

Rapport nr. 158

**Spesialkitosaner som
ingrediens i næringsmidler
Utesting av nytt gelsystem**



Marked

RAPPORTTITTEL

**SPESIALKITOSANER SOM INGREDIENS I
NÆRINGSMIDLER. UTTESTING AV NYTT GELSYSTEM.**

RAPPORTNUMMER	158	PROSJEKTNUMMER	4636
UTGIVER	RUBIN	DATO	April 2008

UTFØRENDE INSTITUSJONER

Advanced Biopolymers AS
Herøya Industripark, bygning 127
3908 Porsgrunn

Kontaktperson: Robert Wahren (robert@corecompetence.se)

SAMMENDRAG OG KONKLUSJONER

Basert på en patentsøkt teknologi har ABC (Advanced Biopolymers AS) utviklet en prosess for fremstilling av spesialkitosaner med full løselighet og funksjonalitet ved biologisk pH, og med reproducerbar kvalitet. Disse kitosanene kan egne seg for bruk innenfor medisinområdet, til kosmetikk og til næringsmidler. Det er gjennomført et prosjekt for å teste ut et nytt gelsystem som inneholder kitosan og pektin, og som kan erstatte dyregelatin. Prosjektet er interessant for den internasjonale næringsmiddelindustrien.

Gelsystemet vil kunne benyttes som ingrediens innenfor mange ulike typer næringsmidler, som brød, mousse, gotteri og mikroinkapsulering av vitaminer, oljer og smaksstoffer. Prosjektet har vært en del av et større samarbeid mellom ABC og den danske næringsmiddelbedriften Danisco, og testene er gjennomført ved NOBIPOL ved NTNU. Forsøkene ga positive resultater, men det viste seg at systemet ikke var tilstrekkelig enkelt og robust for å kunne fungere industrielt. Man har ideer til å få frem et enkelt og pålitelig system, men foreløpig er dette for dyrt.

Stiftelsen RUBIN
Pirsenteret
7462 Trondheim

Telefon 73 54 56 30
Telefax 73 51 70 84
E-mail rubin@rubin.no
www.rubin.no



Physical properties of a mixed system consisting of
Chitosan – Pectin - κ -Carragenan

A Project for ABC A/S

Department of Biotechnology, NTNU

January - April 2007

Study overview:

Introduction

Materials and Methods

Results and Discussion

- 1. The effect of pectin/chitosan particles on a carrageenan gel**
- 2. The effect of 1% pect/chit particles with different mixing ratios on a carrageenan gel**
- 3. Different carrageenan concentration in gels with 1% electroneutral pect/chit particles**
- 4. Fixed carrageenan and electroneutral pect/chit particles, varying K^+ concentration**
- 5. Effect of addition of non-specific ions.**
- 6. Differences between high and low acetylated chitosans**
- 7. The present mixed system compared to mammalian gelatin behaviour**

Conclusions

Recommendations

Introduction:

In a pre-project performed at The Norwegian Biopolymer Laboratory, it had been shown that some combinations of Chitosan – Pectin - κ -Carrageenan exhibited synergistic interactions in the sense that low concentration carrageenan gels had improved gel strength and physical properties that could be of a certain interest in the food manufacturing industry. This was most probably due to coacervate formation of micro/nano particles acting as fillers within the carrageenan network. ABC A/S therefore pursued these effects further by initiating a new research project on this topic. Several possible parameters that could have an impact on the physical properties of this system were studied. A re-examination of the results obtained in the pre-project confirmed the original findings and some essential parameter connections was identified. Based on the obtained results, some basic recommendations are given.

Materials and Methods

The following biopolymers were used in this study:

κ -carrageenan from FMC Biopolymer A/S, Batch # REO 10010

Sugar beet pectin from Danisco, batch # A 40590

Chitosans from ABC A/S, $F_A=0.40$ and $F_A=0.08$

The following procedure was used for the preparation of the mixed gels:

2 (w/w) % sugar beet pectin, pH 4.0

2 (w/w) % chitosan $F_A = 0.4$ or $F_A=0.08$ (as acetate salt), pH 4.5

1(w/w)% κ -carrageenan, pH 5.5

pH in the final mixture was always close to 4.5.

Amount of KCl was adjusted in each case.

- Mix pre-heated solutions of pectin and chitosan in varying proportions (total conc.2%)
- Blend on Ultra Turrax for 30 sec.
- Heat to 90 °C
- Mix preheated κ -carrageenan with equal weight of pectin/chitosan at 90 °C
- Blend on Ultra Turrax for 30 sec.
- Apply mixture to rheometer and measure G' as a function of temperature in a small strain oscillatory modus.

Results and Discussion:

The results presented represent the most significant observations achieved and are presented as combined graphs to highlight these observations.

1. The effect of pectin/chitosan particles on a carrageenan gel

Figure 1 shows the dynamic storage modulus (G') as function of temperature of a 0.5% κ -CGN in the presence and absence of pectin/chitosan coacervate particles. It is quite obvious that the presence of pect/chit coacervates influence on the physical properties; gel strength as well as melting temperature. G' (the dynamic storage modulus representing the elastic properties of the gel) increases with almost two orders of magnitude in this particular system, whereas the melting increases with a few (5-6) degrees centigrade. None of the systems set (gel) before reaching 10 °C. Note that the water content in the mixed system is still as high as 98.5%, suggesting that there might be a direct interaction between the continuous carrageenan network and the pect/chit coacervate particles since the amount of such particles is too low to account for the observed results as a pure filler effect. This result is in accordance with the earlier observations from the pre-project.

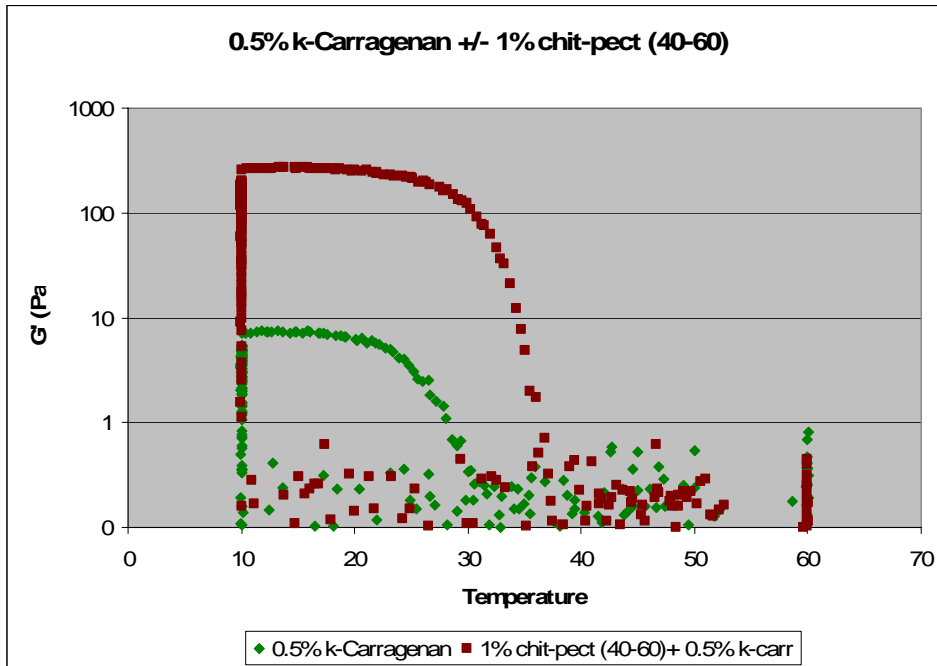


Figure 1 Dynamic storage modulus (G') as function of temperature of a 0.5% κ -CGN in the presence and absence of 1.0% pectin/chitosan coacervate particles. The coacervate particles were made with a mixture of a 0.4 fraction of chitosan and a 0.6 fraction of pectin. The systems are first cooled from 50 °C down to 10, kept there for 1 hour before heating to 60 °C again. Both cooling and heating was performed at a gradient of 1 °C / min.

2. The effect of 1% pect/chit particles with different mixing ratios on a carrageenan gel

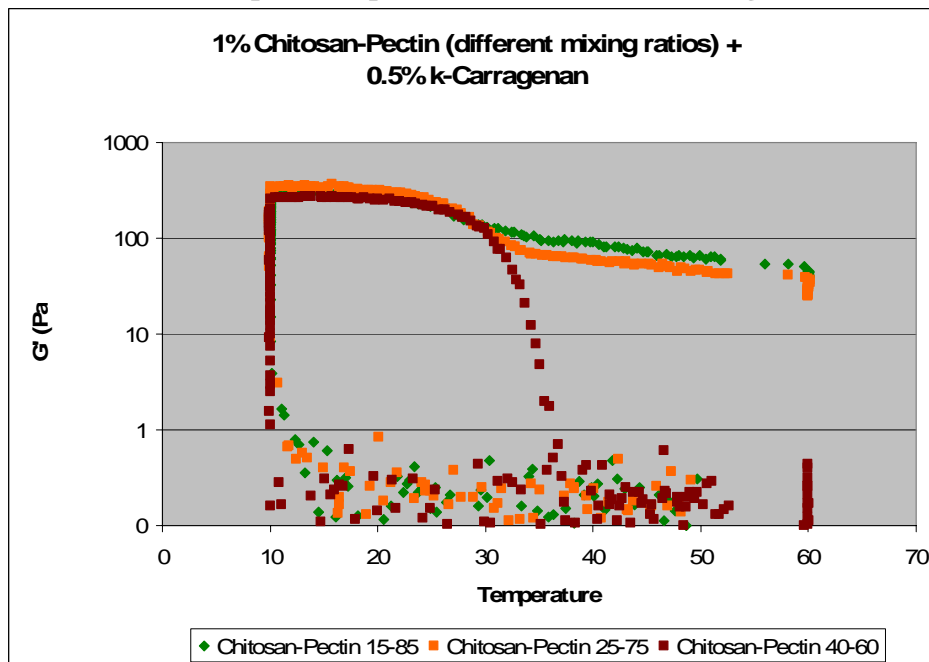


Figure 2 Setting and melting behaviour of a 0.5% κ -carrageenan gel in the presence of 1.0% pect/chit coacervate particles of different mixing ratios.

Figure 2 shows the setting and melting behaviour of a low concentration carrageenan gel in the presence of a fixed amount of pect/chit coacervate particles with different compositions, ranging from a relative large surplus of negative electrostatic charge (15-85) via a slight surplus of negative charges (25-75) to approximately electrostatic neutral complexes (40-60). As can be seen from the graphs, there is little or no difference in the setting behaviour (all types set at 10 °C) and the modulus develop to approximately identical values upon holding at 10 °C. But there is a marked difference in the melting behaviour. As the temperature is increased, the gel containing the electroneutral complexes melts at around 35 °C, whereas none of the other gels, carrying net negatively charged complexes, melts even at as high as temperatures as 60 °C. This particular melting behaviour is most clearly seen when the phase angle, stating the correlation between viscous and solid-like behaviour, is plotted against temperature as seen in figure 3. Since one of the aims of this project was to look for gel systems that can behave like gelatin gels, the use of electroneutral complexes seems to be a pre-requisite if a total melt-down at around body temperature is imperative. Combining this result with data from the pre-project, showing that complexes carrying net positive charge greatly reduce the modulus of the final gel, the importance of using neutral complexes becomes even more important. I will return to this aspect later on.

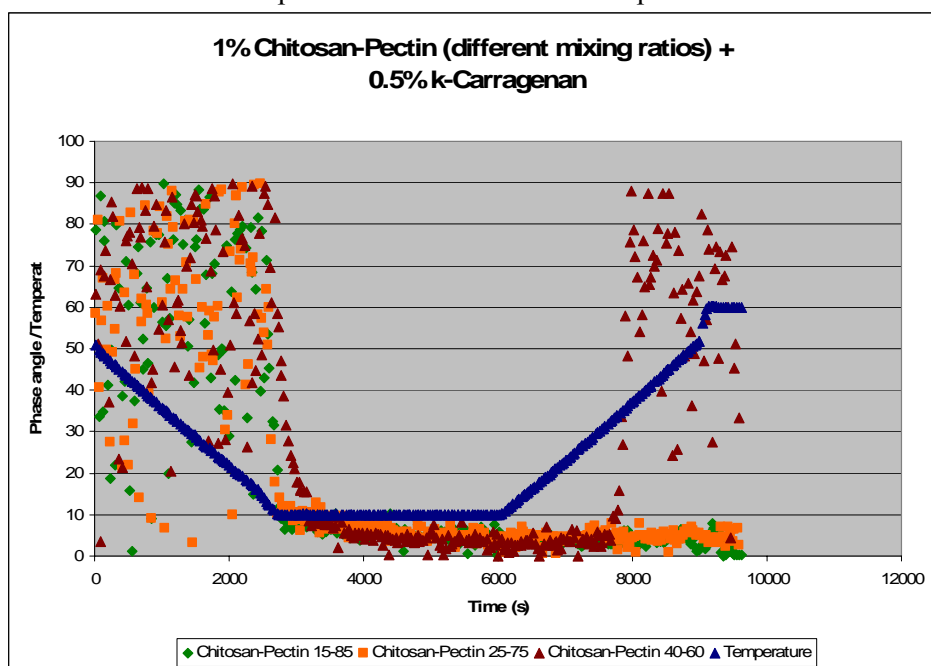


Figure 3 Phase angle as function of temperature for 0.5% κ -carrageenan gel in the presence of 1.0% pect/chit coacervate particles of different mixing ratios.

3. Different carrageenan concentration in gels with 1% electroneutral pect/chit particles

After having found that electroneutral complexes were a pre-requisite for a complete melt-down of these mixed gels, it became logic to look at the effect of carrageenan concentration. Figure 4 shows how the modulus as well as the setting and melting behaviour in gels with a fixed concentration of electroneutral pect/chit complexes (1%) and varying amounts of κ -carrageenan. It is quite obvious that as expected, there is a huge effect on modulus as the carrageenan concentration is increased. As a general rule of the thumb, the modulus is expected to increase proportional to c^2 . In this particular system the increase is somewhat higher, and this is most likely due to the fact that at 0.5% the system is very close to the critical overlap concentration for gelling. In this area, the concentration dependence on modulus is somewhat higher than c^2 . Furthermore, it can be seen that also the gelling and

melting temperatures are influenced by the carrageenan concentration. This suggests that the carrageenan concentration should not be too high if a complete melt-down in the mouth is desired. But still, all gels in this particular concentration range do totally melt (as seen in Figure 5), again most probably a result of the electroneutral particles.

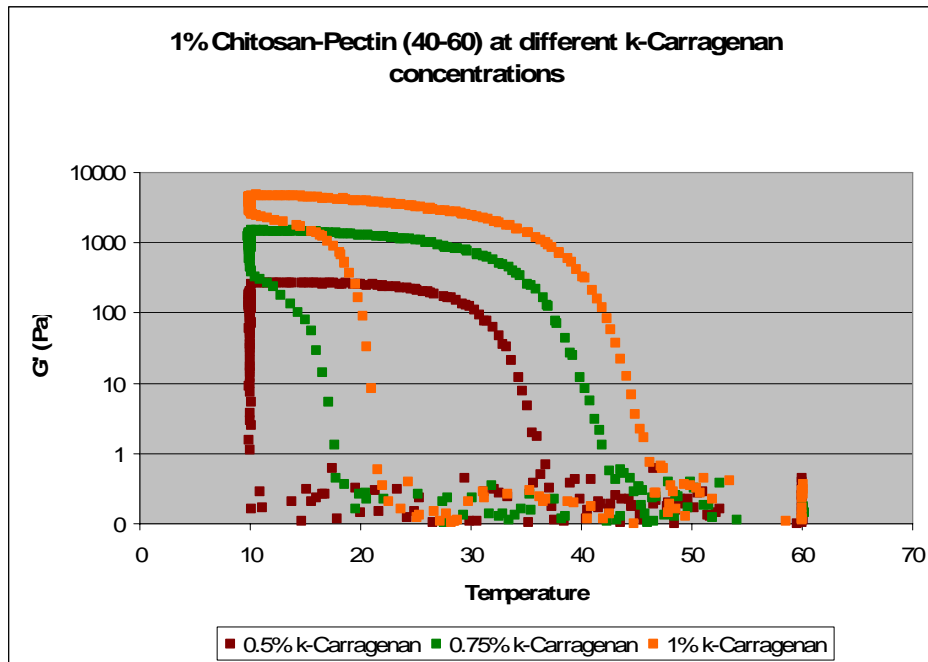


Figure 4 Effect of some physical properties as function of carrageenan concentration in a system with fixed concentration of electroneutral pect/chit coacervates

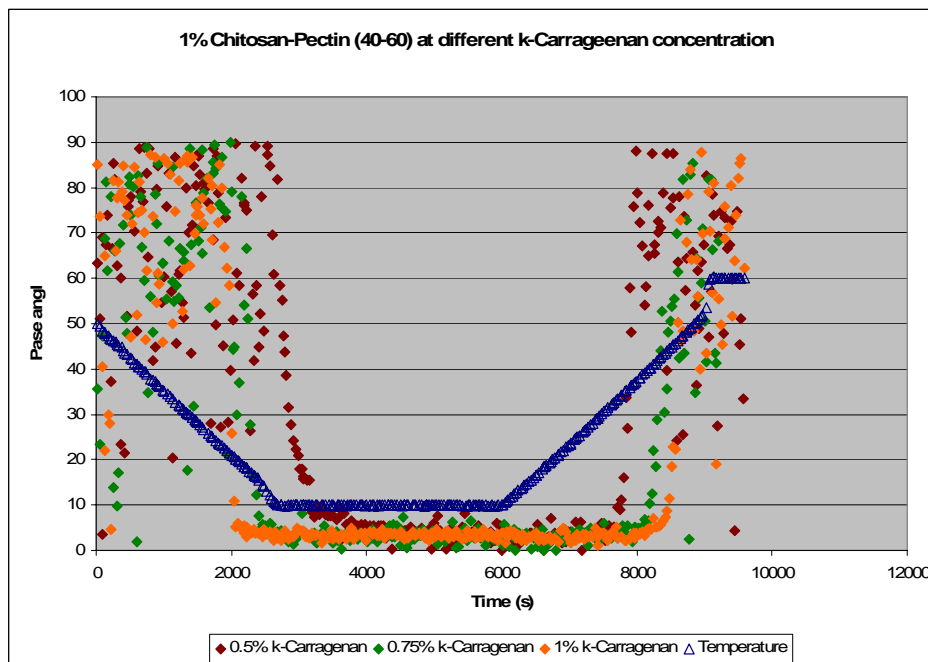


Figure 5 Phase angle as function of carrageenan concentration and temperature in a system with fixed concentration of electroneutral pect/chit coacervates.

4. Fixed carrageenan and electroneutral pect/chit particles, varying K^+ concentration

Since it is well known that potassium is a specific gel promoting ion for κ -carrageenan, it was of a certain interest to see which effect a varying concentration of KCl would have on this mixed system. Figure 6, 7 and 8 shows one example with 0.5% carrageenan. The same picture was also valid for other carrageenan concentrations.

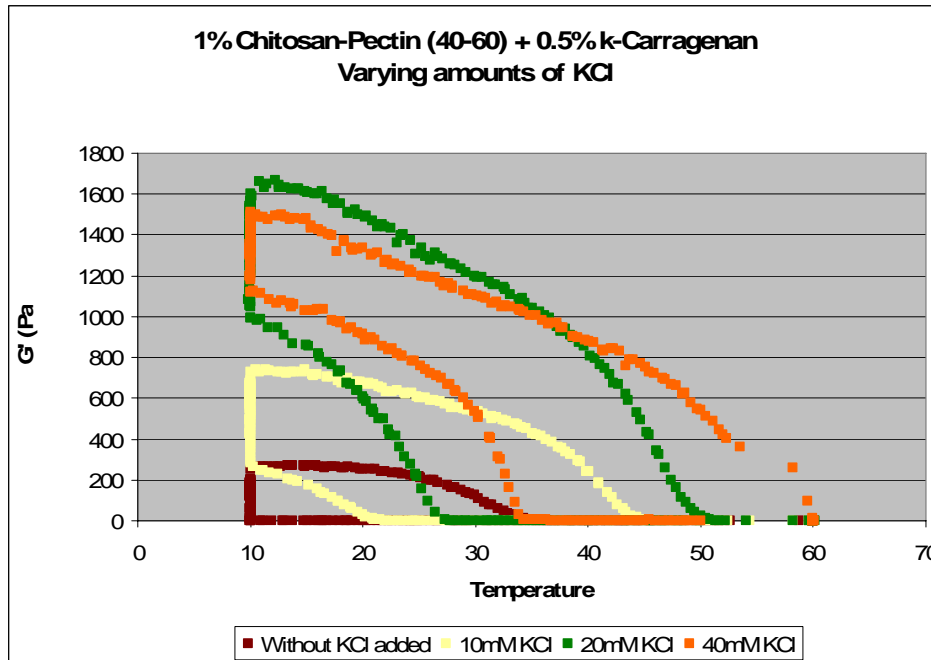


Figure 6 Effect of KCl concentration on a mixed gel system with fixed amounts of carrageenan and electroneutral complexes.

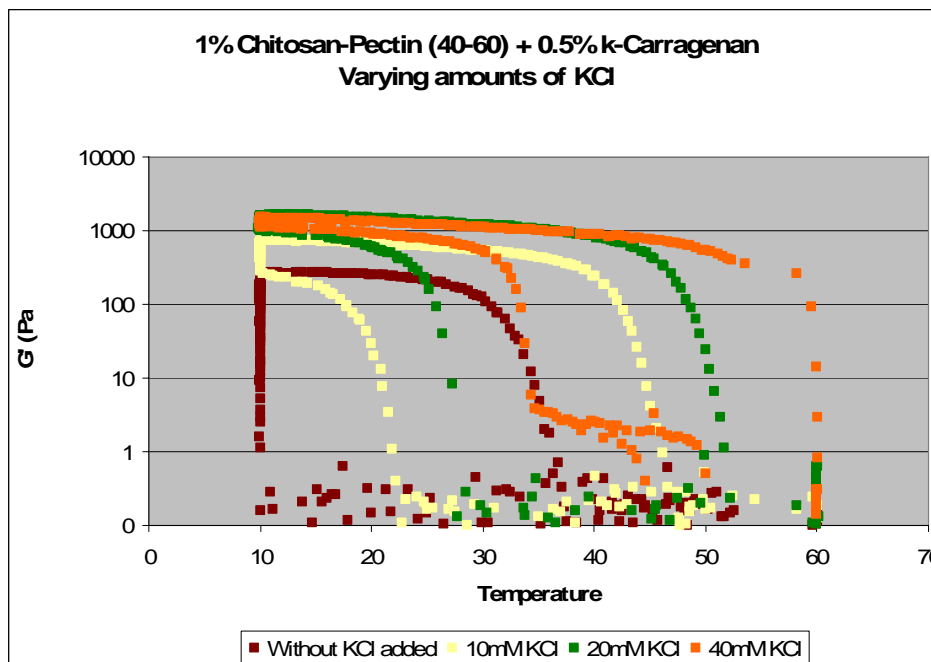


Figure 7 Effect of KCl concentration on a mixed gel system with fixed amounts of carrageenan and electroneutral complexes.

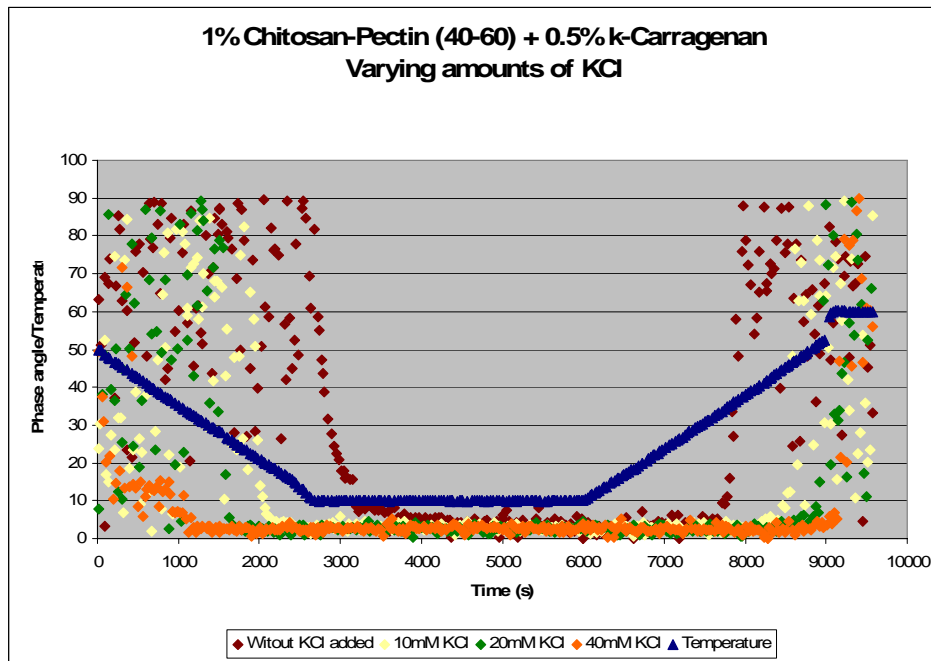


Figure 8 Effect of KCl concentration on a mixed gel system with fixed amounts of carrageenan and electroneutral complexes. Phase angle as function of temperature

It is quite obvious from these figures that increasing the potassium concentration will influence on both modulus as well as gelling and melting temperature. Again, if a melt-in-mouth texture is required, the amount of potassium ions must be controlled.

5. Effect of addition of non-specific ions.

A system containing 0.75% carrageenan with 10 mM KCl and 1% neutral pect/kit coacervates was chosen to look for effects of non-specific ions like sodium. It is well known that Na^+ promotes gelling through a non-specific ionic strength effect. Sodium ions can also, provided that its concentration is high enough, compete with potassium ions and hence also influence on the physical properties of the final gels. The results are presented in Figs 9 and 10. As can be seen from these figures, the maximum gel strength is reached at 100 mM NaCl for this particular system. Any addition beyond this value will reduce the dynamic storage modulus. What is more interesting is that this reduction in modulus at high NaCl concentrations is not accompanied by a reduction in melting temperature, but rather the other way around. The gel containing 250 mM NaCl is the only system that does not melt at all in the temperature regime studied ($> 60^\circ\text{C}$). It may therefore be concluded that this particular system can not be tailored with respect to setting and melting behaviour.

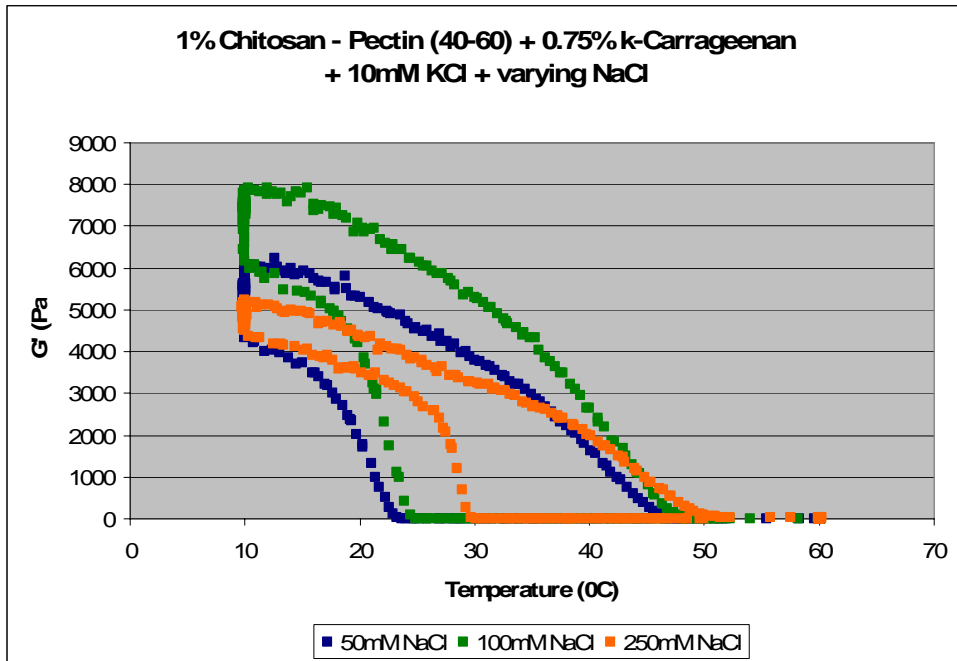


Figure 9 Effect of addition of non-specific ions on the dynamic storage modulus (G') in a fixed carrageenan / pectin / chitosan system.

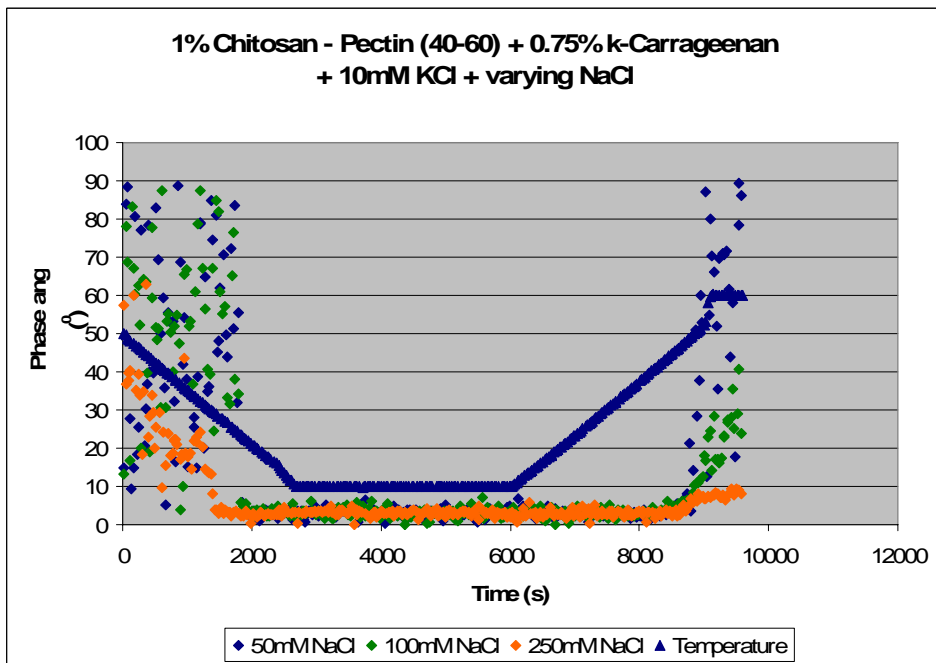


Figure 10 Phase angle as function of temperature in a system with a fixed amount of fixed carrageenan / pectin / chitosan and varying amounts of NaCl.

6. Differences between high and low acetylated chitosans

Figure 11 shows the effect of a fixed addition and composition of pect/chit particles applying one low and one high acetylated chitosan sample. It is quite obvious that at this mixing ratio (40/60), the highly acetylated chitosan will perform considerably better with respect to gel modulus compared to the chitosan low in acetylated units. The difference in modulus is well above one order of magnitude.

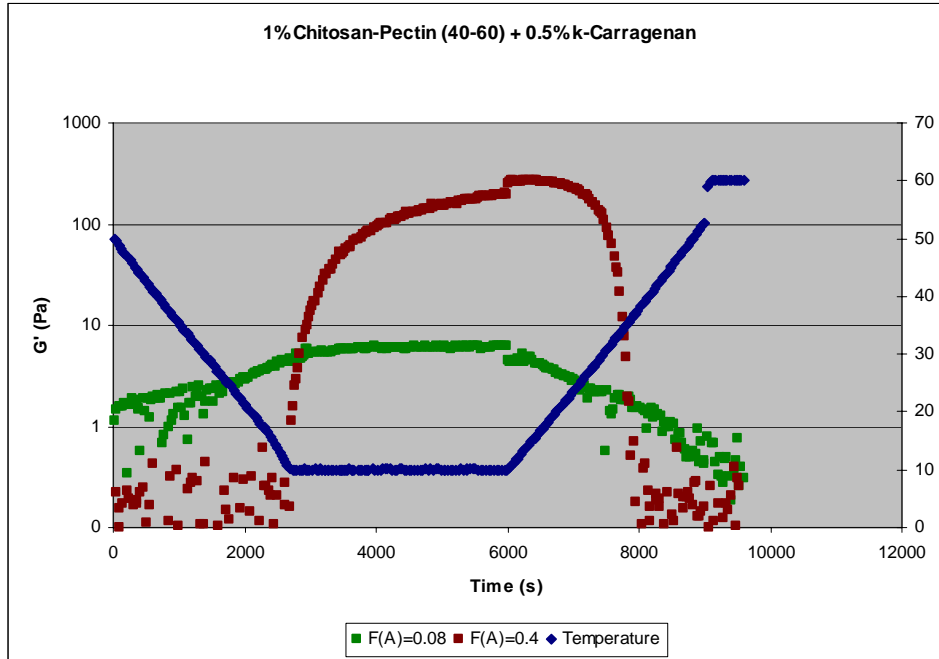


Figure 11 Gel modulus development of a carrageenan gel mixed with a fixed amount and composition of pect/chit coacervate (40-60) particles. Two different chitosans were used

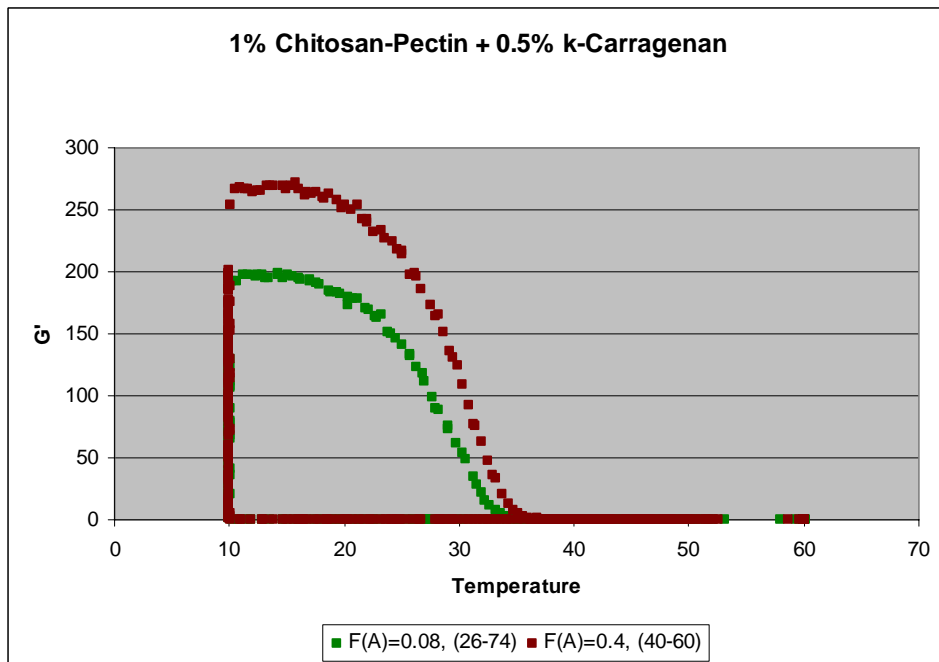


Figure 12 The effect on carrageenan gel modulus adding a fixed amount of pect/chit coacervate particles of an electroneutral nature applying two different chitosans

The reason for this behaviour can be linked to the fact that the coacervate particles made from the highly deacetylated sample in this fixed mixing ratio (40-60) will not be electroneutral but rather have a surplus of positive electrostatic charges. Results from the pre-project showed that such particles will greatly reduce the overall gel strength, probably because they will pull carrageenan molecules out of the network structure and into the complexes. If the composition of the coacervate particles is changed to balance the number of positive and negative charges (26-74) as done in Fig 12, it can be seen that the highly deacetylated chitosan sample start to approach the highly acetylated one. And if the carrageenan concentration is increased to 0.75 % (being further away from the critical gelling concentration), Fig 13 shows that the two different chitosans behave almost identical. For pure watery systems, one possible conclusion may therefore be that as long as the number of electrostatic charges is balanced, the chitosan chemical composition is of less importance.

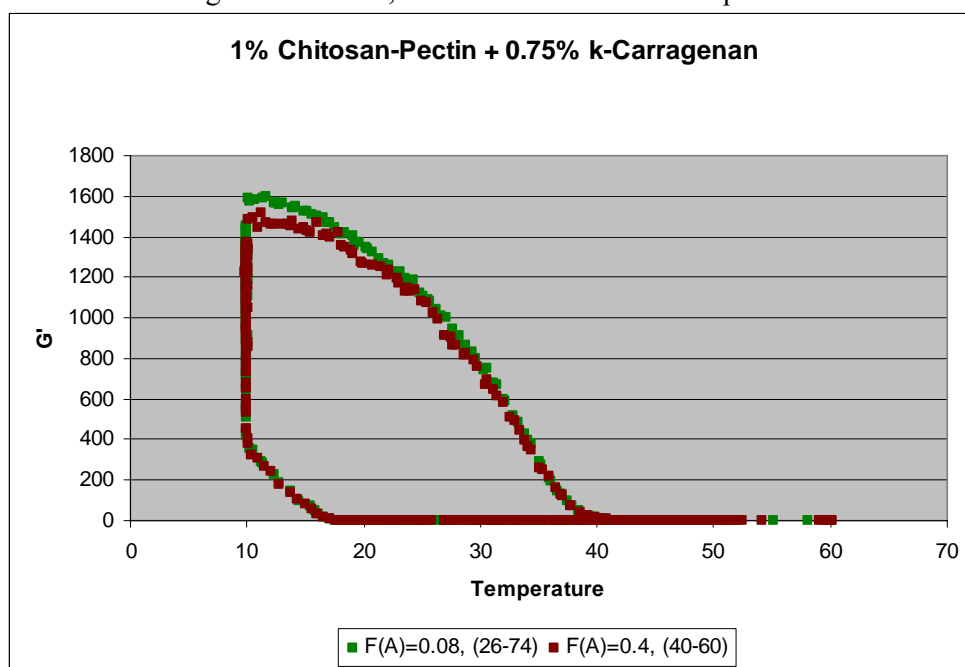


Figure 13 The effect on modulus in a 0.75% carrageenan gel adding a fixed amount of pect/chit coacervate particles of an electroneutral nature applying two different chitosans

There are, however, a few comments to be linked to this conclusion. It should be emphasized that it is probably only valid for

- pure watery gels
- medium to high carrageenan concentration
- low concentration of potassium

We know that highly acetylated chitosans are more surface active than highly deacetylated samples due to the presence of hydrophobic acetyl groups. This has been shown for example in their effectiveness in flocculating oil-in-water emulsions relatively independent upon pH (ABC internal report). This suggests that pect/chit coacervate particles may perform better (increased stability) in mixed phase systems compared to particles made from highly deacetylated chitosans. Any effect in such a context will have to be examined in another research project. And as shown in Fig 14, a difference between the two different chitosans starts to develop again as the content of potassium ions in the system is increased. The reason for this behaviour is at present not clear.

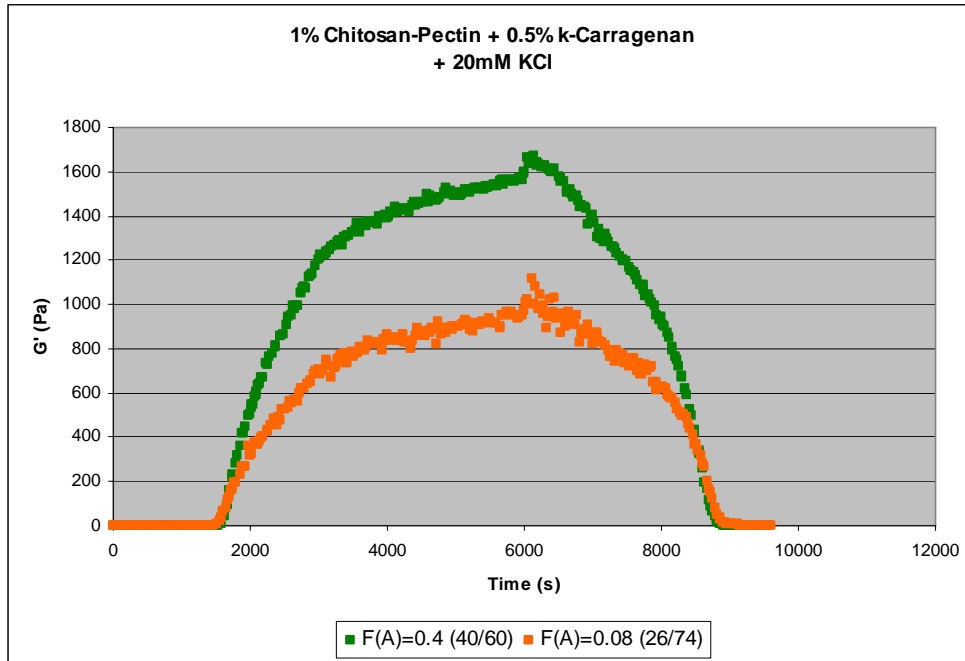


Figure 14 The effect on modulus in a 0.5% carrageenan gel adding a fixed amount of pect/chit coacervate particles of an electroneutral nature and a fixed surplus of potassium ions applying two different chitosans

6. The present mixed system compared to mammalian gelatin behaviour

Figure 15 shows the effect of temperature on one type of the present mixed system compared to typical mammalian gelatin gel behaviour. This figure shows that although the modulus of the mixed gel considerably lower compared to the gelatin gel, it is fully feasible to design / tailor such mixed gels towards a gelatin gel melting behaviour.

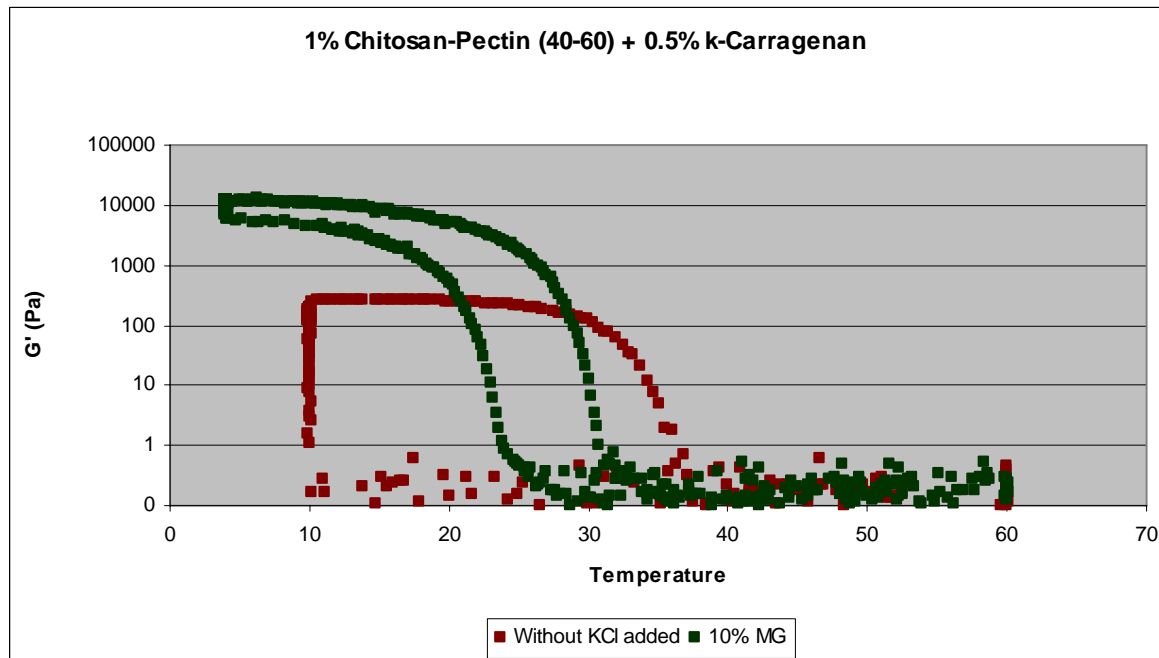


Figure 15 A mixed carrageenan – pectin – chitosan gel system compared to a mammalian gelatin gel as function of temperature

Conclusions

The present system is a carrageenan continuous system containing pectin/chitosan coacervate particles that most probably is linked in some way to the continuous network. If a total melt-down in the mouth is a desired physical property for this system, it seems like electroneutral complexes are required. If a total melt-down of the system should not be required, e.g. that a reduction in modulus by, say, 90% is sufficient to obtain a texture to give consumers acceptance for a given product, then the degrees of freedom with respect to complex composition becomes significantly higher. The only way to obtain information about these requirements is through the aid of educated test panels.

It seems like it will be difficult to try to control setting and melting temperatures through carrageenan and potassium concentration, since both of them will influence both setting and melting behaviour as well as the gel modulus. Other non-specific ions seem to influence more on gel modulus than on setting and melting temperatures.

As long as we are talking about pure watery systems at medium to high carrageenan concentration and low amounts of potassium, it seems like the advantage of using highly acetylated chitosans is limited. If, however, we move towards emulsions, low carrageenan concentrations or higher concentrations of potassium, this picture may change considerably.

It is possible to tailor such mixed systems to mimic the melting behaviour of mammalian gelatin gels.

Recommendations

The present system is rather complex consisting of 3 different biopolymers (carrageenan being the structural component) and with potassium ions as modulator of setting and melting temperatures as well as gel strength. It will work properly as long as one can achieve total control on the mixing regime, i.e. to mix chitosan and pectin prior to the addition of carrageenan. This mixing regime is probably way too complicated to be implemented in a commercial process.

We have therefore also been trying to prepare different dry powder mixtures of all components (also adding neutral agents like sugar to disperse the dry polymer particles) to try to avoid the problem of mixing different polymer solutions. They have all failed. The problem with this approach is of course evident; as soon as the dry polymer particles start to hydrate and dissolve, solubilised chitosan molecules will start to adhere to partially solubilised pectin and carrageenan (and vice versa). The result will be highly suboptimal in the sense that none of the polymers will be fully solubilised but will rather exist as partially un-dissolved particles. There are basically three different theoretical ways around this problem:

1. Dry mixtures with a surplus a salt present, e.g. the total amount of salt that goes into one application. This will reduce the formation of coacervates on the surface of partially dissolved polymer particles due to a screening off of short range attractive forces. The formation of coacervates will most likely occur as the liquid is diluted and the ionic strength is reduced.
2. Dry mixtures at a higher pH value where the molecular weight of the highly acetylated chitosan is reduced in order to obtain solubility above the pK_a -value of the primary amino groups. This approach will prevent an initial formation of coacervates simply because there will be no electrostatic charge on the chitosan molecules. After ensuring complete dissolution of the polymers, the pH of the system can be lowered by the addition of an acid or a lactone so that chitosan regains its electrostatic charge, leading to coacervate formation.

One major problem with both 1. and 2. is that when the formation of coacervates starts, the chitosan molecules will not distinguish between pectin (which it should react with) and carrageenan (which it should not react with because it is the structure forming part of the mixture) since both are polyanions. This may of course lead to reduced gel strength because carrageenan will enter into coacervates instead of network formation.

3. As I see the situation today, the best solution will be to pre-manufacture approximately electroneutral nano-complexes of pectin and chitosan (perhaps a slight negative charge surplus would be recommended). Most likely, these particles can be easily made in diluted solutions and e.g. spray-dried. Such pre-manufactured particles can be used in a much more independent fashion in the sense that a pre-determined amount of this fillers can be added to a given carrageenan (or any gel forming polymer) concentration and hence result in a “tailored” gel strength. A system like this may be patentable. Evidently, such an approach will involve extra efforts; both with respect to investments in equipment as well as in research, but the final result will most likely be considerably more flexible and commercially applicable compared to the system we have been working with this far. The least amount of research needed in the development of a system as proposed, will be to perform some studies on the degree of coacervate re-hydration effectiveness as function of the balance between positive and negative electrostatic charges.
4. Finally, it must be emphasized that as long as insoluble particles are to be used the final product must not be required to be transparent. Possible applications are therefore systems with low to medium gel strength that can be turbid, such as soups, sauces, dressings and dairy products.

NTNU Gløshaugen, May 9, 2007

Kurt Ingar Draget