

Food Chemistry 65 (1999) 493-501

Food
Chemistry

Effect of *Amaranthus* and buckwheat proteins on the rheological properties of maize starch

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Received 12 December 1997; accepted 28 September 1998

Abstract

The relationship between pasting properties (determined with a Rapid Visco-Analyser) of maize starch and the texture of the resulting gel was examined after addition of Amaranthus and buckwheat proteins. An increase in the peak viscosity due to the addition of protein concentrates was observed, and a lesser increase from the addition of protein hydrolysates. The increase in starch pasting viscosity was related to protein solubility, and could be attributed to the starch granule stabilizing action of proteins. The interactions between starch and proteins were further investigated using oscillation and creep/recovery rheological tests. Generally, the proteins weakened starch gel structure, shown by the lower elastic modulus (G') and higher phase degree (δ) compared to gels without any proteins added. The same results were obtained from creep/recovery experiments. It seemed that, since native proteins interact more with the granules, they act as a barrier to the release of amylose molecules; hence the resulting gels became weak. If desired, such effects could be lessened by partially hydrolyzing the proteins. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

The two most abundant and nutritionally important components of cereal flours are starch and protein. The properties of these components determine the functionality of flours, particularly that of wheat flour. Wheat protein, specifically gluten, is unique among other cereal proteins in its extreme influence on the physical properties of wheat dough as well as on the final product. Other cereal proteins do not have the same properties, although their interactions with starch and other ingredients can also affect the physico-chemical state of the food system.

Ring (1985) described a starch gel as a composite material in which swollen gelatinized starch granules reinforce an interpenetrating amylose gel matrix. A gelatinized starch suspension is therefore biphasic in nature, with a continuous phase made up of solubilized amylose and a dispersed phase made up of swollen granules containing the amylopectin molecules. Hansen, Hoseney, and Faubion (1991) reviewed studies claiming that the physical characteristics of starch pastes and gels

depend on the concentration of the granules, the amount of amylose and amylopectin leached from the granules during heating, the shape and swelling power of the granules, the degree of entanglement between amylose and amylopectin, and granule-granule, amylose-granule, and amylopectin-granule interactions. Considering the numerous factors that could influence starch gelatinization and retrogradation, further studies are still being made to elucidate this complex system.

Lindahl and Eliasson (1986) studied the interactions between wheat proteins and different starches based on oscillatory rheological measurements of starch gels. They found an increase in G' of wheat and rye starch gels when gluten was added. However, a decrease in the G' was observed for maize starch while no effect was found on triticale, potato and barley starches. De Gennes (1971) believed that, as starch gelatinizes and proteins denature, it is possible that entanglements develop a network structure which results in a synergistic increase in viscosity. Hamaker, Griffin, and Moldenhauer (1991) also demonstrated the significant influence of starch-granule-associated protein on cooked rice texture. However, Friedman (1995) in a review on starchprotein interactions indicated that the two polymers are probably not miscible.

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In this study, we investigated the effect of Amaranthus and buckwheat proteins on starch gelatinization and retrogradation in a model system using maize starch. The study was part of our overall research on the properties of Amaranthus and buckwheat proteins as functional and nutritional ingredients in food (Bejosano & Corke, 1998a,b).

2. Materials and methods

2.1. Materials

Five different genotypes of *Amaranthus*: K112, K350, K459 and R104 (all A. cruentus) and No. 3 (A. hybridus) (see Bejosano & Corke, 1998b), and one buckwheat commercial grain of Chinese origin (packed by Queenswood Co., United Kingdom) were used to make protein concentrates and hydrolysates. Commercial maize starch (25% amylose) (Kingsford's brand, CPC/AJI Hong Kong Ltd., Hong Kong), and analytical grade sucrose (BDH Chemicals Ltd., Poole, United Kingdom) were used.

2.2. Preparation of isoelectric protein concentrates

Using an alkali wet-milling procedure with subsequent isoelectric precipitation of the proteinaceous liquor, protein concentrates were made out of the five Amaranthus and one buckwheat samples. After acid precipitation of the proteinaceous liquor, a wet curd was obtained which was in an emulsified state having a high oil content. In this condition, conventional drying was found to be very difficult, so the curd was first frozen and then thawed to break the emulsion and to facilitate separation of the water layer. After doing this, a large amount of water could be removed physically. This was followed by drying at 70° C in a forced convection dryer for about 12 h. The coarse pellets formed were crushed finely using a mortar and pestle. The resulting powder was then defatted in petroleum ether (10 h with intermittent shaking; removing the petroleum ether layer; repeated twice). This was followed by airdrying and further grinding using an Udy Cyclone Mill (Udy Corp., Boulder, CO) with 0.5 mm mesh screen.

2.3. Preparation of protein hydrolysates

Partial pepsin hydrolysis of protein concentrates was done as follows. The substrate was prepared by adjusting the protein concentration to 1% (w/v) in 0.01 N HCl (pH 2.0). Ninety-one units of pepsin A (Sigma Chemical Co., St. Louis, MO) per mg solid was added to the suspension at the rate of 0.2 mg ml^{-1} . The mixture was incubated at 37° C with mild shaking for 16 h, after which pH was adjusted to 7.5 and kept at 5° C for 72 h. The pH was then adjusted to 6.5 followed by freeze-drying.

2.4. Starch pasting properties

The effect of the proteins on maize starch pasting properties was determined with the use of a Rapid Visco Analyzer (RVA) (Newport Scientific Pty. Ltd., Warriewood, Australia). Triplicate measurements using a 13 min controlled heating and cooling profile with constant shear were used, wherein the sample was held for 1 min at 50° C, heated at 12[°]C per minute from 50 to 95[°]C, held for 2.5 min at 95° C, cooled at 12 $^{\circ}$ C per min to 50 $^{\circ}$ C, and held for 2 min at 50 $^{\circ}$ C. In each case, 2.5 g maize starch (d.b.) and 25 g accurately weighed distilled water, or protein or sucrose solution (i.e., a 9% w/w starch dispersion) were added to the RVA sample canister. For treatments where proteins were added, this was done by dissolving the protein powder in water to give a concentration of 1.12% (w/v) with a pH of 7.0. When 2.5 g starch is added to 25 ml of this preparation, it gives a final protein concentration of 1.0% (w/w).

2.5. Starch gel texture

The resulting pastes from the RVA experiments were kept in the RVA canister, sealed with Parafilm[®] and kept at 5° C for 24 h. The starch gels were then taken out of the container and were cut into $15 \times 15 \times 10$ mm (length; width; thickness) blocks. Texture analysis of the gel was made using a QTS-25 texture analyzer (Stevens Advanced Weighing Systems, Leonard Farnell and Co. Ltd., United Kingdom). Two methods of compression testing were used. Method 1 used a cylindrical probe (40 mm dia.) which covered the total surface area of the gel block during compression. The test was done at 15% deformation only without gel rupture. Method 2 used a cylindrical metal probe (3 mm dia) . with flat end) that cuts through the gel to 70% deformation, enough to cause gel rupture. A crosshead speed of $0.8 \text{ mm} \text{ sec}^{-1}$ was used and the gram-compression peak force was measured for both methods. Measurements were done at room temperature (approx. 27° C), and were the mean of five repetitions for each of the triplicate samples from the RVA.

2.6. Viscoelastic properties of starch gel

The maize starch gels were cut into 20 mm cylindrical discs (approx. 3 mm thick). The discs were then used for the rheological measurements carried out with a Stresstech controlled stress/strain oscillatory rheometer (Reologica Instruments AB, Lund, Sweden). Oscillation stress sweep was first done according to standard methodology on the gels to determine their linear viscoelastic region. From this experiment, a stress level (in the linear viscoelastic region) was selected which was used in the frequency sweeps. Oscillation frequency sweep was done using frequencies from 0.01 to 7.0 Hz. The elastic modulus (G') , viscous modulus (G'') and phase degree (δ) were measured. The viscoelastic parameters were also determined in the creep/recovery mode using the same stress level as in the oscillation mode. All measurements were done at 27° C.

3. Results and discussion

3.1. Effect of proteins on the pasting properties of maize starch

RVA pasting profiles from cooking the starch suspension (Fig. 1) showed that addition of protein concentrates significantly increased the peak viscosity of maize starch. Protein hydrolysates also affected maize starch pasting properties, but increased peak viscosity to a lesser extent than protein concentrates, suggesting that larger proteins were more effective than smaller ones in increasing viscosity. The strong negative correlation between protein solubility and peak viscosity $(r = -0.72; p = 0.01)$ indicated that the insoluble protein fraction was likely the main factor in the viscosity increase, not the molecular size of the protein per se. Sucrose (1% w/w) had no effect on the pasting profile of the maize starch suspension, showing that the increase

in paste viscosity caused by the proteins was not merely due to an increase in the total solids content. This differed from the findings of Cheer and Lelievre (1983) who showed that, at concentrations below 20%, sucrose increases paste viscosity of wheat starch, but the lowest sucrose concentration they used was 2.5%.

Several studies have proven the correlation between starch paste viscosity and granule swelling behaviour (Bagley & Christianson, 1982; Cheer & Lelievre, 1983; Eliasson, 1986; Takahashi & Seib, 1988; Wong & Lelievre, 1982). Cheer and Lelievre (1983) noted that the increase in starch swelling volume was due to the prevention of granule disintegration and implosion, hence an increase in paste viscosity. Correlating paste viscosity and granule swelling is not straightforward because the continuous phase may also contribute to viscosity (Alloncle, Lefebvre, Llamas, & Doublier, 1989). Nevertheless, Eliasson (1986) believed that the changes in the continuous phase provide a minor contribution to the rheological response compared to the contribution from the dispersed phase.

Chedid and Kokini (1992) used isothermal rheological measurements to study the effects of zein, gliadin, glutelin and glutenin on the gelatinization behaviour of different types of maize starch and 100% potato amylose. They found that, at 55% moisture, addition of all proteins increased the peak viscosity of waxy maize starch (98% amylopectin). However, at 64% moisture, gliadin caused a decrease in viscosity while the others

Fig. 1. Effect of proteins and sucrose on the RVA pasting profile of maize starch.

gave peak viscosities close to that of the maize starchwater suspension. Using 50% amylose maize starch, they found that most of the proteins again increased the peak viscosity at 64% moisture but decreased it at 82% moisture. Moreover, they noted that addition of proteins to 100% amylose generally decreased peak viscosity. They therefore concluded that the effect of proteins depended on the amylose-amylopectin ratio as well as on the amount of water in the system. The study showed that interactions between amylopectin and proteins were involved in the increased viscosity. It was postulated that, above the gelatinization temperature of starch, there is formation of entanglements between amylopectin and protein and that the long side branches of amylopectin enhance the potential for interaction with the hydrophile-compatible portions of the protein molecule.

Thus a picture emerges of the possible effect of an additive on starch paste viscosity during gelatinization. An ingredient might interact with the dispersed phase, with the continuous phase or perhaps with both. It was shown that proteins have more affinity with the dispersed phase and generally, they increase paste viscosity (Chedid & Kokini, 1992). What happens if an additive has an affinity with the continuous phase? Alloncle et al. (1989) demonstrated that hydrocolloids such as galactomannans remain in the continuous phase when added to starch and do not directly affect granule swelling during the pasting process. Nevertheless, they found spectacular increases in paste viscosity when such ingredients were added. The authors explained that, as the granules swell, they absorb large amounts of water thus making the concentration of the galactomannans in the continuous phase increase up to 0.8%. Since such substances are strongly concentration-dependent, the viscosity in the continuous phase dramatically increases. It was also speculated that, as amylose molecules leach out of the granules, a synergistic effect between them and the galactomannans could further contribute to increase in viscosity in the continuous phase.

3.2. Effect of proteins on the texture of maize starch gel

The cooked suspensions were allowed to gel at 5° C for 24 h after which textural measurements were done. In the first method, the gels were compressed at small deformation (15%) which did not break the gel structure. The second method involved 70% deformation and gel rupture. In both tests, the peak force was measured. Compression at small deformation therefore generated lower peak force values. The results indicated that addition of proteins weakened the starch gel texture (Table 1), but there was high experimental error. Using Method 1 (15% deformation), a statistically significant gel-weakening effect could be attributed to only one protein additive (i.e. K112 protein concentrate). In

Table 1

Effect of proteins and sucrose on the RVA peak viscosity of 9% maize starch suspension and the gel compression peak force (GCPF) of the starch gel

Treatment	Peak viscosity	GCPF(g)	
	(RVU)	Method 1 ^a	Method 2 ^b
Control	143	30	72
Sucrose	145	31	74
Protein Concentrate			
K112	159	24	54
K350	149	26	48
K459	156	25	44
R ₁₀₄	162	29	39
No. 3	156	25	37
Buckwheat (BW)	145	31	44
Pepsin Hydrolysate			
K112	147	34	54
K350	150	28	42
K459	150	28	28
R ₁₀₄	155	26	31
No. 3	147	28	50
Buckwheat (BW)	136	34	56
LSD $(p < 0.05)$	13	6	12

^a Method 1—compression at 15% deformation without rupture.

 b Method 2—compression at 70% deformation with rupture.</sup>

Method 2, all the proteins added to the starch suspension gave significant reduction of the gel strength, but the mean effect of protein concentrates was not significantly different from that of hydrolysates. Nevertheless, a positive coefficient $(r = 0.54; p = 0.07)$ was obtained between the gel compression peak force in Method 1 and the solubility of the proteins added to the starch suspension, suggesting that less soluble proteins were probably involved in the gel texture weakening. The sensitivity of the texture methods to test differences between the samples was low, and even the results from the two texture tests were weakly correlated $(r = 0.44$; $p = 0.12$). This is a limitation of the methodology, and the same problem was encountered by Huang and White (1993) using the same type of texture analyzer in evaluating monoglyceride-starch interactions. Despite this problem, differences in the gel texture due to the addition of proteins and of sucrose were detected. Proteins, especially unhydrolyzed ones decreased gel strength but sucrose did not.

3.3. Effect of proteins on the viscoelastic properties of maize starch gel

Tung and Paulson (1995) pointed out the importance of reliably quantifying rheological properties when considering ingredient interactions. Although the textural analysis methods discussed above have a rheological basis, limitations in their use were found. A more

precise characterization of the physical properties of starch gels can be done using a more sensitive instrument such as a rheometer. Starch gels are viscoelastic, having both solid-like (elastic) and liquid-like (viscous) behaviour. Using oscillation stress sweep we were able to determine the linear viscoelastic region of the gels in the range of 1 to 15 Pa. We therefore set the stress to 5 Pa for doing the frequency sweeps. The change in the elastic modulus (G') of the starch gels during oscillation at 0.01 to 7 Hz (Fig. 2) shows that G' was not frequencydependent at the stress level selected. It also shows the effect of the proteins and sucrose on the rigidity of the maize starch gel. The effect of all the proteins studied is presented in Table 2 which gives the G' of the all the gels at 0.1 Hz oscillation frequency. As with the texture analysis, the inherent error was high, but the results did show a trend for the overall effect of the treatments. Part of the variation may be due to non-homogeneity in certain portions of the gel, especially between the top and the bottom parts, as gel formation takes a considerable time to complete, especially in more dilute suspensions. The numerical ranking of the values, according to the treatments, was in the order of concentrate < hydrolysate < control < sucrose. Thus G' of maize starch gels decreased when protein concentrates were added. However, when the proteins were partially hydrolyzed the effect was lessened, and the gel containing sucrose had the highest G' . The effect of

sucrose and protein hydrolysates on the G' of maize starch gel was not statistically significant (Duncan's test, $p < 0.05$). However, the rigidity of the gel with sucrose was significantly higher than for the gels with protein concentrate.

During the RVA test, it was shown that paste viscosity increased in the presence of insoluble proteins. This would be likely due to the protective effect of these proteins on the integrity of the starch granules, making them less affected by the mechanical stress caused by stirring. This allows them to swell more or at least stabilize their swollen state compared to unprotected granules, before breakdown occurs when most of the leaching of amylose molecules takes place. Therefore, after the RVA cycle is completed, it is expected that starch pastes having protein concentrates (with more insoluble protein) would have more intact granules and less amylose molecules leached out into the suspension. Thus, when the gel is formed, it would tend to be less rigid than one with more soluble proteins or without any protein at all. This is because the strength of the gel structure largely depends on the density of amylose network formed.

Wong and Lelievre (1982) measured starch paste viscosity at 30° C. At that temperature they were already measuring the viscosity caused by the gelation that was taking place, hence it might not be an accurate view of the whole pasting process. Their results can be better

Fig. 2. Effect of proteins and sucrose on the elastic modulus (G') of maize starch gel.

Table 2

Effect of proteins and sucrose on the viscoelastic properties $(G'-$ elastic modulus; $G''-$ viscous modulus; δ —phase degree) at oscillation frequency of 0.1 Hz; and on steady state parameters (η —viscosity; J_C—creep compliance; J_R—recoverable compliance) of 9% maize starch gel measured in the creep/recovery experiment

Treatment	G' (Pa)	$G^{\prime\prime}$ (Pa)	δ	η (×10 ⁶ Pa s)	$J_{\rm C}$ (×10 ⁻³ Pa ⁻¹)	$J_R(\times 10^{-4} \text{ Pa}^{-1})$
Maize starch only	1355	43.5	1.82	2.31	1.04	8.07
Sucrose	1480	41.4	1.62	7.02	0.99	8.17
Protein concentrate						
K112	1034	49.8	2.77	0.99	1.68	10.40
K350	1120	49.6	2.53	1.64	1.12	8.43
K459	1095	48.3	2.52	0.95	1.31	10.60
R ₁₀₄	1040	42.6	2.36	1.72	1.19	10.20
No. 3	1036	48.8	2.71	1.51	1.18	10.10
Buckwheat (BW)	974	46.6	2.74	1.14	1.25	9.92
Pepsin hydrolysate						
K112	1260	53.3	2.42	1.64	0.95	7.94
K350	1202	49.3	2.48	2.88	0.80	8.25
K459	1300	43.4	1.91	1.94	1.00	8.93
R ₁₀₄	1160	42.2	2.08	1.65	1.12	9.71
No. 3	1155	43.1	2.14	1.01	1.02	9.49
Buckwheat (BW)	1250	52.1	2.40	1.95	1.18	9.60
LSD $(p < 0.05)$	360	12.4	0.52	-		

interpreted as a relationship between granule swelling and retrogradation. Hansen et al. (1991) used an improved methodology in correlating granule swelling and paste viscosity. Viscosity during sol to gel transformation was monitored from 70 to 20° C. Nevertheless, both studies showed that starch paste viscosities during gelatinization up to early stages of retrogradation, and granule swelling capacities were positively correlated. They were, of course, measuring the differences in starch properties as affected by the method of preparation (i.e., starch concentration and temperature) and by genotypic variation. These studies therefore illustrated that a higher degree of granule swelling results in an increased paste viscosity hence, a stronger gel. However, their observation was quite contrary to our findings, perhaps because we were studying the effect of an additive on a particular starch sample.

On adding proteins, starch paste viscosity was increased but gel rigidity was decreased. These findings are in agreement with those of Takahashi and Seib (1988), who similarly found an increase in paste viscosity resulting in decreased gel firmness with the presence of either native or impregnated lipids in both maize and wheat starch. They asserted that lipids maintained granule integrity; hence they increased the starch paste viscosity, which also resulted in the prevention of amylose leaching out of the granules. Thus, they explained that keeping the amylose molecules inside the starch granules caused reduced gel firmness by lowering the concentration of amylose in the continuous phase. This agrees with our own interpretation except that, instead of lipids, we considered the effect of proteins on starch properties.

Sucrose had a positive effect on the G' of the starch gel, unlike for the RVA results where it had no effect on the pasting profile. This indicated that 1% (w/w) sucrose had no effect on granule swelling during the gelatinization process while proteins did have an effect. Unlike hydrocolloids, a dilute sucrose solution does not form a viscous fluid on its own during heating. However, its molecular structure is probably more compatible with the linear amylose molecules, thus it may have more affinity with the continuous phase. Probably the influence of sucrose on starch properties took effect only during retrogradation. One possibility is that sucrose binds with some amount of water, therefore diminishing the amount of water the amylose matrix had to hold without causing any weakening effect on the network structure and perhaps even causing a synergistic action. Friedman (1995) cited a study claiming that sugars form ``bridges'' between melted crystalline regions and amorphous regions which stabilize the starch gel structure.

The effect of proteins and sucrose on the viscous modulus (G'') of the gel (Fig. 3) showed that, unlike the G' , there appears to be more frequency-dependence in the results. The gels revealed an increasingly more liquid-like characteristic at higher frequencies. No statistically significant differences among the effects of the treatments were detected. Another measure of the liquid-like property of a material is the phase degree (δ) which is calculated as arctan G''/G' . As for G'' , a high δ means a more liquid-like quality. The δ was also frequency dependent (Fig. 4), but the dependence was less pronounced in gels containing proteins although their δ values were higher than those of the gels without proteins at all frequencies. It is therefore clear that proteins

Fig. 3. Effect of proteins and sucrose on the viscous modulus (G'') of maize starch gel.

Fig. 4. Effect of proteins and sucrose on the phase degree (δ) of maize starch gel.

increased the liquid-like behaviour of maize starch gel while sucrose decreased it. Partially hydrolyzed proteins were less effective in increasing the δ of the gels than the native proteins. Basically, gels with higher G' (more rigid) had lower δ values. There was a strong correlation between protein solubility and G' of the gels ($r = 0.63$; $p = 0.03$). There was also a negative correlation between the δ and protein solubility although it was not statistically significant ($r = -0.48$; $p = 0.11$).

A low G' and a high δ value means a weak gel. It also suggests a material with more free water, hence a less efficient network. It is well known that amylose is mainly involved in the gelation process. It was proven that interrupting the interchain association between amylose molecules by inclusion of homologous chain segments of sufficient length would lead to a weakened gel network structure (Biliaderis & Zawistowski, 1990). Hansen et al. (1991) showed that even amylopectin, if a significant amount of it is leached into the continuous phase, would cause detrimental effects on gel formation and rigidity.

It was discussed earlier how proteins were implicated in stabilizing the granules causing a probable reduction in the release of amylose molecules. But it is hardly possible that all proteins would only attach to the dispersed phase, and some of them would likely remain in the continuous phase, contributing further to the gel weakening effect. It would seem that the proteins most involved were the native proteins rather than peptide fragments produced from limited protein hydrolysis, because the gels tended to be less weak in the presence of the latter.

Another indicator of rheological properties of a material is through creep/recovery testing. A creep test is a measure of how a material behaves on application of a sudden stress which is maintained at a constant value for a specified time, while a recovery test measures the behaviour after the removal of stress. With the applied stress (i.e. 5 Pa), gels with proteins showed a more viscoelastic response than the maize starch-water gel as indicated by the less flat curve of the former which with sucrose response, was even diminished (Fig. 5). The creep (J_C) that the gels demonstrated is also a measure of their rigidity. J_C was largest in the gels with

protein concentrate, meaning that they were the least rigid, and were also significantly higher in the gels with the corresponding protein hydrolysate $(t$ -test; $p = 0.04$). The solubility of the added proteins was significantly correlated to the degree of creep (J_C) of the starch gels ($r = -0.79$; $p = 0.002$). This agrees with the observations reached in the oscillation experiment. In the same graph we can also see how the gels behaved when the stress was removed (i.e. during recovery). If the sample were totally elastic, it would be able to perfectly regain its structure; hence its recovery (J_R) would reach the zero level in the graph. Thus, a lower J_R means a more coherent structure. The results show that the gel with sucrose was the most efficient in regaining its structure (lowest J_R) while those with protein concentrate were the least.

In this experiment, the steady state parameters which are extrapolations of the values when stress is equal to zero were also computed (Table 2). The shear rate values during measurement were in the range of 0.7 to 5.2×10^{-6} s⁻¹. Again, the effect of the treatments on the gel steady state parameters can be seen. A higher steady state viscosity (n) denotes a more rigid gel. Here we can see that the gel with sucrose had the highest η , followed by the gel with maize starch and water only. The gels with protein concentrate had the lowest η . These observations again concur with those obtained in the oscillation experiment. The steady state viscosity, creep compliance and recoverable compliance had correlation coefficients of

Fig. 5. Effect of proteins and sucrose on the creep/recovery of maize starch gel.

0.75 ($p = 0.002$); -0.59 ($p = 0.03$); and -0.75 ($p = 0.002$) respectively, with G' .

4. Conclusion

This study showed that, when both Amaranthus and buckwheat proteins were added to 9% (w/w) maize starch, the pasting viscosity of the suspension was increased considerably. The increase was lower when hydrolysates were added than with protein concentrates. From the established relationship between pasting viscosity and granule swelling during gelatinization, it is concluded that the observations were due to the proteins exerting a stabilizing effect on starch granule integrity. From the texture analysis of the starch gels and from oscillatory rheological and creep/recovery measurements, there were clear indications that the increase in paste viscosity caused by proteins resulted in a weaker gel texture. It appears that the phenomenon was due to the preferential interactions between starch granules and proteins during gelatinization and retrogradation. We found that such interactions could be controlled by limited protein hydrolysis.

Acknowledgements

Financial support was received from the Hong Kong Research Grants Council and the University of Hong Kong Committee on Research and Conference Grants.

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