Biopolymer Microstructure and Rheology

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ABSTRACT

There is an increasing need to control rheological properties of food by use of biopolymers. New developments are being made in order to tailor make structures with specific sensory attributes for example in low fat products. The aim of this paper is to demonstrate relationships between structure and sensory properties of single as well as complex mixed biopolymer systems.

The dimension of biopolymer networks span over a broad range. The widths of a gels strand can vary from 10^{-9} to 10^{-6} m and the pore size in the range 10^{-8} to 10^{-8} to 10^{-4} m. Changes from one extreme to the other can be achieved by small changes in experimental conditions. Mixtures of biopolymers give opportunities to create structures with a wide variety of rheological properties. Mixtures of two biopolymers can form discontinuous, bicontinuous or coarcervate structures where the two components merge into one single network.

In order to understand the gel properties we need to understand how biopolymers associate and aggregate into a network structure. Much work has been focused on events taking place at the onset of gelation. More information is needed on how secondary aggregation and phase separation phenomena affects rheological properties of complex biopolymer gels.

BIOPOLYMER GELS

Variations in network structure

Substantial changes in the network structure can be achieved for one single biopolymer just by small changes the experimental conditions. Fig. 1a and b illustrate the variation in pore dimensions and strand thickness of β-lactoglobulin gels obtained at two different pH (1). Fig. 1a shows a light micrograph of a particulate gel

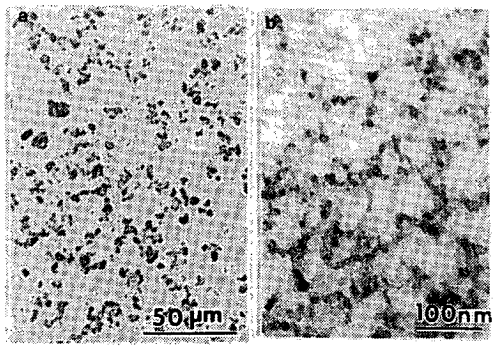

Figure 1 a and b. Sections of β-lactoglobulin gels a) Light micrograph of a gel at pH 5, b) TEM micrograph at pH 7.5 (2).

with pores and strands with dimensions in microns. Fig. 1b shows a fine-stranded gel structure of the same gel visualised in the transmission electron microscope, where the dimensions are in the nanometer regime. For β-lactoglobulin particulate gels are formed in the isoelectric region at pH 4-6 and fine stranded gels above and below this pH range. The range for particulate gels is

slightly broader in the presence of salt and for partially denatured commercial samples. The rheological properties between the two types of network structures differ considerably. When comparing rheological properties it is very important to take both small and large deformation tests into account. Small deformations obtained by oscillatory measurements in shear show that the storage modulus was higher for particulate gels than for fine stranded gels at corresponding concentrations (2). The frequency dependence was slightly higher for particulate gels indicating a higher degree of aggregation than in the fine stranded gels. However the viscoelastic properties do not reveal the enormous differences in gel structure between the two types of gels. The complexity in rheological behaviour is demonstrated by the fracture properties shown in Fig. 2 (3). The particulate gels at pH 4.5-6 vary in fracture stress but have the same stress at fracture, whereas the fine stranded gels below and above this region vary substantially in their rheological behaviour at large deformations. The fine-stranded gels at pH 6.5 and 7.5 can be extended to large deformations, whereas the gel formed at pH 3 is very weak and brittle. The differences in behaviour is to a large extent dependent on the structure of the fine strands. At pH 3 the fine strands are stiff and brittle, whereas they are more curled and elastic at pH above the isoelectric region (3). The results illustrate the complexity in relationships between structure and rheological properties, where different parts of the structure can contribute to the rheological behaviour in different ways.

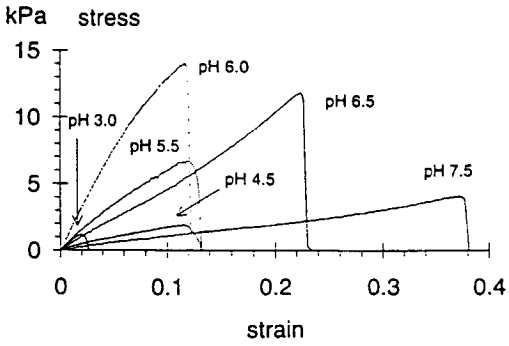

Figure 2. Stress-strain curves in tension of 12 % β-lactoglobulin gels (3).

Fine stranded gels

Fine stranded gels are generally referred to as transparent gels and electron microscopy is necessary to obtain information about the fine structural characteristics of such gels. Even this group of gels displays a wide variety of structures and sometimes a complex rheological behaviour. The simplest case is a fine-stranded homogeneous gel. The β-lactoglobulin gel at pH 7 shown in Fig. 1b is one example. The calcium form of κ-carrageenan shown in Fig. 3 is another (4). The appearance between the two figures differs because the preparation techniques for electron microscopy differs. The micrograph shown in Fig. 1b is a thin section of a gel prepared by chemical fixation, dehydration, plastic embedding and sectioning. The micrograph shown in Fig. 3 is a replica of a monolayer of the supramolecular structure making up the gel network. The resolution obtained by the latter technique is higher but this can only be obtained by spreading a monolayer at concentrations that most often are far below the concentrations necessary for gel formation (5).

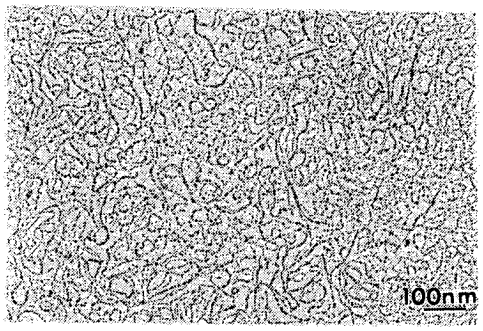

Figure 3. The supramolecular structure of Ca-κ-carrageenan in 30 mM $CaCl_2$ (4).

The strands in the β-lactoglobulin gels are formed by aggregation of globular protein molecules on denaturation. The strands in carrageenan gels are formed by alignments of helices below the coil-helix transition temperature. Typical of these homogeneous flexible fine-stranded gels compared to more aggregated and inhomogeneous gels gel are relatively low storage modulus and very low phase angles at concentrations well above that necessary for gel formation.

Fine-stranded gel can have a far more complex structure than that shown i Fig. 3. A completely different structure of carrageenan gels can be achieved just be changing the cation. Potassium has a vary specific effect on κ-carrageenan and promotes helix formation as well as self-association and aggregation of helices (5). The structural effects are very dependant on potassium concentration, whereas variation in the calcium content does not influence

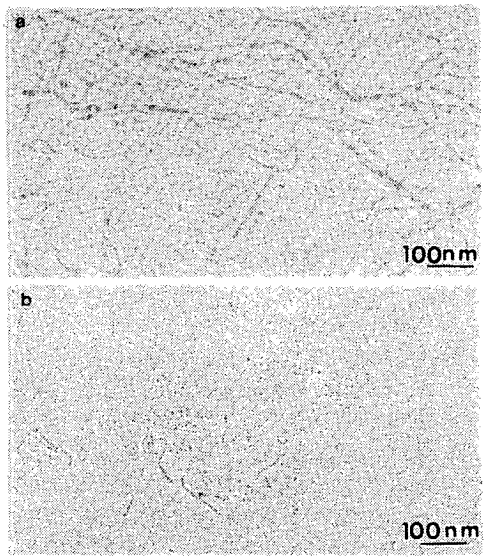

Figure 4a and b. Supermolecular structures of 1 % K-κ-carra-geenan in 100 mM KCl. a) without any other biopolymer b) after addition of locust bean gum (6).

the gel structure providing the concentration is above that necessary for gel formation and below that of salting out (4, 5). Fig. 4a shows the structure of K-κ-carrageenan in the presence of 100mM KCl. At 50-200 mM KCl, helices associate and aggregate into the coarse supermolecular strands shown in Fig. 4a. In the background a fine-stranded structure interpreted as dimers of double helices can be seen. Thus a *mixed* gel structure can be obtained by one single biopolymer! Mixed gel structures generally give rise to strong gels. This is illustrated in Fig. 5, where the storage modulus of 1 % K-κ-carrageenan in 200 mM KCl is far higher than that of the calcium form of κ-carrageenan with a more homogeneous fine-stranded structure. The balance between the fine and coarse aggregated network of K-κ-carrageenan gels depends on the potassium concentration and has a strong impact on the rheological properties (4, 5).

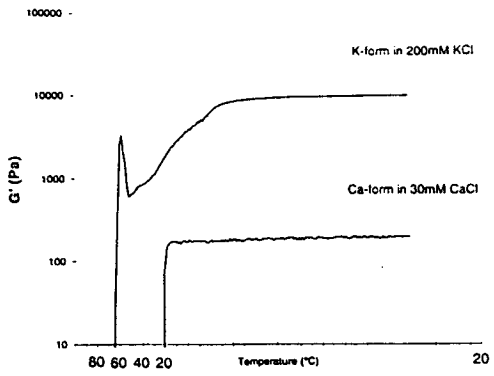

Figure 5 The storage modulus of K- and Ca - forms of κ-carrageenan biopolymer.

Mixed gels

This far examples have been given on how the gel structure of one biopolymer can be manipulated by a change in the experimental conditions such as variations in pH or the ionic composition of the system. At present there is a lot of interest in synergistic effects that can be achieved by mixing biopolymers. There are several types of mixed gels. Phase-separated gels can either be discontinuous similar to that of an emulsion or bicontinuous. Two biopolymers can merge into one network and form a complex coarcevate structure. There are interpenetrating networks where two network structures interact by mutual entanglements and there are cases where synergism is obtained by physical interaction between two biopolymers (6). The way two biopolymers act in a mixed system has a strong impact on the rheological properties of the mixed system.

When other biopolymers are added to κ-carrageenan it has been found that they can adsorb to the carrageenan helices and thereby influence the self-association of carrageenan helices into the coarse supermolecular superstrands This has been demonstrated for both caseins and locust bean gum (6, 7). Locust bean gum has a random coil structure and cannot form gels except under special concentrations and shearing conditions. Fig. 4a shows the coarse supermolecular structure of K-κ-carrageenan in 100 mM KCl alone. When locust gum is added these molecules adsorb onto the κ-carrageenan helices and prevent them from self-association. The result is striking. Fig. 4b shows exactly the same carrageenan system as Fig. 4a after addition of locust bean gum and the coarse supermolecular strand can no longer be seen. The rheological impact due to interactions between these two molecules is enormous. The storage modulus can increase more than 100 times and gels can form at concentrations where carrageenan does not gel by itself. The rheological response depends on factors such as the ratio of the two biopolymers as well as on the mannose to galactose ratio of the locust bean gum used. Apart from the effects of locust bean gum on the degree of self-association of κ-carrageenan, further studies are needed to elucidate other interaction effects between the two biopolymers that might have a bearing on the rheological properties of the mixed system.

In a recent study it has been demonstrated that bicontinuous, discontinuous and coarcevate structures can be obtained by the same mixture of gelatin and whey protein by varying the process and thereby the route of gel formation of the two biopolymers (8, 9). β-lactoglobulin is the main gel forming whey protein and a whey protein isolate has the same type of gel forming behaviour as the previously discussed β-lactoglobulin. Gelatin form fine-stranded gels, where the strands are composed of aligned triple helices.

Whey protein form gels on heating, whereas gelatin form gels on cooling. The whey protein form particulate as well as fine-stranded gels on heating with a similar

appearance to the structures shown in Fig. 1. If gelatin is present the gelatin network form in the pores of the whey protein network. This is a bicontinuous gel structure, where the rheological properties follow those of one of the components depending on their individual strength. Under many practical conditions the rheological behaviour is dominated by the particulate network structure. One important feature is that this type of bicontinuous structure cannot be melted due to the whey protein network.

Quite different structures were obtained if high pressure or combinations of high pressure and temperature treatments were used. In the pH region, where a bicontinuous structure with particulate whey protein formed on heating, a discontinuous phase-separated gel was formed on high pressure heating as illustrated in Fig. 6. Gelatin makes up the continuous phase, whereas whey protein makes up the darkly stained inclusions. This gel has completely different properties than the previously discussed bicontinuous structure. First of all, it melts like gelatin on heating. This can be quite important in low fat applications , where the melting behaviour of fat is to be mimicked.

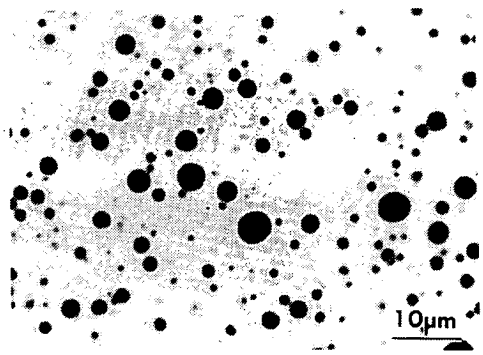

Figure 6. Light micrograph of a gelatin continuous mixed gel; 12 % whey protein and 3 % gelatin (8).

There are also other differences in the rheological characteristics and one example is given in Fig. 7. The frequency dependence is higher and thus dominated by the particulate network in the bicontinuous structure (9) When gelatin is making up the continuous phase the frequency dependency is low, which is typical for a fine-stranded type of gel. We can also see that there is a substantial difference in the magnitude of the storage modulus between the two types of gels.

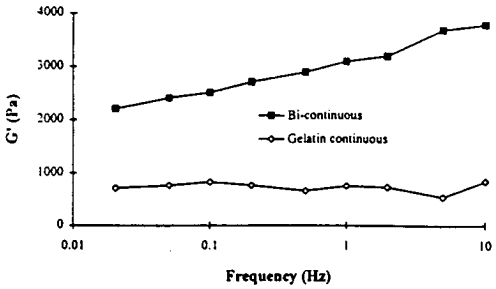

Figure 7. Frequency dependence for a discontinuous and a bicontinuous mixed 12 % whey protein and 3 % gelatin gel (9).

Secondary aggregation

Most fundamental studies of biopolymer gels have concerned events taking place at the onset of gelation such as denaturation of proteins or coil-helix transitions of polysaccharides and gelatin. In order to understand the gel properties we need information on how proteins aggregate and polysaccharide helices associate into a gel network. Secondary aggregation phenomena and structural rearrangements often plays an important role for the final gel properties but have this far been too little studied. One reason for this is that we need to further develop rheological techniques in order to elucidate structural events taking place in an already formed network structure.

Phase transitions and many structural rearrangements are accompanied by a maximum in the phase angle and the loss modulus. Often these events takes place so

fast during the onset of gelation that they are difficult to follow. Sometimes changes take place more slowly in an already formed gels due to cooling of a heat-set gel or melting of a gel formed on cooling. In a recent study the effect of shear in the vicinity of the gel point of pure and mixed whey protein gels has been studied (10). Fig. 8 shows that moderate shear results in a considerable increase in the storage modulus. It is interesting to note that the shear induced enhancement is most pronounced after cooling of the heat set gel. It is difficult to say whether this increase in storage modulus is due to a strengthening of an already existing network or if structural rearrangements take place.

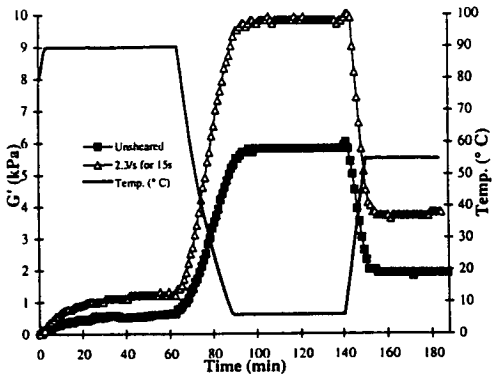

Figure 8. Changes in the storage modulus on thermal treatments of sheared and unsheared 12 % whey protein gels at pH 5.4 (10).

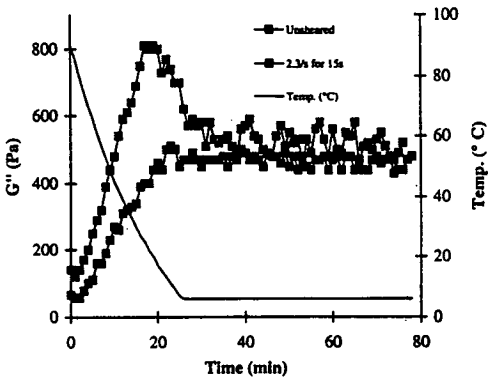

Figure 9. Changes in the loss modulus on cooling of a sheared and an unsheared 12 % whey protein gels at pH 5.4 (10).

More information can be obtained by also studying the changes in the loss modulus. Fig. 9 shows that the shear enhanced storage modulus is accompanied by a maximum in the loss modulus, which is not the case of the unsheared gel. Both curves levels off towards the same end value. The difference in behaviour indicates that some structural rearrangements take place on cooling in the sheared system which does not happen to the same extent in the unsheared sample.

This example also illustrate another important aspect of structure and rheology. Shear was applied just before gel formation and had some impact on protein aggregation. However, the most substantial effects on the viscoelastic properties were observed on cooling of the already set gel. Thus small effects due to variations in processing parameters such as shear may have significant effects on the rheological properties later on in the process chain during food production.

Structural rearrangements often involve local aggregation phenomena that lead to an increased degree of phase separation and/or a more coarsely aggregated system. An example of such a phenomenon can be seen

in Fig. 5. An initial peak in the storage modulus can be observed, whereafter the storage modulus increases to a value above that of the peak value. This peak has been interpreted as a transition from a fine stranded gel structure to aggregation of helices into coarse superstrands and the formation of a mixed structure with a high final storage modulus. The interpretation is based on microscopy findings, where structures have been frozen in different states of the transition (5). Rheologically the transition is accompanied by an increase in the phase angle as the gel becomes more inhomogenous. The presence of a mixed gel also results in complex melting behaviour with local maxima in the phase angle. However, interpretations of transitions in the storage modulus are controversial, because aggregation can also lead to syneresis and a change in volume. Measuring systems for oscillation in shear are not well suited for transitions involving changes in volume. At present most rheologists try to avoid the study of such systems. However, these systems are quite common in mixed biopolymer systems and it would be worth while to develop new techniques, where the rheological measurement allows for and makes it possible to measure volume changes during structural rearrangements in complex biopolymer systems. One possibility is to explore the possibilities of axial oscillation, where the normal force is kept constant throughout the measurement. This technique is mainly used for synthetic polymers but work is going on to develop this technique also for biopolymer systems.

Small and large deformations

Many examples have already been given that illustrate the complexity of rheological behaviour of single and mixed biopolymers. Small deformation tests can give completely different information than large deformation tests and information from more than one test is necessary if we are to understand how structure relates to the rheological behaviour of a system. It has been demonstrated that fine stranded and particulate gels can behave quite differently. Even small variations of one type of structure can lead to considerable differences in the rheological behaviour of a gel.

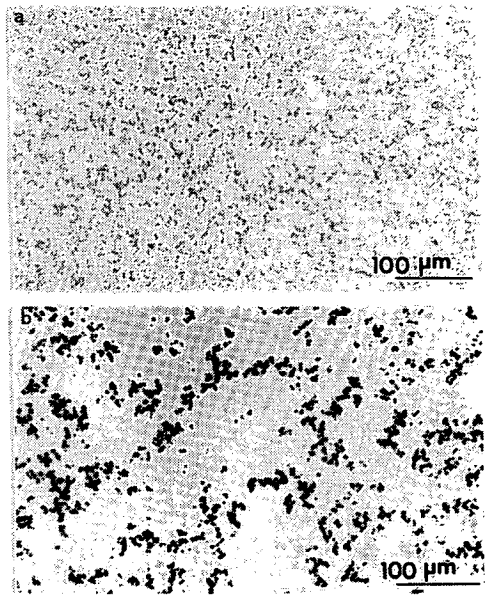

Figure 10 a-b. 10 % β-lactoglobulin at pH 5.3 formed at a) 12 °C/min and b) 1 °C/min (11).

The rate of heating has a strong impact on gel structures, where aggregation is an important part of the gel-forming mechanism. Fig. 10 a-b show light micrographs of 10% β-lactoglobulin gels at pH 5.3 formed at two different heating rates. From these micrographs it can be seen that the two gels vary considerably with regard to pore size. Large deformation tests sense the weakest part of the structure and the gel with the bigger pores gave rise to a lower stress at fracture than the gel with the smaller pores (11). This is illustrated in Fig. 11. The pore size corresponds fairly

well with the size of the measured natural notch, x. modulus for the gel heated at the lower heating rate (11).

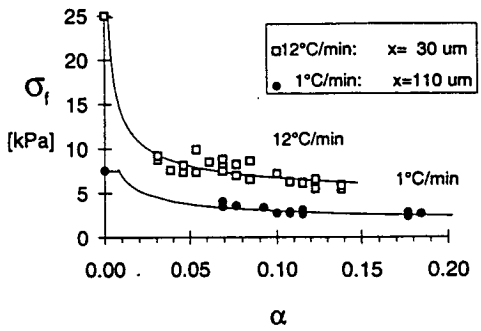

Figure 11. The notch-sensitivity of the fracture stress of 12 % β-lactoglobulin gels at pH 5.3 The heating rates and size of the inhomogeneities,x, are inserted (11).

The opposite behaviour was found in small deformation tests, shown in Fig. 12, where oscillation in shear gave the highest storage modulus for gels heated at the lowest heating rate. Small deformation tests sense different structural characteristics than large deformation tests. The strands characteristics and the mode of aggregation of protein particles are the most important structural attributes for small deformation tests of particulate gels. The particles making up the gel heated at high heating rates are smaller than those heated at low heating rates. They are aggregated like "string of beads" making up more flexible strands than the gels heated more slowly. In the latter gels, protein particles are fused together in forming thicker and stiffer strands (11). The difference between the two strands is schematically depicted in Fig. 13.

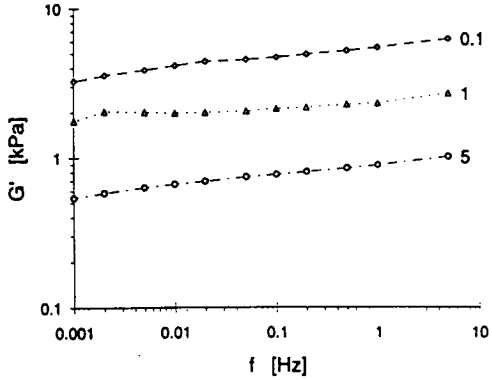

Figure 12. The frequency dependency of 10 % β-lactoglobulin gels at pH 5.3 formed at different heating rates (11).

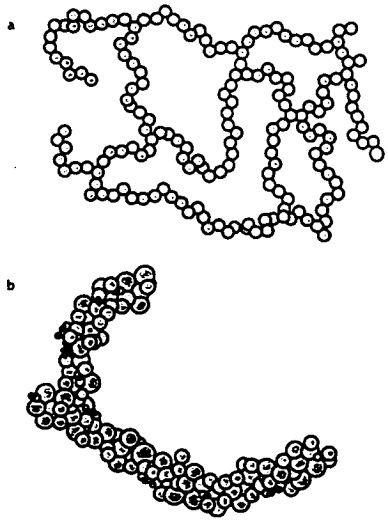

Figure 13. A schematic representation of the strand microstructure of particulate β-lactoglobulin gels at pH 5.3 formed at a) fast heating rates (5-12 °C/min) and at b) slow heating rates (0.1-1 °C/min) (12).

Structure and perceived texture

The example of how the particulate gel structure respond differently to small and large deformation tests indicates that relationships between structure and the sensory perceived texture is far from trivial. It is therefore very dangerous to rely on just

one type of rheological test, when evaluating the texture of a product. However, tools for evaluating the impact of structure on rheological parameters and perceived texture are becoming available, which opens up an interesting field of research. Image analysis can be used to quantify structure parameters. Access to computer power and multivariate techniques makes it possible to establish relationships also for complex model systems and even real food products. Fig. 10-12 have demonstrated the effects of heating rate on pure β-lactoglobulin gels at pH 5.3. The same effects were found for whey protein isolate containing a mixture of proteins , but where β-lactoglobulin is the most important gel forming protein (13). The pore size was determined by image analysis and evaluated statistically according to an experimental design. Fig. 14 illustrates an important aspect of this approach. There is an interactive effect between pH and heating rate. This means that the heating rate is of significant importance for the pore size at pH 5.4 but not at pH 4.6. Such effects are important to establish in real food systems, where several factors can be varied at the same time.

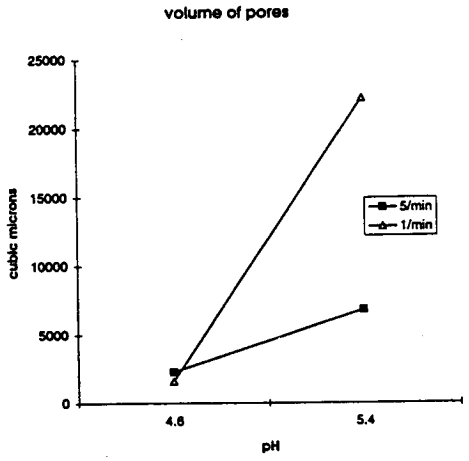

Figure 14. The interaction effect between pH and heating rate on the volume of pores estimated at a magnification of 20x (13).

Whey protein gels were taken as a very simple model system for studies of relationships between structure and perceived texture (14). It was found that structure parameters that influenced large deformation tests such as pore size also influenced sensory attributes when a large force were applied on the sample during analysis such as cutting and chewing. Similarly it was found that there were a number of sensory attributes that were more related to the strand characteristics of the gels. These attributes were found when small deformations were used during sensory analysis such as light pressure on the gel with the forefinger. These relationships between structure, instrumental rheology and sensory analysis of texture are very promising. However this area of research is still in its infancy. The biopolymer gels presented here are very simple compared to the complex colloidal structures of real food products. In future research we can add to the complexity by clarifying the importance of other structural components and increase our understanding of the sensory perception of texture. Hopefully new rheological instrumental techniques will also be available which will expand the possibilities to study rheological changes in combination with other techniques.

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