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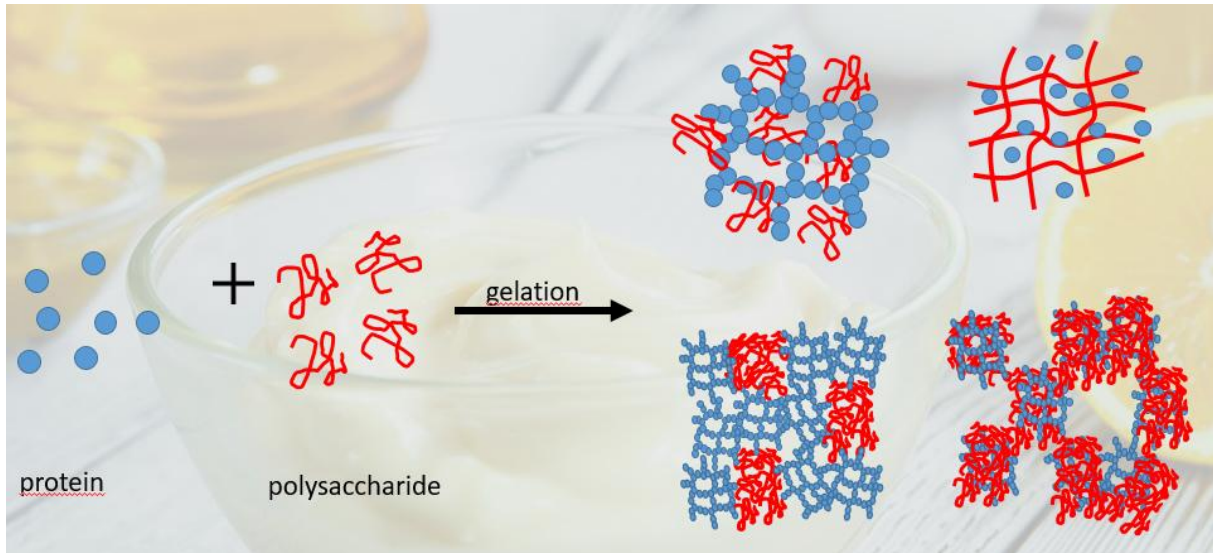
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# 1 Mechanical properties and microstructure of (emul)gels formed by mixtures 2 of proteins and polysaccharides

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5 TOC :



6

## 7 Abstract

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

18

## 19 Introduction

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

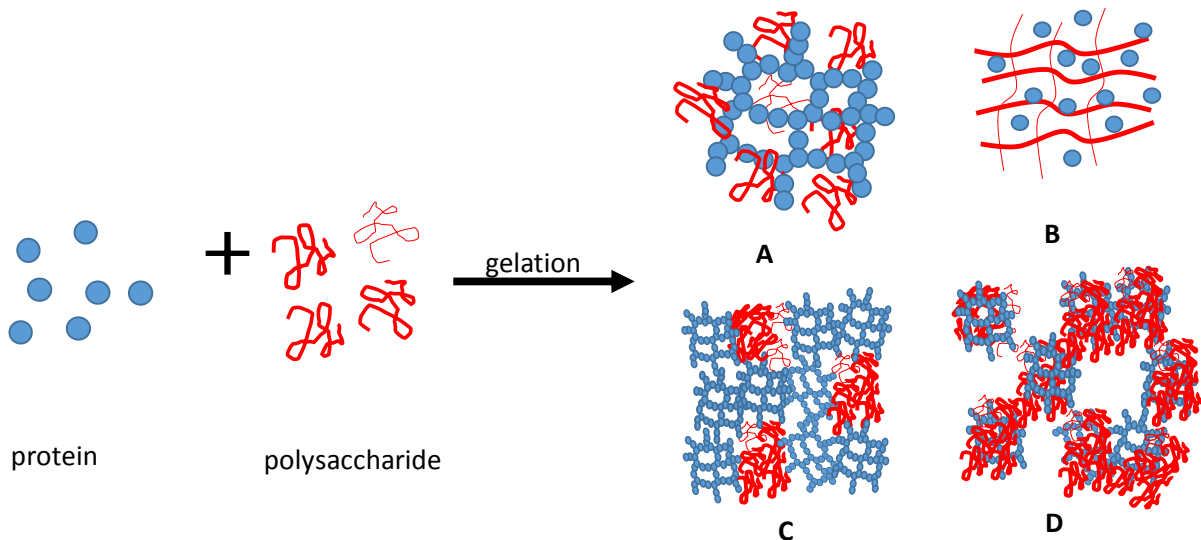
29 Therefore, we will only briefly mention the general features here and refer to other reviews for more  
30 details.

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

52 Gelation involves the formation of crosslinks between the macromolecules leading to the formation  
53 of a system spanning network that needs to be sufficiently strong to resist gravity. In mixtures the  
54 network can be formed by crosslinks between the proteins, between the polysaccharides or both  
55 [5]. It is also possible that the proteins bind to the polysaccharides. Gelation can be induced by  
56 various methods [6]. Gelation of globular proteins is induced by denaturation exposing hydrophobic  
57 groups leading to the formation of crosslinks between denatured proteins [7]. Denaturation is most  
58 often induced by heating (leading to so-called heat-set gels), but can also be achieved by applying  
59 high pressure. Other gelation methods can be applied at low temperatures (cold-set gels) and involve  
60 adjusting the pH closer to the pI or adding salt, both of which reduce electrostatic repulsion. Using  
61 these methods most crosslinks are physical though disulfide bridging can also occur. Covalently cross-  
62 linked protein gels can be formed by adding enzymes such as transglutaminase. Gelation of  
63 polysaccharides often involves addition of salt combined with cooling, but in a few cases it is induced  
64 by heating [8]. The gelation rate and the microstructure of pure protein and polysaccharide gels  
65 depends on the type of protein and polysaccharide, but also on the environmental conditions such as  
66 temperature, type and concentration of salt, pH and polymer concentration.

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

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



85  
 86 Figure 1 Schematic drawing of different types of mixed protein/polysaccharide gels: unbound  
 87 polysaccharide homogeneously distributed throughout the protein network (A) or vice versa (B);  
 88 segregative (C) or associative (D) microphase separated gel.

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

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

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

122 Here we review the literature on gels formed by protein/polysaccharide mixtures published between  
123 2021 and early 2023 focussing on food related systems. A range of different techniques have been  
124 used to study different aspects of the gels, but in this review only the mechanical properties and the  
125 microstructure of the gels will be considered. The stiffness of the gels can be characterized by the  
126 storage shear modulus ( $G'$ ) determined in the linear response regime. Another mechanical property  
127 that is often determined is the hardness, also called gel strength, defined as the maximum force  
128 during compression, which may, however, depend on the geometry that is used and the rate of  
129 compression. Confocal laser scanning microscopy (CLSM) is a non-invasive method to determine the  
130 microstructure of the gels with a resolution of approximately 0.1  $\mu\text{m}$ . Scanning electron microscopy  
131 (SEM) has a higher resolution, but requires severe treatment that often perturbs the gel structure as  
132 was nicely illustrated recently by Zhang et al. [9] who showed both CLSM and SEM images of the  
133 same gels at different scales. SEM images showed large structures at low resolution for gels that  
134 CLSM showed were perfectly homogeneous on these length scales, see Fig. 1. Li et al. 2022 also  
135 noticed the difference between the microstructure of casein gels as observed by SEM and CLSM. The  
136 microstructure of the gels influences its mechanical properties, but there is no straightforward  
137 relationship between the two, because the latter are determined by the flexibility of the structure  
138 and resistance of the crosslinks to stress that cannot be seen in a static image of the structure.  
139 Nevertheless, investigation of the microstructure is important, because it determines the visual  
140 aspect of the gels and to a large extent their water and/or oil retention capacity.

## 141 **1. Gels**

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

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

161

### 162 *1.1 The effect of polysaccharides on heat-set protein gels*

163 In almost all studies covered by this review a fixed heating protocol was used consisting of heating up  
164 to a relatively high temperature varying between 80°C and 95°C, holding the temperature between  
165 10 and 50 min followed by cooling. Generally, it is not explained why a particular heating protocol  
166 was chosen and it is likely the mechanical properties of the gels will depend to some extent on the  
167 heating protocol that was chosen as was shown by Zhang et al. [9]. Serabi et al. [11] studied the  
168 influence of adding basil seed gum (BSG) on gels formed by heating whey protein isolate (WPI)  
169 solutions at pH 7 and 0.05 M NaCl. WPI contains a mixture of globular proteins, mostly  $\beta$ -  
170 lactoglobulin (b-Ig) and  $\alpha$ -lactalbumin. Segregative phase separation was observed in mixtures  
171 before heating at higher BSG concentrations. For a given heating rate, gelation occurred at a lower  
172 temperature if more BSG was present and stiffer gels were formed. In mixtures of b-Ig and the  
173 polysaccharide Levan at pH 7 segregative phase separation was found that was favored by increasing  
174 the molar mass of the polysaccharide or by adding 0.1 M NaCl [12]. During a heating ramp, b-Ig  
175 solutions gelled at a lower temperature when salt was added, because the aggregation rate is faster  
176 when electrostatic repulsion is screened. The apparent gelation temperature depends, of course, on  
177 the heating ramp rate, but at a given rate it decreased with increasing Levan concentration and more  
178 so when its molar mass was higher.  $G'$  measured after keeping the samples 20 min at 90°C was much  
179 larger in the presence of salt, in part due to the faster gelation rate and in part due to the different  
180 structure of the gels. Addition of Levan led to an increase of  $G'$  up to a certain concentration, but  $G'$   
181 decreased again when more Levan was added. It was suggested that segregative phase separation  
182 induced by adding either Levan or BSG increased the gel stiffness by locally increasing the protein  
183 concentration, but strong phase separation at higher Levan concentrations weakened the gel  
184 perhaps by reducing the connectivity between protein rich domains.

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

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

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

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

237 An investigation of the effect of adding 0.5 % w/v dextran or dextran sulfate (DS) on gels formed by  
238 heating egg white protein at pH 4, 7 and 9 showed little effect of dextran on G', but in the presence  
239 of DS a significant decrease at pH 4 and an increase at pH 7 [9, 25]. The hardness defined as the  
240 maximum force during compression was much smaller both in the presence of dextran and DS at pH  
241 4, and somewhat larger in the presence of DS at pH 7. In this study the authors varied the holding  
242 time at 90°C and showed that the hardness increased significantly with increasing holding time. The

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

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

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

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

275

## 276 *1.2 The effect of polysaccharides on cold-set protein gels*

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



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

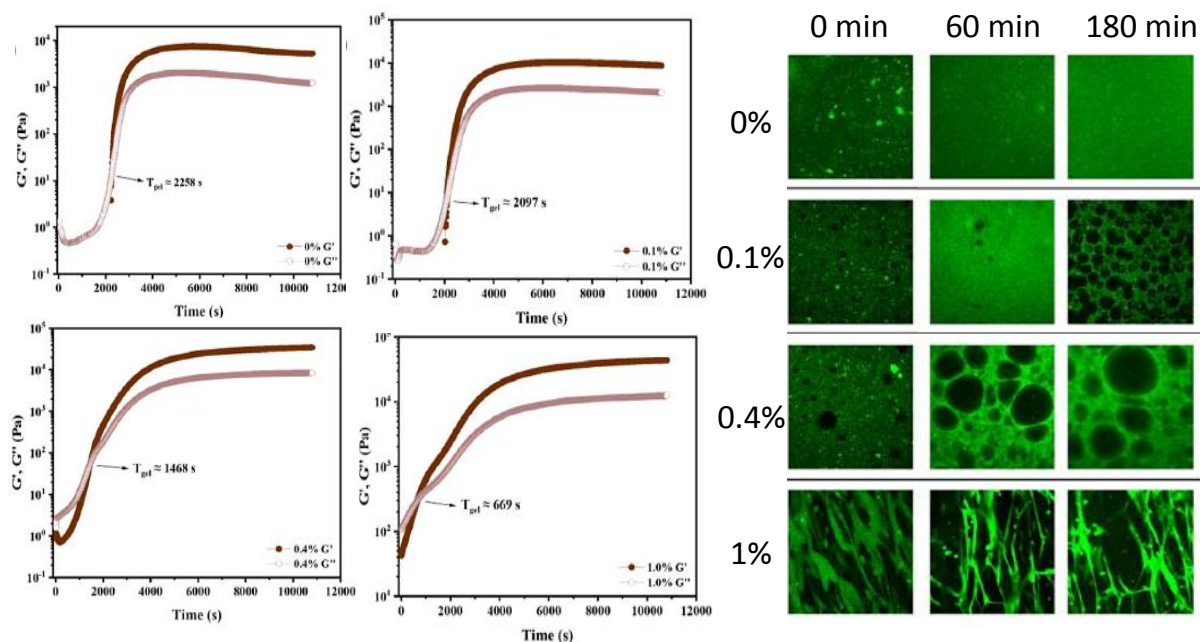
290 An alternative method to obtain proteins gels at low temperatures is to use gelatin or casein  
291 proteins. The stiffness of a gelatin gel increases with time at a rate that increases with decreasing  
292 temperature. Ji et al. [32] added hydroxy propyl methylcellulose (HPMC) to gelatin in an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

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

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

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

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



338  
 339 Fig. 2 Evolution of the storage and loss moduli during acidification of casein solutions with different  
 340 amount of ALG added. CLSM images taken at different time during acidification are also shown.  
 341 (reproduced with modifications from [9]).

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

347

### 348 1.3 The effect of proteins on polysaccharide gels

349 If the proteins don't crosslink, gels can still be formed by physical crosslinking of the polysaccharides  
 350 in the mixtures. Hu et al. [39] investigated gelation of k-car in the presence of native b-lg or in the  
 351 presence of b-lg aggregates in the form of nanoparticles or fibrils formed by preheating at pH 5.8 and  
 352 pH 2, respectively. The effect of the proteins was investigated at pH 9 where both components are  
 353 negatively charged and at pH 4 where the proteins have a net positive charge and form a complex  
 354 with the still anionic k-car. At pH 9 gelation of k-car during cooling was little influenced by the  
 355 presence of the proteins, but at pH 4 the proteins perturbed the coil-helix transition of k-car and as a  
 356 consequence  $G'$  decreased strongly with increasing protein concentrations. This effect was stronger  
 357 when b-lg was added in the form of fibrillar aggregates suggesting a more extensive interaction of  
 358 the k-car chains with the latter. A reduction of  $G'$  caused by complexation of native b-lg was also  
 359 observed by Taheri et al [40] for the anionic polysaccharide cress seed mucilage at pH 3.5. Lin et al.  
 360 [41] heated mixtures of peanut proteins with k-car or gellan at 90°C under stirring. Gels were  
 361 subsequently formed by cooling to room temperature. These gels melted again upon heating to 80°C.  
 362 As might be expected,  $G'$  and the hardness of the gels increased with increasing polysaccharide

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

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

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

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

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

397 Finally, Yao et al. [48] added gelatin to a solution of *Artemisia sphaerocephala* Krasch polysaccharide  
398 (ASKP). By itself ASKP slowly formed a gel when  $\text{FeSO}_4$  was added at pH 4, but the gelatin by itself did  
399 not gel at the conditions studied. Addition of gelatin slowed down gelation of the polysaccharides at  
400 25°C and reduced both  $G'$  and the hardness measured after 3 days. The influence of gelatin on the  
401 polysaccharide gelation was attributed to complexation caused by opposite charge interactions,  
402 which implies that it will strongly depend on the pH.

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

## 408 2. Emulgels

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

### 430 2.1 Influence of the preparation method of the emulsions

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

442 The second way for obtaining emulsions stabilised by protein/polysaccharide complexes involves  
443 preparing protein/polysaccharide complexes *ex situ* and dispersing them in the aqueous phase,  
444 which is then emulsified with oil. The *ex situ* method makes it possible to better study and optimise  
445 the characteristics of the complexes before they are used as stabilisers, although their interaction  
446 with the interface and their further processing may ultimately affect these characteristics. Liu et al.  
447 [58] compared the two processes using hemp protein isolates (HPI) with various polysaccharides. The  
448 emulsions prepared *in situ* proved to be more stable over time than those prepared *ex situ*, but less  
449 so with temperature or salt addition, which was attributed to differences in the interface structures.  
450 However, the exact composition of this interface and its degree of stratification as a function of the  
451 production process of the emulsion have not been reported. Using sodium caseinate/pectin  
452 mixtures, Liao et al. [59] also concluded that emulsions prepared *in situ* were more stable, while  
453 specifying the electrostatic nature of the pectin/caseinate interaction mechanism as evidenced from

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

## 457 *2.2 From emulsions to emulgels*

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

472 In the previous example, gelation of the continuous phase is induced by the addition of calcium ions  
473 or protons. However, it can also occur without using any external crosslinking agents as described  
474 hereafter. Sun et al. [62] prepared emulgels using fibrillar aggregates of WPI (WPIF) complexed with  
475 arabic gum that were insoluble between pH 4.6 and 6. For an oil volume fraction of 60%, stable  
476 emulsions were obtained either as liquids with WPI/AG complexes or as emulgels with WPIF/AG  
477 complexes, which was explained by the fibrils' greater propensity to complex AG. The result was the  
478 formation of bundles, which promoted the formation of a network within the emulsion. In terms of  
479 sensory analysis, these emulgels are on a par with commercial mayonnaises. Similarly, Dong et al.  
480 [63] produced emulgels by complexing ovotransferrin fibrils and pectins with a pearl necklace  
481 structure. With the complexes, the moduli of emulgels formed by mixing with canola oil and  
482 candelilla wax were increased by more than one order of magnitude compared with their  
483 counterparts stabilised by fibrils alone. However, an anisotropic shape of the complexes is not a  
484 prerequisite for achieving emulgels as evidenced by Su et al. [64]. They produced complexes based  
485 on beta-lactoglobulin microgels and PGA whose hydrophilicity increased with the proportion of PGA  
486 and which were used to stabilise triglyceride emulsions. PGA stiffened WPI microgels, which  
487 improved the emulsion stability and led to the formation of emulgels when the oil volume fraction  
488 was increased. Some emulgels resisted centrifugation and their microstructure, measured by  
489 confocal microscopy, indicating the presence of a PGA network in the continuous phase.

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

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

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

510

## 511 **Conclusions and outlook**

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

530

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532

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