin minerals content (Table 1). Preliminary research showed, that preheated egg white low-mineral isolate dispersions don’t gel at the concentration below 6%. The aim of the present research was to obtain calcium-ion-induced-gels of egg white isolate and to investigate their physicochemical properties and microstructure.

Materials and Methods

Material Egg white isolate - EWI (88.1% protein) was a gift from Kewpie Corporation (Tokyo, Japan). Protein concentration was determined by the Kjeldahl method (AOAC, 1995) and mineral analysis was performed by an atomic absorption spectrometry using a Varian Spectra 280 FS (Varian, Inc., Palo Alto, USA). Egg white isolate mineral composition was compared in Table 1 to average content of calcium, magnesium, sodium and potassium in dried egg white albumin (DEWA) presented by U.S. Department of Agriculture (i).

Gelation Egg white isolate dispersions (5.50; 5.75 and 6.00% w/w) were made by hydrating in distilled water. Dispersions were heated in water bath for 30 min at 80°C. After heating dispersions were cooled down to room temperature using tap water. The dispersions were used for rheological measurements. Preliminary dynamic rheological research revealed that 5.50% w/w of the protein concentration was the concentration at which no gelation was observed (Fig. 1) and this solution was used for calcium ions induced gelation. A 1 M calcium chloride solution was added to obtain 5, 10, 15, 20, 25 and 30 mM Ca\(^{2+}\) concentration. Obtained gels were stored at 7°C for 20 hrs, and were equilibrated at 21°C for 2 hrs and subjected to evaluations of their physicochemical properties and microstructure.

Ultrasound viscosity Ultrasound viscometer Unipan type 505 probe was immersed into the gel samples (UNIPAN, Warsaw, Poland) and the values of (dynamic viscosity) \(\times\) (density) in mPas x g/cm\(^3\) were measured. This is dynamic (shear) viscosity of a fluid i.e. its resistance to shearing flows and it should be distinguished from dynamic viscosity from low-strain dynamic rheology i.e. complex viscosity with elastic and viscous portions. Viscosity was measured at 10 min after the salt added at 21°C. The results are the average of six measurements.

Dynamic rheology Dynamic rheological measurements were performed using RS300 (ThermoHaake, Karlsruhe, Germany) rheometer equipped with serrated parallel steel plate geometry (35 mm diameter, 2 mm gap size) to reduce the risk of the slippage. Gelation of pre-heated EWI dispersions with added calcium salts was monitored immediately after the salt addition at 21°C for 10 min. Storage modulus (\(G'\)) and loss modulus (\(G''\)) was measured at 0.1 Hz and a strain of 0.01. Gels stored at 7°C for 20 hrs, and equilibrated at 21°C for 2 hrs were analyzed using frequency sweeps in the 0.1 – 10 Hz range in the linear viscoelastic region (evaluated in the present study by strain sweep performed prior to the frequency sweep measurement).

Texture profile analysis (TPA) A two-bite penetration test was performed using the TA-XT2i Texture Analyzer (Stable Micro Systems, Godalming, UK). Gels were prepared in beakers (diameter 40 mm and height 60 mm), stored at 7°C for 20 hrs, and equilibrated at 21°C for 2 hrs. The gel samples were analyzed using a 10 mm diameter steel cylinder probe operated at a crosshead speed of 1 mm s\(^{-1}\) and penetration distance of 30 mm.

<table>
<thead>
<tr>
<th></th>
<th>Ca (mg/kg)</th>
<th>Mg (mg/kg)</th>
<th>Na (g/kg)</th>
<th>K (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kewpie</td>
<td>198 ± 11</td>
<td>214 ± 19</td>
<td>1.57 ± 0.09</td>
<td>1.59 ± 0.06</td>
</tr>
<tr>
<td>DEWA</td>
<td>890</td>
<td>720</td>
<td>12.38</td>
<td>11.16</td>
</tr>
<tr>
<td>% DEWA</td>
<td>22.2</td>
<td>29.7</td>
<td>12.7</td>
<td>14.2</td>
</tr>
</tbody>
</table>

Table 1. Comparison of minerals content in Kewpie egg white isolate and dried egg white albumin – DEWA (i)

Fig. 1. Influence of protein concentration on changes in storage and loss moduli for EWI dispersion preheated for 30 min at 80°C and cooled down to 21°C.
Hardness, adhesiveness, springiness, cohesiveness and chewiness were evaluated. Hardness was the peak force of the first penetration cycle. Adhesiveness was the negative area for the first penetration cycle representing the work necessary to pull the probe from sample. Springiness was the height that the gel recovered during the time that elapsed between the end of the first penetration and the start of the second penetration. Cohesiveness was the ratio of positive force during the second penetration to positive force of the first penetration cycle. Six measurements were carried out for each test.

**Surface roughness** Surface roughness of gels was observed using an optical profilometer GT Contour Surface Metrology (Veeco, Tucson, USA). Surface roughness was determined using Vision64 computer program (Veeco, Tucson, USA). The following surface roughness parameters were calculated: \( R_q \) - quadratic mean of the surface roughness and \( R_t \) - maximum roughness height.

**Scanning electron microscopy (SEM)** The microstructure of the gels was investigated using SEM. Samples of the gels were fixed in 2.5% glutaraldehyde solution in 0.1 M sodium cacodylate buffer, dehydrated in serial dilutions of ethanol and acetone and dried at the critical point in liquid carbon dioxide. Samples were coated with gold using a vacuum evaporator EMITECH K550x (Emitech, Ashford, United Kingdom). Preparations were viewed and photographed using a scanning electron microscope VEGA II LMU (Tescan, Canberra, USA).

**Statistical analysis** Statistical analysis of the results was performed 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 confidence level of \( p \leq 0.05 \) based on the least significant difference.

**Results and Discussion**

**Rheological properties** Egg white isolate dispersions which were preheated and cooled to room temperature were immediately put onto the oscillatory rheometer. Changes in storage and loss moduli in time are shown in Fig. 1. For 5.50% protein concentration no gelation was observed. Loss moduli had higher values than storage modulus. Sample was a viscous liquid. At 5.75% protein concentration a gelation point was observed as a cross-point was observed for storage and loss moduli (Ikeda *et al*., 2001; Solowiej *et al*., 2008). Obtained gel was very weak with both moduli below 1 Pa. The increase in protein concentration up to 6.00% caused gelation right after the analysis started as at time zero equal values of \( G' \) and \( G'' \) were noted. After 10 min. storage modulus was several times higher than loss moduli - a gel was formed. Based on these observations, protein concentration 5.5% was chosen to study calcium–induced-gels. Fig. 2 shows values of the product of (dynamic viscosity) \( \times \) (density) of calcium-induced-gels measured 10 minutes after the salt solution was added. There was an exponential increase in viscosity with increased calcium concentration.

There was no statistically significant increase in the viscosity for 5 and 10 mM of calcium. It is in good agreement with dynamic rheology data presented in Fig. 3. Very similar curves of changes of storage and loss moduli were observed for the gels obtained in the presence of 5 and 10 mM Ca\(^{2+}\). Gelation point was observed right after the measurements started. Changes in \( G' \) and \( G'' \) support the data from the viscosity measurements, as there was an increase in these values with increased calcium concentration. At 30 mM Ca\(^{2+}\) strong gels were formed with the storage modulus at 640 Pa. Rheological properties of obtained gels were evaluated after they were stored at 7°C for 20 hrs. Frequency sweeps data is presented in Fig. 4.
Increased calcium concentration produced gels with higher values of storage and loss moduli. There was an increase in storage and loss moduli with the frequency, which is characteristic for so-called weak gels. At higher frequency (i.e. smaller deformation time) weak gel samples had less time for relaxation and behaved as more rigid material.

**Textural properties** Texture of obtained gels was measured using modified texture profile analysis method (Table 2). Double penetration test was used to evaluate several textural attributes. An increased calcium concentration caused an increase in hardness of induced gels, except for 5 and 10 mM Ca$^{2+}$ at which almost equal values were observed. It is in agreement with the measurements of viscosity and storage modulus of the gels. Probably somewhere above 10 mM of Ca$^{2+}$ there is a critical value of calcium concentration, which induces gelation at investigated protein content. Increased calcium concentration caused increase in adhesiveness of the gels. Adhesiveness is calculated as an energy needed to overcome attractive forces between the gel and all contact surfaces of the probe. In the present method of double penetration the surface of the contact between gel and the probe is much larger than in a traditional double compression method, which makes it more sensitive for evaluation of this particular texture parameter. Increased calcium concentration resulted in higher chewiness of the gels. It should be expected, that the energy needed to chew the gels was higher for harder and more adhesive gels. There was a linear correlation between (dynamic viscosity) x (density) of the gels and chewiness, hardness and adhesiveness with the coefficient of determination equal: 0.99, 0.98 and 0.82 accordingly. Similar correlations was found earlier for magnesium induced whey protein gels (Tomeczynska-Mleko et al., 2014). The highest correlation was noted for chewiness. For gels with higher chewiness, more energy is needed to overcome frictional forces between gel particles, which are responsible for higher viscosity.

**Microstructure** Microstructure investigated using scanning electron microscopy was presented in Fig. 5. Increased calcium concentration resulted in more aggregated structure. More aggregated structure of heat-induced egg white gels with increased Ca$^{2+}$ ions concentration were observed by Croguennec et al. (2002). More aggregated microstructure of the gels influences not only the rheological and textural properties, but also their surface properties.

**Surface roughness** The gels roughness was described using two different parameters: $R_t$ represents the distance between the highest peak and the lowest valley on the measured gel surface; $R_q$ is a quadratic mean of the surface roughness and is given by the standard deviation of the vertical values for the gel area. Gels with more aggregated microstructure had surfaces with higher values of roughness parameters. There was a linear correlation between these surface roughness parameters ($R^2 = 0.995$) (Fig. 6). Samples obtained at 25 and 30 mM calcium had similar roughness and the microstructural observation of the gels support this similarity (Fig. 5). Chen et al. (2006) observed that 200 mM NaCl produced whey protein gels with rougher surface than gels obtained without the salt addition. Hongsprabhas and Barbut (1997) found that salt-induced gels of whey protein isolate showed the maximum strength at 30 mM calcium concentration. Maltais et al. (2009) observed an ordered, filamentous structured gel at low salt concentration (10 mM CaCl$_2$) while a particulate, unordered gel structure was found at higher salt concentration (20 mM CaCl$_2$). In these protein gels, the structure becomes rougher with increasing salt concentration at some concentration range. This phenomenon has been also observed in polysaccharide gels induced by cations. It is well known that the addition of small amount of cations increases
the elastic modulus of polysaccharide gels, but the excessive addition decreases the modulus (Morris et al., 2012). In other words, the elastic modulus of polysaccharide gels as a function of added salt shows a maximum at a certain concentration of salt. Morris et al. (1995) attributed this phenomenon to the formation of inhomogeneous structure. The rough surface observed in the present work may be also related to the formation of the inhomogeneous structure. Introduction of xanthan causes smoothing of heat-induced whey protein gels surface (Nayebzadeh et al., 2006). There was no research so far on roughness of egg white gels. Surface roughness of the gels can influence their application as matrices for the release of active ingredients (Mleko et al., 2010).

In conclusion, this research shows for the first time ion-induced gelation of EWI. Application of low minerals EWIs enables to obtain cold-set gels with different, engineered microstructure. These kinds of gels, because of different porosity and surface roughness, can be used as matrices for the release of active ingredients with planned release time.

### References


