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Ability of Various Proteins to Form Thermostable Gels with Propylene Glycol Alginate

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ABSTRACT

Propylene glycol alginate forms gels with proteins in alkaline conditions (pH 9·3 to 10·5). The ability of gelatin, casein, soya isolate, whey protein concentrate, egg albumin and bovine serum albumin to form 'protein–alginate' gels was investigated. Conditions were varied so as to alter the structures of the protein and observe the effect on gel formation. These conditions include changing pH, heat, the action of nitrous acid, SDS, mercaptoethanol, hydrogen bond breakers and changing concentration of the protein. The extent of gel formation was indicated by its rigidity modulus. The tertiary structure of the protein appears to be a very important factor in the gel formation. The gels are readily brought down to pH values more suitable for foods, and are stable to heating at 95°C and to freeze–thawing.

INTRODUCTION

In a patent (Agfa, 1964) it is claimed that an aqueous solution of propylene glycol alginate (PGA) can react with gelatin in alkaline solution to produce gels which are resistant to breakdown at high temperatures and which swell only to a limited extent in water. These

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features are advantageous for the hardening of a photographic film and ought to be useful to the food industry, particularly if proteins other than gelatin could be used and if the stability was maintained at a more useful pH. The idea would be to bind the alginate ester to proteins which are unattractive and may be inedible, to form attractive, palatable foods with the addition of flavour and texture. A patent along these lines has been put forward by Unilever (1976) for using waste fish protein.

Except for patents, the only other publications on these gelatin–alginate gels are by McDowell (1970), Ranganayaki (1977), Wilson (1978), Chan (1979), and Stainsby (1980). Ranganayaki developed a simple means of preparing homogeneous gels, thus preparing the way for further investigation of their physical and mechanical properties. She also showed that gelatins which have had their amino groups blocked chemically could not form a gel with PGA. Wilson presented evidence strongly suggesting that the crosslinking is due to the formation of amide bonds between uncharged amino groups on the protein and esterified carboxyl groups on the polysaccharide. Chan measured the absolute rigidity modulus of gelatin–alginate gels at various temperatures and times after setting. McDowell reviewed the nature of the reactions with amino and hydroxyl groups, on both large (gelatin, starch, polyvinyl alcohol) and small molecules, and considered potential uses in the cosmetic and pharmaceutical industries. Stainsby reviewed the information then available for linking polysaccharides and proteins by covalent bonds to form edible gels.

The main objectives of the present investigation are to see whether other proteins can form similar gels with PGA, and the structural requirements for gel formation. The thermal stabilities—both to heating and to freezing—are also examined.

The proteins selected for these experiments range from gelatin, which has a highly disordered structure, to serum albumin which is a tightly coiled-up globulin containing 17 S—S bonds. Among the proteins with intermediate structures are sodium caseinate which is disordered like gelatin but which is believed to be partially aggregated (though not like casein in a micelle), and soya isolate, the two major proteins of which consist of several globular subunits held together by hydrogen, hydrophobic and disulphide bonds. Egg white (albumen), which is a solution of many proteins, mostly globular, was also examined. Other soluble proteins studied were whey proteins, consisting mainly of β-lactoglobulin and α-lactalbumin. The latter is a single polypeptide chain

with four intramolecular S—S bonds. The lactoglobulin may be considered as two polypeptide strands, each compactly folded and held by S—S bonds. Like soya, the sub-units become dissociated in alkaline conditions.

MATERIALS AND METHODS

PGA (Manucol Ester E/RE) was obtained from Alginate Industries Ltd, Great Britain. The proteins used were in the form of air-dried powders: gelatin, two stocks of fat-free sodium caseinate, Old (O) and New (N), (BDH); Promine D soya isolate; whey protein concentrate (WPC) Nos 56 and 64, from cheddar whey and Solac, a New Zealand product obtained after casein manufacture (all from Express Dairies); WPC No. 4, said to be of superior quality, manufactured as a partial replacement for egg white; egg albumen (BDH) and the purest form of bovine serum albumin (Sigma). WPC Nos 56 and 64 contained approximately 58 and 59% protein, of which about one-fifth was caseinate. The rest of the powder was mostly lactose (about 25%) and fat (8–9%). Solac WPC contained 63·5% protein (one-ninth as caseinate), 25% lactose and less than 0·5% fat. No analyses were given for WPC No. 4.

Preparation of gels

Unless otherwise stated a 6% protein solution was made in 0·1M sodium carbonate/bicarbonate buffer at the pH value stated for each experiment. To this was added an equal volume of 4% aqueous solution of PGA (brought to pH 7·0, if a gel containing few bubbles was required). After mixing an homogeneous gel would form. The effect of temperature on the time of formation was noted. With gelatin the temperature should not fall below 35°C, or a normal gelatin gel will also form in a competing reaction.

Prolonged heating at 95°C

The beaker containing gelatin–PGA gel was placed in a 95°C water bath for 6 h.

Treatment with nitrous acid

10 ml water were added to 0·6 g gelatin and allowed to soak for 1 h. To this were added 0·5 ml glacial acetic acid and 1 ml 60 % w/v $NaNO_2$ solution. After standing for 30 min the gelatin was filtered and washed several times with distilled water. 10 ml buffer (pH 9·7) were added, warmed to dissolve and then 2 g of KSCN were added. The solution was then added to 10 ml 4 % PGA.

Treatment with sodium dodecyl sulphate (SDS)

0·4M SDS was added to the gelatin solution before mixing with the PGA solution. SDS of a final concentration of 0·2M was also allowed to diffuse into protein–alginate gel over a period of 2 days.

Treatment with disulphide bond breakers and hydrogen bond breakers

For some proteins, the effect of adding, final concentration, 1M KSCN, 4M urea or 1 % mercaptoethanol on gel formation was also studied.

Measurement of gel strength

Gel strength was measured as an absolute rigidity modulus, using the Saunders & Ward (1954) U-tube apparatus. Carbon tetrachloride was used instead of mercury to monitor the deformation of the gel. Immediately after mixing, the PGA and protein solutions were passed into the U-tube so that the gel formed there. No slippage was noticed when air pressure was applied. Deformations of up to 9 cm, in the capillary tube, were used. When the pH of the gel was changed a different method, using an Instron, was needed (see later).

RESULTS AND DISCUSSION

Gelatin–alginate gels

6 % gelatin forms a good strong gel with 4 % PGA at pH 9·6. Table 1 shows the time of gel formation at various temperatures.

TABLE 1
Time of Gelatin–Alginate Gel Formation at Various Temperatures

Temperature (°C)	*Time of formation* (*s*)
45	10–15
35	25
25	35
15	150
6	225

The time of formation was taken as the time when a high-speed magnetic stirrer, used to obtain thorough mixing, causes the surfaces to bulge severely. Continuous mixing is not necessary for the formation of an homogeneous gel, but in this case the stirrer was allowed to stir continuously so that the change in viscosity of the gelatin–alginate mixture could be detected by eye.

Prolonged heat treatment

Constant observations showed that the gel did not dissolve or melt. At the end of the heat treatment, the gel was slightly weaker than before, as judged by pressing with a finger. It still held its shape fairly well but had changed from colourless to a translucent brown colour (non-enzymic browning).

Treatment with nitrous acid

The treatment with nitrous acid converts all free amino groups of gelatin to hydroxyl groups before reacting with PGA. The α-NH_2 groups react quantitatively in 3–4 min at room temperature, while the more numerous ε-NH_2 groups complete their reactions in about $\frac{1}{2}$ h. No bubbles were seen when gelatin was dissolved in the carbonate/bicarbonate buffer, showing that the amount of acidic gelatin added was slight and not enough to alter the pH of the buffer. When the solution was added to 10 ml 4% PGA, no gel formed.

When a formol titration was carried out on nitrous acid-treated gelatin, it was found that only 5% of the initial free amino groups remained after treatment.

Treatment with SDS

When SDS (final concentration 0·2M) was present, 10 ml 6% gelatin and 10 ml 4% PGA formed a very weak gel of pourable consistency, although the gel became very firm on standing for 2 days. When SDS, final concentration 0·2M, was allowed to diffuse into PGA–gelatin gel over a period of 2 days, the gel did not weaken showing that hydrophobic bonds are not important in the final gel strength.

Caseinate–alginate gels

A 6% solution of sodium caseinate 'O' forms a gel readily with PGA at pH 9·6. 'N' did not gel so readily. Doubling the concentration of 'N' to 12% results in a thixotropic gel even before the addition of PGA. A firm gel formed on the addition of PGA.

The effect of pH on time of formation of gel

Sodium caseinate 'O' was selected for this experiment because it is the only protein apart from gelatin which forms a good strong gel with PGA without the need to add other chemicals and, unlike gelatin, does not form a gel on its own.

The results are shown in Table 2. For caseinate, the pH has to be above 9·25 before a gel can form and preferably in the region above 9·5.

Soya–alginate gels

Table 3 summarises the results of the experiment.

To form a good strong soya–alginate gel, a final soya concentration of at least 6% is required. At 3% final soya concentration a higher pH value (9·7) needs to be used, whereas pH 9·2 was sufficient for 6% soya gels. In

TABLE 2
Time of Gel Formation in the Presence of Carbonate/Bicarbonate Buffers at Different pH Values

pH:	9·25	9·3	9·4	9·45	9·55
Time of gel formation (min):	55	31	5	4	2

TABLE 3
Effect of pH on Gel Formation

Final soya concentration in gel (%)	*(Carbonate buffered) pH of gel*	*Description*
3	9·5	Weak gel formed in $\frac{1}{2}$–1 h
3	9·7	Slightly stronger gel
6	9·2	Fairly firm gel — Time of formation decreased, brittleness increased.
6	9·8	Firm gel — After $\frac{1}{2}$ h, the strength of all the gels was almost the same.
6	10·1	Firmer gel
6	10·5	Gel formed quickly and disintegrated quickly

the absence of carbonate/bicarbonate buffer, a higher pH had to be used before any gel formed and, even so, the gel was lumpy.

The higher the pH the higher the proportion of uncharged amino groups and hence the more favourable the reaction with PGA, although too alkaline a pH would result in rapid destruction of not only the PGA molecule, by β-elimination, but the protein molecule as well. Alkaline pH also causes denaturation of protein molecules by electrostatic repulsion and breakdown of disulphide bonds (Friedman, 1973), and though this may also favour reaction with PGA it is evident that the dominating effect is the breakdown of polysaccharide.

Whey–alginate gels

All the four kinds of WPC dissolve readily in buffer and form gels at 3% final concentration with PGA in the absence or presence of denaturing agents. Unlike those from gelatin, all these gels melted when heated and did not solidify again on cooling, perhaps because the conditions at pH 9·7 and 85 °C were too severe. On storage for 1 year all the WPC lose their ability to form gels with PGA. (Some of the lysine may have undergone Maillard reaction.) The ability to form gels can be partly restored by treating with mercaptoethanol at 60 °C. WPC which has not undergone prolonged storage forms even stronger gels after treatment with mercaptoethanol and heat. The denaturing action of mercaptoethanol and heat disorganises the proteins so that they become more like gelatin and casein. Increasing the pH or WPC concentration did not enable stored WPC to form gels with PGA.

Egg albumen–alginate gels

6% egg albumen powder in pH 9·7 buffer does not form a gel with 4% PGA. Neither does 50% liquid egg white (approximately 6% albumen) in the same buffer. The globular structure of most of the proteins in egg albumen was thought to prevent reaction. When a similar albumen solution was denatured at 40°C for 1 h in 1M KSCN and 1% mercaptoethanol, a gel formed spontaneously without the addition of PGA solution. That is, denaturation has been followed by agglutination/coagulation.

With a higher concentration of KSCN or urea (10M), aggregation could be seen, and a weak gel then formed with PGA after standing for 1 h.

Increasing the concentration of egg albumen, and increasing the pH from 9·2 to 9·9, did slightly improve the gel formation with alginate.

Bovine serum albumin

Serum albumin did not form gels with PGA at pH 9·6. However, in the presence of 1% mercaptoethanol and 1M KSCN at 40°C, a stiff gel formed on the addition of PGA. When this was dialysed for 4 days to remove all denaturants, the gel remained whole.

Development of gel strength

The development of strength, after setting, is shown in Fig. 1, from which it is evident that by far the strongest gel is formed using gelatin. Figure 2 compares the strengths of gelatin gels with and without added PGA. The gelatin–PGA gels normally include gelatin–gelatin associations which enhance the strength, but these have been disrupted (by thiocyanate) in Fig. 2. This demonstrates that most of the strength in the gelatin–PGA system is due to covalent crossbonding, and not to hydrogen bonding. When only covalent bonding is present, as in Fig. 2, the dependence of strength in gelatin concentration is very marked indeed. In contrast (Fig. 3) the dependence on polysaccharide concentration reaches a limit. This is not really surprising as the ratio of uncharged amino groups to ester groups, on mixing (i.e. before the inevitable alkaline de-esterification) is at most 1 to 50 at pH 9·6, so changing the protein content has much more effect than changing the PGA content.

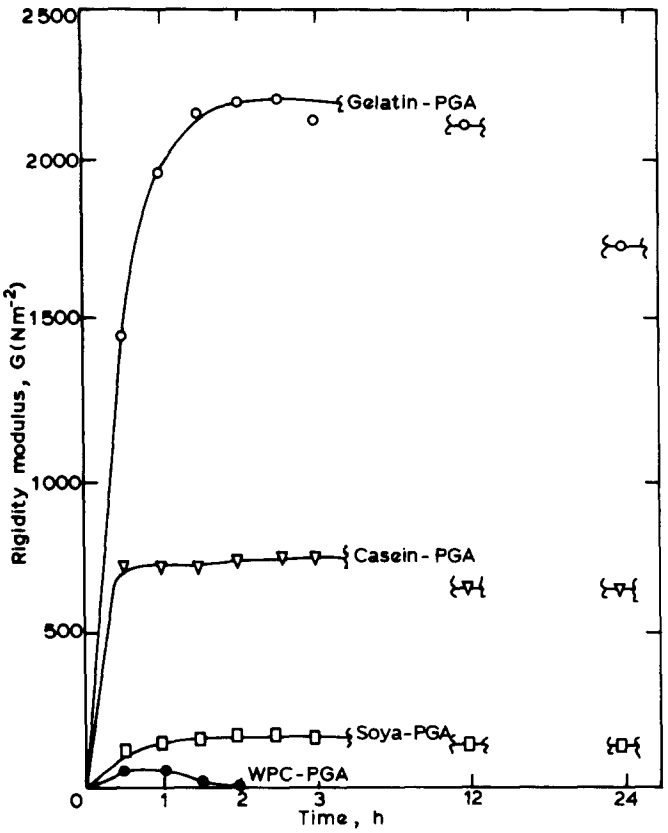

Fig. 1. Rigidity moduli at different times for four different gels at room temperature. (All gels made with 3 g protein and 2 g PGA per 100 g water.)

The maximum strength, in these rather alkaline conditions is reached in a few hours (Fig. 1) and then slowly declines. The most probable cause of the decline is alkaline depolymerisation of polysaccharide forming the gel network. Titration of alginate ester with alkali in a pH-stat at room temperature, in the absence of protein, shows that about 25% of the uronic acid groups remain esterified even after 1 day. Depolymerisation, at the glycosidic bonds adjacent to such groups, by β-elimination is therefore bound to occur and to continue to fragment the protein–PGA network during storage, i.e. during these rigidity measurements, unless the pH is changed to prevent degradation.

Gelatin–PGA gels feel rather rubbery and like agar gels. This is shown in the rigidity measurements—on application of the load (air pressure)

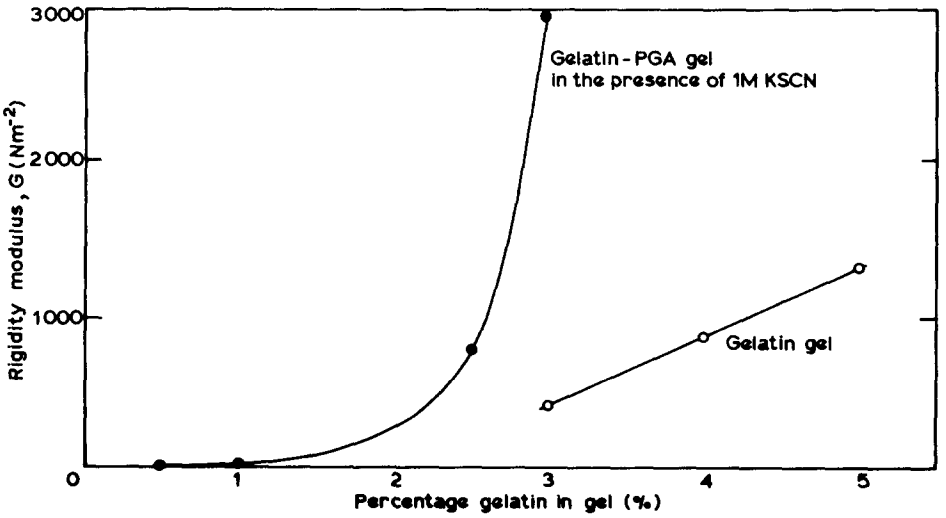

Fig. 2. Effect of gelatin concentrations on rigidity modulus of gelatin and gelatin–PGA gels after 2 h mixing.

there is an instantaneous response (deformation) which is independent of time and fully recoverable when the load is removed. Such behaviour is quite unlike that of a normal gelatin-in-water gel, which creeps under load, i.e. the initial deformation is followed by a further (smaller) time-dependent deformation and both can be recovered over a long period of time, on removing the load. The lack of creep in a PGA–gelatin gel is consistent with the view that gelatin–gelatin interactions are of little significance and that the polysaccharide–protein network is covalently linked, resembling lightly vulcanised rubber.

All the other proteins so far examined differ from gelatin in that their gels with PGA show creep. The deformation of a caseinate–PGA gel, for example, very rapidly rose to 9·4 cm movement along the capillary tube and then continued to rise to 9·8 cm over 6 min. For this reason the results presented in Fig. 1 refer to deformations after 30 s (9·5 cm in this example).

Lastly, denaturation of globular proteins can increase this 30-s rigidity modulus by a factor of 100. WPC 4, for example, after disorganisation by mercaptoethanol and heat and reaction with PGA, gives a modulus of over $1700\,N\,m^{-2}$ as compared with $17\,N\,m^{-2}$ for the undenatured WPC 4–PGA gel. Soya protein is most readily denatured by alkali.

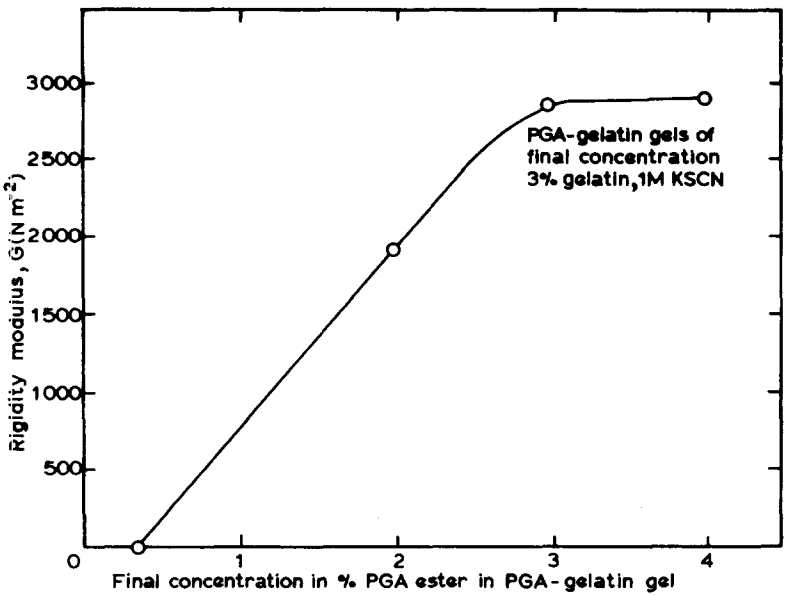

Fig. 3. Effect of PGA concentration on rigidity modulus of gelatin–PGA gel.

Properties after changing the pH

Protein–PGA gels have to be made in mildly alkaline conditions and in this condition they are quite unacceptable as foods. However, they are readily brought down to more acceptable values for pH, by washing with weak acids (acetic, citric, lactic) or by dialysis. During these processes they swell to an extent that depends mainly on the change in the nett charge on the protein and the change in ionic strength as the original buffer is removed.

In the following experiments the pH has been reduced by soaking in citric acid/disodium phosphate buffers, ionic strength approximately 0·15M, once the gel had reached maximum firmness (*c.* 2 h). As expected swelling continued, at a reducing rate, over several days, and after 3 days the rate had fallen to about 5% a day. (Very thin strips, washed in acid, reach this condition in about 20 min.) A convenient time of 4 days soak was chosen.

Except in rather acidic conditions, pH 3 or less, the gels remained transparent even though the protein had passed its normal isoelectric

TABLE 4
Heat Treatment of Protein–PGA Gels

Treatment	*Gelatin*	*Casein (0)*	*Whey Solac*	*PGA–protein gels* *Whey Nos 56 and 64*	*New whey WPC 4*	*New whey + mercaptoethanol at 60°C*	*Egg albumen + mercaptoethanol KSCN at 60°C*
Soaking in phosphate buffer 0·15M ionic strength, pH 7·0	Gel swelled	Gel swelled, remained firm still holds shape	Swelled, still holds shape	Disintegrates	Partially disintegrates	Swelled but still firm and holds shape	Disintegrates
Heating at 85°C for 15 min	Remained solid, did not melt	Did not melt	Did not melt	Dissolved completely (passed into solution)	Passed into solution	Did not melt	Dissolved completely
On cooling	Still a gel but less firm than before	Gel became firmer on cooling but not as firm as before	Still a gel but less firm than before	Did not reform into gel	Did not reform into gel	Still a gel but less firm than before	Did not reform into gel

point. When acidic opaqueness was produced it was due to insolubilisation of entrapped alginic acid, mainly. Thymol was added to retard microbiological growth in the more usual range (pH 4–7) for foods.

Three related properties have been examined—heat stability (at 95 °C), freeze/thaw stability and gel strengths. Table 4 summarises typical results for gels, at pH 7, heated at 95 °C and then cooled. Though some firmness was lost, particularly for gels now holding much more water, the gels are at least as stable as in the mildly alkaline conditions in which they are made.

Freezing and storage when frozen almost always reduces the textural quality of foods, and one way in which this is observed is through a decrease in water-binding. PGA/gelatin gels were therefore frozen at −18 °C for 2 days, brought back to room temperature and the % loss of water determined after centrifugation using a bench centrifuge (approximately 2000 × *g*). Some loss occurred, but was too small to determine precisely with the samples used (typical initial weight 30 g). Similar results were obtained for PGA/casein gels.

A better guide to the extent of damage is given by the rigidity modulus. The Saunders and Ward U-tube method cannot be used, as the gels do not now adhere to the walls of the tube, so compression in an Instron was studied. Cuboids were cut, about twice as wide as they were tall. The height was determined and the area calculated from the weight and density of each gel piece. After carefully placing the gel on the lower platen, and bringing down the overhanging platen, the gel was compressed at the slowest possible speed (0·5 mm min^{-1}), and the load/deformation recorded. A total reduction in height of approximately 1 % was used, being reached in at least 4 stages. The slope of the linear plot of crosshead displacement against load gave Young's modulus for the sample, making the reasonable assumption that the change in height in these experiments is balanced by a corresponding change in area of contact, without significant barrelling. The rigidity modulus is then taken as one third of the Young's modulus as the gels are homogeneous and isotropic. Confidence in the method is given by the following values for an alginate–gelatin gel at pH 9·6, 3 h after setting: Saunders and Ward value = $2{\cdot}8 \times 10^3$ Nm^{-2}; Instron value = $3{\cdot}0 \times 10^3$ Nm^{-2}.

Using the Instron method for gels taken to pH values in the range pH 4–7, no significant changes in modulus were found after defrosting. More substantial losses in moduli occurred at pH 3·0, however. The

magnitude of the modulus, for a given protein–alginate system, depends sharply on pH—rising almost linearly from $1 \times 10^3\,Nm^{-2}$ at pH 7 to $3 \times 10^3\,Nm^{-2}$ at pH 4 for gelatin–alginate gels, for example. This is due to the pH dependence of the water-holding power of the gels. If the moduli are re-calculated in terms of a constant protein content—as if there were no differences in swelling—then the values are almost independent of pH in the range most useful for foods.

CONCLUSION

When aqueous solutions of the ester and protein are mixed, gel formation proceeds smoothly provided the pH lies within fairly narrow limits. Above pH 10 the reaction is too rapid and gross inhomogeneities result, whilst below pH 9 there is no reaction at all. Several competing reactions also occur, including de-esterification and depolymerisation of the polysaccharide and degradation of the protein. The gel thus contains entrapped alginic acid and fragments of the starting materials. Through the appropriate choice of temperature and pH the gelling reaction can be optimised and the time for setting, as well as the ultimate strength, can be controlled. Temperatures in the range 40–50 °C, and pHs in the range 9·3–9·6, are generally convenient.

The disordered protein, gelatin, is particularly effective for forming network gels with alginate ester, and the disordered protein caseinate—which is partially self-aggregated—is the next best of those studied. Proteins with compact globular structures are not good at forming such gels, even though the reactive groups are on their surfaces, but the ability is considerably improved if disordering (by heat or chemicals) precedes reaction with the ester.

For use in foods, the pH of a protein–alginate ester gel must be lowered once the optimum strength has been reached. The gels then retain their stability to heating and to freeze/thawing. The water-holding power of the gels depends on the final pH and ionic strength, and this in turn governs the strength of the system. The formation of a network gel by covalent linkage of polysaccharide and protein, via an amide bond (Wilson, 1978), ensures that the system has useful thermal stability and unusual elastic properties. The ease of formation and useful properties, including adhesiveness, should encourage consideration of these systems in fabricated foods.

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