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A Three Component Interaction among Starch, Protein, and Free Fatty Acids Revealed by Pasting Profiles

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A three way interaction among starch, protein, and lipid that affects the Rapid Viscoanalyzer (RVA) paste viscosity profile was revealed using a model system composed of isolated sorghum starch, whey protein isolate, and free fatty acids (FFAs) (20:2:1, w/w/w). A prominent cooling stage viscosity peak in the RVA profile was produced when all three components were present in the system, while there was no viscosity peak when either protein or FFA alone was combined with starch. The magnitude of the cooling stage viscosity peak differed with addition of palmitic, oleic, or linoleic acids to starch and protein. Amylose was the major functional molecule of the starch component. Addition of both protein and FFA to starch substantially reduced starch solubility after gelatinization, while solubility was less affected by single addition of FFA and was not affected by protein. Nonspecificity of this interaction phenomenon was demonstrated by similar results using maize starch and other soluble proteins.

KEYWORDS: Starch; protein; free fatty acid; interaction; paste viscosity

INTRODUCTION

Interactions among food ingredients play an important role in the texturization and mechanical properties of food products. The study of food ingredient interactions can supply meaningful information for the food industry and enhance the understanding of functionalities of food ingredients in real food systems. Studies of two component interactions are common in the literature, such as starch−protein (*1*), starch−lipid (*2*), and protein−lipid (*3*) interactions. Only a few studies have been reported on the functionality of food ingredients in a food system with more than two components (*4*, *5*). However, to our knowledge, there are no reports of a true three component interaction.

Starch, proteins, and lipids are the three major food components in cereal-based food products, and interactions among them in a food system are of importance to functionality and quality. The impetus for our research on a three way interaction among these components arose from an observation of an unusual amylogram profile found in aged sorghum flour pastes. While a typical amylogram has only one viscosity peak in the heating stage that is caused by starch gelatinization, aged sorghum flour had a second high viscosity peak in the cooling stage (Zhang and Hamaker, unpublished data). A pronounced 2-fold increase in cooling stage viscosity correlated to the liberation of free fatty acids (FFAs) from triacylglycerols in stored sorghum flour. A high viscosity cooling stage peak was not produced when isolated starch alone was mixed with FFAs (only a slight increase in cooling stage viscosity resulted), suggesting that protein played a role in the production of the high viscosity peak. In this study, we present evidence for the first time of a three way interaction among starch, protein, and lipid that alters starch paste viscosity profiles. In a companion paper to this, the actual three component complex is identified (*6*).

The three way interaction investigated here was based on a model starch pasting system where starch was the primary component and protein and FFAs were the minor components. A Rapid Viscoanalyzer (RVA) was used as a tool to examine changes of starch functionality. Sorghum starch from a normal genotype was used as the primary component, while whey protein isolate and FFAs (palmitic, oleic, and linoleic—primary constituents of cereal triacylglycerols) were used as the minor components.

MATERIALS AND METHODS

Sorghum cultivar P721N was harvested from the Purdue University Agronomy Farm in 1997 and was conditioned (27 °C, 67% relative humidity) for 2 weeks to approximately 13% moisture content. Starch was isolated from whole sorghum grains according to a general toluene procedure for starch isolation (*7*). Starch was defatted with 85% methanol for 16 h at room temperature. Whey protein isolate (WPI-BioPro: protein 97.9% (db), fat 0.5%, ash 1.6%, moisture 4.8%) was from Davisco Foods International Inc. (Eden Prairie, MN). Palmitic (C16:0), oleic (*cis*-9-octadecenoic acid, C18:1), and linoleic acids (*cis*-9,*cis*-12-octadecenoic acid, C18:2) were from Sigma Chemical Co. (St. Louis, MO).

RVA Operation. A RVA (model 4, Newport Scientific Inc., Australia) was used to obtain paste viscosity profiles. Starch (2.00 g), FFAs (100 mg), and protein (200 mg) were used for analysis according

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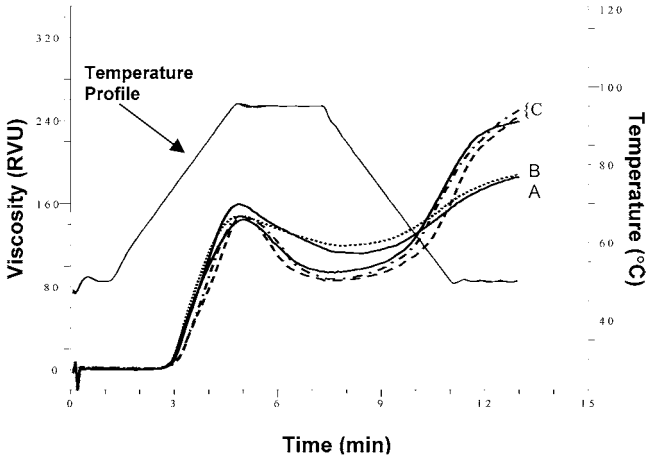

Figure 1. RVA profiles of starch in the presence of whey protein or FFA. Starch control (A), starch + whey protein (B), and starch + FFA (palmitic, oleic, and linoleic acids) (C).

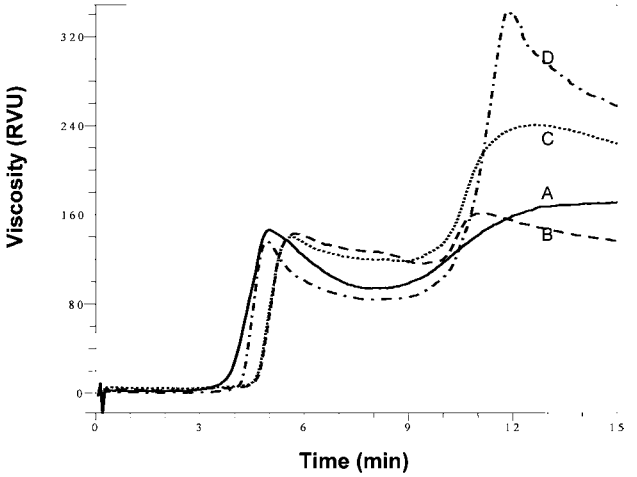

Figure 2. RVA profiles of starch in the presence of whey protein and FFA. Starch control (A), starch + protein + palmitic acid (B), starch + protein + oleic acid (C), and starch + protein + linoleic acid (D).

to standard method 1 from the RVA manual. In this procedure, the starch-based slurry is subjected to a temperature regime of increase from 50 to 95 °C, a holding period at 95 °C, and a decrease from 95 to 50 °C with a subsequent holding period at 50 °C. Different amounts of protein (0, 20, 50, 80, 120, and 200 mg) and FFA (0, 50, 75, and 100 mg) were used to determine the minimum amounts of these components needed to produce the RVA cooling stage viscosity peak. The final mixture weight was 25.0 g with addition of purified water (Barnstead 3 module E-pure, organic free, Dubuque, IA). Continuous RVA cycling experiments involved running of the RVA repeatedly through the complete heating and cooling profile. Starch was always present in the first cycle according to the above procedure; whey protein and/or FFAs were added after the first or second RVA cycles. For the RVA operations that were designed to reveal the relationship between the amount of minor components added and the cooling stage viscosity peak, protein was always added in the first cycle with starch, and FFA was added in the second cycle.

Water Absorption and Starch Solubility. The effect of FFAs and whey protein on sorghum starch swelling and solubility was measured based on the following procedure. Different combinations of starch (5%, w/v), whey protein (10% of starch, w/w), and different FFAs (5% of starch, w/w) were used. Samples were incubated in a water bath (85 and 100 °C) with periodic stirring for 20 min, quickly cooled to room temperature in a cold water bath, and held further for 30 min. Samples were then centrifuged at 14 000*g* for 20 min. The supernatant was measured for total carbohydrate content by the phenol−sulfuric acid method (*8*) to measure starch solubility, and the precipitate was used to measure water absorption (water absorption = weight of the precipitate/original weight of starch; starch solubility = 100 × (carbohydrate amount in supernatant/carbohydrate amount from starch control sample at 100 °C in the supernatant)).

RESULTS AND DISCUSSION

Cooling Stage Viscosity Peak in RVA Profiles of Starch. A cooling stage viscosity peak, which was absent from normal starch RVA profiles, appeared only when starch, protein, and FFA were present together in the system (**Figures 1** and **2**). FFAs alone with sorghum starch produced an increase in starch pasting viscosity at the cooling stage. However, it did not result in the appearance of a RVA cooling stage viscosity peak (**Figure 1**). Addition of whey protein alone to starch had no effect on starch pasting behavior. In the presence of whey protein, linoleic acid was the most and palmitic acid was the least effective in producing the cooling stage viscosity peak (**Figure 2**). While amplitudes of the ternary component cooling stage viscosity peaks were not in each case higher than those of the binary FFA−starch cooling stage viscosities, it was the peak itself that distinguished the three component system. Cooling stage paste viscosity differences relating to the three FFAs were possibly associated to their degree of unsaturation (0, 1, and 2 cis double bonds in palmitic, oleic, and linoleic acids, respectively). The presence of the cis double bonds causes somewhat less effective and less stable complex formation (*9*, *10*). Perhaps linoleic acid, with its two cis double bonds, produced a more extended three component complex that resulted in higher viscosities than its more saturated counterparts. Another common property of the three component system RVA profile was that the first viscosity peak resulting from starch granule gelatinization was delayed as compared to systems containing only starch or starch with either protein or FFA. These findings present evidence that a three way interaction existed among starch, protein, and FFAs that changed starch pasting properties.

Occurrence of the cooling stage viscosity peak was not unique to sorghum starch or whey protein. Normal maize starch showed the same RVA profile pattern in the presence of whey protein and FFAs (not shown). Other proteins, such as bovine serum albumin and egg white protein, also produced the cooling stage viscosity peak when mixed with sorghum starch and FFAs. Thus, the three way interaction appears to be a common phenomenon occurring among different starches, proteins, and FFAs.

No cooling stage viscosity peak resulted in a waxy starch−whey protein−FFA three component system (not shown). Therefore, the main starch molecule involved in the three way interaction was indicated to be amylose, and amylose−FFA complexation was suggested in the three way interaction.

Component Addition Sequence and the Three Way Interaction. The three way interaction among starch, protein, and FFAs was further studied by continuous RVA cycling with minor components added prior to each cycle and in different combinations. These experiments followed a finding that the interaction phenomenon was reproducible in a second RVA cycle with paste viscosity approximately equal to that of the gelatinization peak viscosity of the first cycle followed by reformation with subsequent cooling. Their purpose was to examine the effect of physical states of starch (native granule or gelatinized starch) and protein (native or denatured) on the interactions that occurred to produce the viscosity cooling stage peak and to better understand the nature of the interaction. Considering the stipulation that starch must always be in the system as the primary component, the possible choices were limited to the following: for two continuous RVA cycles, (i)

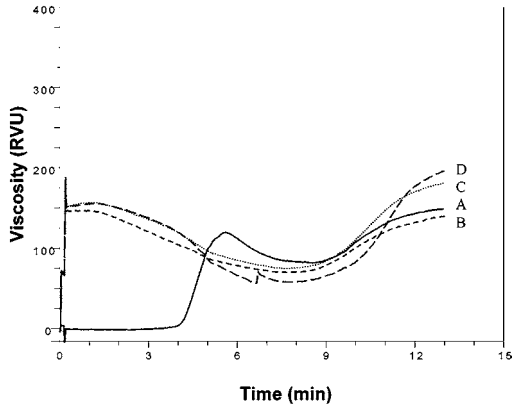

Figure 3. Profile of starch with continuous RVA cycling in the presence of FFAs. (A) Starch control; (B) starch control second cycle; (C) A + oleic acid second cycle; and (D) A + linoleic acid second cycle.

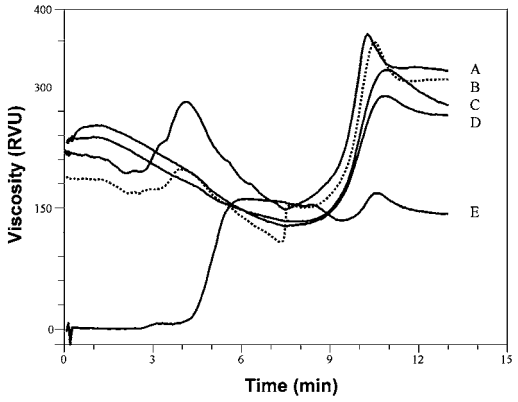

Figure 4. RVA profiles of starch–whey protein–palmitic acid in different mixing sequences. SP1F2 (A), S1PF2 (B), SF1P2 (C), S1F2P3 (D), and SPF (E) in one cycle (S, P, and F are for starch, protein, and FFA; the number *n* is the *n*th RVA cycle).

starch in the first cycle, protein and FFA added in the second cycle (S1PF2); (ii) starch and protein in the first cycle, FFA added in the second cycle (SP1F2); and (iii) starch and FFA in the first cycle, protein added in the second cycle (SF1P2). For three continuous RVA cycles, there was only one interaction sequence tested as follows: (i) starch in the first cycle, FFA added in the second cycle, and protein added in the third cycle (S1F2P3). A RVA second cycle control with added oleic and linoleic acids showed only a slight increase in cooling stage paste viscosity in the presence of the FFAs (**Figure 3**).

Figure 4 shows the results of addition of palmitic acid and whey protein in continuous RVA cycling experiments. The highest viscosity cooling stage peaks were obtained when palmitic acid was added prior to the second RVA cycle but in the presence of protein (SP1F2 and S1PF2). Reduction in cooling stage peak viscosity was observed when palmitic acid was added prior to whey protein in SF1P2 and S1F2P3. Much lower cooling stage peak viscosity was found when starch, protein, and palmitic acid were added together prior to the first RVA cycle (**Figure 4**, profile E; also seen in **Figure 2**). Comparison with other profiles in **Figure 4** (components added in sequential cycles) suggests that starting with gelatinized starch resulted in higher reactivity with FFA than ungelatinized starch granules. Of the three FFAs tested, this effect was most pronounced with palmitic acid. Therefore, the sequence of addition of whey protein and palmitic acid, the minor components, had a substantial effect on the cooling stage peak viscosity.

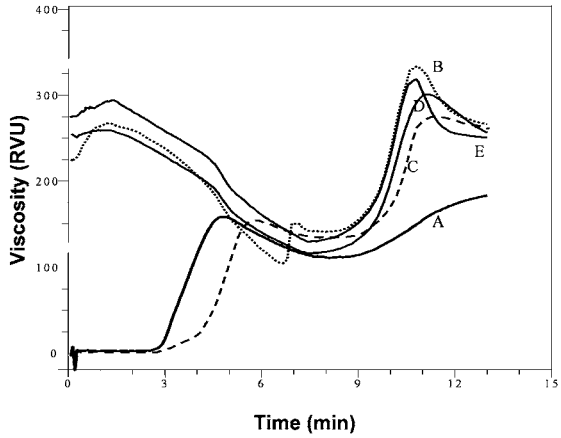

Figure 5. RVA profile of starch–whey protein–oleic acid in different mixing sequences. Starch control (A), S1PF2 (B), SF1P2 (C), SP1F2 (D), and SPF (E) in one cycle (S, P, and F are for starch, protein, and FFA; the number *n* is the *n*th RVA cycle).

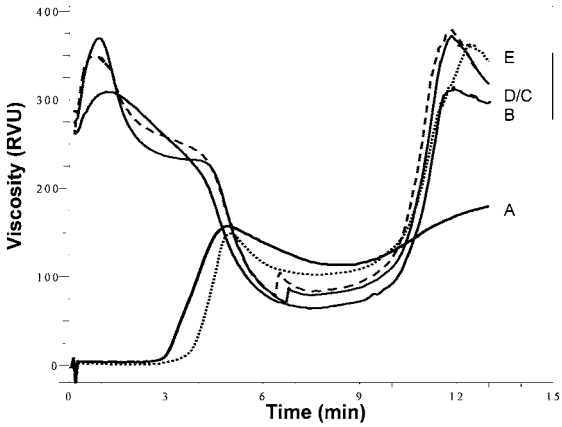

Figure 6. RVA profile of starch–whey protein–linoleic acid in different mixing sequences. Starch control (A), SF1P2 (B), SP1F2 (C), S1PF2 (D), and SPF (E) in one cycle (S, P, and F are for starch, protein, and FFA; the number *n* is the *n*th RVA cycle).

Comparably high viscosity cooling stage peaks were produced in different continuous RVA cycling operations when oleic (**Figure 5**) and linoleic acids (**Figure 6**) were used, and there were less substantial differences in the cooling stage peak viscosities among different sequential RVA operations. Similar to palmitic acid, the first peak representing starch gelatinization was delayed in the three component system, and the cooling peak viscosity was lower when FFA was added in the cycle before whey protein. Large differences in magnitude of cooling stage peaks were observed among the three FFAs used when all three components were added prior to RVA analysis; linoleic acid caused the highest viscosity peak following by oleic and palmitic acids (**Figure 2**).

The effect of the three way interaction was also seen in the finding that starch solubility was substantially reduced when starch, protein, and FFA were added together in the system, while solubility was less affected by FFA addition to starch and was not affected by protein (**Table 1**). Of the FFAs tested, palmitic acid caused the greatest reduction in starch solubility.

These results suggest that the observed difference in three way interaction that occurs when the components are mixed in one RVA cycle vs many cycles is related to the physical states of the different components. It is reasonable to suppose that molecules in different physical states have different accessibilities as well as different potential energies and reactivities that result in different three way interaction patterns. The use of

Table 1. Water Absorption and Relative Solubility of Sorghum Starch Affected by Whey Protein and FFAs at 85 and 100 °C

	water absorption		solubility (%)	
	85 °C	100 °C	85 °C	100 °C
starch (S) control	10.0	15.5	56.5	100.0
S + whey protein (W)	10.3	15.0	57.6	98.7
S + palmitic acid	7.8	15.0	39.6	83.5
S + W + palmitic acid	9.1	14.1	17.9	26.2
S + oleic acid	9.3	15.1	32.6	80.1
S + W + oleic acid	9.1	13.7	25.9	57.3
S + linoleic acid	10.3	15.0	79.0	97.7
S + W + linoleic acid	10.5	13.2	38.5	35.0

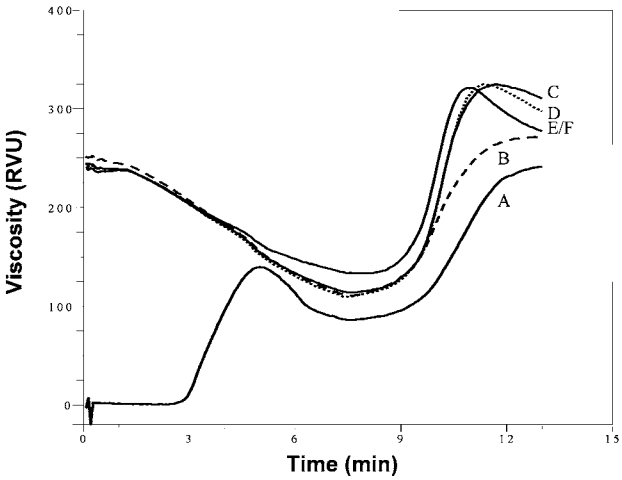

Figure 7. RVA profiles of starch with 100 mg of palmitic acid and different amounts of whey protein. Whey protein contents (in mg): 0 (A), 20 (B), 50 (C), 80 (D), 120 (E), and 200 (F).

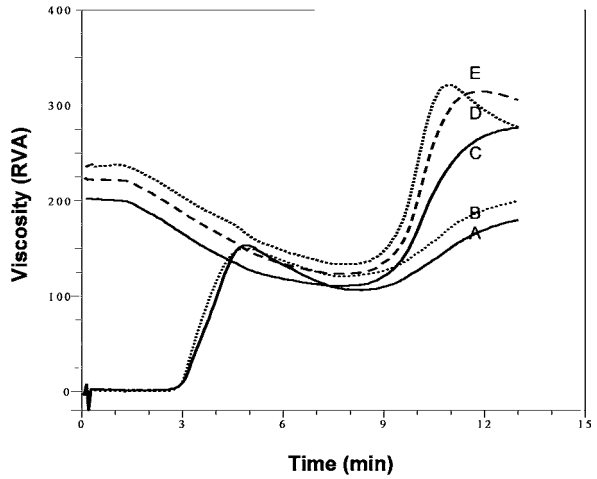

Figure 8. RVA profile of starch with different amounts of palmitic acid and 200 mg of whey protein. Palmitic acid contents (in mg): starch control (A), 0 (B), 50 (C), 75 (D), and 100 (E).

different FFAs in different interaction sequences may provide additional opportunities to manipulate the starch functionality.

Amount of Minor Components and the Cooling Stage Viscosity Peak. The minimum amount of whey protein and palmitic acid to create the cooling stage viscosity peak was determined when other components were kept constant (**Figures 7** and **8**). Protein and FFA were incrementally increased in sequential RVA cycles. Similar interaction patterns appeared both when protein (200 mg) was held constant and FFA was increased incrementally and when FFA (100 mg) was held constant and protein was increased incrementally. Cooling stage viscosity increased as the third component was introduced until a threshold was reached; a peak appeared in the profiles at 50 mg of whey protein and 75 mg of palmitic acid. Cooling stage viscosity was increased and maintained with little breakdown at the threshold amounts (20 mg of whey protein, 50 mg of palmitic acid). At higher levels of either whey protein or palmitic acid, paste viscosity increased progressively earlier during the cooling period with higher amounts added, followed by a marked paste viscosity breakdown forming the characteristic second RVA peak. For oleic and linoleic acids, the threshold point for whey protein was 120 mg when oleic acid or linoleic acid was kept constant at 100 mg (not shown). The threshold point of oleic and linoleic acids was the same as that of palmitic acid (50 mg) in the system when whey protein was kept constant at 200 mg.

CONCLUSION

A three way interaction among starch, protein, and FFA was identified by starch functionality changes observed during RVA cycling, with a prominent cooling stage viscosity peak formed in the presence of all three components. Amylose was the major functional molecule of the starch component. The sequence of component addition to the system affected the magnitude of the cooling stage viscosity peak. Palmitic, oleic, and linoleic acids produced different interaction patterns with starch and whey protein, which showed that the molecular structure of the FFA affects the three way interaction pattern. Quantitatively, a specific amount of minor component (soluble protein and FFA) was required for the production of the cooling stage viscosity peak. This study supplies a theoretical, and perhaps practical, basis to manipulate the texture of food products through food component interaction.

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