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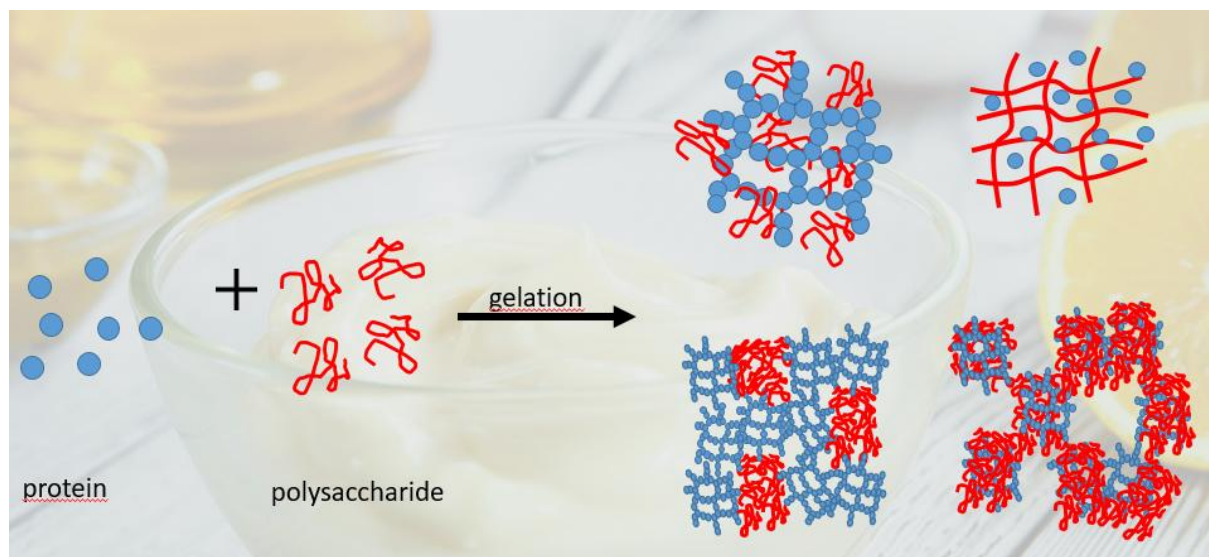
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# Mechanical properties and microstructure of (emul)gels formed by mixtures of proteins and polysaccharides

Christophe Chassenieux\* and Taco Nicolai

IMMM UMR CNRS6283, Le Mans Université, Avenue O. Messaien, 72085 Le Mans cedex, France.

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## Abstract

It is now well established that the combination of proteins and polysaccharides makes it possible to obtain textures that can be used by the food industry. However, consumer demand for more environmentally friendly and healthier foods is leading to the development of new ingredients (particularly plant proteins) for which the know-how acquired on animal proteins cannot be directly transposed. The aim of this review is to take stock of the work published between 2021 and 2023 on gelation of polysaccharide/protein mixtures on their own or in the presence of oil. The emphasis is on structural and rheological studies of these (emul)gels. In addition to the composition and variability of the ingredients, there are a multitude of formulation parameters (pH, salt, temperature, etc.) and shaping parameters (pressure, homogenisation, etc.), which makes it difficult to compare the different studies in order to conclude on general structure/properties relationships.

## Introduction

Gels formed by mixtures of proteins and polysaccharides have been studied intensively in the past and continue to raise much interest. One motivation for these studies is to obtain textures that are desirable in food products, but that are difficult to obtain using only proteins. The current tendency to replace animal proteins by plant proteins further stimulates this research since it is often more difficult to obtain desired food textures with the latter. The general features of the interaction between proteins and polysaccharides in aqueous solution as well as those of the formation of gels by these biopolymers are by now well-known and have been extensively reviewed [1-4]. The objective of this review is not to discuss again in detail these general features, but rather to give an overview to the most recent literature in this area and in particular for food grade systems.

Therefore, we will only briefly mention the general features here and refer to other reviews for more details.

The behavior of aqueous mixtures of proteins and polysaccharides depends on the type of interactions between the two types of biopolymers. If repulsive interactions dominate, segregative phase separation tends to occur with the formation of a phase rich in protein separated from a phase rich in polysaccharides. If attractive interactions dominate, associative phase separation can occur between a phase rich in both types of macromolecules and a dilute aqueous phase. This process is called coacervation if the association is sufficiently weak that the concentrated phase remains liquid. If the association is strong, complexes of proteins and polysaccharides are formed that may form large macroscopic flocs. Association is often driven by opposite charge interactions, though hydrophilic and hydrophobic interactions may also play a role. The net charge of proteins varies from strongly negative at high pH to strongly positive at low pH with a net zero charge at their isoelectric point (pI). Therefore, opposite charge driven association of proteins with anionic polysaccharides such as  $\kappa$ -carrageenan (k-car) occurs at  $\text{pH} < \text{pI}$  and with cationic polysaccharides such as chitosan at  $\text{pH} > \text{pI}$ . However, this association can happen even when the proteins and polysaccharides have the same net charge close to the pI where the proteins have both negative and positive patches. We note that when the proteins and polysaccharides form complexes, segregative phase separation can sometimes still occur between the complexes and polysaccharides or proteins if the latter are present in excess. Liquid-liquid phase separation leads to the formation of domains of the minority phase suspended in the continuous majority phase that grow leading eventually to macroscopic phase separation. The rate at which this process occurs depends on the strength of the interactions, but also on the viscosity of the phases. Phase separation leads to loss of the mixing entropy and is therefore more likely to occur if the molar mass of the components is higher.

Gelation involves the formation of crosslinks between the macromolecules leading to the formation of a system spanning network that needs to be sufficiently strong to resist gravity. In mixtures the network can be formed by crosslinks between the proteins, between the polysaccharides or both [5]. It is also possible that the proteins bind to the polysaccharides. Gelation can be induced by various methods [6]. Gelation of globular proteins is induced by denaturation exposing hydrophobic groups leading to the formation of crosslinks between denatured proteins [7]. Denaturation is most often induced by heating (leading to so-called heat-set gels), but can also be achieved by applying high pressure. Other gelation methods can be applied at low temperatures (cold-set gels) and involve adjusting the pH closer to the pI or adding salt, both of which reduce electrostatic repulsion. Using these methods most crosslinks are physical though disulfide bridging can also occur. Covalently cross-linked protein gels can be formed by adding enzymes such as transglutaminase. Gelation of polysaccharides often involves addition of salt combined with cooling, but in a few cases it is induced by heating [8]. The gelation rate and the microstructure of pure protein and polysaccharide gels depends on the type of protein and polysaccharide, but also on the environmental conditions such as temperature, type and concentration of salt, pH and polymer concentration.

In mixtures, gelation of proteins and/or polysaccharides will be influenced by the interaction between the two macromolecules leading to gels with different microstructures, see figure 1. If the interaction between the proteins and the polysaccharides is weak, both components are homogeneously mixed with either one or both components crosslinking with itself. If the interaction between the two components is strong the gelation rate is very important, because phase separation occurs during the crosslinking process. If crosslinking is slow compared to phase separation, the latter can become macroscopic before the network fills the whole space. If phase separation is sufficiently slow it can be arrested on a microscopic scale by crosslinking one or both components

into a space filling network. In the case of segregative phase separation the network will then contain domains of the other phase that can still be liquid. The size and volume fraction of the inclusion domains depends on the composition and the relative kinetics of crosslinking and phase separation, which in turn depend on the type of proteins and polysaccharides, their composition in the mixture and the environmental conditions. In the case of associative phase separation, the network will contain domains with low concentrations of either component. It is obvious that gels with a wide range of properties can be obtained with mixtures if we consider that the properties of pure protein and polysaccharide gels already depends on their type, concentration and environmental conditions. These properties will furthermore be influenced by the presence of other ingredients such as oil or sugar.

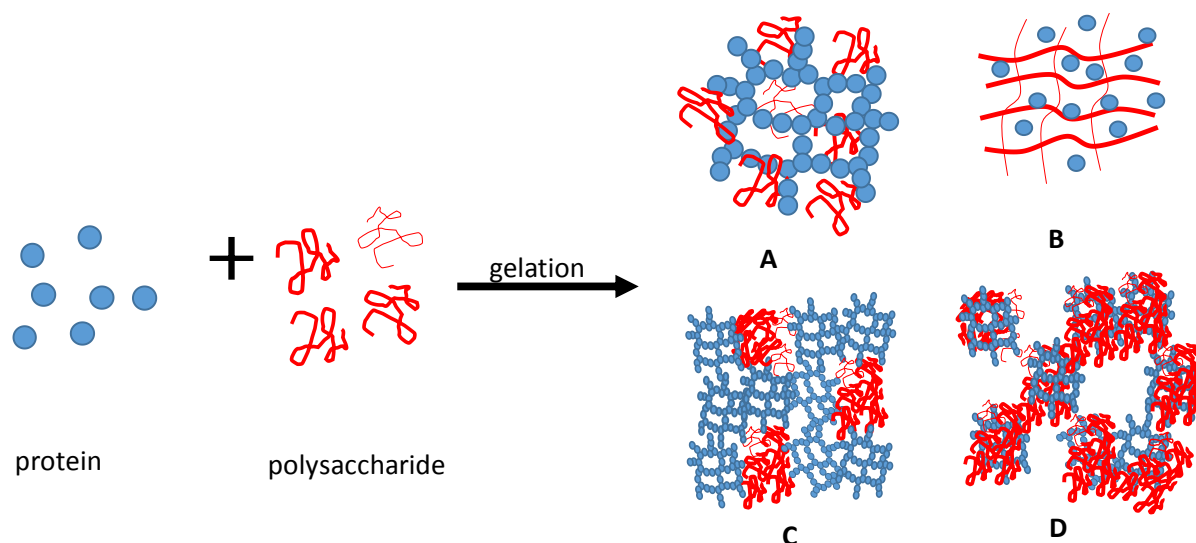


Figure 1 Schematic drawing of different types of mixed protein/polysaccharide gels: unbound polysaccharide homogeneously distributed throughout the protein network (A) or vice versa (B); segregative (C) or associative (D) microphase separated gel.

Even if we consider only food applications, there are dozens of different protein and polysaccharides types one can choose from, which means that the number of different binary mixtures is enormous. In fact, most often the protein component is itself a mixture of different types that can interact with each other in ways that depend on the environmental conditions. Currently, it is not possible to predict the properties of a mixed gel at a particular environmental condition from its composition even if the properties of the individual components are known. Therefore, each mixture of interest needs to be investigated separately. Full understanding of a particular mixture requires elucidating the effects of varying the concentration, the composition, the pH, the ionic strength, the processing conditions such as the heating temperature and duration, and other parameters of potential interest. No single study can provide such a full understanding. For a few systems several studies focussing on different conditions have been published, but even that has not resulted in full understanding of their properties at all conditions. For most mixtures that have been investigated current knowledge is patchy with results obtained only at particular concentrations, compositions and environmental conditions. An additional complication is that, as was mentioned above, the protein component is often itself a mixture that can vary in composition. This is in particular a problem for plant proteins for which the composition and also the state (native vs aggregated) of the proteins strongly depends on the extraction method. With such systems it is difficult even to compare studies of nominally the same mixtures reported by different research groups. A similar difficulty arises for nominally the

same polysaccharide, which can vary not only in the molar mass distribution, but also in configuration such as the degree of acetylation or methylation.

Given the huge range of systems that can be investigated, the choice to study a particular system has to be motivated by its potential for application, which includes considerations of cost, nutritional value and environmental sustainability. The aim can be to investigate the potential of a particular type of polysaccharide or protein, e.g. because it is produced in particular areas, is claimed to have health benefits, or can potentially valorise waste products. The aim can also be to produce gels with certain dynamic mechanical properties that give a desired texture to a particular industrial food product. Another issue, which has attracted much attention in recent years, is the possibility to incorporate functional ingredients, such as flavors and antioxidants, that require protection and controlled release at desired locations, e.g. in the mouth or rather in the intestines. As was mentioned above, the properties of any chosen mixture will depend on the pH, the salt concentration and the processing method. This can be an advantage in that the conditions can be varied in order to optimize the desired properties, but it can also be a constraint if particular conditions are required e.g. for reasons of taste or industrial production methods.

Here we review the literature on gels formed by protein/polysaccharide mixtures published between 2021 and early 2023 focussing on food related systems. A range of different techniques have been used to study different aspects of the gels, but in this review only the mechanical properties and the microstructure of the gels will be considered. The stiffness of the gels can be characterized by the storage shear modulus ( $G'$ ) determined in the linear response regime. Another mechanical property that is often determined is the hardness, also called gel strength, defined as the maximum force during compression, which may, however, depend on the geometry that is used and the rate of compression. Confocal laser scanning microscopy (CLSM) is a non-invasive method to determine the microstructure of the gels with a resolution of approximately 0.1  $\mu\text{m}$ . Scanning electron microscopy (SEM) has a higher resolution, but requires severe treatment that often perturbs the gel structure as was nicely illustrated recently by Zhang et al. [9] who showed both CLSM and SEM images of the same gels at different scales. SEM images showed large structures at low resolution for gels that CLSM showed were perfectly homogeneous on these length scales, see Fig. 1. Li et al. 2022 also noticed the difference between the microstructure of casein gels as observed by SEM and CLSM. The microstructure of the gels influences its mechanical properties, but there is no straightforward relationship between the two, because the latter are determined by the flexibility of the structure and resistance of the crosslinks to stress that cannot be seen in a static image of the structure. Nevertheless, investigation of the microstructure is important, because it determines the visual aspect of the gels and to a large extent their water and/or oil retention capacity.

## 1. Gels

Gels of protein/polysaccharide mixtures can form by crosslinking the proteins, the polysaccharides or both. The effect of mixing on the properties can be gauged by comparing with the equivalent pure protein and polysaccharide systems. Globular proteins in aqueous solution tend to aggregate when denatured by heating and form a gel if the concentration is sufficiently high. These gels persist and are usually even stiffer after cooling. Globular protein gels can also be formed at room temperature if the globular proteins are aggregated by preheating and the electrostatic repulsions are subsequently reduced by reducing the pH or adding salt. Two types of non-globular proteins, gelatin and casein that are often present in food products, can also form gels without heating. Gelatin solutions are liquid above 30°C and gel reversibly below 25°C. Casein solutions gel when the pH is reduced in-situ to close to its  $pI \approx 4.5$ . In-situ acidification is usually done by adding glucono delta lactone (GDL) which degrades slowly in time releasing protons. Gelation of certain polysaccharides such as gellan,

k-car, alginate and pectin can be induced by cooling, acidification and/or adding adding salt [5, 8, 10]. Others such as guar and xanthan form increasingly viscous solutions with increasing concentration that may appear as gels at higher concentrations. In following, we will first review investigations of the effect of adding polysaccharides on the properties of protein gels formed at high and low temperatures and then the effect of adding protein on the properties of polysaccharide gels. The review of purely aqueous systems will be followed by an overview of the use of protein/polysaccharide mixtures on the stability of oil in water emulsions and the properties of emulsion gels.

### *1.1 The effect of polysaccharides on heat-set protein gels*

In almost all studies covered by this review a fixed heating protocol was used consisting of heating up to a relatively high temperature varying between 80°C and 95°C, holding the temperature between 10 and 50 min followed by cooling. Generally, it is not explained why a particular heating protocol was chosen and it is likely the mechanical properties of the gels will depend to some extent on the heating protocol that was chosen as was shown by Zhang et al. [9]. Serabi et al. [11] studied the influence of adding basil seed gum (BSG) on gels formed by heating whey protein isolate (WPI) solutions at pH 7 and 0.05 M NaCl. WPI contains a mixture of globular proteins, mostly  $\beta$ -lactoglobulin (b-Ig) and  $\alpha$ -lactalbumin. Segregative phase separation was observed in mixtures before heating at higher BSG concentrations. For a given heating rate, gelation occurred at a lower temperature if more BSG was present and stiffer gels were formed. In mixtures of b-Ig and the polysaccharide Levan at pH 7 segregative phase separation was found that was favored by increasing the molar mass of the polysaccharide or by adding 0.1 M NaCl [12]. During a heating ramp, b-Ig solutions gelled at a lower temperature when salt was added, because the aggregation rate is faster when electrostatic repulsion is screened. The apparent gelation temperature depends, of course, on the heating ramp rate, but at a given rate it decreased with increasing Levan concentration and more so when its molar mass was higher.  $G'$  measured after keeping the samples 20 min at 90°C was much larger in the presence of salt, in part due to the faster gelation rate and in part due to the different structure of the gels. Addition of Levan led to an increase of  $G'$  up to a certain concentration, but  $G'$  decreased again when more Levan was added. It was suggested that segregative phase separation induced by adding either Levan or BSG increased the gel stiffness by locally increasing the protein concentration, but strong phase separation at higher Levan concentrations weakened the gel perhaps by reducing the connectivity between protein rich domains.

In another study *Lycium barbarum* polysaccharides (LBP) were added to WPI [13]. Also in this study the WPI concentration was fixed at 12%, but the effect of 3 different polysaccharide concentrations at 3 pH values was investigated. WPI gels were formed by heating. Addition of 0.1 % LBP increased  $G'$ , but 0.5 % had less effect and adding 1% decreased  $G'$  with respect to pure WPI gels. The values of  $G'$  and the quantitative effect of LBP depended on the pH with stiffer gels being formed at pH 5.2, close to the pI of WPI, than at pH 3 and pH 7. Babaei et al [14] studied the effect of adding alginate or k-car with or without  $\text{CaCl}_2$  on gels formed by heating WPI solutions. The system was studied at one particular pH (6.7) and WPI concentration (12 %) with and without 0.25 % polysaccharide and with and without  $\text{CaCl}_2$  (30 mM). Addition of either polysaccharide increased the gel hardness and the addition of  $\text{CaCl}_2$  increased it further. It is not clear from this study and the one by He et al. [13] whether the interactions between the polysaccharides and the whey proteins were attractive or repulsive.

Jiang et al. [15] studied WPI (10 wt%) mixed with 2 wt% *mesona chinensis* polysaccharide (MCP) at different pH. After heating, a stable self-supporting gel was formed at pH 10, but not at lower pH down to pH 4 implying that MCP inhibits gelation of WPI at lower pH. These authors did not compare the mixed systems with equivalent pure WPI so that the effect of adding MCP cannot be judged. The same research group investigated the effect of adding  $\text{CaCl}_2$  to this mixture at pH 10 [16]. They found that adding  $\text{CaCl}_2$  reduced the stiffness and the hardness of the gels, whereas CLSM showed that the microstructure became more heterogeneous. Guo et al. [17] observed that  $G'$  and the hardness of gels formed by a mixture of gellan and WPI varied with the pH. However, no comparison was made with the equivalent pure WPI gels.

Turning to globular plant proteins, Johansson et al. [18] found that replacing a small fraction of faba bean proteins by either polysaccharide fibres or by starch led to an increase of  $G'$  of gels formed by heating. The gels containing more fibres of starch were more heterogeneous with microdomains rich in fibre or starch, which could explain that they had a lower fracture stress and strain. Addition of konjak glucomannan (KGM) significantly increased  $G'$  of SPI (10%) gels formed by heating [19] and CLSM image showed segregative microphase separation between the two components. Adding low methoxy pectin to pea protein isolate (15%) gels formed at pH 6.5 by heating led to an increase of  $G'$  with increasing pectin concentration and the gel was more heterogeneous [20]. Large protein aggregates were formed in solutions with pectin suggesting complexation between the two components.

Jiang et al. [21] and Lu et al. [22] studied the influence of adding k-car on gels formed by heating solutions of oyster protein isolate (OPI) and SPI, respectively. The elastic modulus and the hardness of the gels increased strongly with increasing k-car concentration. Lu et al. [22] showed that  $G'$  of the mixed gels decreased sharply upon heating and increased again upon cooling, which is caused by the reversible gelation of k-car. It is clear, therefore, that for these mixtures the mechanical properties of the mixed gels were increasingly dominated by the gelation of k-car during cooling. Unfortunately, no results for equivalent pure k-car gels were reported, which would allow one to determine the effect of the protein on the k-car gel. The stiffness of k-car depends not only on its concentration, but also on the type and concentration of ions that are present [23], information about which should therefore be provided. CLSM images showed that the SPI/k-car mixed gels were heterogeneous. It was suggested that complexation occurred in spite of the fact that both components were negatively charged at pH 7 at which the measurements were done, but at higher k-car concentrations also segregative micro phase separation was thought to occur. Lei et al. [24] compared the effect of adding k-car or guar gum on gels formed at pH 8 by heating solutions of phyco cyanate (16% w/v), a protein extracted from *Spirula*.  $G'$  of the gels measured at 25°C increased with the addition of more k-car up to the maximum added (0.4%). With guar  $G'$  increased at 0.1%, but decreased when larger amounts were added. The effect was stronger for k-car, perhaps because it gelled in the mixtures, but it is difficult to conclude since no comparison with pure polysaccharide solutions was made. CLSM images showed that the pure protein gels had a heterogeneous microstructure which appeared less heterogeneous in the presence of 0.4 % k-car and more heterogeneous with 0.4 % guar.

An investigation of the effect of adding 0.5 % w/v dextran or dextran sulfate (DS) on gels formed by heating egg white protein at pH 4, 7 and 9 showed little effect of dextran on  $G'$ , but in the presence of DS a significant decrease at pH 4 and an increase at pH 7 [9, 25]. The hardness defined as the maximum force during compression was much smaller both in the presence of dextran and DS at pH 4, and somewhat larger in the presence of DS at pH 7. In this study the authors varied the holding time at 90°C and showed that the hardness increased significantly with increasing holding time. The

negative effect at pH 4 was attributed to extensive complex formation at this pH and the positive effect at pH 7 to weak attractive electrostatic attraction [26]. CLSM images showed that the addition of DS led to more homogeneous gels, which explains why these gels were less turbid.

Zhuang et al. [27] and Zhang et al. [28] studied the effect of adding KGM on gels formed by heating myofibrillar protein (MP) solutions at pH 7. The mixtures contained a high concentration of salt (0.6 M). Zhuang et al reported for MP at 4% that adding KGM improved the hardness up to 1%, but decreased at higher concentrations, whereas Zhang et al found that both  $G'$  and the hardness increased up to the highest concentration tested of 2%. CLSM images showed segregative microphase separation between the components. Zhang et al. [28] quantified the synergistic effect of KGM on MP gelation by comparing the evolution during heating of the shear moduli of the mixture with that of the sum of the individual components. They also quantified the microstructure of the gels in terms of a fractal dimension derived both from the rheology and from CLSM images. However, different values were obtained from the different techniques and their physical meaning is not clear for these microphase separated systems.

The effect of adding either guar gum, locust bean gum or xanthan on heat induced gelation of quinoa protein isolate was studied at pH 7 in the presence of 50 mM NaCl [29]. CLSM showed that addition of polysaccharide led to formation of larger protein aggregates and after heating to a more heterogeneous gel. However, since the polysaccharides were not labelled it is not clear the CLSM images whether associative or segregative microphase separation had occurred.  $G'$  and the yield stress of the heat set gels increased with increasing polysaccharide concentration up the highest tested (0.2%). The effect depended on the type of polysaccharide being smallest for guar gum and largest for xanthan.

Almost all investigations discussed so far showed an increase of the stiffness when more polysaccharide was added up to a certain amount, but in some cases larger quantities of polysaccharide caused a decrease of  $G'$ . Quantitatively, the effects depend on the particular mixture and the conditions at which the gels were produced. In none of these studies the equivalent pure polysaccharide solution was studied, which means that it is difficult to tell whether in some cases the improvement was not simply caused by gelation of the polysaccharide. This is particularly obvious in the study by Jiang et al. [21] who added k-car, which can form a strong gel by itself. In cases where both proteins and polysaccharides form a crosslinked network one should consider the influence of proteins on the polysaccharide network as well as the influence of the polysaccharides on the protein network.

### *1.2 The effect of polysaccharides on cold-set protein gels*

As was already mentioned, globular protein solutions can also form gels at low temperatures if they are denatured and aggregated by preheating and subsequently the electrostatic repulsion is reduced by acidification or adding salt. Brito et al. [30] gelled preheated SPI solutions (15 wt%) at 10°C by adding  $\text{CaCl}_2$ . Addition of small quantities of xanthan (0.1%) or locust bean gum (0.2%) led to stiffer gels with a lower creep compliance. Bu et al. [31] preheated a mixture of tenebrio molito protein and low methoxy (LM) pectin at pH 3.4 where the two components formed complexes. They subsequently induced gelation by adding  $\text{CaCl}_2$  and  $G'$  was found to increase with increasing  $\text{Ca}^{2+}$  concentration. Solutions of LM pectin gel by themselves in the presence  $\text{Ca}^{2+}$  and the preheated denatured proteins could perhaps also form a gel by themselves in the presence of  $\text{Ca}^{2+}$ . Therefore, equivalent pure protein and polysaccharide solutions need to be investigated before one can draw conclusions about the effect of complexation on the gels. Adding pectin to preheated pea protein



aggregates was found to induce microphase separation [20]. A gel containing elongated pectin rich microdomains could be formed by enzymatically crosslinking the proteins while applying a shear.

An alternative method to obtain proteins gels at low temperatures is to use gelatin or casein proteins. The stiffness of a gelatin gel increases with time at a rate that increases with decreasing temperature. Ji et al. [32] added hydroxy propyl methylcellulose (HPMC) to gelatin in an attempt to increase the melting temperature. HPMC has the particularity to form a gel above about 55°C. It was found that gelatin and HPMC phase separate, but the mixtures gel at 25°C when the gelatin rich phase is continuous. The gelatin rich phase contained an unknown amount HPMC, which led to a small increase of the melting temperature by 3°C, but significantly reduced  $G'$ . The microstructure of the mixed gel varied with the HPMC content, but there was no clear relationship between the microstructure and  $G'$ . Interestingly, the viscosity measured at the same temperature after a heating and cooling ramp was different showing that for such a phase separating system the history of the sample is important.

Addition of a small quantity of hsian-tsao gum (HG) to gelatin solutions slowed down the gelation rate at 22°C, but at larger quantities it increased the gelation rate [33]. The presence of HG did not influence  $G'$  much. The yield stress upon compression increased a little with small amounts of HG, but decreased when a larger amount was added. CLSM showed a heterogeneous structure for the mixed gels, but there was no clear relationship between the microstructure and the mechanical properties. The authors concluded that HG formed complexes with gelatine by association of opposite charges, but that also microphase separation occurred between gelatin and HG rich phases. To complicate matters further, heating to 80°C influenced the properties at 22°C. Zhang et al. [34] added polysaccharides extracted from mulberry leaves to gelatin. They observed that complexes were formed, in particular at higher pH even though both components were negatively charged. Adding mulberry polysaccharides reduced the shear dependent viscosity and the elastic modulus measured at 25°C, in a manner that depended not only on the pH, the ionic strength and the quantity of added polysaccharide, but also on the manner in which the polysaccharides were extracted.

The polysaccharides used in these 3 studies did not gel by themselves at lower temperatures contrary to mixtures with k-car studied by Makarova et al.[35]. Much stiffer, but also more fragile gels were formed in the presence of k-car. SAXS measurements showed that different structural heterogeneities were present in the gels with and without k-car. Most likely the properties of the k-car dominated the properties of the mixed gel, but no results were reported for the equivalent pure k-car gels so that the effect of the proteins on the k-car gel remains unknown.

Another type of protein that gels at room temperature is casein, which is the main protein component of milk and is present in milk as colloidal particles called casein micelles. Aqueous solutions of casein gel after reducing in-situ the pH towards the pI of the proteins. Li et al. [36-38] studied the influence of adding the anionic polysaccharides ALG, gellan or the neutral polysaccharide methyl cellulose on the properties of casein gels at 8% formed after adding GDL. The hardness of the gel increased with increasing ALG or MC concentration up to a maximum at 0.4% decreasing at higher concentrations. In the presence of gellan the hardness increased progressively up to the highest concentration tested (0.6%). Gelation was faster when more polysaccharide was present, which in the case of ALG was shown to be caused by a faster decrease of the pH and CSLM images of the gels showed an increasingly heterogeneous microstructure with protein poor domains surrounded by protein rich domains, see fig. 2. At low concentrations casein was shown to form complexes with ALG, which inhibited large scale flocculation of the caseins below pH 5.5 and similar complexation is also expected with the gellan. The authors suggested that segregative phase separation occurred at high pH followed by complexation of the anionic polysaccharides with the

proteins at lower pH. However, since the polysaccharides were not labeled it was not possible to observe their distribution in the gels as a function of the pH. It is remarkable that the three types of polysaccharide had a similar effect on the casein gels even though the anionic polysaccharides formed complexes with the proteins, whereas the neutral MC is not expected to do so.

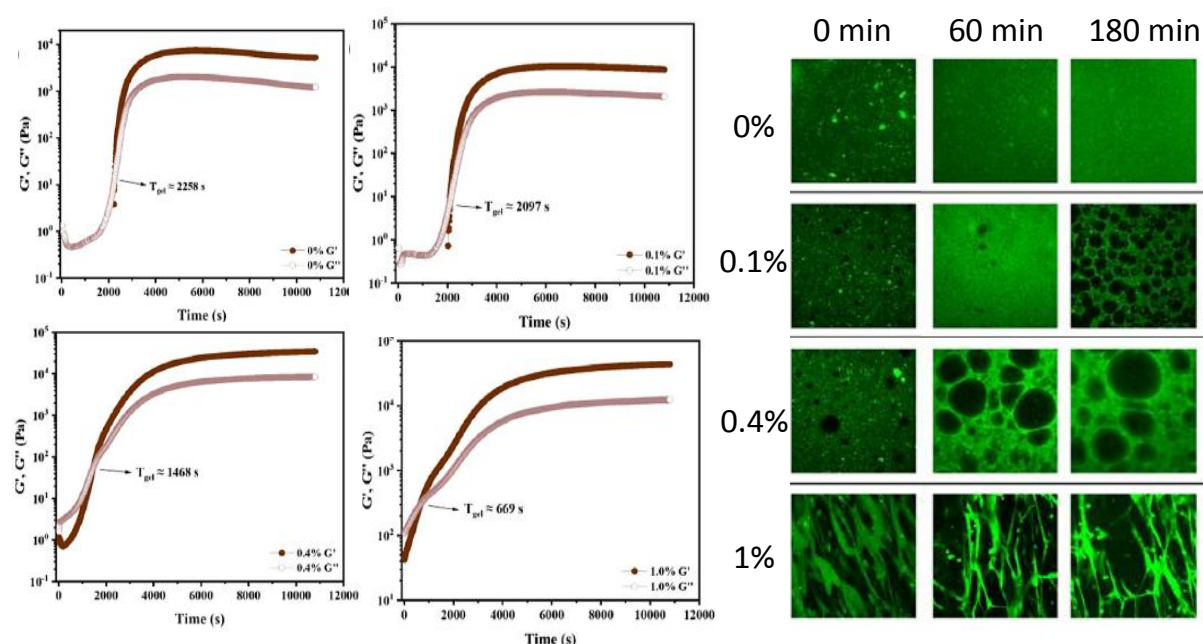


Fig. 2 Evolution of the storage and loss moduli during acidification of casein solutions with different amount of ALG added. CLSM images taken at different time during acidification are also shown. (reproduced with modifications from [9]).

It appears that cold-set gels formed by globular protein aggregates or casein also become stiffer when polysaccharides are added in modest quantities. However, gelatin gels appear to become less stiff, at least when the polysaccharide itself does not form a strong gel as was the case in mixtures with k-car studied by Makarova et al. [35]. It seems that the added polysaccharides interfere with the crosslinking of gelatin.

### 1.3 The effect of proteins on polysaccharide gels

If the proteins don't crosslink, gels can still be formed by physical crosslinking of the polysaccharides in the mixtures. Hu et al. [39] investigated gelation of k-car in the presence of native b-lg or in the presence of b-lg aggregates in the form of nanoparticles or fibrils formed by preheating at pH 5.8 and pH 2, respectively. The effect of the proteins was investigated at pH 9 where both components are negatively charged and at pH 4 where the proteins have a net positive charge and form a complex with the still anionic k-car. At pH 9 gelation of k-car during cooling was little influenced by the presence of the proteins, but at pH 4 the proteins perturbed the coil-helix transition of k-car and as a consequence  $G'$  decreased strongly with increasing protein concentrations. This effect was stronger when b-lg was added in the form of fibrillar aggregates suggesting a more extensive interaction of the k-car chains with the latter. A reduction of  $G'$  caused by complexation of native b-lg was also observed by Taheri et al [40] for the anionic polysaccharide cress seed mucilage at pH 3.5. Lin et al. [41] heated mixtures of peanut proteins with k-car or gellan at 90°C under stirring. Gels were subsequently formed by cooling to room temperature. These gels melted again upon heating to 80°C. As might be expected,  $G'$  and the hardness of the gels increased with increasing polysaccharide

concentration, but no comparison was made with equivalent pure polysaccharide gels so that it is not possible from this study to judge the effect of the proteins on the polysaccharide gelation. The reversible gelation was exploited to use these mixtures for 3D-printing.

Kazemi et al. [42] studied the effect of adding WPI on gelation of gellan solutions induced by adding  $\text{FeSO}_4$ . The measurements were done at pH 7 where both components are negatively charged and not expected to form complexes. As might be expected  $G'$  was larger when the fraction of gellan was larger at a given total polymer concentration. Here also, no comparison was made with pure gellan gels

The influence of adding native amarynth protein isolate (API) or bean protein isolate (BPI) on xanthan gels was studied by Cortez et al. [43]. Whereas pure xanthan gels were formed at pH 8 and 9, in the presence of API, gels were formed between pH 7 and 9 and in the presence of BPI close to pH 7. The proteins by themselves did not form a gel. The effect was attributed to association between the two types of biopolymers. In a follow up study the authors found that if the pH was decreased in-situ using GDL, API/xanthan gels were also formed at pH 4 and pH 5.5 [44]. Most likely, the network is broken if the pH is decreased by adding a strong acid under stirring and a suspension of aggregates is formed. The hardness of the gels was found to be much larger below the pI of the protein at pH 4 then above the pI at pH 5.5. This was attributed to strong electrostatic attractive interaction at pH 4, whereas hydrophobic interactions were thought to be more important at pH 5.5 For a given total biopolymer concentration of 1%, the hardness of the gels was found to decrease with increasing API and decreasing xanthan content at pH 4. Similar results were obtained for mixtures of BPI and xanthan below (pH 4) and above (pH 4.5) pI, but the effect of the pH on the hardness and  $G'$  was much smaller [45]. At pH 4.5 there was a significant decrease of the hardness with increasing protein content. Unfortunately, no comparison was made with pure xanthan gels.

Methyl cellulose solutions gel during heating above approximately 50°C and the gels melt during cooling below about 40 °C. Soy globulins were found to form complexes with methylcellulose already at room temperature, which led to a decrease of  $G'$  of the gel formed at 70°C with increasing protein up to 0.4% [46]. At higher protein concentrations tested up to 1.6%,  $G'$  increased again, but did not reach the value of pure MC.

Protein hydrolysates derived from scallop were found to form complexes with the polysaccharides in mixtures of xanthan and k-car at a weight ratio 3:2 [47]. Gels were formed at total biopolymer concentration of 1.5% with  $G'$  increasing with increasing polysaccharide content. For a given mixture,  $G'$  increased with decreasing pH between pH 9 and pH 3, which could perhaps be related to stronger electrostatic attractive interaction between the proteins and the anionic polysaccharides. No comparison was made with gels of just the polysaccharide mixture at the same conditions.

Finally, Yao et al. [48] added gelatin to a solution of *Artemisia sphaerocephala* Krasch polysaccharide (ASKP). By itself ASKP slowly formed a gel when  $\text{FeSO}_4$  was added at pH 4, but the gelatin by itself did not gel at the conditions studied. Addition of gelatin slowed down gelation of the polysaccharides at 25°C and reduced both  $G'$  and the hardness measured after 3 days. The influence of gelatin on the polysaccharide gelation was attributed to complexation caused by opposite charge interactions, which implies that it will strongly depend on the pH.

The main conclusion that can be drawn from these studies is that if the added proteins form complexes with the polysaccharides they interfere with gelation of the latter leading to gels that are less stiff. Quantitatively, the effect will be different for each type of polysaccharide and it depends on the type and morphology of the proteins as well as the pH. Most likely, it will also depend on the ionic strength, which was not considered in these studies.

## 2. Emulgels

Proteins, either individually or in the form of aggregates, can also be combined with polysaccharides to effectively stabilise water/oil interfaces. The first objective is to improve the stability of emulsions over time or as a function of external parameters (pH, temperature, freeze-drying, etc.) compared with the performance of emulsions prepared with the two ingredients considered separately. This involves combining i) the rapid adsorption kinetics of individual proteins at these interfaces or ii) the Pickering stability provided by their aggregates and iii) the (electro)steric stabilisation provided by the polysaccharides [49]. Here again, numerous protein/polysaccharide combinations have been explored in the recent literature, not to mention variations in pH, temperature, concentration, composition, nature of the oil or processing conditions of the formulations considered. Therefore, it is quite difficult to rationalise the results as a whole, because the performance of each new type of stabiliser is not always evaluated in the same way, which makes it impossible to obtain a proper comparison. Another option is to produce emulgels, i.e. gelled emulsions that can be considered as soft solids whose textural, nutritional and sensory properties can be optimised to lead to cheese [50] or meat analogues [51] or as fat replacers. In both cases, in response to the concerns of vegan consumers, ingredients derived from plants for both oils and proteins provide interesting alternative. New functions can also be envisaged for these emulsions, such as better resistance to oxidation of the oil phase thanks to thicker interfaces acting as a physical barrier [52-54] or the possibility of using them as “inks” for 3D printing of foods, which requires specific rheological profiles [55]. Before discussing the recent literature on the use of protein/polysaccharide complexes to obtain stable emulsions and emulgels, we will consider briefly the influence of their preparation method on their properties.

### 2.1 Influence of the preparation method of the emulsions

Emulsions involving protein/polysaccharide complexes can in fact be obtained in two different ways. The first, known as *in situ*, involves preparing a primary emulsion stabilised by proteins and then adding the polysaccharide which will adsorb onto the primary droplets. It has been illustrated for instance by Shi et al. [56] who have studied fish oil emulsions stabilised with caseinates, to which sulphated fucan was added. Emulsions formulated in this way are stable if sufficient polymer is added to saturate the interfaces and prevent the formation of bridges between oil droplets. The salt and pH stability of the resulting emulsions is superior to that of primary emulsions, particularly for high molar mass polymers, which is attributed to a thicker interface. The latter limits the flocculation of oil droplets which increases the specific surface area of the oil phase resulting in increased digestion rate. The increase in stability as a function of pH of complex-stabilised emulsions has also been reported by Juyang et al. [57] for sodium caseinate/pectin mixtures.

The second way for obtaining emulsions stabilised by protein/polysaccharide complexes involves preparing protein/polysaccharide complexes *ex situ* and dispersing them in the aqueous phase, which is then emulsified with oil. The *ex situ* method makes it possible to better study and optimise the characteristics of the complexes before they are used as stabilisers, although their interaction with the interface and their further processing may ultimately affect these characteristics. Liu et al. [58] compared the two processes using hemp protein isolates (HPI) with various polysaccharides. The emulsions prepared *in situ* proved to be more stable over time than those prepared *ex situ*, but less so with temperature or salt addition, which was attributed to differences in the interface structures. However, the exact composition of this interface and its degree of stratification as a function of the production process of the emulsion have not been reported. Using sodium caseinate/pectin mixtures, Liao et al. [59] also concluded that emulsions prepared *in situ* were more stable, while specifying the electrostatic nature of the pectin/caseinate interaction mechanism as evidenced from

ITC measurements. However, these authors stressed the need to control the order and speed of addition of the components: the primary emulsion should be added to the pectin rather than vice versa, in order to limit flocculation of the oil droplets by bridging.

## *2.2 From emulsions to emulgels*

Emulgels can be obtained by gelation of the continuous phase in the same manner as in the pure aqueous systems discussed above. Lin et al. [60] formed emulgels from mixtures of SPI and alginate by calcium ions that cause crosslinking of alginate. Here again, the order of addition has a considerable influence on the microstructure of the emulgels due to the presence of flocculated oil droplets when the polysaccharide is added to the primary emulsions. The gelling kinetics are also slower and the final modulus of the emugel lower in the latter case due to the entrapment of some alginate chains between the flocculated droplets. When alginates were combined with casein micelles instead, the results were very different. Despite the lack of affinity between the two partners, their mixtures with oil can also give rise to emulgels after acidification as was reported by Li et al. [61] In this peculiar case the order of addition is of less importance, casein micelles rather than alginate stabilize oil/water interface inducing the location of oil droplets mainly in the casein phase. Upon acidification, the casein phase displays gelation and eventually when the pH is low enough, the interaction between alginate and casein becomes attractive. The structure of these emulgels is controlled by the casein/alginate ratio rather than by the way the mixtures proceeded.

In the previous example, gelation of the continuous phase is induced by the addition of calcium ions or protons. However, it can also occur without using any external crosslinking agents as described hereafter. Sun et al. [62] prepared emulgels using fibrillar aggregates of WPI (WPIF) complexed with arabic gum that were insoluble between pH 4.6 and 6. For an oil volume fraction of 60%, stable emulsions were obtained either as liquids with WPI/AG complexes or as emulgels with WPIF/AG complexes, which was explained by the fibrils' greater propensity to complex AG. The result was the formation of bundles, which promoted the formation of a network within the emulsion. In terms of sensory analysis, these emulgels are on a par with commercial mayonnaises. Similarly, Dong et al. [63] produced emulgels by complexing ovotransferrin fibrils and pectins with a pearl necklace structure. With the complexes, the moduli of emulgels formed by mixing with canola oil and candelilla wax were increased by more than one order of magnitude compared with their counterparts stabilised by fibrils alone. However, an anisotropic shape of the complexes is not a prerequisite for achieving emulgels as evidenced by Su et al. [64]. They produced complexes based on beta-lactoglobulin microgels and PGA whose hydrophilicity increased with the proportion of PGA and which were used to stabilise triglyceride emulsions. PGA stiffened WPI microgels, which improved the emulsion stability and led to the formation of emulgels when the oil volume fraction was increased. Some emulgels resisted centrifugation and their microstructure, measured by confocal microscopy, indicating the presence of a PGA network in the continuous phase.

Formation of emulgels may circumvent the poor efficiency of plant derived proteins to stabilize emulsions. This has been exemplified for SPI and various polysaccharides (pectin, kappa and iota carrageenan) by Ge et al [65] who heated soy bean oil emulsions above the denaturation temperature of SPI at pH4. At these conditions, electrostatic interactions take place between the polysaccharides (to a bigger extent with k-car) and the denatured SPI and the anchoring of the latter at the oil interface resulting in the formation of emulgels with tunable rheological properties depending on the polysaccharide.

In conclusion, protein and polysaccharide complexes can form a new type of stabilisers of emulsions that is much more effective than their individual components. However, the number of combinations

is enormous given that other parameters such as composition, temperature, etc. can also be modified to a large extent. When starting from a protein-stabilised primary emulsion, the polysaccharide phase must be added in a controlled manner to avoid flocculation of the oil droplets. Emulgels can be derived from these emulsions by cross-linking the continuous phase, which can overcome certain disadvantages presented in particular by some vegetable protein isolates which are unable to stabilise emulsions over time. Emulgels can also be formed starting from emulsion stabilised by a mixture of proteins and polysaccharide displaying segregative properties. These emulgels meet the requirements in terms of rheological profiles for 3D printing of food that requires easy flow during extrusion followed by fast setting after extrusion. Here we have focused our review on emulsions based on oil but should be noted that recent literature also reports the use of protein/polysaccharide complexes to stabilise water-in-water emulsions [66, 67].

## Conclusions and outlook

The interactions between proteins and polysaccharides can be used to direct their association and, ultimately, the characteristics of the resulting (emul)gels from a structural and rheological/mechanical point of view. However, it is difficult to make comparisons between systems, as different formulation and process parameters are involved and the intrinsic variability of natural ingredients complicates the painting an overall picture. Much research has been done on an increasing range of different systems at particular conditions driven by potential applications. However, it remains a difficult task to predict behaviour of a particular mixtures at particular conditions based on the behaviour of the individual components using general knowledge of the interactions between proteins and polysaccharides. More systematic research on well-defined systems with typical behaviours and covering a wide range of conditions may help to acquire more general knowledge of the behaviour of (emul)gels formed by mixtures of these two types of biopolymers. It is important to include the behaviour of the pure systems for comparison with that of the mixtures. The type (associative or segregative) and extent (relative volume fractions) of phase separation should be clearly identified. The choice of experimental conditions such as heating time and duration should not be chosen arbitrarily, but should be motivated based on preliminary studies in which they are varied. Nevertheless, it is clear that faced with new consumer demand for healthier, environmentally-friendly foods, the use of in particular plant proteins in combination with polysaccharides has excellent potential, eg in providing vegan alternatives for cheese and meat.

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