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Starch-free fatty acid complexation in the presence of whey protein☆

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Abstract

The effect of whey protein on starch-free fatty acid (FFA) complexation was studied in a model system composed of sorghum starch, whey protein, and different FFAs (palmitic, oleic, linoleic, and lauric acids) in a weight ratio of 20:2:1(w/w/w). Whey protein decreased the enthalpy of the melting of the starch–lipid complex by 20–30% for the FFAs except linoleic acid, and increased the reformation exothermic enthalpy by 150–350% in the DSC cooling cycle. The large difference between enthalpies upon heating and cooling in the starch–FFA sample was diminished by the addition of whey protein. X-ray diffraction data showed more pronounced crystalline order of V-type starch–FFA complexes when whey protein was present. A previously described cooling stage viscosity peak, formed due to starch–FFA–protein complexation, paralleled formation of the better defined V-type crystallite of the starch–FFA complex. Whey protein also significantly decreased the amount of starch–FFA complexation in a dilute system. The effect of protein on starch–FFA complexation was related to the formation of a three-component complex composed of starch, FFA, and protein previously identified in our laboratory.

Keywords: Starch-free fatty acid complex; Protein; Interaction

1. Introduction

A three-way interaction among starch, protein, and free fatty acid (FFA) was previously discovered by our group through the observation of a large Rapid ViscoAnalyzer (RVA) cooling stage viscosity peak that occurred only when all three components were present (Zhang & Hamaker, 2003). Further work revealed a distinct three-component complex that was identified through size-exclusion chromatography (Zhang, Maladen, & Hamaker, 2003). The large water-soluble complex contained amylose, soluble protein, and FFA, and furthermore involved an amylose–FFA complex as shown by a thermal endothermic transition near 100 °C. However, the mechanism of the starch–protein–FFA interaction was unclear.

Although there was no evidence in the literature to support the existence of a starch–protein–lipid three-way interaction, there have been several studies showing a cooling stage viscosity peak. Takahashi and Seib (1988) found a cooling stage viscosity peak in an amylogram of wheat starch when wheat starch lipids were added. They speculated that the cooling stage viscosity peak was due to starch–lipid interaction. Osman and Dix (1960) also found the same phenomenon when they heated cornstarch with a variety of mono-fatty acid esters. Later, Kim and Seib (1993) found a cooling stage viscosity peak in noodle flours and breadcrumb that they indicated was also due to a starch–polar lipid interaction. Although the mechanism of the production of the cooling stage viscosity peak was not addressed, starch and lipids were the common components in all of these studies. Complexation between starch amylose and lipids is well documented in the literature (Brisson, Chanzy, & Winter, 1991; Mikus, Hixon, & Rundle, 1946; Zobel, French, & Hinkle, 1967), and results in V-type crystallites with relatively high melting temperature. Thus, starch–FFA complexation is an important subject for our investigation of the three-way interaction mechanism. In this study, starch–FFA complexation was examined in the presence of whey protein, and the effect of protein on starch–FFA interaction was addressed as a first step to resolve the three-way interaction mechanism.

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2. Materials and methods

A normal sorghum cultivar, P721N, was harvested from the Purdue University Agronomy Farm in 1997 and was conditioned (27 °C, 67% relative humidity) for two weeks to approximately 13% moisture content. Starch was isolated from whole sorghum grains according to a general toluene procedure for starch isolation (Badenhuizen, 1964). Starch was defatted with 85% methanol for 16 hr at room temperature. Whey protein isolate (WPI-BioPro, 97.5% protein) was from Davisco Foods International Inc. (Eden Prairie, MN). Palmitic acid (C16:0), oleic acid (*cis*-9-octadecenoic acid, C18:1), linoleic acid (*cis*-9,*cis*-12-octadecenoic acid, C18:2), and lauric acid (C12:0) were purchased from Sigma Chemical Co. (St Louis, MO).

2.1. Rapid viscosity analysis

A RVA (Newport Scientific Inc. Australia) was used to investigate the interaction among starch, protein and lauric acid, and to prepare RVA paste samples. Starch (2.00 g) was mixed with FFAs (100 mg) and/or protein (200 mg), and the RVA was run according to standard method 1 from the RVA manual. Purified water was added to a final weight of 25.0 g. Controls of starch alone and starch with whey protein were also prepared in the same way.

2.2. Differential scanning calorimetry

A differential scanning calorimeter (DSC 2920, TA Instruments, New Cartle, DE) was used to examine the thermal properties of the starch–FFA complexes. Paste samples taken at the end of the RVA cycle were quickly frozen in liquid nitrogen and freeze-dried. Dried paste (3–5 mg) was mixed with distilled water (1:3, w/w) and hermetically sealed in aluminum pans. Experiments were carried out at a heating rate of 10 °C/min from 20 to 130 °C, and then cooled down to 20 °C at the same rate.

2.3. X-ray diffraction

A Kristalloflex diffractometer (Siemens, Germany), set at 40 KV, and −20 mA, was used to examine the crystalline order of starch–lipid complexes. The same freeze-dried samples as used in DSC experiments were first ground to pass a No. 50 sieve (300 μm pore diameter), mounted on a sample carrier, and then scanned at a rate of 2°/min from 2θ 4° to 30° in the diffractometer at room temperature.

2.4. Starch and FFA complexation in a dilute system

A dilute system was used to examine starch–FFA complexation in the presence of different concentrations of whey protein and FFAs. The starch concentration was 0.5% (w/v); whey protein concentrations were 0, 5, 10, and 15% (w/w, based on starch); and FFA concentrations were 1.25, 2.5, 3.75, and 5% (w/w, based on starch). Starch (0.5%) was first cooked for 20 min in a boiling water bath; then appropriate amounts of protein and/or FFA's were added to the hot starch suspension. The mixture was then cooked for another 20 min. After overnight cooling at room temperature, the mixture was centrifuged at 14,000*g* (20 min, 25 °C), 100 μl of supernatant (essentially amylose as determined by Zhang, Maladen and Hamaker, 2003) was diluted to 3 ml with purified water, and 0.4 ml iodine solution (2% KI, 0.2% I_2) was added. The blue value was measured at 620 nm. The starch–FFA complex index was calculated based on the difference of OD_{620} between pure starch (amylose) and other samples (starch + FFA or starch + FFA + protein). The OD_{620} of a sample was divided by the OD_{620} of the starch sample alone, and that fraction was subtracted from 1.0, and the difference multiplied by 100. Therefore, starch that was highly complexed with FFA would bind little iodine and have a complex index approaching 100%.

3. Results and discussion

3.1. Starch–FFA complexation during RVA pasting

Differential scanning calorimetry (DSC) results showed that complexation of starch with FFAs occurred during the pasting process as evidenced by the endothermic transitions at about 100 °C, and that whey protein reduced the endothermic enthalpy of the starch–FFA complexes, except when using linoleic acid (Table 1, Fig. 1). An increase was observed in the exothermic enthalpy during the DSC cooling process when protein was added to the starch–FFA mixtures indicating reformation of the starch–FFA complex. Note that DSC tracings failed to show a protein denaturation transition, likely due to the low concentration of the whey protein. As shown in the literature (Bulpin, Welsh, & Morris, 1982), amylose forms a thermally reversible inclusion complex with FFAs; the endothermic peak at ~100 °C upon heating is due to the melting of amylose–FFA complex; and the exothermic peak at ~70 °C upon cooling is due to amylose–FFA complex reformation. The exception of linoleic acid during melting may be due to its unique structure with two double bonds in *cis* conformation. Peak temperatures and values of enthalpy of melting of amylose–FFA complexes were shown to be different for various free fatty acids, and linoleic acid had the lowest melting enthalpy (Akuzawa, Sawayama, & Kawabata, 1995; Raphaelides & Karkalas, 1988). The endothermic enthalpy of starch–FFA complex melting was in the range of 4–9 J/g (based on starch) for the starch + FFA samples, and 4–6 J/g (based on starch) for three-component samples. The enthalpy of the exothermic peak was 1–3.5 J/g (based on starch) for starch + FFA samples, and 4–6 J/g (based on starch) for the three-component samples. The results show clearly that the presence of

Table 1
Differential scanning calorimetry results of freeze-dried cooked starch paste samples

Samples	Process (H/C)[a]	Onset temperature (°C)	Peak temperature (°C)	Enthalpy (ΔH) (J/g starch)	
				Average	SD.
Starch (S)	H	92.3	102.3	1.19	0.10
	C	83.5	80.0	1.04	0.08
S + lauric	H	91.3	101.8	9.13	0.15
	C	81.9	75.2	3.34	0.09
S + (W[b]) + lauric	H	92.0	100.9	5.84	0.33
	C	79.2	74.8	5.02	0.15
S + palmitic	H	88.5	101.7	8.78	0.34
	C	79.3	75.4	3.58	0.13
S + W + palmitic	H	95.2	104.3	4.63	0.41
	C	82.9	78.3	5.60	0.32
S + oleic	H	86.8	99.1	7.73	0.26
	C	78.8	73.6	2.32	0.10
S + W + oleic	H	88.6	101.1	6.03	0.61
	C	82.9	77.5	5.75	0.31
S + linoleic	H	83.4	95.1	4.08	0.31
	C	68.3	65.4	1.09	0.12
S + W + linoleic	H	77.9	95.4	4.55	0.58
	C	74.7	67.7	3.80	0.29

[a] H: heating from 30 to 130 °C; C: cooling from 130 to 30 °C.
[b] W: whey protein.

protein in the system decreased the melting enthalpy suggesting a lesser amount of starch–FFA complex, but increased the exothermic enthalpy suggesting a higher amount of starch–FFA complex reformation in the cooling cycle.

There is another way to compare the data in Table 1. A large difference was found between the enthalpy values of endothermic and exothermic peaks for starch + FFA samples, however, the magnitude of this difference was diminished in the three-component samples. For example, the difference between endothermic and exothermic enthalpies of the palmitic acid + starch sample was 5.2 J/g, while the addition of protein reduced the difference to 1.0 J/g. The amount of starch–FFA complex in the presence of whey protein was about equal when dissociated upon heating and reformed during cooling. Thus, the starch–FFA complex dissociation and reformation was quantitatively similar in the presence of protein.

The protein effect of decreasing endothermic enthalpy indicates that starch–FFA complexation is decreased by the addition of protein during the pasting process. This result was likely due to binding of some FFA molecules to the protein component, leading to formation of the three-way complex during the RVA pasting process that was previously described by our group (Zhang & Hamaker, 2003; Zhang et al., 2003). This is also supported by results from the literature showing that β-lactoglobulin, which accounts for 70% of whey protein, binds FFA in aqueous solution through ionic and hydrophobic interactions (Pérez & Calvo, 1995).

3.2. The order of starch–FFA complexes formed during pasting

The V-type X-ray diffraction pattern is characteristic of the starch–lipid complex, and the shape of the X-ray diffraction pattern reflects the order of the starch–lipid complex crystallite. Thermal crystallization behaviors of amylose–lipid complexes are affected by crystallization conditions (such as moisture content and temperature). The complex can exist in various states of aggregation. Biliaderis and Galloway (1989) showed two distinct forms of starch-lipid complexes: I (low T_m) and II (high T_m) depending on the crystallization temperature. Form I, which was formed through rapid nucleation with a random distribution of the basic complex structural elements, lacked a well-defined V-pattern although it still had a high endothermic enthalpy in DSC analysis. On the contrary, crystalline form II had a well-defined V-pattern with an ordered lamellar arrangement of the inclusion complexes.

Whey protein increased the order of the starch–FFA complex as shown in the diffraction patterns in Fig. 2.

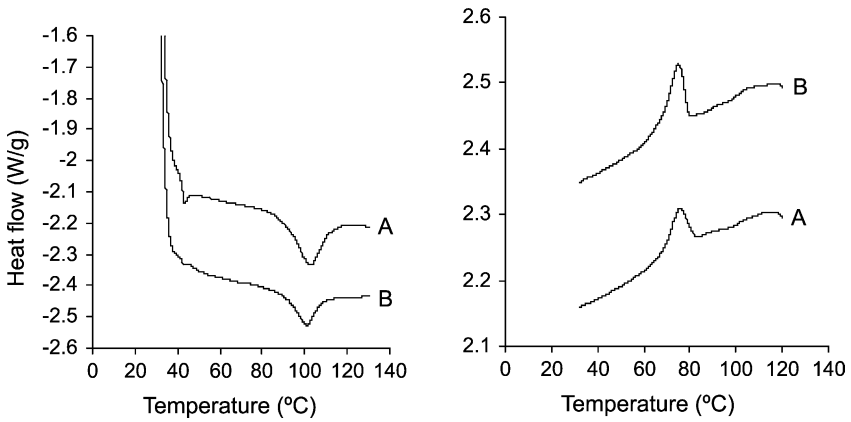

Fig. 1. The differential scanning calorimetry profile of freeze-dried starch pastes during heating (left) and cooling (right) processes. (A): starch + FFA, (B): starch + whey + FFA.

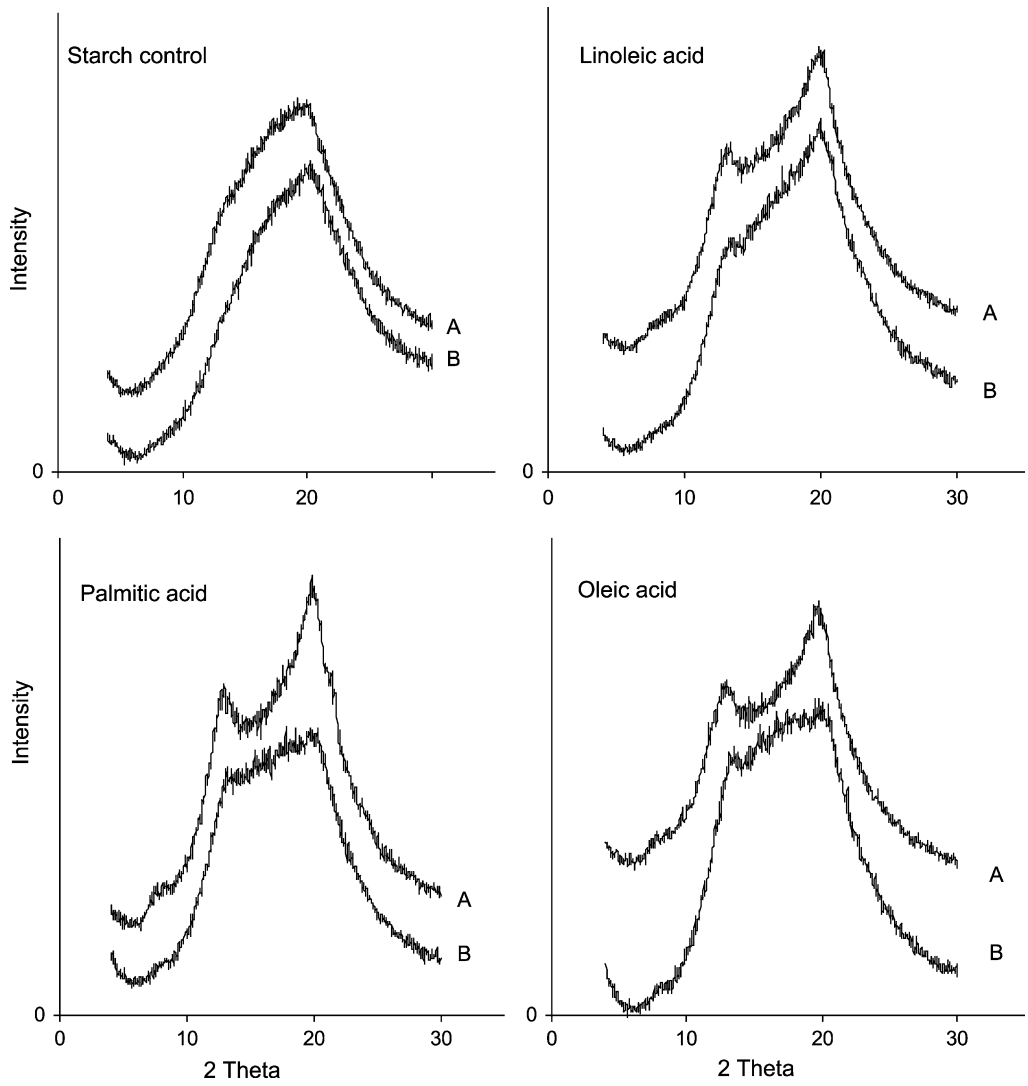

Fig. 2. The X-ray diffraction patterns of starch pastes formed in the presence of FFA and whey protein. (A): starch + FFA + protein, B: starch + FFA, except for controls.

Samples composed of starch, protein and FFA (palmitic, oleic, and linoleic acid) had better-defined V-patterns compared to the samples composed of only starch and FFA. The complex formed during the RVA cooling cycle in samples with only starch and FFA had a higher endothermic enthalpy compared to that when protein was present (Table 1), and may represent a form I complex through rapid nucleation in a very short time (5–6 min). On the other hand, the starch–FFA complex formed when protein was present may belong to the form II complex type that has a well-defined V-pattern with an ordered lamellar arrangement of the inclusion complexes.

Interestingly, the pronounced V-pattern produced when whey protein was present coincided with formation of a cooling stage viscosity peak in RVA profiles (as shown in Zhang and Hamaker, 2003). Lauric acid behaved somewhat differently from the other FFAs used in that both an ordered V-pattern and RVA cooling stage peak were found in the amylose–lauric acid complex alone. However, addition of whey protein did appear to enhance further the V-pattern and viscosity of the peak (Fig. 3). Thus, an ordered V-type starch–lipid complex is related to the formation of the RVA cooling stage viscosity peak.

The result of X-ray diffraction and DSC analyses show that protein decreased the formation of starch–FFA complexation during the pasting (RVA) process, but increased the order of the lamellar arrangement of the inclusion complexes. The ordered starch–FFA complex with protein present is also more quantitatively thermally reversible than starch–FFA complex alone. Further evidence is provided by the following experiment where the starch–FFA complexation was done in a dilute system.

3.3. Protein effect on starch–FFA complexation in a dilute system

The experiment presented here is based on the simple phenomenon that starch-lipid complexation decreases the

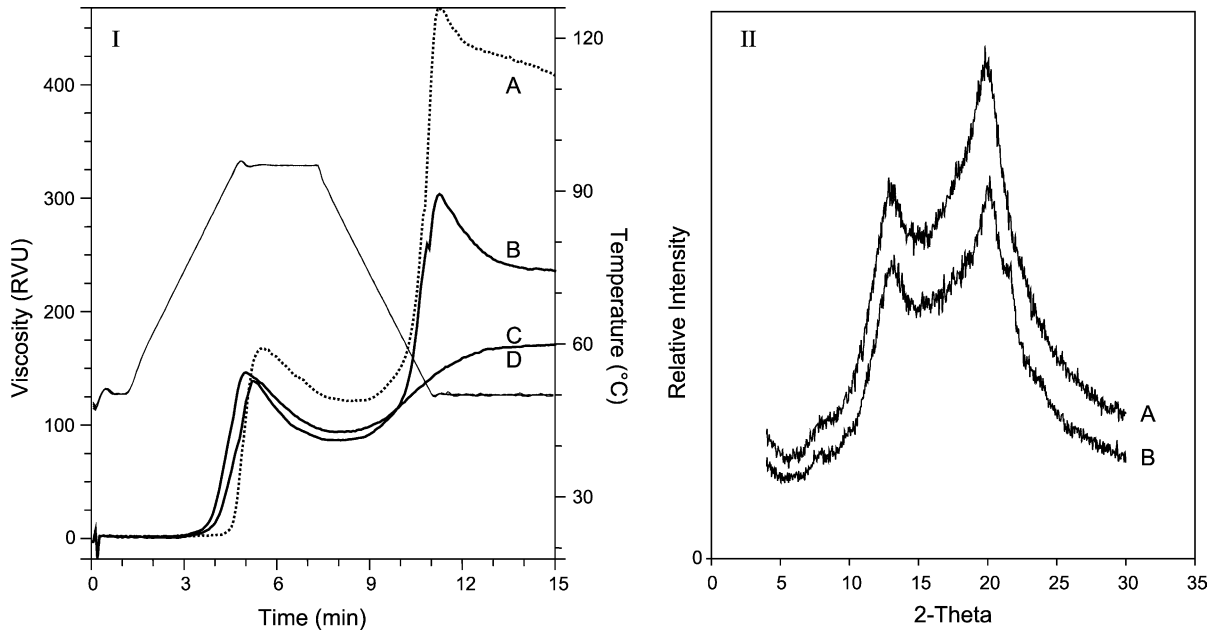

Fig. 3. The RVA pasting profile (I) and X-ray diffraction pattern (II) of starch in the presence of whey protein and lauric acid. (A): starch + whey protein + lauric acid, (B): starch + lauric acid, (C): starch + whey protein, (D): starch control.

iodine binding capacity of starch molecules. Different amounts of protein and FFA were used in the system to examine the iodine-binding capacity of starch molecules under different conditions in order to observe changes in starch–FFA complexation in the presence of whey protein.

When only FFA was in the system with starch, the complexation index (CI in the graph) increased (indicating less iodine binding and higher amylose–FFA complexation) as the concentration of FFA increased until a plateau was reached at 5% for most FFAs (Fig. 4A). In the presence of 10% whey protein (w/w, based on starch), the starch–lipid complexation index was dramatically lower than without protein, although the index increased slightly with increasing FFA concentration (Fig. 4B). In the presence of 5% FFA (w/w, based on starch), the complexation index decreased (seen as an increase in *y*-axis values) as the concentration of protein increased until it reach a plateau at about 10% protein (Fig. 4C). Thus, protein caused a reduction in amylose–FFA complexation and supports the DSC results shown in Table 1.

We speculate that a competitive binding mechanism may be responsible for the decrease in starch–lipid complexation

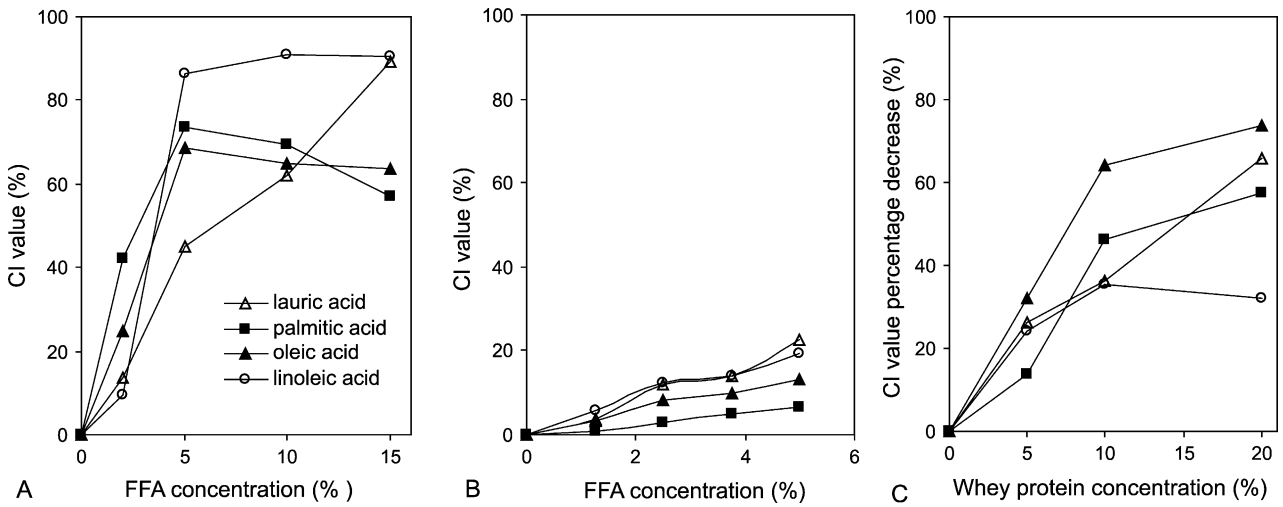

Fig. 4. Starch and free fatty acid complexation. (A): without whey protein, (B): with whey protein (10%), (C): varying whey protein concentration with 5% FFA. The concentration of whey protein and FFAs were based on starch content (w/w). The concentration of starch was 0.5% in water, and concentrations of whey protein and FFAs were based on starch.

when protein is present, if the FFA concentration is below the amount required to saturate amylose. However, it is also possible that a unique characteristic of the three-component complex results in the decrease in starch–FFA complexation and increase in structural order of starch–FFA complex.

4. Conclusion

Starch–FFA complexation in the presence of whey protein was studied using a model system. Starch–FFA complexation was decreased in pastes by the presence of whey protein; but the crystalline order of the V-type starch–FFA complex was better defined. This ordered starch–FFA complex with protein present was quantitatively thermally reversible as measured by melting and reformation enthalpies. Formation of the cooling stage viscosity peak appeared to be related to the presence of the ordered V-type starch–FFA complex. It is unclear what the mechanism might be to explain the observed phenomenon. However, the formation of the three-component complex upon paste cooling, as described in other papers from our laboratory, indicates that whey protein may restrict the random movement of the basic structural element of the amylose–FFA complex, and that may in turn assist the folding of these basic structural elements to form a more ordered amylose–FFA complex and decrease the opportunity for further complexation between amylose and FFA. In light of the commonality of the components tested, it would appear likely that such interactions are common in cooked starchy foods and may influence their functionality.

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