DEVELOPMENT OF A NEW PRODUCT: EGG WHITE ALBUMEN GELS WITH DIFFERENTIATED MAGNESIUM RELEASE

Marta Tomczyńska-Mleko

Institute of Plant Genetics, Breeding and Biotechnology
University of Life Sciences in Lublin

Abstract

Commercial egg white isolates (EWI) or concentrates contain substantial quantities of minerals and they easily form heat-induced gels. An innovative, low mineral EWI was used in the present research to investigate the influence of magnesium ions on the gelation process and on the release of these ions in simulated gastric conditions. Heat-induced gelation of egg white proteins was monitored by using an ultrasound viscometer and an oscillatory rheometer. Samples behaved as weak gels with the storage modulus about 5 times larger than the loss modulus. Both methods revealed two phases of gelation. More elastic behavior of gels with an increased magnesium ion concentration can be explained by a higher number of bonds between protein molecules observed in transmission electron microscopy images. An increased concentration of magnesium ions in gel caused an increased release rate of these ions in simulated gastric conditions, which was probably caused by a higher surface roughness of the gel. Higher roughness of the gel surface enlarges the contact area and enables faster hydrolysis. The egg white isolate gels with a high bioavailability organic form of magnesium could be used as food supplement with a regulated magnesium release rate in the human stomach.

Key words: magnesium, controlled release, egg products, gelation, rheological properties.

dr Marta Tomczyńska-Mleko, Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Akademicka Street 15, 20-950 Lublin, Poland, phone: 48 81 445 68 96, fax: 48 81 445 60 31, e-mail: martamleko@tlen.pl

INTRODUCTION

Egg white protein (EW) has been used for decades as an excellent functional ingredient. The most important functional properties are gelation and foaming. The most abundant protein in EW is ovalbumin, which constitutes 54% of total protein. Ovalbumin is a globular protein with a molecular weight of 45.5 kDa. Its properties determine the gelation of EW. Heat-induced gels have a very important role in food technology, medical and material science. They provide a matrix for water and air holding, delivering nutrients and flavors, and regulating rheological properties (GLIBOWSKI 2009). Heat-induced gelation of egg proteins is a multi-stage process, which involves, as the first stage, the unfolding of native proteins in solution with the exposure of inner hydrophobic regions. Denatured proteins progressively pass to an aggregated network that forms a sol state, which eventually reaches the final state of three-dimential gel (TOTOSAUS et al. 2002). Gel networks are formed when the protein concentration exceeds a critical concentration. Aggregation involves noncovalent and intermolecular disulfide (covalent) interactions (BROERSEN et al. 2006). Gelation of ovalbumin occurs at pH regions not so far from the pI and at appropriate concentrations of NaCl, where intermolecular hydrophobic interactions and electrostatic repulsive force are well balanced (SUN et al. 2002). In the absence of salt, more than 99% of ovalbumin was estimated to be in a monomeric form after incubation at 80°C for 5 min (TANI et al. 1997). Heated ovalbumin at pH 7 forms linear aggregates of monomeric ovalbumin, with very little branching at low ionic strength, but with strong branching at 0.1 M NaCl (WEIJERS et al. 2002). Magnesium and calcium ions improved the firmness of ovalbumin gels owing to the screening effect and increased crosslinking between proteins at pH values above the isoelectric point (ARNTFIELD 1996). Cation binding generally increased with an increasing salt concentration, and Mg^{+2} bound to a greater extent than Ca^{+2} at the higher levels studied (SAVOIE, ARNTFIELD 1996).

Polymeric systems have been used lately as controlled drug delivery materials (MLEKO et al. 2010). In previous research, whey protein aerated gels were obtained as a potential matrix for active ingredients release (TOMCZYŃSKA-MLEKO 2010, 2013). TAHERI et al. (2012) produced egg white nanoparticles suitable for use as drug carrier systems. ROSENZWEIG et al. (2013) used egg albumin to develop and characterize floating, stomach-retentive matrix tablets, which would deliver polyphenols in a controlled release manner. Egg albumin-based tablets had higher resistance to mechanical forces than cellulose-derived polymers. Magnesium deficiency (hypomagnesaemia) is frequent in both underdeveloped and developed countries. In the latter ones, coffee drinking depletes an organism of magnesium. Another cause is that edible parts of new, high-yielding varieties of cereals and vegetables are much poorer in minerals, including magnesium, than the old, low-yielding ones (GRZEBISZ 2011). Deficiency of magnesium may result in many disorders

(Cieślewicz et al. 2013), hence oral magnesium supplementation is recommended. Magnesium ions in the form of organic compounds are better tolerated and cause lesser laxative effect than inorganic ones (Badran, Cretin 2009). Studies on magnesium bioavailability in Mg-depleted rats showed that organic magnesium was better available than inorganic one (Coudray et al. 2005). Sahin et al. (2005) observed that supplementation with Mg-proteinate was more protective than Mg-oxidate in reducing the negative effects of heat stress in quail. Magnesium acts as a cofactor or an activator of many critical enzymes for reactions involving ATP that energize all major metabolic pathways, and for those purposes organic Mg was found to be much more effective than the inorganic form (Liu et al. 2007). Kłobukowski et al. (2014) proved high bioavailability of calcium from natural dairy products.

All research on the influence of ions on gelation process was performed using powder ovalbumin containing low concentrations of salts. Commercial egg white isolates or concentrates have substantial quantities of minerals and they easily form heat-induced gels. Investigation of the influence of salts on such material is not fully meaningful. Lately, a new low-mineral egg white isolate has been produced by Kewpie Corporation (Tokyo, Japan), which is suitable for use in model study concerning the development of gels with different ions release properties. The aim of this study has been to investigate EW heat-induced gels with different magnesium ions concentrations as matrices for magnesium release in the stomach.

MATERIAL AND METHODS

Egg white isolate, EWI (88.1% protein), was obtained from Kewpie Corporation (Tokyo, Japan). The protein concentration was determined by the Kjeldahl method. Mineral analysis of the isolate was performed by atomic absorption spectrometry using a Varian SpectrAA 280 atomic absorption spectrometer with a 4.0 mA cathode lamp (Varian, Inc., Palo Alto, USA) (Bubnič et al. 2011). The results were compared with the content of calcium, magnesium, sodium and potassium in dried egg white albumin (USDA 2010) – Table 1.

Egg white isolate dispersions (6% w/w) were made by hydrating in 10, 30 or 50 mM Mg^{2+} solutions (from magnesium chloride). The dispersions were heated in a water bath for 60 min at 75°C. They were heated in glass tubes (20 mm diameter) and an ultrasound viscometer Unipan type 505 probe was immersed into the samples (UNIPAN, Warsaw, Poland). Every 5 minutes, the value of viscosity x density in mPas g cm^{-3} was measured. Preliminary tests showed that 6% protein EWI dispersion obtained in distilled water and heated under the above conditions did not form gel. Gels stored at 7°C for 20 h were equilibrated at 21°C for 2 h and subjected to the determination of their physicochemical properties and microstructure.

Dynamic rheological measurements were performed on a RS300 (ThermoHaake, Karlsruhe, Germany) rheometer with a serrated parallel steel plate geometry (35 mm diameter, 2 mm gap size) to limit the potentiality of sliding effects. Gelation of EWI dispersions was monitored at 75°C for 60 min, with the storage modulus and loss modulus measured at 0.1 Hz and strain 0.01. Gel samples from water bath were cut (35 mm diameter and 3 mm thick) with a scalpel and analyzed by frequency sweeps at the 0.1-10 Hz range in the linear viscoelastic region (previously identified by strain sweeps).

Samples of the gels were fixed by immersion in 2.5% glutaraldehyde solution in 0.1 M 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 a vacuum evaporator EMITECH K550x (Emitech, Ashford, United Kingdom). Afterwards, they were viewed and photographed under a scanning electron microscope VEGA II LMU (Tescan, Canberra, USA).

Samples of the gels were also cut with a scalpel into 5 mm side cubic pieces, fixed for 3 h at 4°C with 3% (v/v) glutaraldehyde and 2% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7) and then washed with phosphate buffer. The samples were post-fixed in 0.5% (w/v) OsO_4 for 1 h at 4°C. Afterwards, they were dehydrated in serial dilutions of ethanol and acetone and dried at the critical point in liquid carbon dioxide. Dried gels were embedded in graded series of EPON (Embed 812, EMS, England) diluted in propylene oxide. The blocks were polymerized at 60°C for 12 h, then thinly sliced (90 nm thick) using an ultramicrotom Reichert Ultracut S (Leica Microsysteme, Wien, Austria). Ultrathin cuts were stained using Reynolds lead citrate and uranyl acetate. Microscopic observations were carried out under an FEI Tecnai Spirit G2 microscope (FEI, The Netherlands) at the acceleration voltage of 120 kV.

The surface of the gels was observed using an optical profilometer GT Contour Surface Metrology (Veeco, Tucson, USA). Surface roughness was determined with the aid of a Vision64 computer program (Veeco, Tucson, USA).

The gels were also cut into 10 mm edge cubes using a scalpel. Approximately 15 g batch of each sample was used for tests in an artificial stomach. A sample was placed in an artificial stomach composed of a 1 dm^3 cylindrical bioreactor (Schott glass, 15 cm in diameter) with a heating mantle, magnetic stirrer, automatic pH control system and a water bath. The bioreactor was filled with 0.5 dm^3 of hydrochloric acid solution at pH 2.3. and the pepsin from porcine stomach mucosa (2000 U mg^{-1} protein, Sigma-Aldrich, St. Louis, USA) was added in an amount of 1.6 g. The hydrolysis process of the gels was carried out for hours at an automatically controlled pH 2.3 and a temperature of 37°C. Samples of liquid from the artificial stomach were taken for analysis every 5 min. The magnesium content in the samples was determined by atomic absorption spectrometry using a Varian Spectra 280 FS (Varian, Inc., Palo Alto, USA).

The charts in this article present mean values from three replications fitted to a power law model using the Microsoft Excel Program.

RESULTS AND DISCUSSION

The preliminary research showed that 6% egg white isolate dispersions did not gel when heated at 75°C for 60 min. It was probably caused by a low concentration of minerals in the egg white isolate powder. Table 1 compares basic mineral concentrations in the isolate and in dried egg white (USDA 2010). Concentrations of sodium, potassium, calcium and magnesium in the isolate made up 12.7%; 14.2%; 22.2% and 29.7% of the DEWA mineral concentrations, respectively. In some previous results, the "zero" sample with no

Table 1

Comparison of the content of minerals in Kewpie egg white isolate and dried egg white albumin – DEWA (USDA 2010)

Material	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (g kg^{-1})	K (g kg^{-1})
Kewpie	198 $\pm$ 11	214 $\pm$ 19	1.57 $\pm$ 0.09	1.59 $\pm$ 0.06
DEWA	890	720	12.4	11.2
% of DEWA	22.2	29.7	12.7	14.2

salts added always formed heat-induced gels (HOLT et al. 1984, CROGUENNEC et al. 2002). This implies that the results obtained by other researchers show the influence of salts on gels, which would have beeen created at the "native" salt concentration as well. The preheated egg white protein isolate dispersion obtained at 6.5% concentration and cooled down to room temperature formed gel when rested on a plate of the rheometer (Figure 1). No such effect was observed for 6% protein dispersion. For egg white isolate dispersions used in the present research, an addition of minerals was necessary for heat-induced gelation to enhance the screening effect and to diminish repulsive forces between protein molecules. As a result, proteins randomly aggregated to form gels resulting from the transition to favorable protein-protein interactions from protein-water interactions (CHOI et al. 2000). Heat-induced gelation of egg white proteins was monitored with an ultrasound viscometer. The dynamic viscosity x density value was read every 5 min, and the results are presented in Figure 2. Different rates of the viscosity change were noted up to 30 min time, and after an elapse of 30 min, with a larger increase in viscosity during the second phase of heating. An increase in viscosity during the up to 30 min was probably caused by aggregation and formation of increasingly larger protein aggregates. Larger aggregates raise viscosity (VARDHANABHUTI, FOEGEDING 1999). After 30 min. of heating, aggregates probably started to form a three-dimensional gel structure and further heating induced more hydrophobic and ionic interactions, which resulted in the growing density of the gel structure. A higher magnesium ion concentration resulted in the
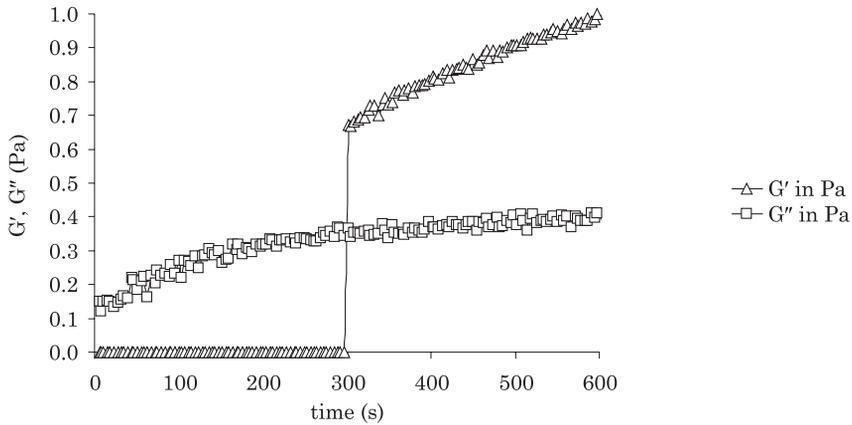
Fig. 1. Changes in storage and loss moduli for 6.5% protein EWI dispersion preheated for 1 h at 75°C and cooled down to 22°C
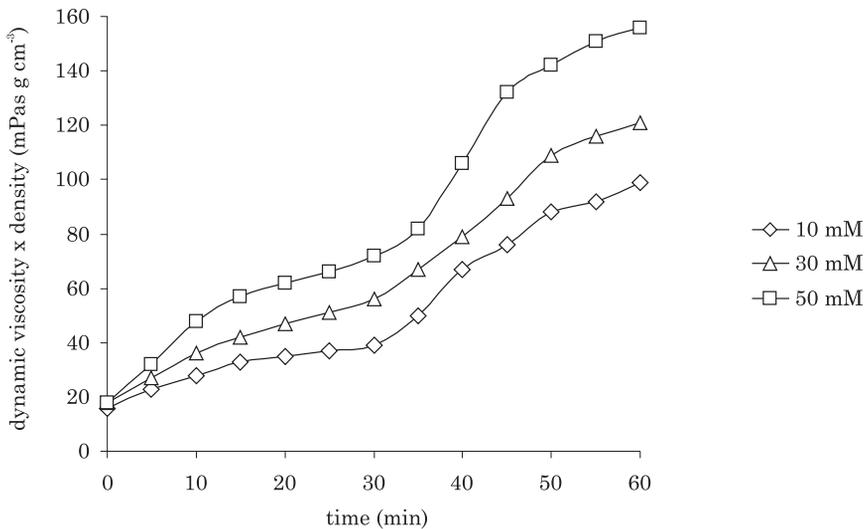

Fig. 2. Influence of concentrations of magnesium ions on changes in the dynamic viscosity x density of 6.0% protein EWI dispersion heated for 1 h at 75°C

higher viscosity of the forming gel. A similar behavior was observed using an oscillatory rheometer. Two different phases of the storage modulus increase were observed (Figure 3). The time of the aggregation phase was shorter (about 12-15 min) as the gelation process was monitored using parallel plates with the gap size 2 mm. Heat transfer between the plates was faster than in glass tubes 20 mm in diameter. An increase in the magnesium ion concentration caused an increase in the storage modulus, as a more elastic structure was being formed. The gels were analyzed with the frequency sweep test (Figure 4). An increase in frequency caused an increase in the storage
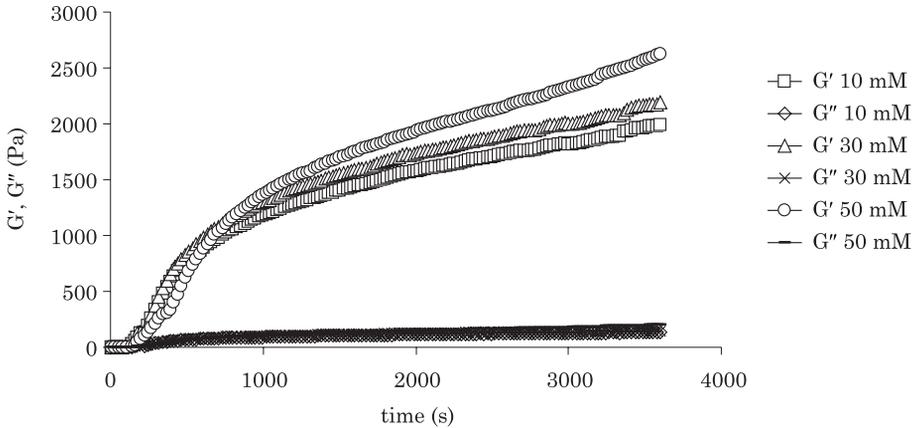
Fig. 3. Influence of concentrations of magnesium ions on changes in storage and loss moduli of 6.0% protein dispersion EWI heated for 1 h at 75°C

modulus. A sample behaved like weak gel as the storage modulus was about 5 times larger than the loss modulus. To investigate changes in viscoelasticity of the samples, the same results were presented as the relationship between the frequency and the loss tangent (Figure 5). At a lower frequency, the loss tangent decreased and the samples behaved as more elastic ones. A higher frequency (shorter deformation time) meant that samples had less time for relaxation and the Debora number increased, which reflected a more solid material (ABU-JDAYIL et al. 2013). In general, from the frequency of about 1 Hz, no changes in the loss tangent were obsetved. Higher frequency oscillation movements with higher energy did not break the structure of gel. More elastic behavior of the gels observed concurrently with an increased magnesium ion concentration can be explained by a higher number of bonds
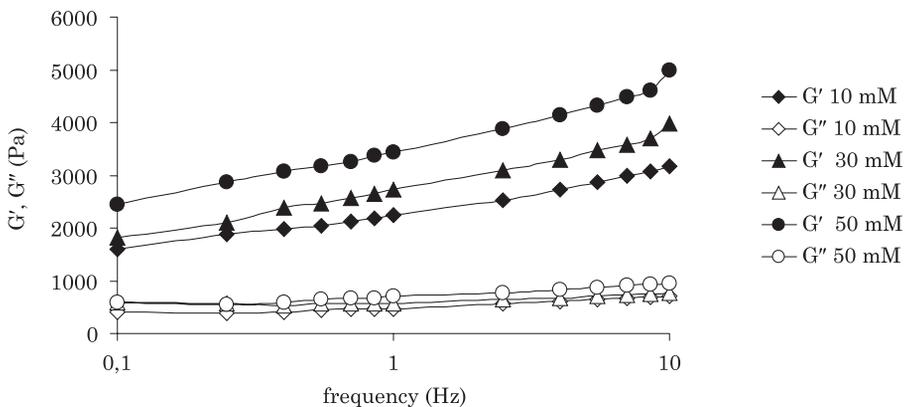

Fig. 4. Changes of storage and loss moduli at frequency sweep of 6% protein EWI gels obtained at different magnesium ion concentrations
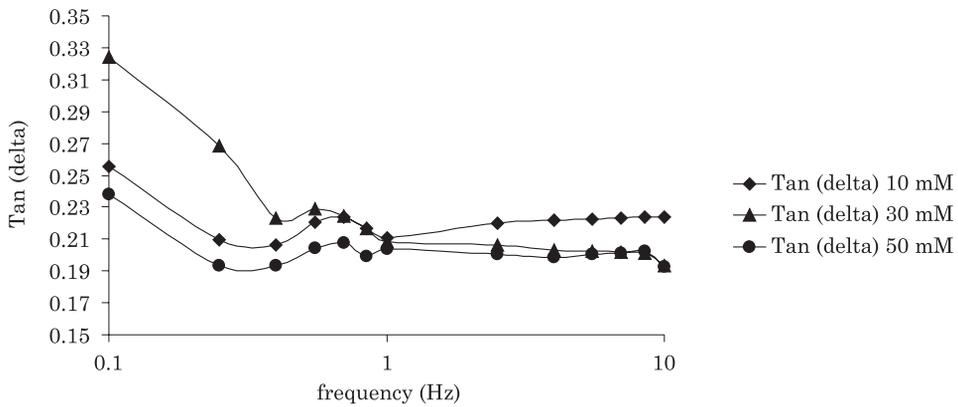
Fig. 5. Changes of the tangent of the phase angle at frequency sweep of 6% protein EWI gels obtained at different magnesium ion concentrations

between protein molecules (Figure 6 *a-c*). A higher concentration of divalent magnesium ions probably caused a stronger screening effect, which facilitied more powerful hydrophobic interactions. At higher ion concentrations, stronger ionic interactions were possible with more magnesium bridges being formed. This resulted in a more aggregated structure, as Figure 6 *A-C* depicts. Similar changes were observed for calcium ions by CROGUENNEC et al. (2002).

The gels could be used as food supplements for sportsmen and physically active people. Egg white isolate is a very good source of protein with a high content of essential amino acids (VANNIEKERK, VANHEERDEN 1993). Recently, whey protein gels have also been used as matrices for active ingredient release (GUNASEKARAN et al. 2006, TOMCZYŃSKA-MLEKO, MLEKO 2014). YOSHINO et al. (2004) proved that heat-coagulated egg white showed good peptic digestibility at the pH range from 1.5 to 2.5, unlike protein in raw egg white, which was almost resistant to the enzyme at all tested acidic pH ranges. The release of an active substance from a matrix can be described by the following semi-empirical equation:

$$M_t/M_\infty = kt^n,$$

where:

M_t – mass of an active ingredient released after time t,

M_∞ – total mass of an active ingredient,

t – time,

k – kinetic constant,

n – exponent.

The value of n as the power function exponent indicates the growth dynamics of an active ingredient in the stomach solution, while the value of k shows the concentration of the active ingredient extrapolated to time zero. The combination of these coefficients represents the the release process intensity. RITGER and PEPPAS (1987) suggested that the coefficient k is a con-
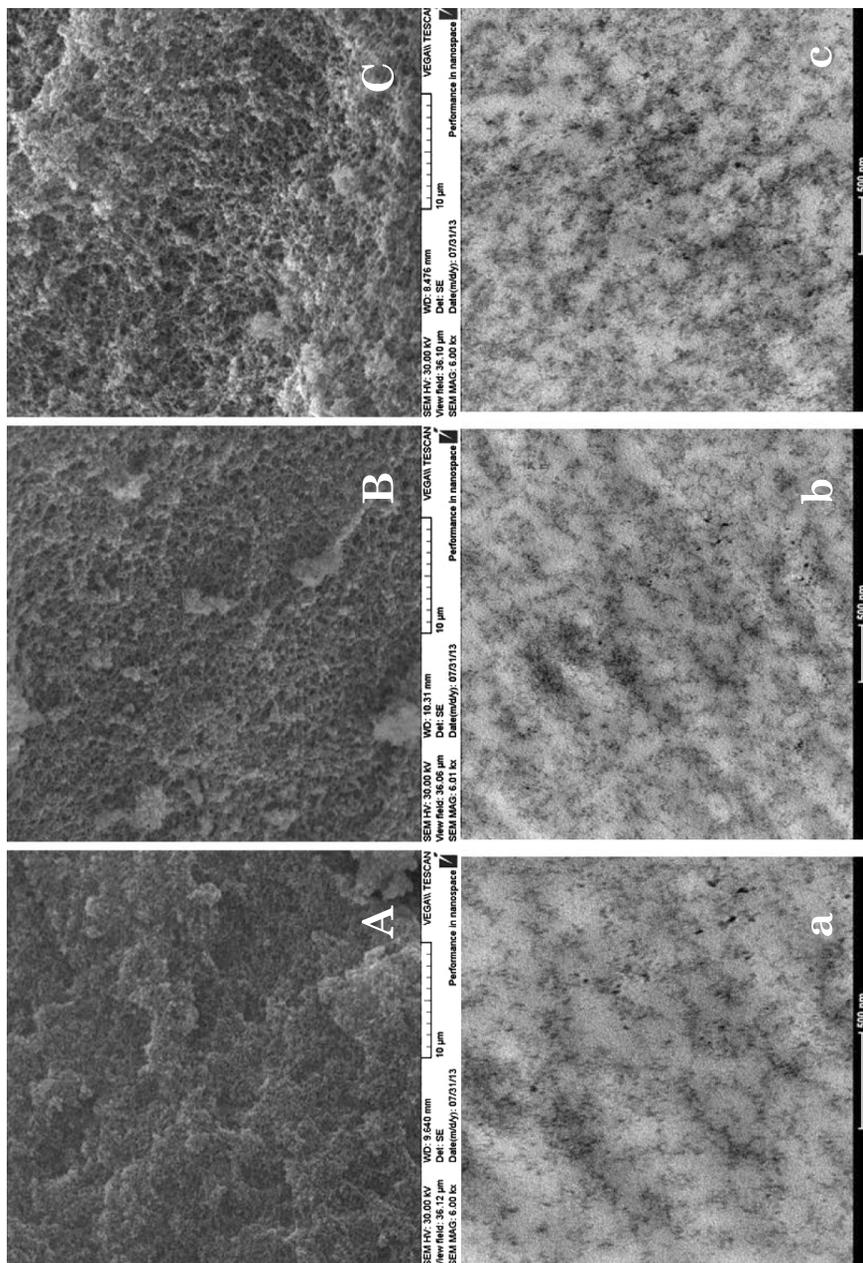
Fig. 6. Scanning electron micrographs (capital letters) and transmission electron micrographs (small letters) for 10 mM (A, a); 30 mM (B, b) and 50 mM (C, c) magnesium ion concentrations of 6% protein EWI gels

stant characteristic of the matrix, wile n is a coefficient characteristic of the type of a release mechanism. The release of an active substance from a matrix can be treated as a combination of diffusion of the active ingredient from the matrix and the particle transport through polymer chains. The value of "n" depends on the shape of samples and when it is close to 0.44 for cubes, it indicates the mechanism of diffusion while the value of about 0.87 implicates the mechanism of transport through polymer chains (KOSMIDIS et al. 2003). Figure 7 shows release of magnesium ions in simulated gastric conditions. Values of n obtained for the investigated egg white gels with magnesium are close to 0.4 what indicates that diffusion mechanism was responsible for magnesium ions release from the gel. As the chart shows a relative increase in the magnesium concentration, it is possible to compare the release rate for different gels. An increased concentration of magnesium ions in gel increased the release rate. It is probably connected with different microstructure for the gels with different magnesium content. At higher magnesium ion concentrations, a more aggregated microstructure of the gels is observed (Figure 6 A-C). Figure 8 shows that higher magnesium ion concentrations produced gels with a rougher surface structure i.e. with a higher quadratic mean of the surface roughness R_q and maximum roughness height R_a. Higher aggregation of proteins in gels form a surface with higher roughness. There is a linear correlation between the quadratic mean of the surface roughness and the maximum roughness height ($R^2 = 0.99$). CHEN et al. (2006) found that whey protein gel without NaCl addition had a very smooth surface, with R_q and R_a of 0.20 and 0.18 μm, respectively. At an increased 200 mM NaCl concentration, a much rougher surface with high R_q and R_a (2.39 and 1.91 μm, respectively) was observed. At a higher concentration of magnesium chloride, larger aggregates were formed (Figure 6 A-C), which resulted in higher
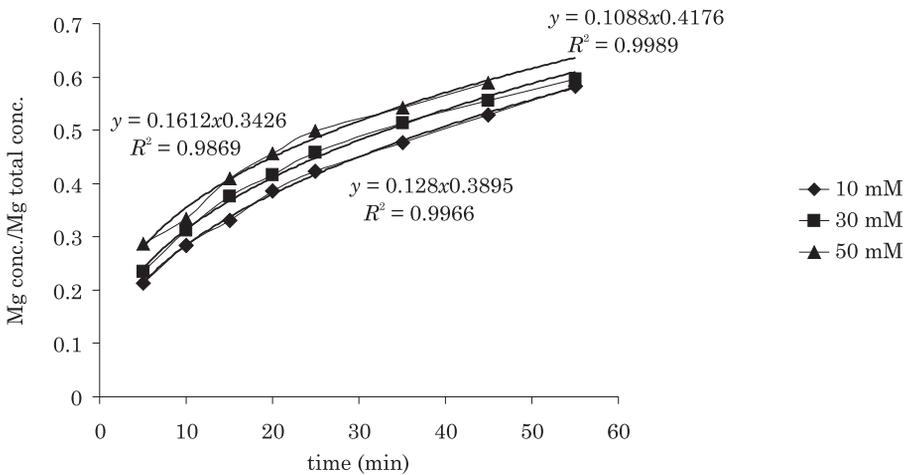

Fig. 7. Relationship between M_t/M_∞ and time for magnesium ion release in simulated gastric conditions from 6% protein EWI gels obtained at different magnesium ion concentrations
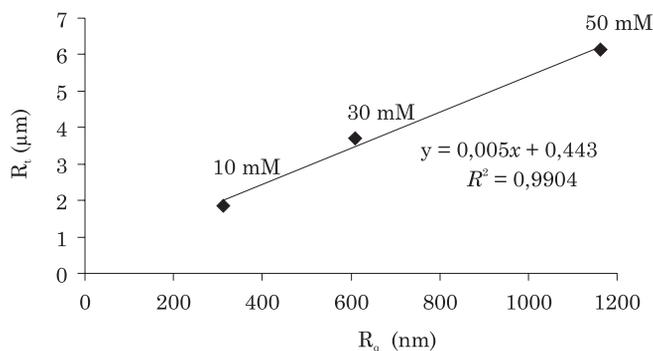
Fig. 8. Influence of magnesium ion concentrations on the quadratic mean of surface roughness R_q and maximum roughness height R_a for 6% protein EWI gels

roughness of the gel surface. NAYEBZADEH et al. (2006) observed that xanthan gel acted as a surface smoothing agent for heat-induced whey protein gels. Whey protein aggregates formed a rough structure and xanthan gel filled the empty spaces between protein aggregates. A higher surface roughness of gel probably favours hydrolysis process caused by pepsin. It increases the contact area and accelerates hydrolysis.

CONCLUSIONS

1. By varying the concentration of ions, it was possible to produce gels with different aggregation and different surface roughness.

2. A higher concentration of magnesium resulted in gels with the higher release of these ions in an artificial stomach.

3. Higher roughness of the gel surface increases the contact area and accelerates hydrolysis.

4. The egg white isolate gels with the high bioavailability organic form of magnesium could be used as a food supplement with a regulated magnesium release rate in the human stomach.

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