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Microstructure and rheology of whey protein isolate-iota carrageenan mixed gels

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The small angle X-ray scattering (SAXS) method was applied to examine the whey protein isolate (WPI) – iota carrageenan mixed gel structures. The results obtained by means of the SAXS method were compared with the rheology of these gels. The SAXS curves of different WPI + iota carrageenan gel structures obtained at different pH were versatilely analysed by fulfilling the power law scattering requirements. The fractal dimensions of individual gel structures were estimated. The formation of different protein gel structures, which depended on pH values as well as on the amount of iota-carrageenan in a sample, was confirmed by SAXS curves appearances and fractal dimension. It was found that the fractal dimension of individual protein gels were changed after the addition of a small amount of iota-carrageenan. The correlation between shear strain and stress values and structure parameters obtained by the SAXS method was observed.

1. INTRODUCTION

Proteins and polysaccharides are widely used hydrocolloids in the food industry [1]. One of the main functional properties of proteins is their ability to form gels during heating in suitable conditions [2]. The ability of proteins to form gels as well as the type of protein gel structures formed depends on the equilibrium between the repulsive and attractive forces, which, in turn, depend on the existing pH value, protein concentration, and ionic strength of the protein suspensions [3].

Polysaccharides, especially carrageenan, are widely used in the dairy industry as stabilising, thickening and gelling agents. Carrageenans are anionic polysaccharides extracted from red seaweed. They are highly sulphated and the different types of carrageenan vary in the number and positions of sulphate groups on the galactose dimer. The two most common types of gel forming carrageenans are iota and kappa carrageenan [4].

Gelation of mixed aqueous solutions of polysaccharides and proteins has recently received much attention owing to the commercial availability of several new food ingredients and their use in creating products with specific textures e.g. whey protein desserts [5, 6]. Mixed gels are formed from blends containing more than one gelling biopolymer; their classification comprises three types: interpenetrating, coupled, and phase-separated networks [7]. In many cases, the entropic contribution is often greater than the enthalpic one, which is why the phase separation of biopolymers is generally the rule [8].

The three-dimensional structure of protein gels determines many functional properties of food such as texture or water- and fat-binding ability as well as the diffusion properties, etc.. A number of researchers have focused their attention on this aspect of protein gel structures. One of the methods that enable the examination of protein gel structures is the small angle X-rays scattering (SAXS).

The scattering intensity of radiation at small angles $I(q)$ depends on the electron density difference between the scattering phases and the surroundings as well as on the size and shape of scattered objects which may have dimensions in the range of 10-10000 Å and on the concentration of the examined objects i.e. the possibility of occurrence of the secondary interference between them [9].

For many systems it is difficult to establish the exact influence of the shape, size and concentration effects by the SAXS method. Normally, the approach is to search and obtain a system in which only one of the above criteria plays the dominant role. For example, during the examination of the globular structure of protein gels, it is possible to eliminate the intermolecular scattering effect by a dilution process and only then to estimate a number of parameters which describe the shape and size of examined particles.

On the other hand, one can utilise the differences that exist in the scattering curves by assuming that in various samples the interference of scattering radiation occurs for a given scattering body with a definite protein concentration. Intermolecular interferences have a significant effect on the scattering profiles of SAXS (as well as on the small angle neutrons scattering – – SANS); this effect depends not only on the particle concentration but also on the type of spatial distribution [10]. The latter property can be used in examining the structure of many systems, e.g. the structure of protein gels [11].

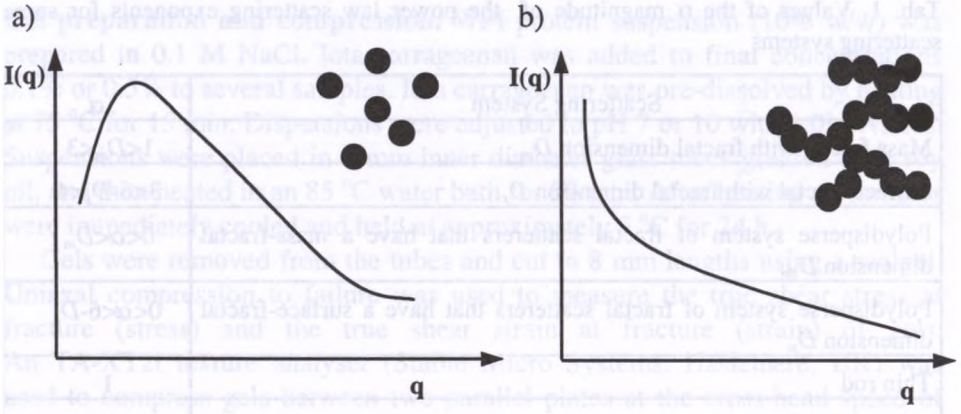

Fig. 1. An example of scattering curve shape of two structures which differed in spatial distribution of primary particles

In Figure 1, the scattering curve profiles for two exemplary different structures are shown. The two structures analysed were formed by identical primary particles but differed in their spatial particle distributions. Such two structures can also describe certain structures of protein gels.

In the last few years, new developments have given rise to a new interpretation of SAXS scattering curves for different systems. It has been confirmed that SAXS scattering effect can be used to estimate a wide range of q values in the form of power law for large numbers of disordered, complex and condensed systems [12]:

$$I(q) = I_o \cdot q^{-\alpha}$$

where:
I_o, α – constants
$q = (4\pi\sin\theta)/\lambda$ – scattering vector modulus
2θ – scattering angle
λ – wavelength

An interesting information can be obtained on the basis of α-coefficient values. The values of this coefficient allow identify a type of structure in the examined system. This also concerns the fractal systems and the determination of electron density profile changes at the interface between scattering objects and surroundings. In Table 1, the α-coefficient values for various types of structure are shown. The presented α-coefficient values are obtained with the use of point-collimated beams; for a slit-collimated camera, these values should be reduced by a factor of 1.

Tab. 1. Values of the α magnitude of the power law scattering exponents for some scattering systems

Scattering System	α
Mass fractal with fractal dimension D_m	$1<D_m<3$
Surface fractal with fractal dimension D_s	$3<6-D_s<4$
Polydisperse system of fractal scatterers that have a mass-fractal dimension D_m,	$0<\alpha<D_m$
Polydisperse system of fractal scatterers that have a surface-fractal dimension D_s,	$0<\alpha<6-D_s$
Thin rod	1
Thin plane lamina	2
Non-fractal scatterer with smooth boundary	4
Porous solid with smooth pore boundaries and a continuous power-law density transition with a power-law-transition exponent β	$4<4+2\beta<6$

For the last few years, various experimental techniques in rheology, microscopy, scattering, and gel permeability have been applied to investigate fractal structures in protein gels [13-20].

Recent investigations of examined condensed protein materials (e.g. protein gels) by the SAXS method have concentrated on the differentiation of various kinds of protein gel structures [14, 21-23] and estimations of fractal dimensions [24].

As shown above, the SAXS method allows for do determine fractal dimensions if the power law scattering conditions are fulfilled and if the α-exponent attains optimal values. Nevertheless, it is practically impossible to provide the ideal power law scattering conditions. They are affected by both the statistical experimental errors and the nature of the examined material.

The aim of this work was to analyse the profile of the SAXS curve for heat-induced WPI gels obtained at different pH and with different amounts of iota carrageenan. The results were then compared with the rheology of mixed gels.

2. MATERIAL AND METHODS

In this study, Iota carrageenan (Sigma Chemical Co., St. Louis, USA) and whey protein isolate (WPI), (BIPRO, Davisco Foods, USA), 91.87% protein content, were used.

Gel preparation and compression. WPI protein suspension (10% w/w) was prepared in 0.1 M NaCl. Iota carrageenan was added to final concentrations 0.1% or 0.5% to several samples. Iota carrageenan was pre-dissolved by heating at 75 °C for 15 min. Dispersions were adjusted to pH 7 or 10 with 1.0M NaOH. Suspensions were placed in 8 mm inner diameter glass tubes greased with soy oil, and then heated in an 85 °C water bath for 30 min. After heating all samples were immediately cooled and held at approximately 5 °C for 24 h.

Gels were removed from the tubes and cut to 8 mm lengths using a scalpel. Uniaxal compression to failure was used to measure the true shear stress at fracture (stress) and the true shear strain at fracture (strain) of gels. An TA-XT2i texture analyser (Stable Micro Systems, Haslemere, UK) was used to compress gels between two parallel plates at the cross-head speed of 1 mm/s. Six samples of gels were evaluated during each treatment. Each treatment was repeated three times. Gels were treated as incompressible materials, and the 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: σ_c = Force $[1-(\Delta h/h)]/\Pi r^2$, where r is the initial gel radius. The shear stress at fracture (σ_{cs}) was calculated as: $\sigma_{cs} = 0.5(\sigma_c)$ [25].

SAXS measurement. The measurement of small angle X-rays scattering (SAXS) was carried out using Kratky's camera (produced by Military Technical Academy, Warsaw, Poland) with a linear focusing Cu tube. The monochromatic rays were obtained by applying a Ni filter, a linear amplifier, and a high impulse analyser. The geometry of the SAXS camera and other conditions of the SAXS experiments allowed for treating the obtained scattering curves as slit-smeared data for a beam with an infinite length. The background scattering curves (for the empty cuvette) were each time subtracted from the scattering curve for the investigated sample [26]. The measurements were performed at the room temperature.

3. RESULTS

SAXS measurements. Figure 2 shows the SAXS scattering curves for the WPI protein gel samples obtained at pH=7 with and without iota carrageenan. It can be observed that the SAXS curve without iota carrageenan demonstrates the greatest scattering with a quite indistinct peak. The presence of a peak on the SAXS curve for globular protein gels confirms the regular distribution of globules in the gel structure. This would indicate that the structure of gel in this sample is very complicated, because many globular gels in the form of coral

thread-like structures were formed [27] whereas a certain number of them remained in the form of loosely isolated globules.

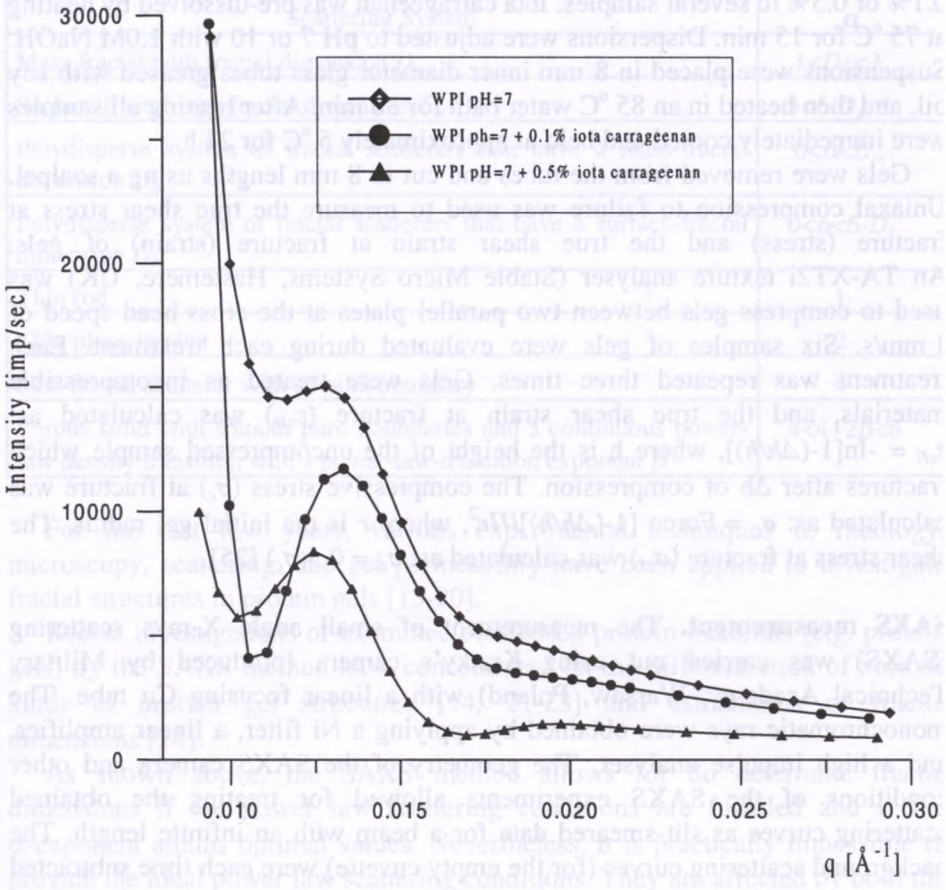

Figure 2. The SAXS curve for the WPI gels at pH =7

The 0.1% increase in iota carrageenan significantly increases the peak value on the SAXS curve but it can also be observed that the SAXS scattering intensity is much lower for this sample. This means that the difference of electron density between the gel globules and the matrix decreases and that the distribution of globules has become more regular in the gel with iota carrageenan. A further increase of iota carrageenan up to 0.5% continues to decrease the SAXS scattering; the peak also remains less distinctive. This means that an increase in iota carrageenan causes a further decrease of the electron density difference between globules and the matrix as well as a decrease in regularity of globule distribution.

Figure 3 shows the same scattering curves that are charted in Figure 2 but in the log-log scale. This method of presentation allows to determine whether scattering curves fulfil the scattering power law, i.e. whether they yield a rectilinear run. As Figure 2 shows, the curves have a rectilinear run with a varying slope within the significant range of q (from approximately 0.02 to 0.11). The α factor of the scattering power law has been calculated on the basis of the slope of the curves. The obtained data are shown in Table 2. (The SAXS measurements were made using slit-collimated equipment, which is why 1 was added to the SAXS curve slope values in order to determine α).

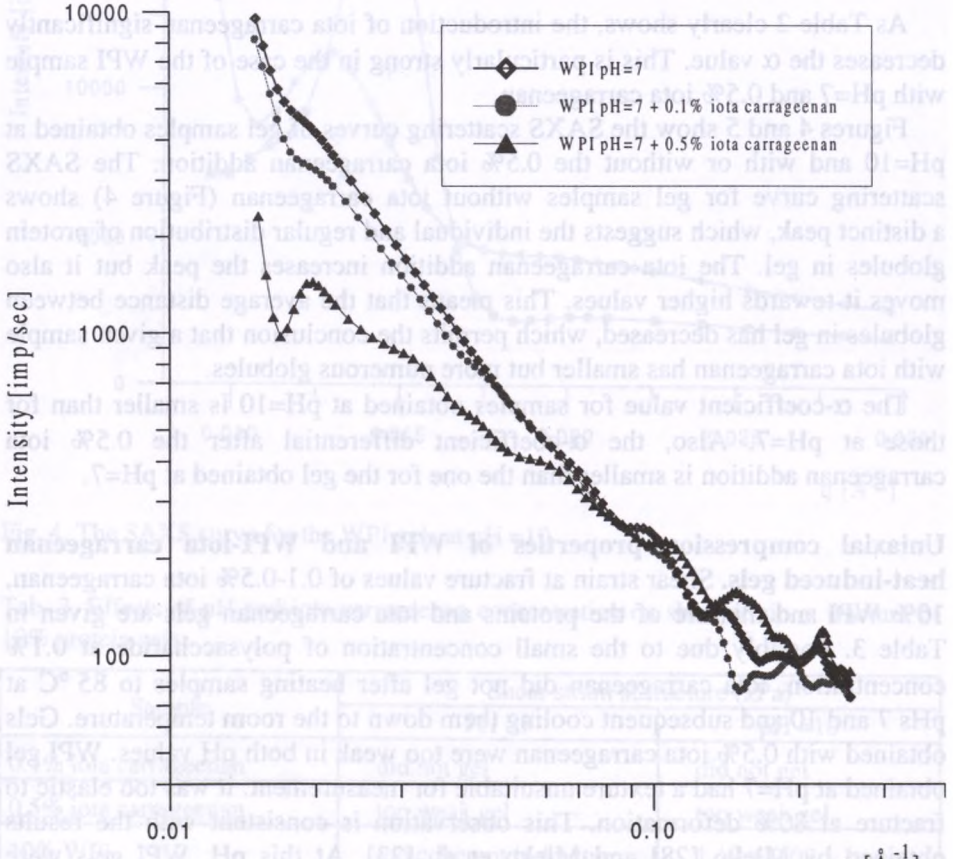

Fig. 3. The profile of logI vs. logq plot dependence for WPI gels at pH = 7

Tab. 2. Values of the α magnitude for investigated samples

Sample	α
WPI pH=7	2.91
WPI pH=7 + 0.1% iota carrageenan	2.75
WPI pH=7 + 0.5% iota carrageenan	2.14
WPI pH=10	2.71
WPI pH=10 + 0.5% iota carrageenan	2.49

As Table 2 clearly shows, the introduction of iota carrageenan significantly decreases the α value. This is particularly strong in the case of the WPI sample with pH=7 and 0.5% iota carrageenan.

Figures 4 and 5 show the SAXS scattering curves of gel samples obtained at pH=10 and with or without the 0.5% iota carrageenan addition. The SAXS scattering curve for gel samples without iota carrageenan (Figure 4) shows a distinct peak, which suggests the individual and regular distribution of protein globules in gel. The iota carrageenan addition increases the peak but it also moves it towards higher values. This means that the average distance between globules in gel has decreased, which permits the conclusion that a given sample with iota carrageenan has smaller but more numerous globules.

The α-coefficient value for samples obtained at pH=10 is smaller than for those at pH=7. Also, the α-coefficient differential after the 0.5% iota carrageenan addition is smaller than the one for the gel obtained at pH=7.

Uniaxial compression properties of WPI and WPI-iota carrageenan heat-induced gels. Shear strain at fracture values of 0.1-0.5% iota carrageenan, 10% WPI and mixture of the proteins and iota carrageenan gels are given in Table 3. Possibly due to the small concentration of polysaccharide at 0.1% concentration, iota carrageenan did not gel after heating samples to 85 °C at pHs 7 and 10 and subsequent cooling them down to the room temperature. Gels obtained with 0.5% iota carrageenan were too weak in both pH values. WPI gel obtained at pH=7 had a texture unsuitable for measurement: it was too elastic to fracture at 80% deformation. This observation is consistent with the results obtained by Mleko [28] and Mleko et al. [23]. At this pH, WPI gels were defined as particulate/fine-stranded [28] or fine-stranded [29]. The addition of 0.1% carrageenan to WPI gels at pH 7 causes a decrease in shear strain at fracture. For the higher concentration of iota carrageenan (0.5%), a coagulum was obtained.

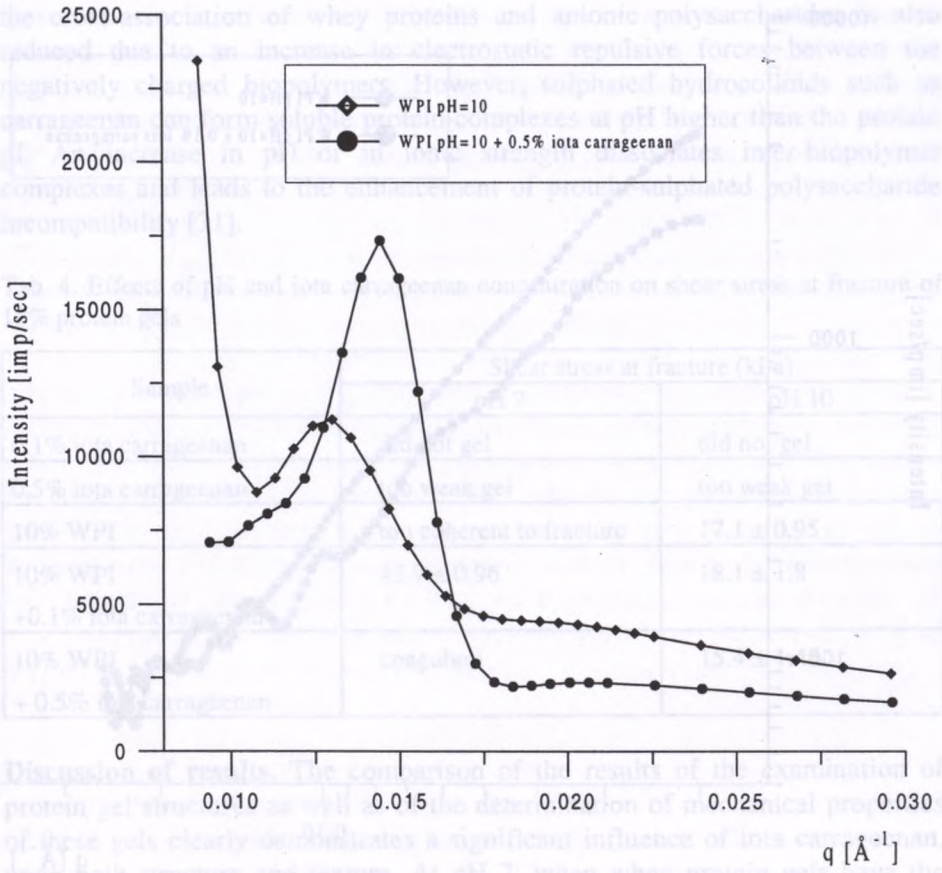

Fig. 4. The SAXS curve for the WPI gels at pH =10

Tab. 3. Effects of pH and iota carrageenan concentration on shear strain at fracture of 10% protein gels

Sample	Shear strain at fracture (kPa)	
	PH =7	pH =10
0.1% iota carrageenan	did not gel	did not gel
0.5% iota carrageenan	too weak gel	too weak gel
10% WPI	too coherent to fracture	1.10 ± 0.08
10% WPI +0.1% iota carrageenan	1.49 ± 0.03	1.38 ± 0.14
10% WPI + 0.5% iota carrageenan	Coagulum	1.45 ± 0.07

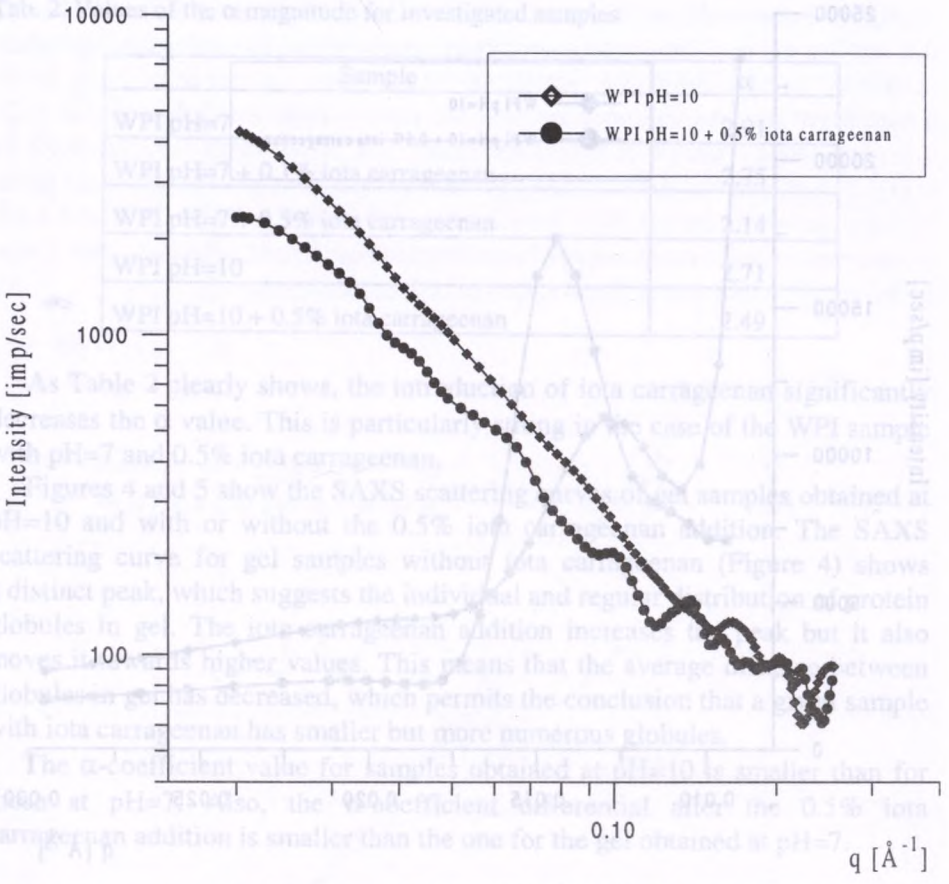

Fig. 5. The profile of logI vs. logq plot dependence for WPI gels at pH = 10

Sanchez et al. [30] observed the deleterious effect of high xanthan concentration (0.5%) on the mechanical properties of WPI gels at pH 7.0. At pH 10 carrageenan caused an increase in shear strain at fracture with an increase in the polysaccharide concentration.

Gel with the highest shear stress value was obtained at pH=7 for 0.1% iota carrageenan and 10% WPI mixed system (Table 4). At pH 10, the presence of the same concentration of polysaccharide causes an increase in shear stress of mixed gels whereas at a higher carrageenan concentration shear stress decreased (Table 4). Phase separation probably occurred in mixed WPI-iota carrageenan gels, which is consistent with the investigations carried out by Syrbe [29], who observed such a type of interactions for solutions of anionic hydrocolloids-whey protein at pH 6-7. The segregation in these systems occurs after the whey protein is denaturated by a thermal or high pressure treatment. At pH higher than the whey protein pI, the protein self-association is reduced;

the cross-association of whey proteins and anionic polysaccharides is also reduced due to an increase in electrostatic repulsive forces between the negatively charged biopolymers. However, sulphated hydrocolloids such as carrageenan can form soluble protein-complexes at pH higher than the protein pI. An increase in pH or in ionic strength dissociates inter-biopolymer complexes and leads to the enhancement of protein-sulphated polysaccharide incompatibility [31].

Tab. 4. Effects of pH and iota carrageenan concentration on shear stress at fracture of 10% protein gels

Sample	Shear stress at fracture (kPa)	
	pH 7	pH 10
0.1% iota carrageenan	did not gel	did not gel
0.5% iota carrageenan	too weak gel	too weak gel
10% WPI	too coherent to fracture	17.1 ± 0.95
10% WPI +0.1% iota carrageenan	43.9 ± 0.96	18.1 ± 1.8
10% WPI + 0.5% iota carrageenan	coagulum	15.4 ± 1.45

Discussion of results. The comparison of the results of the examination of protein gel structures as well as of the determination of mechanical properties of these gels clearly demonstrates a significant influence of iota carrageenan, upon both structure and texture. At pH 7, when whey protein gels have the mixed (particulate/fine-stranded) structure, an increase of iota carrageenan by 0.1% causes a change of structure to isolated globules. A further increase in the polysaccharide to 0.5% causes a clear decrease in scattering; the peak on the SAXS curve is smaller compared to that on the curve for a sample with a lower concentration of iota carrageenan. Also, the peak is moved in the direction of lower q values. Such results may suggest that the polysaccharide mainly enters the gel matrix, which results in the decreased scattering, and, at higher concentrations, affects the creation of larger isolated protein globules. This suggestion is clearly confirmed by the changes in the protein gel texture accompanying the increased polysaccharide concentrations (Tables 3 and 4). At higher pH, WPI gel has the fine-stranded structure comprising isolated protein globules. An increase in iota carrageenan at this pH causes an increase in the peak on the SAXS curve while the peak moves towards lower q values. This suggests that the globules created in such conditions are smaller compared to globules in pure protein gel. This structural change in gels may explain an

increase in shear strain at fracture at pH 10 and with polysaccharide compared to pure WPI gels (Table 3). However, at pH 10 iota carrageenan appears to have little influence on the hardness of gels (Table 4).

The examination of structure clearly shows that the analysed samples possessed properties of mass fractals (compare Tables 1 and 2) [24]. An increase in polysaccharide contents caused a decrease in fractal dimensions; this was particularly clear at pH 7. The changes in fractal dimensions may be connected with the changes in structure of investigated gels. At pH 7, significant changes in the rheology were observed; at higher pH's such changes were small (Tables 3 and 4). This clearly correlates with the changes in fractal dimensions obtained from the SAXS measurements (Table 2).

The present analysis constitutes an introduction into the examination of the influence of polysaccharide upon the structure and the rheology of whey protein gels. Considering the obtained results it is possible to state that even a small increase in iota carrageenan contents causes significant changes in the mechanical properties of gels, which was clearly reflected in the results obtained using the SAXS method. However, a more detailed interpretation of the obtained data, and particularly the analysis of the correlation between the SAXS scattering curves and the rheology, requires further researches.

REFERENCES

[1] Kilara A., *Interactions of Ingredients in Food Systems: An Introduction.* in: *Ingredint interations, effects on food quality*. A.G. Gaonkar (Ed.), Marcel Dekker, New York, 1-12, 1995.
[2] Ziegler G.R., Foegeding E.A. , *Adv. Food Nutr.*, 34, 203, (1990).
[3] Mulvihill D.M., Kinsella J.E., *Journal of Food Science*, 53,231, (1988).
[4] Thomas W., *Thickenning and Gelling Agents for Food*. A. Imeson (Ed.), Blackie Academic & Professional, London, 45-59, (1997)
[5] Mleko S., *Milchwissenschaft*, 52(5), 262 (1997)
[6] Mleko S., Gustaw W., *Milchwissenschaft*, 55(3), 149 (2000).
[7] Morris V. 1986, *Multicomponent Gels in Gums and Stabilisers for Food Industry*. G.O. Philips & D.J. Wedlock & P.A. Williams (Eds), Elsevier, London , 87-99, 1986.
[8] Doublier J. L., Garnier C., Renard D., Sanchez C., *Current Opinion in Colloid & Interface Science*, 5, 202 (2000).
[9] Guinier A., Fournet G., *Small-Angle Scattering of X-rays*. John Wiley & Sons, New York, 1955.
[10] Porod G., *Small Angle X-Rays Scattering*, Glater O., Kratky O. (Eds), New York, Academic Press, 17, 1982.

[11] Eastoe J., *New Physico-Chemical Techniques for the Characterisation of Complex Food Systems*. Ed. E. Dickinson, 268, 1995.
[12] Schmidt P.W., *Modern Aspects of Small-Angle Scattering*, H Brumberger (Ed.), NATO ASI Ser., C451,1, 1995.
[13] Bremer L.G.B., van Vliet T., Walstra P., *J. Chem. Soc. Faraday Trans.*, 1, 85, 3359 (1989).
[14] Gimel J.C., Durand D., Nicolai T., *Micromolecules*, 24 , 583 (1994).
[15] Hagiwara T., Kumagai H., Matsunaga T., Nakamura K., *Biosci. Biotech. Biochem.*, 61, 1663 (1997).
[16] Dziuba J., Smoczyński M., Dziuba Z., Smoczyński L., *Milchwissenschaft*, 52, 448 (1997).
[17] Ikeda S., Foegeding E.A., Hagiwara T., *Langmuir*, 15, 8584 (1999).
[18] Verheul M., Roefs S.P.F.M., *Food Hydrocoll.*, 12, 17 (1998).
[19] Verheul M., Roefs S.P.F.M., Mellema J., de Kruif K.G., *Langmuir*, 14, 2263 (1998).
[20] Marangoni A.G., Barbut S., McGauley S.E., Marcone M., Narine S.S., *Food Hydrocoll.*, 14, 61 (2000).
[21] Renard D., Axelos M.A.V., Boue F., Lefebvre J., *Chim. Phys.*, 93, 998 (1996).
[22] Matsumoto T., Chiba J., *J. Chem. Soc. Faraday Trans.*, 86, 2877 (1990).
[23] Mleko S., Li-Chan E., Pikus S., *Food Res. Inter.*, 6, 427 (1997).
[24] Aymord P., Gimel J.C., Nicolai T., Durand D., *J. Chim. Phys.*, 93, 987 (1996).
[25] Hamann D., *Structure failure in solid foods. In Physical Properties of Food.* M. Peleg and E. Bagley (Eds), AVI Publishing Co., 351, 1983.
[26] Glatter O., Kratky O., *Small Angle X-ray Scattering.* O.Kratky, O. Glatter (Eds), London, Academic Press, 1982.
[27] Pikus S., Gustaw W., Kobylas E., *Pol. J. Food Nutr. Sci.*, 9/50, 61 (2000).
[28] Mleko S., 1996, Pol. *J. Food Nutr. Sci.*, 5/1, 63 (1996).
[29] Syrbe A., *Polymer incompatibility in aqueous whey protein and polysaccharide solutions: phase separation phenomena and microgel particle formation.* PhD thesis. Munich: Munich Technical University, 1997.
[30] Sanchez C., Schmitt C., Babak V., Hardy J., *Nahrung*, 41(6), 336 (1997).
[31] Grindberg V., Tolstoguzov V., *Food Hydrocoll.*, 11, 145 (1997).

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