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Influence of the concentration of locust bean gum on the gelling ability of whey peptic hydrolysates

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Abstract

The gelling ability of whey proteins can be changed by limited hydrolysis and by the presence of other components such as polysaccharides; depending on the environmental conditions it can either be improved or impaired.

In this work the effect of LBG on the heat-set gelation of aqueous whey protein hydrolysates (10 % w/w) from pepsin was assessed at pH 7.0 by small deformation rheology. Whey protein concentrate (WPC) and hydrolysates with a degree of hydrolysis (DH) of 1.5, 2.5 and 4.9 % were used. Different LBG concentrations were tested: 0, 0.1, 0.3 and 0.55 % (w/w).

The behaviour of gels from whey proteins or whey protein hydrolysates towards the presence of LBG was very similar. The evolution of the viscous and storage moduli followed the general behaviour reported for many biopolymer heat-set gelation processes including whey proteins gelation. The increase in the LBG concentration generally led to a decrease in the gel strength. However, for whey proteins a small amount of LBG (0.1 %) leads to a big enhancement in the gel strength probably due to an increase in the protein concentration of the protein enriched phase. Further increases in the LBG concentration led to a decrease in the gel strength.

The gelation process is very sensible to environmental conditions and to processing and often leads to rather coarse data. The factorial planning used allowed validating conclusions using fewer experiments than those needed if no planning had been used, while still getting statistical significance out of the results. However, as many factors are involved, the modelling of the process was not straightforward.

1 Introduction

The gelling ability of whey proteins can be changed by limited hydrolysis; depending on the environmental conditions it can either be improved or impaired.

The functionality of whey proteins can also be changed by the presence of other components. For instance, protein-polysaccharide complexes exhibit many functional properties able to provide new food texturization and stabilization methods (Schmitt and others, 1998).

Synergistic effects have been found between whey proteins and several polysaccharides such as galactomannans, xanthan or carrageenan (Croguennoc et al.,

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2001; Turgeon and Beaulieu, 2001; and many others). The effect of limited proteolysis in the interaction with polysaccharides is hardly ever mentioned.

Locust bean gum (LBG) is a galactomannan (non gelling neutral polysaccharides found in the endosperm of *Leguminosae*) widely used in the food industry as a thickening agent (Pollard and Fischer, 2006).

Rheological studies are useful to evaluate the gelling ability of biological macromolecules; in particular, they allow accessing the structure of the gel, evaluating its texture, controlling the gelling behavior or complementing the information provided by sensory methods. In fact, as gelation is essentially a phase transition from liquid to solid, monitoring the changes in mechanical properties is important. Small amplitude oscillatory shear techniques can be used to monitor continuously the evolution of the viscoelastic properties, avoiding any modification of the molecular structure caused by shear. This is an advantage over other rheological tests.

In this work the effect of LBG on the gelation of aqueous whey protein hydrolysates (10 % w/w) from pepsin was assessed at pH 7.0.

2 Materials and methods

All chemicals used were of analytical grade and supplied by Sigma, Co (St. Louis MO, USA). Pepsin from hog stomach with an activity of 2540 units/mg $_{protein}$ (one unit will produce a ΔA_{280} of 0.001 per min at pH 2.0 at 37 °C, measured as TCA-soluble products using hemoglobin as substrate in a reaction final volume of 16 mL and 1 cm light path) was obtained from Sigma Chemical Co.

A commercial whey protein concentrate (WPC) powder (Lacprodan 80, batch Q500246) kindly supplied by Arla Food Ingredients (Viby, Denmark) was used for the experiments. According to the suppliers, the WPC dry basis protein content was 82 % (5.5 % moisture), the ash content was 3.5 % max., the lactose content was 7 %, and fat content was 8 %. max. Locust bean gum (> 75 % galoctomanan content) was kindly supplied by Danisco Portugal (Faro, Portugal).

Locust bean gum was purified by precipitation with isopropanol as described by da Silva and Gonçalves (1990).

Whey protein concentrate (WPC) and hydrolysates with a degree of hydrolysis (DH) of 1.5 (P1.5), 2.5 (P2.5) and 4.9 % (P4.9) were used. Different LBG concentrations were tested: 0, 0.1, 0.3 and 0.55 % (w/w).

Dynamic oscillatory tests were performed in a controlled stress rheometer AR2000 (TA Instruments, Delaware, USA) fitted with a parallel plate geometry (40 mm diameter, gap 800 μm). Each sample was equilibrated during 5 min; this step was followed by a frequency sweep ("mechanical spectrum") from 100 to 0.1 Hz at a strain of 5 %. Then a temperature ramp from 20 to 80 ºC was applied, at a rate of 2 º C.min^{-1}, after which the temperature was maintained at 80 ºC for 3 h. At the end of this time sweep the sample was cooled back to 20 ºC, at the same constant rate (2 ºC.min^{-1}). The mechanical properties of the resulting gel were monitored at 20 ºC for 1 h.

A full factorial design was used considering two factors (LBG concentration and degree of hydrolysis) and three levels for each factor and a quadratic model was adjusted with Design Expert 6.0.6 (Stat-Ease, Inc. Minneapolis). Two replicates of the experiments with WPC (DH = 0) were used to estimate errors and determine if the lack of fit of the chosen model was significant. Further refinement of the empirical model was made by excluding the factors that were found to be insignificant, one at a time, as the exclusion of one factor may influence the other.

3 Results and discussion

The evolution of rheological parameters follows the general behaviour reported for

many biopolymer heat-set gelation processes including whey proteins gelation (see for instance Paulsson et al., 1990; Gosal and Ross-Murphy, 2000). Initially *G"* is slightly higher than *G'* because of the liquid nature of the sample and the absence of pre-aggregated protein molecules (Figure 1).

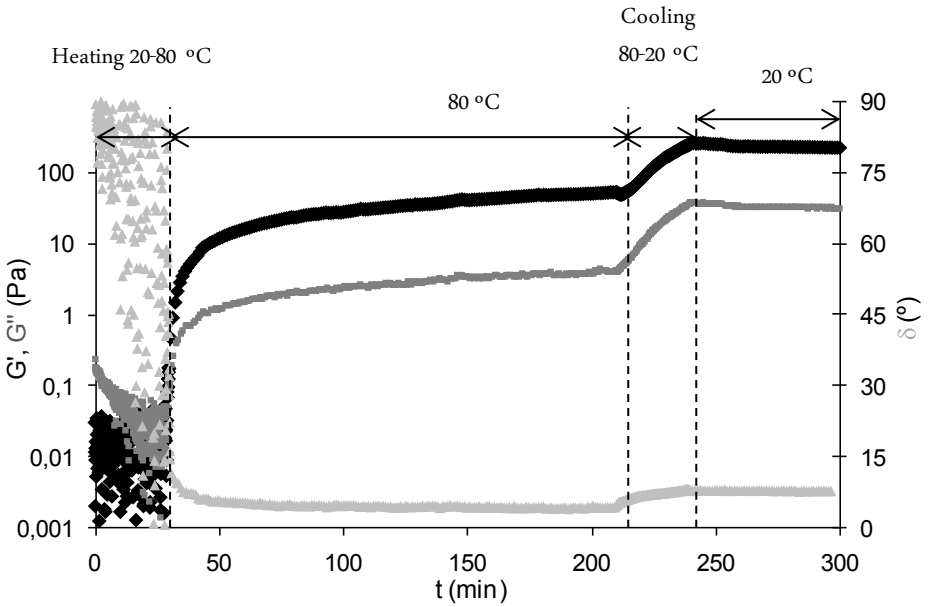

Figure 1 Gelling ability of whey protein concentrate (10 % w/w): black – *G'*; dark grey – *G"*; light grey – δ

As the temperature rises both moduli decrease until the gelation treshold is achieved (either before the end of the temperature ramp or during the time sweep step). As this point approaches, a sudden increase in the values of *G'* and *G"* is observable. However *G'* rises much faster and the crossover *G'-G"* point was considered the gelling point. By the same time the values of the loss angle decrease even more markedly, sign of the increase of the elastic behaviour.

Table 1 Influence of the LBG concentration and hydrolysis degree on the gelling ability of whey protein hydrolysates

Hydrolysate (% w/w)		LBG (% w/w)	*DH* (%)	*G'* (Pa)	*G"* (Pa)	*tan* δ	*Tg* (ºC)	*tg** (s)
WPC	10	0	0	204±33	28.6±3.8	0.14±0.00	79.5±0.0	-
WPC	10	0.1	0	1436±334	207±47	0.14±0.00	75.2±0.1	-
WPC	10	0.3	0	596±420	97.9±69.1	0.17±0.00	76.0±1.4	-
WPC	10	0.55	0	119±7	30.7±1.1	0.26±0.03	78.9±0.6	-
WPC	10	0.80	0	108	39.0	0.36	80	326
P1.5	10	0	1.5	657	92.8	0.14	80.0	36.0
P1.5	10	0.1	1.5	588	105	0.18	77.6	-
P1.5	10	0.3	1.5	118	19.4	0.17	77.9	-
P2.5	10	0	2.5	138	24.8	0.18	73.7	-
P2.5	10	0.1	2.5	107	16.2	0.15	72.6	-
P2.5	10	0.3	2.5	27.8	5.00	0.18	72.4	-
P2.5	10	0.55	2.5	29.5	11.4	0.39	78.7	-
P2.5	10	0.8	2.5	31.5	13.0	0.41	80	745
P4.9	16.5	0	4.9	18.4±18.4	3.35±3.04	0.20±0.04	80.0±0.0	0.0±0.0
P4.9	16.5	0.1	4.9	284	43.2	0.15	74.9	-
P4.9	16.5	0.3	4.9	207	37.3	0.18	72.8	-
P4.9	16.5	0.55	4.9	76.0±17.1	23.8±2.3	0.32±0.04	80.0	35.9±0.1

The increase in the storage modulus and the reduced phase angle indicate the formation of viscoelastic gels. *G'* continues to increase after the gel point as more and more protein reinforces the weak tridimensional network initially formed, enhancing its elasticity.

Although the overall gelation patterns were similar for all tested samples the corresponding gelling parameters (*G'*, *G''*, δ, *Tg*, *tg*) were quite different (Figure 2 and Table 1).

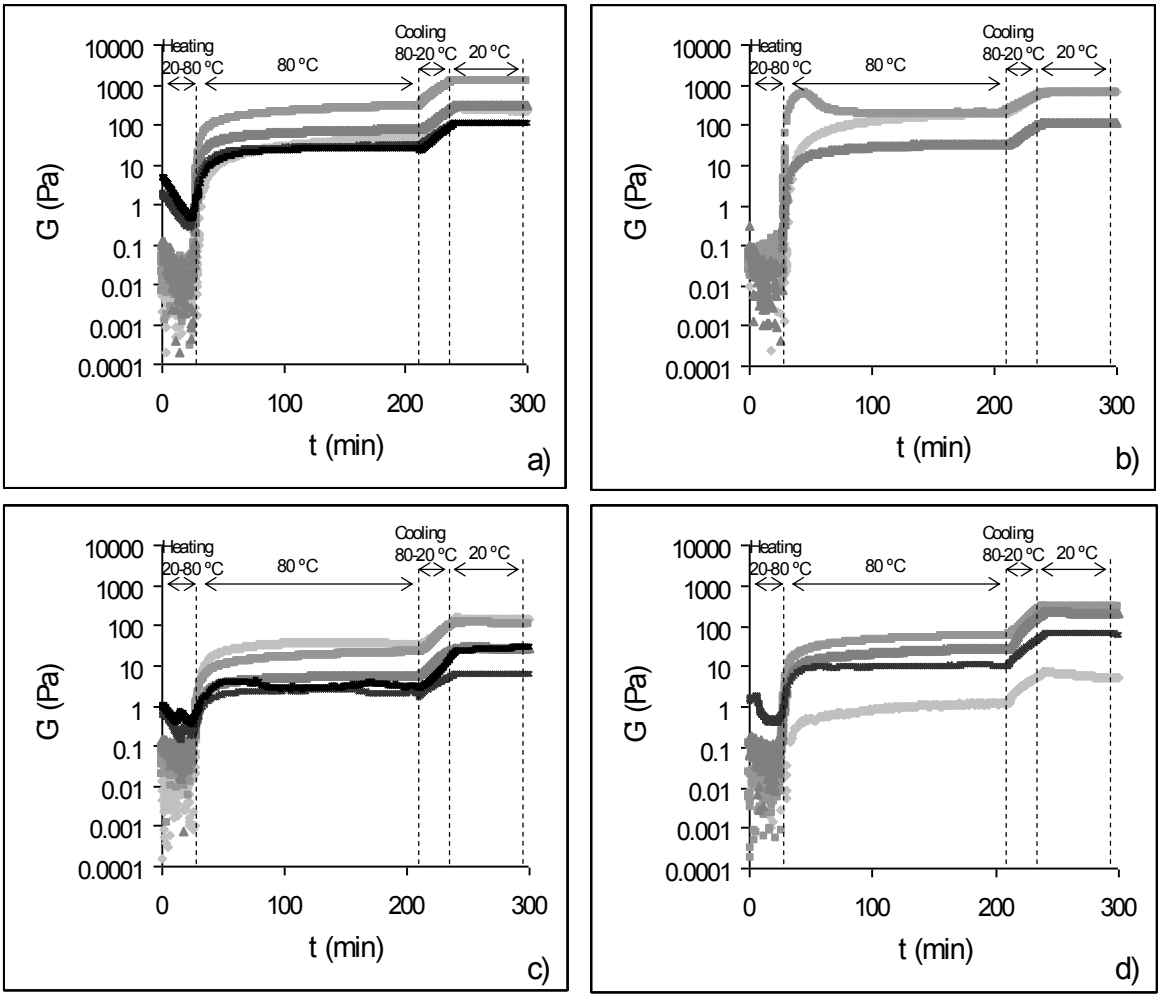

Figure 2 Influence of the LBG concentration on the gelling ability of whey peptic hydrolysates: the darker the colour the higher the LBG amount (0, 0.1, 0.3, 0.55, 0.8): a) WPC 10 % (w/w); b) P1.5 10 % (w/w); c) P2.5 10 % (w/w); d) P4.9 16.5 % (w/w)

In the case of hydrolysates alone, *G'* was higher for P1.5 (657 Pa) followed by P2.5 (138 Pa). These two were stronger than WPC at this concentration (*G'* = 204 Pa) indicating that they were stiffer. They were also more elastic as the loss angle was smaller. Apparently pepsin is effective in improving the gelling ability of whey protein gels for low degree of hydrolysis, possibly because β-Lg (the main gelling protein) is resistant to pepsin. In fact, P1.5 still has all the β-Lg intact and P2.5 still has 96 % of intact β-Lg. This improvement might be due either to the presence of low molecular weight hydrophilic peptides which can reduce electrostatic repulsions between intact β-Lg molecules enhancing protein-protein interaction or to the partial unfolding of α-La and BSA exposing their hydrophobic residues, therefore improving their individual gelling ability and/or allowing for a better interaction with the intact β-Lg.

LBG alters the microstructure of whey protein gels by modifying the equilibrium between aggregation and segregation. The gelation time was also decreased. The behaviour of gels from whey proteins or whey protein hydrolystates towards the presence of LBG was very similar. The increase in the LBG concentration generally led

to a decrease in the gel strength. However, for whey proteins a small amount of LBG (0.1 %) leads to a big enhancement in the gel strength probably due to an increase in the protein concentration of the protein enriched phase. Further increases in the LBG concentration led to a decrease in the gel strength.

When analysing the influence of the concentration of LBG on gelling properties (*G'*, *G"*, tan δ and temperature of gelation), the differences between the hydrolysates and WPC with no polysaccharide and the hydrolysates with 0.1 % of LBG were usually sharp (an example is presented in Figure 3); this difficults modelling with a simple quadratic function.

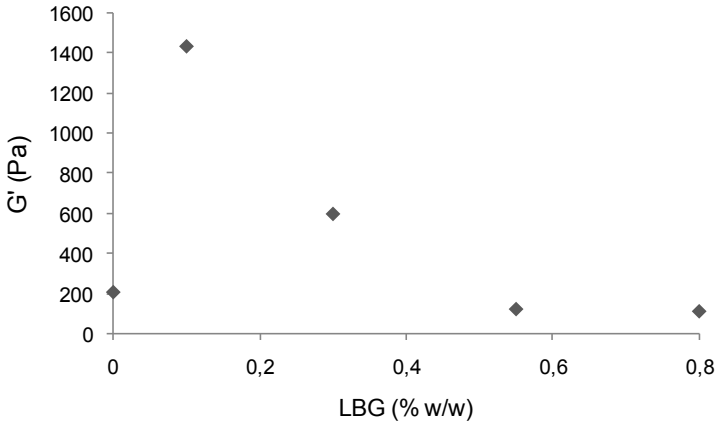

Figure 3 Influence of the LBG concentration on the final storage modulus of whey protein gels (10 % w/w)

Therefore the statistical analyses and empirical modelling were performed only with the data from mixed systems for the LBG range of 0.1 – 0.55 %. A quadratic model as described by Eq. 1 was used.

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_{11} x_1^2 + b_{22} x_2^2 + b_{12} x_1 x_2 + residual \qquad \text{Eq. 1}$$

By means of an analysis of variance it was concluded that all the adjusted models are significant. The effect of LGB concentration and of the degree of hydrolysis on peptic hydrolysates gelation was negative for *G'* and *G"*, though an interaction factor has also to be considered. For tan δ and the temperature of gelation a minimum value exists in the studied range of LBG concentration, while a maximum exists in the studied range of *DH* for the temperature of gelation.

Table 2 Statistical analysis of the influence of the LBG concentration and hydrolysis degree on the gelling ability of 10.0 % (w/w) whey peptic hydrolysates

Variable	Regression coefficients						Regression quality	
	b0	*b1*	*b2*	*b11*	*b22*	*b12*	*PF* (%)	Lack-of-fit
G' (Pa)	1619	**-2886**	-618	-	-	1086	0.20	Not significant
G" (Pa)	235	**-393**	-87.3	-	-	146	0.31	Not significant
$tan\ \delta$ (º)	0.193	-0.470	-0.012	1.09	-	0.102	0.05	Not-significant
T_g (ºC)	75.8	-10.4	5.40	31.3	-2.50	-	0.51	Not-significant

Items in **bold** correspond to non significant model terms that could not be withdrawn from the model because they were required to support hierarchy.

The gelation process is very sensible to environmental conditions and to processing and often leads to rather coarse data. The factorial planning used allowed validating conclusions using fewer experiments than those needed if no planning had been used, while still getting statistical significance out of the results. However, as many factors are involved, the modelling of the process was not straightforward. A simple quadratic function was generally not enough to accurately describe the system behaviour.

4 Conclusion

WPC mild hydrolysis (up to 2.5%) ameliorates the gelling ability, but affects the WPC synergism with LBG (no max found in the G' dependence on LBG content).

As a general conclusion, it can be stated that it is possible to make all kinds of different gels (fast gelling, slow gelling, hard and stiff, or weak gel) by manipulating the protein concentration, the degree of hydrolysis and the amount of LBG. It is important though to master the mechanism of phase separation in order to be able to design the adequate conditions for the desired texture.

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