Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2003
Volume 6
Issue 2
Series FOOD SCIENCE AND TECHNOLOGY

GUSTAW W., TARGOŃSKI Z., GLIBOWSKI P. MLEKO S., PIKUS S. 2003. THE INFLUENCE OF XANTHAN GUM ON RHEOLOGY AND MICROSTRUCTURE OF HEAT-INDUCED WHEY PROTEIN GELS **Electronic Journal of Polish Agricultural Universities**, Food Science and Technology, Volume 6, Issue 2.
Available Online http://www.ejpau.media.pl

THE INFLUENCE OF XANTHAN GUM ON RHEOLOGY AND MICROSTRUCTURE OF HEAT-INDUCED WHEY PROTEIN GELS

Waldemar Gustaw[1], Zdzisław Targoński[1], Paweł Glibowski[1], Stanisław Mleko[1], Stanisław Pikus[2]
[1] *Department of Food Technology, Agricultural University of Lublin, Poland*
[2] *Faculty of Chemistry, University of Marie Curie –Skłodowska, Lublin, Poland*

ABSTRACT

Whey protein isolate (WPI) – xanthan gum heat-induced gels were obtained at pH range 5-10. The rheological properties of WPI (3%) – xanthan gum (0.1 and 0.5%) were examinated. The highest apparent viscosity had mixtures obtained at pH 7 and 0.1% polysaccharide concentration. At higher xanthan gum concentration mixtures obtained at pH 5 and 7 had similar apparent viscosity. The shear stress at fracture of WPI (10%) – xanthan gum mixed gels reached a maximum (17.9 kPa) at pH 7 and 0.3% addition of polysaccharide. TEM and SAXS methods, used to examine microstructure of mixed gels showed phase separation in WPI – xanthan gum mixed systems.

Key words: protein, polysaccharide, TEM, SAXS, phase separation, gel.

INTRODUCTION

Proteins and polysaccharides are both present in many kinds of food systems. These biopolymers contribute to the structure, texture and stability of food, through their thickening and gelling behaviour.

Whey proteins are utilized as functional ingredients in many foods because of their ability to form gels with desirable nutritional, sensory and physicochemical characteristics [14]. The heat-induced gelation of whey

protein-water systems has been described as a two-stage process in which the denaturation of native protein is followed by subsequent aggregation. The aggregates form a three-dimensional network that entraps water trough capillary forces. The physicochemical properties of these gels are primarily determined by structural organization and interactions of the protein molecules, and these characteristics are altered by changes in environmental conditions, such as solvent composition or temperature [4].

Xanthan gum (XG) is water-soluble polysaccharide produced by Xanthonomas campestris. XG consists of 1,4-linked β-D-glucose residues, having a trisaccharide side chain attached to alternate D-gucosyl residues. The backbone of the polymer is similar to cellulose. The side chains are β-D-mannose-1,4→ β-D-glucuronic acid-1,2-α-D-mannose, where the internal mannose is mostly O-acetylated and the terminal mannose may by substituted by a 4,6-linked pyruvic acid ketal [7].

In aqueous solutions, XG shows a conformational transition from a disordered chain conformation at elevated temperatures and low ionic strength to an ordered shape at physiologically relevant temperatures and salt concentrations [11]. Xanthan solutions show thickening properties, with a pseudoplastic behaviour, which is very stable in wide range of pH and temperature [6].

Mixed gels are formed from blends containing more than one gelling agent and may be classified into three types: interpenetrating, coupled and phase-separated networks [10]. Interpenetrating networks represent the simplest situation, where two components gel separately and form independent networks. Coupled networks are formed in the presence of favourable intermolecular interactions between the different types of biopolymers, however phase separated gels are formed by incompatible polymers.

In food industry protein – polysaccharide mixtures have been used in stabilization of oil-in-water emulsions [18], and modification of rheological properties of solutions and gels [13,14]. However, expanding of protein – polysaccharide mixed systems utilizations by the food industry, a better knowledge about interactions between these biopolymers is required.

In this context, we studied the effect of xanthan gum concentration and pH on the rheology and microstructure of whey protein gels.

METHODS

Whey protein isolate (WPI) – BIPRO from Davisco Foods, USA (91.9% protein) and xanthan gum (XG) (Sigma Chemical Co., USA) were used.

Preparing of solutions and gels

Stock solutions of WPI and XG were prepared separately. WPI solutions were prepared at 6 or 20% (w/w) protein in 0.1 M NaCl by stirring for 2 h at ambient temperature. Solutions of XG (0.2-1.0% w/w) were prepared in 0.1 M NaCl by stirring for 120 min at ambient temperature. Stock solutions were mixed in the ratio of 1:1and pH was adjusted in the range 3-10 with 1.0 M NaOH or 1.0 M HCl.
Mixed gels were prepared as previously. Solutions were poured into glass tubes (8 mm inner diameter) lubricated with soya oil, and heated in water bath at 85°C for 30 min. After heating, samples were immediately cooled and stored for 24 h at 5°C.

Rheological measurements

Rheological properties of whey protein – carrageenan mixed solution were investigated using Brookfield RV II (Brookfield Engineering Laboratories, Inc., Stoughton, MA, USA) viscometer equipped with a coaxial measuring system. Temperature was maintained by Fisherbrand FBH 600 thermostat (Fisher Scientific, Schwerte, Germany). The apparent viscosity vs. temperature measurements were performed at 5 1/s. Solutions were heated from 20°C to 85°C, held for 5 min, than cooled back to 20°C.

Texture measurements

Obtained mixed whey protein – carrageenan gels were removed from the tubes and cut to 8 mm lengths using a scalpel. Uniaxal compression to failure was used to measure true shear stress at fracture (stress) and true shear strain at fracture (strain) of gels. A TA-XT2i texture analyzer (Stable Micro Systems, Haslemere, UK) was used to compress gels between two parallel plates at crosshead speed of 1mm/s. Six measurements were done for each of the 3 replications. Gels were treated as incompressible materials, and true shear strain at fracture (ε_{cH}) was calculated as: $\varepsilon_{cH} = -\ln [1-(\Delta h/h)]$, where h is the height of the uncompressed sample which fractures after Δh of compression. The compressive stress (σ_c) at fracture was calculated as: $\sigma_c = \text{Force}\ [1-(\Delta h/h)]/\pi r^2$, where r is the initial sample radius.

SAXS measurement
The protein and protein - polysaccharide suspensions (prepared as previously) were transferred into a cuvette and heated for 30 min at 85°C in a water bath. The measurement of small angle X-rays scattering (SAXS) was performed by a Kratky's camera (Military Technical Academy, Warsaw, Poland) using a linear focusing Cu lamp. The monochromatic rays were obtained by applying Ni filter, linear amplifier and a high impulse analyzer. The measurements were performed at a room temperature.

Transmission electron microscopy
Gels samples were cut into 1mm^3 pieces and fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 4 h. After 3 rinses in this buffer, the samples were post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) for 2 h, dehydrated in graded series of ethanol, embedded in Spurr resin (Polyscience Inc., Warrington, USA) and polymerized in vacuum oven overnight at 70ºC [16]. Samples were examined with a BS-500 Tesla transmission electron microscope.

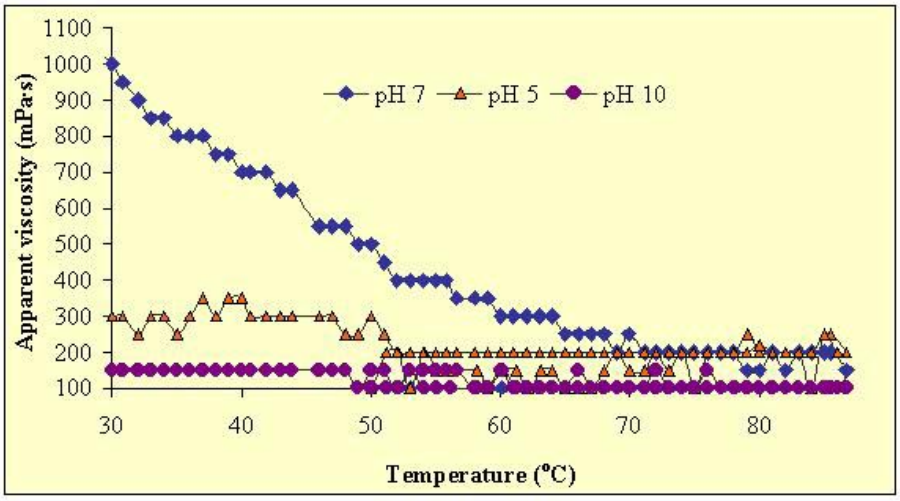
Statistical analysis
The data were analyzed using the Student's t-test by Stat 1 (ISK, Skierniewice, Poland).

RESULTS

The effect of process conditions on the behaviour of biopolymers, like proteins and polysaccharides is a one of the major research area [21]. Depending on the type of process, biopolymers become subjected to different conditions, for example high temperature, ionic strength, flow conditions, etc. These conditions are often very important for the properties of the final product [20].

Figure 1 presents the apparent viscosity curves for WPI (3%) – xanthan gum (0.1%) mixtures obtained at different pH. Biopolymer mixture obtained at pH 7 had the highest apparent viscosity. As the samples were held at 85°C for 5 min, an increase in apparent viscosity was observed. This was probably caused by aggregation of whey proteins due to formation of disulfide bonds and hydrophobic interactions. Cooling down resulted in steady increase in apparent viscosity of mixture obtained at pH 7, from 150 mPa · s at 85°C to above 1000 mPa · s at about 30°C.

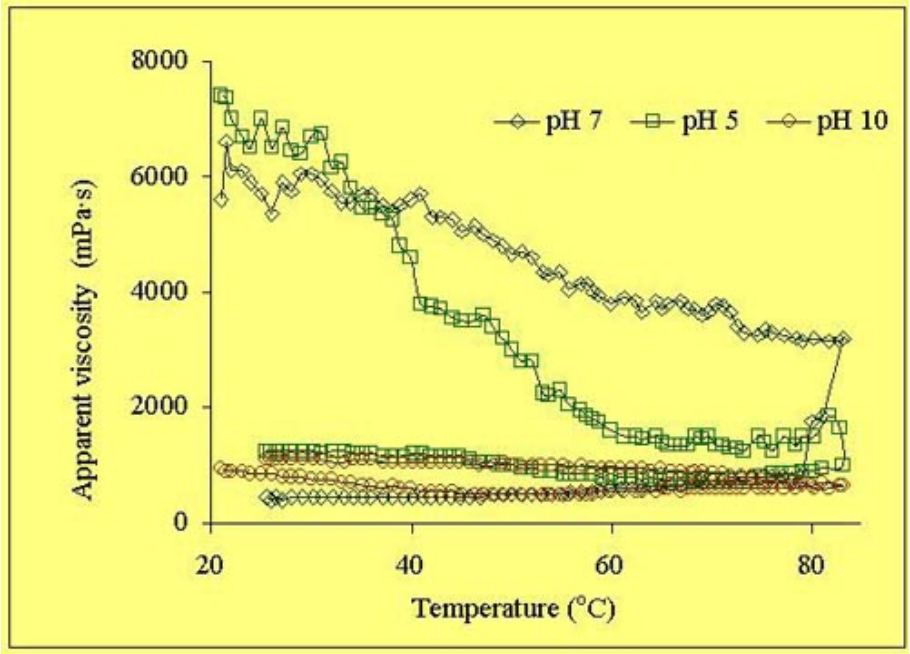
Fig. 1. Effect of pH on apparent viscosity of WPI (3%) – 0.1% xanthan gum mixed solutions as a function of temperature



Higher xanthan gum concentration (0.5%) caused an increase in apparent viscosity of the mixtures (Fig. 2). In the previous research the viscosity of WPI solutions increased with xanthan concentration and showed pronounced shear-thinning behaviour [1]. Heating at 85°C resulted in an increase in apparent viscosity of the mixtures obtained at pH 7 and 5, to values 3100 and 1600 mPa · s, respectively. At higher xanthan gum concentration (0.5%) the biopolymer mixture obtained at pH 5 had the highest apparent viscosity. This was probably caused by the increased size of protein aggregates in presence of xanthan gum or by formation of protein – polysaccharide complexes at this pH. At pH 10 WPI – xanthan gum mixed solutions had very small values of apparent viscosity, which was caused by existence of phase separation between biopolymers. Stading and Hermansson [17] found that pH had significant effect on structure of WPI solutions, especially aggregation

of the main WPI protein - β-lactoglobulin. Xanthan gum solutions are stable at wide range of pH [4]. Walkenstrom et al. [21] suggested that the increase apparent viscosity of WPI solutions after addition of xanthan gum, was caused by the increase of the whey protein aggregation. Other workers suggested that synergy of WPI and xanthan on rheology of mixtures is caused by phase separation of dissimilar biopolymers through volume exclusion phenomena [16,18].

Fig. 2. Effect of pH on apparent viscosity of WPI (3%) – 0.5% xanthan gum mixed solutions as a function of temperature



Shear stress at fracture values of 10% WPI and WPI – xanthan mixed gels obtained at pH 5-10 are shown in Table 1. At pH 5 and 6 WPI gels did not fracture at 80% deformation, and strong syneresis was observed. At pH 7 and 8 WPI gels did not fracture either, however they have different texture in comparison to WPI gels obtained at pH 5 and 6. These gels were very coherent and had low syneresis. Texture of WPI gels obtained at pH range from 6 to 8 was changed on "rupturing", after addition 0.1% xanthan gum. At pH 8 mixed polysaccharide-protein gels were the strongest, however gels obtained at pH 7, 9 and 10 had similar values of shear stress at fracture (Table 1). Sanchez et al. [16] obtained the strongest WPI-xanthan gum mixed gel at pH 7.5 and 0.05 % polysaccharide concentration.

Table 1. Changes in shear stress at fracture of 10% WPI and WPI – xanthan gum gels at different pH and polysaccharide concentration

Shear stress at fracture (kPa)						
XG (%)	**pH 5**	**pH 6**	**pH 7**	**pH 8**	**pH 9**	**pH 10**
0.0	--	--	--	--	13.46^{bc}±2.69	14.25^{b}±3.10
0.1	--	9.98^{b}±0.61	12.48^{c}±1.90	12.94^{c}±2.91	12.52^{c}±1.62	12.43^{c}±0.75
0.2	--	--	14.60^{d}±1.01	14.64^{d}±1.65	11.81^{c}±1.04	8.19^{b}±0.70
0.3	--	--	17.9^{d}±0.6	10.18^{c}±0.53	7.73^{b}±1.07	8.25^{b}±0.91
0.5	--	--	7.30^{a}±0.75	10.07^{b}±0.68	8.19^{a}±0.50	8.04^{a}±0.63

a-g Means within lines with different superscripts significantly different $P<0.05$,
(---) texture unsuitable for measurements

At alkaline range of pH, addition of xanthan gum caused decrease of shear stress at fracture of WPI gels. The increase of polysaccharide concentration to 0.2% caused the increase of WPI gels strength obtained at pH 7 and 8, however at pH 9 and 10 decrease of strength of WPI gels was observed. The shear stress at fracture reached a maximum at pH 7 and 0.3% addition of polysaccharide (17.9 kPa). The further increase of xanthan gum to 0.5% caused decrease of WPI gels strength at the whole pH range.

Mixed WPI-xanthan gum gels had higher values of true shear strain at fracture as polysaccharide concentrations increased, especially at alkaline pH range (Table 2). At pH 7 and 8 the effect of addition of xanthan gum on WPI gels was insignificant. Addition of 0.9% xanthan to heat – induced gels of β-lactoglobulin caused decrease in rigidity and elasticity [22].

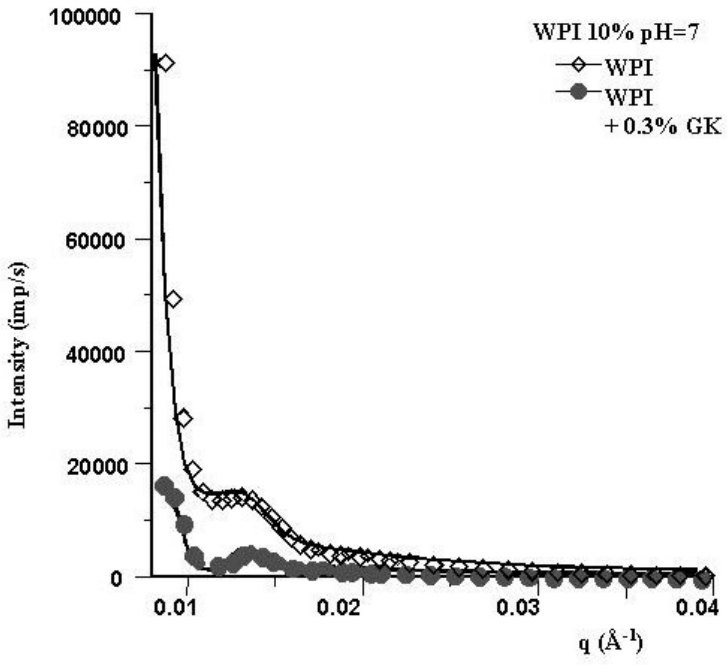
Table 2. Changes in shear strain at fracture of 10% WPI and WPI – xanthan gum gels at different pH and polysaccharide concentration

Shear strain at fracture						
XG (%)	**pH 5**	**pH 6**	**pH 7**	**pH 8**	**pH 9**	**pH 10**
0.0	--	--	--	--	0.90^{b}±0.01	1.10^{b}±0.08
0.1	--	1.21^{c}±0.16	0.88^{b}±0.17	0.94^{b}±0.04	0.93^{b}±0.01	1.13^{c}±0.07
0.2	--	--	1.03^{b}±0.10	0.93^{b}±0.07	1.06^{b}±0.03	1.23^{c}±0.08
0.3	--	--	0.92^{b}±0.02	0.95^{b}±0.05	1.08^{c}±0.13	1.20^{d}±0.06
0.5	--	--	0.91^{a}±0.10	1.10^{ab}±0.16	1.07^{ab}±0.16	1.19^{b}±0.13

a-g Means within lines with different superscripts significantly different P<0.05,
(---) texture unsuitable for measurements

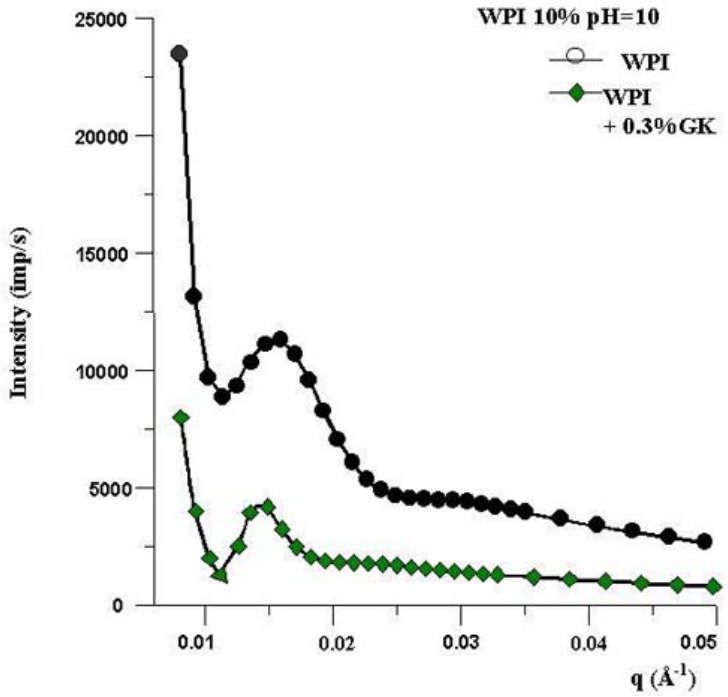
In Figure 3, scattering curves of SAXS for WPI and WPI - xanthan gum mixed gels obtained at pH 7, with polysaccharide concentration of 0.3% are shown. A clear difference may be seen in individual curve profiles. The highest value of scattering intensity was observed for 10% WPI gel, SAXS curve profile shows a maximum with inclination at q ≈ 0.013 $Å^{-1}$. The shape of curve of WPI gel at pH 7 suggests the existence of a particulate structure [3]. Pikus et al. [12] suggested that such curve shape indicates that the structure is very complicated, and probably is formed by coral-thread like structure and loosely isolated globules. The SAXS curve for WPI-xanthan gum mixed gel showed a very small scattering intensity, but it had a low maximum at q ≈ 0.013 $Å^{-1}$. The presence of a peak on the SAXS curve for globular protein gels confirms the regular distribution of globules in the gel structure [12]. Addition of xanthan gum caused the decrease of the difference of electron density between the gel globules and the matrix and the distribution of globules has become more regular in mixed gel. A very small scattering intensity obtained for mixed biopolymer gel suggests that the protein globules of the gel have undergone an aggregation. Formed particles are to large to allow scattering at small angles. Pikus et al. [13] reported similar results for WPI – iota carrageenan mixed gels obtained at pH 7.

Fig. 3. The SAXS curve for WPI (10%) and WPI - xanthan gum(0.3%) mixed gels obtained at pH 7



At pH 10 SAXS scattering curve for gel without polysaccharide shows a distinct peak (Fig. 4), but scattering intensity is low in comparison with WPI gels obtained at pH 7. This shape is characteristic for a structure consisting of separated, spherical particles of similar size [3]. Similar SAXS results were observed earlier by Pikus et al. [12,13]. Mixed WPI - xanthan gum gel obtained at pH 10 produced similar scattering curves to WPI gel but had lower scattering intensity. Much lower scattering intensity was observed for the mixed gel formed at pH 7 than for the gel obtained at pH 10. Similar behaviour of mixed whey protein – kappa carrageenan gels was observed earlier by Mleko et al. [9]. At this pH, both biopolymers are negatively charged, so repulsive forces are predominant. Results of the examination of protein and mixed protein-polysaccharide gel structures clearly demonstrated a significant influence of xanthan gum on WPI structure gels.

Fig. 4. The SAXS curve for WPI (10%) and WPI - xanthan gum(0.3%) mixed gels obtained at pH 10



Microscopic studies (TEM) of WPI-xanthan gum mixed gels obtained at different pH (Fig. 5 and 6), showed large difference in the gel network structure. Micrographs of gels containing whey protein and xanthan gum obtained at pH 7 showed inclusions of polysaccharide beads in the protein network (Fig. 5). This indicates the existence a phase separation in this system. Similar results were obtained for β-lg/xanthan gum mixed gel at pH near neutral [22].

Fig. 5. Electron micrograph of the WPI (10%) – xanthan gum (0.3%) mixed gel obtained at pH 7

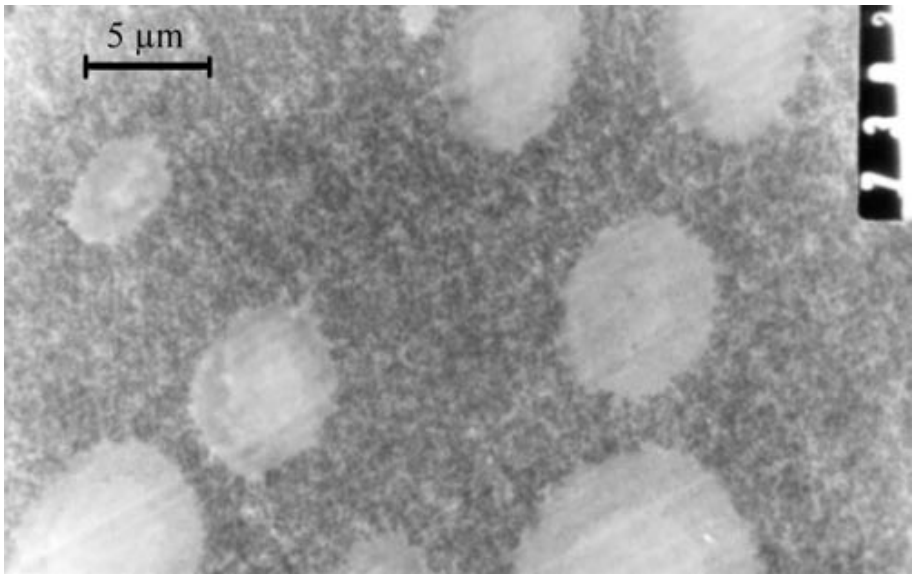
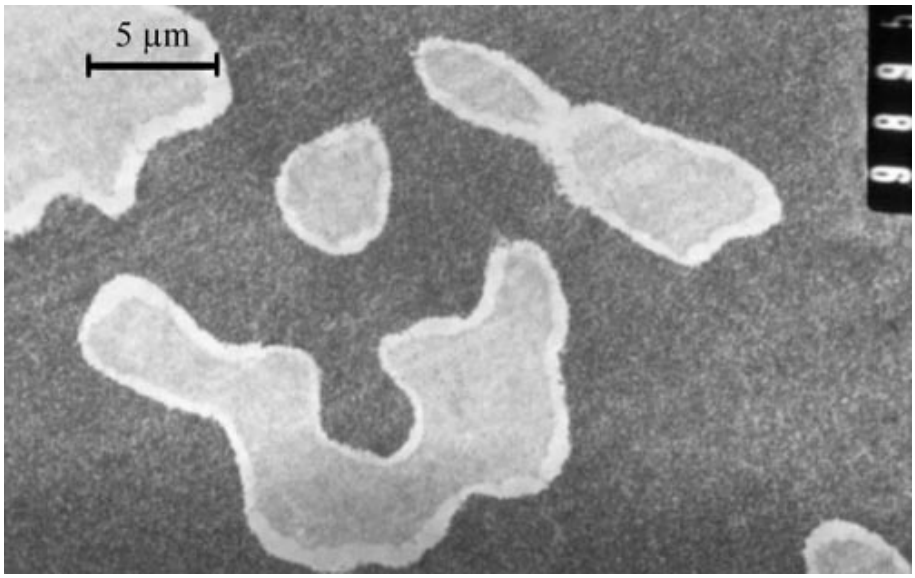


Fig. 6. Electron micrograph of the WPI (10%) – xanthan gum (0.3%) mixed gel obtained at pH 10



At pH 10 extensive phase separation occurred, with higher concentration of each biopolymer in separated phases (Fig. 6).

Interaction between xanthan gum and whey protein isolate were evident in investigated pH range. Addition of xanthan gum caused the increase of viscosity and strength of whey protein isolate solutions and gels, especially at pH 7. A clear difference can be seen in microstructure of xanthan-WPI mixed gels and WPI gels. At pH 10 microstructure of the mixed gels differences were more clear. At this range of pH, above protein pI, when these two macromolecules are mixed together in water, phases separation occurs, leading either to separate phases or concentration of both macromolecules in one phase [19]. Zasypkin et al. [22] and Syrbe [18] observed phase segregation for protein – polysaccharide mixed systems following protein denaturation caused by thermal or high pressure treatment. However, sulphated polysaccharides as carrageenans can form soluble complexes at pH above the isoelectric point of the protein [5]. Walkenstrom et al. [20] suggested that in mixed WPI – xanthan system, polysaccharide inhibits aggregation behaviour of whey proteins. As results of this sterical inhibition, weaker gels with more homogenous network were found compared to pure whey protein gel.

At polysaccharide concentrations 0.2 – 0.3%, a positive effect of the addition of xanthan gum on the rheological properties WPI gels was observed. Microstructure of these gels was more homogenous than at higher polysaccharide concentrations. Probably at lower xanthan gum concentrations (0.2-0.3 %) effect of thermodynamical incompatibility between biopolymer molecules was beneficial for WPI gels properties, however at higher polysaccharide concentration (0.5%) phase separation had deteriorating effect. Dickinson and McClements [2] suggested that even when a mixed biopolymer system is thermodynamically unstable, phase separation may not be observed on the experimental time scale. Phase separation of mixed biopolymer systems can have a detrimental effect on product quality, however, it can be utilized to create foods with novel appearances and textures [16].

CONLUSIONS

1. Xanthan gum caused an increase of apparent viscosity of WPI solutions, especially at 0.5% polysaccharide concentration and pH 7.
2. The optimal level of xanthan gum concentration and pH, which produced gels with the highest shear stress was 0.3% at pH 7.
3. SAXS method showed significant difference between microstructure of WPI and WPI-xanthan gum mixed gels. In xanthan gum presence, protein aggregates were larger, especially at pH 7.
4. Microscopic analysis showed existence of phase separation between whey proteins and xanthan gum in mixed gels obtained at pH 7 and 10.

REFERENCES

1. Bryant C.M., McClements D.J., 2000. Influence of xanthan gum on physical characteristics of heat-denatured whey protein solutions and gels. Food Hydrocoll. 14, 383-390.
2. Dickinson E., McClements D.J., 1995. Advances in food colloids. Blackie Academic & Professional, London.
3. Eastoe J., 1995. Small-angle neutron scattering and neutron reflection. In: E. Dickinson (Ed.). New physico-chemical techniques for the characterization of complex food systems, Blackie Academic & Professional, Glasgow.
4. Gosal W., Ross-Murphy S., 2000, Globular protein gelation. Curr. Opinions Colloid Interface Sci. 5, 188-194.
5. Grindberg V., Tolstoguzov V., 1997. Thermodynamic incompatibility of protein and polysaccharides in solution. Food Hydrocoll. 11, 145-158.
6. Gustaw W., Achremowicz B., Mleko S., 1999. Effect of NaCl on rheological properties of chosen hydrocolloids and their mixtures. Żywn. Nauka Technol. Jakość 1(18), 38-48 [in Polish].
7. Jansson P.E., Kenne L., Lindberg B., 1975. Structure of the extracellular polysaccharide from Xanthomonas campestris. Carbohydr. Res. 45, 275-282.
8. Le Bon C., Nicolai T., Durand D., 1999. Growth and structure of aggregates of heat-denaturated β-lactoglobulin, 34, 451-465.
9. Mleko S., Li-Chan E., Pikus S., 1997. Interactions of κ-carrageenan with whey proteins in gels formed at different pH. Food Res. Int. 30(6), 427-433.
10. Morris V., 1986. Multicomponent gels. In: G. O. Philips, D.J. Wedlock & P.A. Williams (Eds.). Gums and stabilisers for the food industry. Elsevier, London.
11. Norton J., Goodall D., Frangou S., Morris E., Rees D., 1984, Mechanism and dynamics of conformational ordering in xanthan polysaccharide. J. Mol. Biol. 175, 371-394.
12. Pikus S., Gustaw W., Kobylas E., 2000, Examination of heat-induced milk proteins gel structures by the small angle X-rays scattering (SAXS) method. Pol. J Food Nutr. Sci. 4, 61-68.
13. Pikus S., Gustaw W., Mleko S., Kobylas E., 2001, Microstructure and rheology of whey protein isolate – iota carrageenan mixed gels. Ann. Univ. Mariae Curie Skłodowska Sect. AA 12, 171-183.
14. Puyol P., Perez M.D., Calvo M., 1999. Rheological properties of commercial whey protein samples from the MADGELAS survey. Int. J. Food Sci. Technol. 34, 565-572.
15. Reynolds E., 1963. The use of the lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17, 208-216.
16. Sanchez C., Schmitt C., Babak V., Hardy J., 1997. Rheology of whey protein isolate-xanthan mixed solutions and gels. Effect of pH and xanthan concentration. Nahrung 41, 336-343.
17. Stading M., Hermansson A-M., 1990. Viscoelastic behaviour of β-lactoglobulin gel structures. Food Hydrocoll. 4(2), 121-135.
18. Syrbe A., Bauer W., Klostermayer H., 1998. Polymer science concepts in dairy systems – an overview of milk protein and food hydrocolloid interaction. Int. Dairy J. 8, 179-193.
19. Tolstoguzov V., 1991. Functional properties of proteins and role of protein-polysaccharide interaction. Food Hydrocoll. 4, 429-468.
20. Walkenstrom P., Nielsen M., Windhab E., Hermansson A-M., 1999. Effects of flow behaviour on the aggregation of whey protein suspensions, pure or mixed with xanthan. J. Food Eng. 42, 15-26.
21. Walkenstrom P., Panighetti N., Windhab E., Hermansson A-M., 1998. Effects of fluid shear and temperature on whey protein gels, pure and in mixture with xanthan. Food Hydrocoll. 12, 469-479.
22. Zasypkin D., Dumay E., Cheftel J., 1996. Pressure- and heat-induced gelation of mixed β-lactoglobulin/xanthan solutions. Food Hydrocoll. 10, 203-211.

Waldemar Gustaw, Zdzisław Targoński, Paweł Glibowski, Stanisław Mleko
Department of Food Technology
Agricultural University of Lublin
Akademicka 13, 20-950 Lublin, Poland
Fax: +48 81 533-35-49
e-mail: wgustaw@agros.ar.lublin.pl