

Sorghum (*Sorghum bicolor* L. Moench) Flour Pasting Properties Influenced by Free Fatty Acids and Protein¹

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ABSTRACT

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A second unusually high viscosity peak appeared at the cooling stage (50°C) of a Rapid Visco-Analyser (RVA) profile of short-term stored (two months at room temperature) whole grain sorghum flour, while freshly ground flour had a typical pasting curve with one viscosity peak at the 95°C holding period. The formation of the second viscosity peak was caused by liberation of free fatty acids (FFA), mainly palmitic (15.6%), oleic (41.9%), and linoleic (37.9%) acids from stored flour. After the flour samples were pretreated with pepsin or the protease thermolysin, the second peak disappeared in the presence of FFA while the high viscosity was partially retained, indicating that flour protein was another essential component to the production of the actual peak. Effects of dithiothreitol

(DTT), pH, and NaCl on RVA profiles of stored flour suggested that disulfide-linked protein and electrostatic interaction are required for the peak production. In the presence of sufficient FFA, similar cooling stage viscosity peaks appeared in the RVA profiles of flour samples from maize, rice, millet, and wheat; thus, the effect was not unique to sorghum flour. Coinciding with previously reported findings from our laboratory of a three-component interaction and discernable complex in a model system, a similar three-component (starch, protein, and FFA) interaction was revealed in natural flour systems resulting in formation of an unusual and notably high cooling stage viscosity peak. Practical applications and an interaction mechanism are discussed.

Interactions among food components play an important role in determining the rheological properties of foods during processing and texture of the finished food products. The study of food component interactions aids in understanding of functionalities of food components and can supply meaningful information to the food industry regarding process optimization and novel product development. Starch, protein, and lipids are three major food components and their functionalities determine not only the nutritional values of the products, but also their textural property and shelf life. The amylose-lipid complex, formed by interaction between starch and lipids, has been well documented (Liu et al 1997; Tufvesson et al 2003) and has significant effects on the functionality of starch. It can reduce starch retrogradation (Matsunaga and Kainuma 1986), decrease starch digestibility (Guraya et al 1997), and affect the thermal and mechanical properties of starch (Biliaderis and Tonogai 1991). Protein, as another important biopolymer, rarely interacts with starch directly in regular food systems due to thermodynamical incompatibility (Grinberg and Tolstoguzov 1997). However, protein can bind lipids through electrostatic and hydrophobic interactions to affect final product quality (Cornell and Patterson 1989). Recently, a three-component interaction among starch, protein, and free fatty acids (FFA) was discovered in our laboratory using a model system composed of isolated starch, whey protein, and FFA (Zhang and Hamaker 2003), and a water soluble three-component complex was identified through chromatography (Zhang et al 2003). A further study showed that the starch-FFA inclusion complex became more ordered in the presence of protein and that the crystalline order of the starch-FFA complex was related to a high viscosity peak at the cooling stage of paste RVA profiles of the model system (Zhang and Hamaker 2004).

Cereal flour contains starch, protein, lipids, and other minor components including secondary metabolites, vitamins, and minerals. Thus, cereal flour is an ideal natural and practical food system for studying multiple food component interactions. Sorghum (*Sorghum*

bicolor L. Moench) is one of the important food cereals providing energy, protein, vitamins, and other nutrients to millions of the poorest people living in semiarid tropical regions of the world. The utilization of sorghum as food in most parts of Africa and part of Asia (Murty and Kumar 1995) still mostly involves traditional processes. Thick porridges made from sorghum flour are commonly consumed in sorghum-growing regions of Africa and their viscosity or texture is a primary determinant of porridge quality (Aboubacar et al 1999).

Our investigation into the interactions between lipids and starch, as well as protein, in a flour system originated from the observation of unusual amylograph profiles found in stored sorghum flours. A normal amylograph profile has only one paste viscosity peak occurring during the gelatinization of starch, while the amylograph profile of stored whole grain sorghum flour had two distinct peaks: one at the heating stage and the other at the cooling stage. Moreover, the magnitude of the cooling stage paste viscosity was substantially increased over that of freshly ground flour. Traces of such a peak in the cooling stage can be found in a few reports on wheat (Takahashi and Seib 1988; Xu et al 1992), where the additional peak was speculated to be a result of interaction between starch and polar lipids.

Amylography was once the common method to measure the pasting properties of cereal flour or starches. Since the introduction of the Rapid Visco-Analyser (RVA), its high sensitivity, automated operation, and small sample size has quickly made it an ideal method to measure the pasting properties of cereal samples. The peak viscosity, breakdown, and setback provide an RVA curve, and the shape of the curve reflects the physicochemical property of the tested samples (Shibanuma et al 1996). The food component changes due to storage and interactions with other constituents such as in starch-lipid complexation (Nelles et al 2000) can be easily tested through RVA analysis, so RVA was chosen in the current study as the major tool to study the pasting properties of cereal flours.

The purpose of this investigation was to reveal how cereal components interact in cereal flour systems to alter RVA cooling stage viscosity profiles and produce the observed second viscosity peak. Textural changes due to flour storage are important to product quality and, additionally, identification of flour component interactions that can be used to manipulate texture could be valuable to processors of starch-based foods. This study was conducted principally on sorghum flour and starch, although this phenomenon was not unique to sorghum.

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MATERIALS AND METHODS

Sample Preparation and Starch Isolation

Sorghum cultivar P721N was harvested from the Purdue University Agronomy Farm and was conditioned (27°C, 67% rh) for two weeks to ≈13% moisture content. A flour sample from whole grain sorghum (P721N) was prepared by milling through a 0.5-mm screen using a sample mill (Cyclotec model 1093, Tecator, Sweden), and was stored in a glass jar at room temperature. Defatted sorghum flour was prepared by petroleum ether extraction until the solvent became clear. Residual solvent in flour was evaporated in a ventilation hood. Starch was isolated from whole sorghum grains according to a general toluene procedure for starch isolation (Badenhuizen 1964). After the protein was removed by 10% toluene, 85% methanol was applied for 16 hr with stirring at room temperature to extract the remaining polar lipids. Starch samples were dehydrated with 70, 90, and 100% ethanol, and dried at 50°C for one day. Palmitic acid (C16:0), oleic (cis-9-octadecenoic acid, C18:1), linoleic (cis-9, cis-12-octadecenoic acid, C18:2), dithiothreitol (DTT), protease thermolysin (type x from *Bacillus thermoproteolyticus rokko*, 36 units/mg solid) and pepsin (type A from porcine stomach mucosa, 51 U/mg solid) were from Sigma.

Pasting Viscosity Analysis

A Rapid Visco-Analyser (model 4, Newport Scientific, Australia) was used to obtain paste viscosity profiles. Flour (2.5 g) or starch (2.0 g) was used for analysis according to standard method 1 from the RVA manual (also Approved Method 61-02, AACC International 2000). In this procedure, the starch- or flour-based slurry is subjected to a temperature regime of increase from 50 to 95°C, a holding period at 95°C, and a decrease to 50°C with a subsequent holding period at 50°C. The final weight was 25.0 g with added purified water (Barnstead 3 module E-pure, organic free, Dubuque, IA). When the effect of pH on the RVA profile was tested, the pH was adjusted with sodium hydroxide or hydrochloric acid, and the pH was additionally measured after RVA analysis by diluting the paste with 20 mL of purified water. As to the effect of salt and reducing agent on the pasting property of stored sorghum flour, different amounts of NaCl (0.1–1.0M) or DTT (5–100 mM) were added into the RVA cup with flour samples without preincubation before RVA analysis.

Protein effect on the RVA profiles of sorghum flour was also tested using thermolysin, a thermal-stable protease, and pepsin. For thermolysin treatment, 2.5 mg of thermolysin was mixed with stored whole grain sorghum flour (2.5 g) and water (filled up to 25 g) and incubated for different times (0–30 min) at 50°C before RVA operation. For pepsin treatment, defatted sorghum flour (2.5 g in 50 mL of water at pH 2.0) was treated with pepsin based on the procedure of Zhang and Hamaker (1998). The residue was washed three times with purified water (40 mL) followed by centrifugation at 5,000 × g. Flour residue was used for RVA analysis.

Thin Layer Chromatography (TLC) of Lipids

Nonpolar lipids from sorghum flour (10 g) stored for 0, 1, and 2 months, were extracted with 100 mL of petroleum ether for 1 hr with continuous stirring, and then centrifuged at 4,000 × g for 10 min. The liquid was concentrated to 5 mL by nitrogen sweeping in a hood. A 5-μL sample was used for TLC. Samples were fractionated by TLC on silica gel with petroleum ether/diethyl ether/acetic acid (80:20:1) as the developing solvent. After the development of the chromatogram, individual lipid fractions were identified by exposure to iodine vapors.

Lipase-Catalyzed Hydrolysis of Corn Oil

Free fatty acids were obtained using the procedure of Rahmatullah et al (1994) with modification. A mixture of corn oil (10 g) and potassium phosphate buffer (pH 7.0, 0.08M, 20 mL) containing a lipase preparation (Type III, 0.1 g, Sigma) from *Candida*

rugosa was stirred in a 50-mL flask at room temperature for 2 hr, and then the reaction mixture was extracted three times with 10 mL of diethyl ether. The three extracts were combined and washed with water (2 × 5 mL) containing 20% methanol (v/v). The organic solvent was evaporated in a nitrogen stream. The product from lypolysis was dissolved in 10 mL of hexane and extracted with 40 mL of NaOH (1N in 50% ethanol). The aqueous phase was extracted with hexane (2 × 10 mL). The hexane phase was acidified with 10 mL of 6N HCl, extracted again with diethyl ether (3 × 20 mL), and washed with water (2 × 10 mL). The solvent was removed to yield free fatty acids.

Free Fatty Acid Analysis

Total nonpolar lipids were extracted with petroleum ether from sorghum flour that was stored for six months. Liberated FFA were separated from total lipids by TLC, and the FFA fraction was scraped off and eluted out from the gel with petroleum ether. A gas chromatograph (HP5890 series II, autosampler 7673, HP 3365 ChemStation; Hewlett-Packard Co., Avondale, PA) equipped with a DB 225 or DB 23 column (30 m, 0.53 mm, i.d., 0.5 μm film thickness) (J&W Scientific Co., Folsom, CA) was used to measure the free fatty acid profile. The method used for fatty acid methyl ester preparation and analysis was based on the procedure of Li and Watkins (1998).

RESULTS AND DISCUSSION

Lipid Changes in Sorghum Flour After Storage

There are starch and nonstarch lipids in cereal flour, and lipids extracted with petroleum ether are mainly of the nonstarch, nonpolar type (Azudin and Morrison 1986). TLC analysis of petroleum ether-extracted lipids from whole grain sorghum flour showed that the concentration of triacylglycerols decreased while that of FFA increased during relatively short-term storage (two months) at room temperature (Fig. 1B). The main liberated FFA after storage (six months) were palmitic acid (16:0), oleic acid (18:1), and linoleic acid (18:2) (Table I). This FFA profile is similar to fatty acids in triacylglycerols of freshly ground whole grain sorghum flour, showing that there was no selective release of certain FFA in sorghum flour during storage. The result is consistent with those of Tan and Morrison (1979) that palmitic, oleic, and linoleic acids are the major nonstarch lipids in cereal flours.

FFA and RVA Cooling Peak

After two months of storage, an unusual peak with high viscosity (Fig. 1A) appeared in the cooling stage of the RVA profile that correlated to liberation of free fatty acids in sorghum flour. The RVA profile of freshly ground sorghum flour (0 month) was

TABLE I
Free Fatty Acid Composition of Sorghum Flour
and Liberated Free Fatty Acids (FFA) Profile After Storage

Fatty Acid	Composition (% of total lipids)	Liberated FFA (% total liberated FFA)
14:0 (myristic)	0.03	0.0
16:0 (palmitic)	11.34	15.66
16:1n-7 (palmitoleic)	0.63	0.0
17:0 (margaric)	0.07	0.0
18:0 (stearic)	1.25	2.55
18:1n-9	35.03	41.94
18:1n-7	1.356	1.92
18:2n-6 (linoleic)	46.42	37.92
18:3n-3 (α-linolenic)	2.45	0.0
20:0 (arachidic)	0.17	0.0
20:1n-9 (gondoic)	0.29	0.0
22:0 (behenic)	0.11	0.0
22:5n-6	0.10	0.0
22:6n-3 (docosahexaenoic)	0.25	0.0

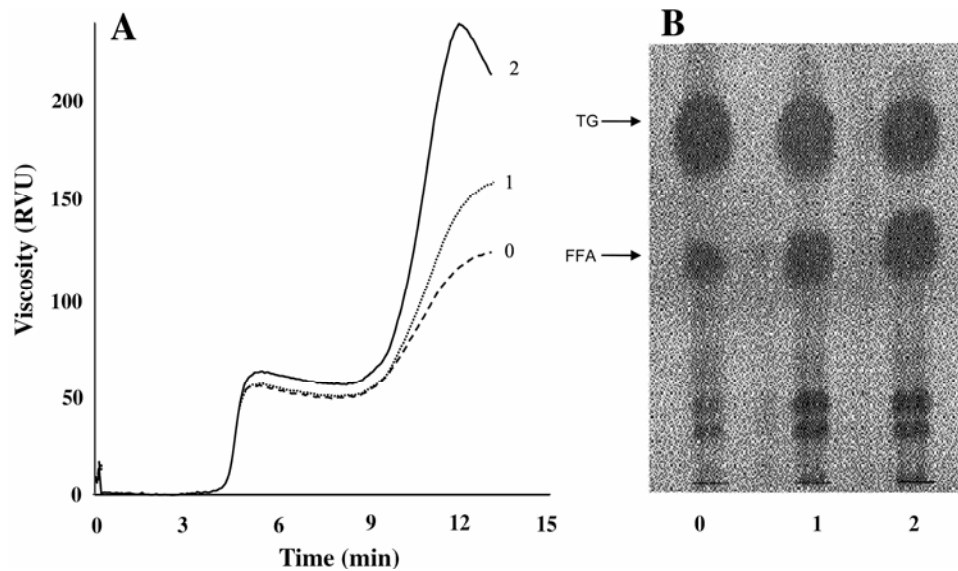


Fig. 1. A, RVA profiles of whole grain sorghum (cv. P721N) flour from different storage times (0, 1, 2 months) held at room temperature. B, Thin layer chromatographic results of lipids extracted from these samples (lane numbers indicate months); TG, triacylglycerols; FFA, free fatty acids.

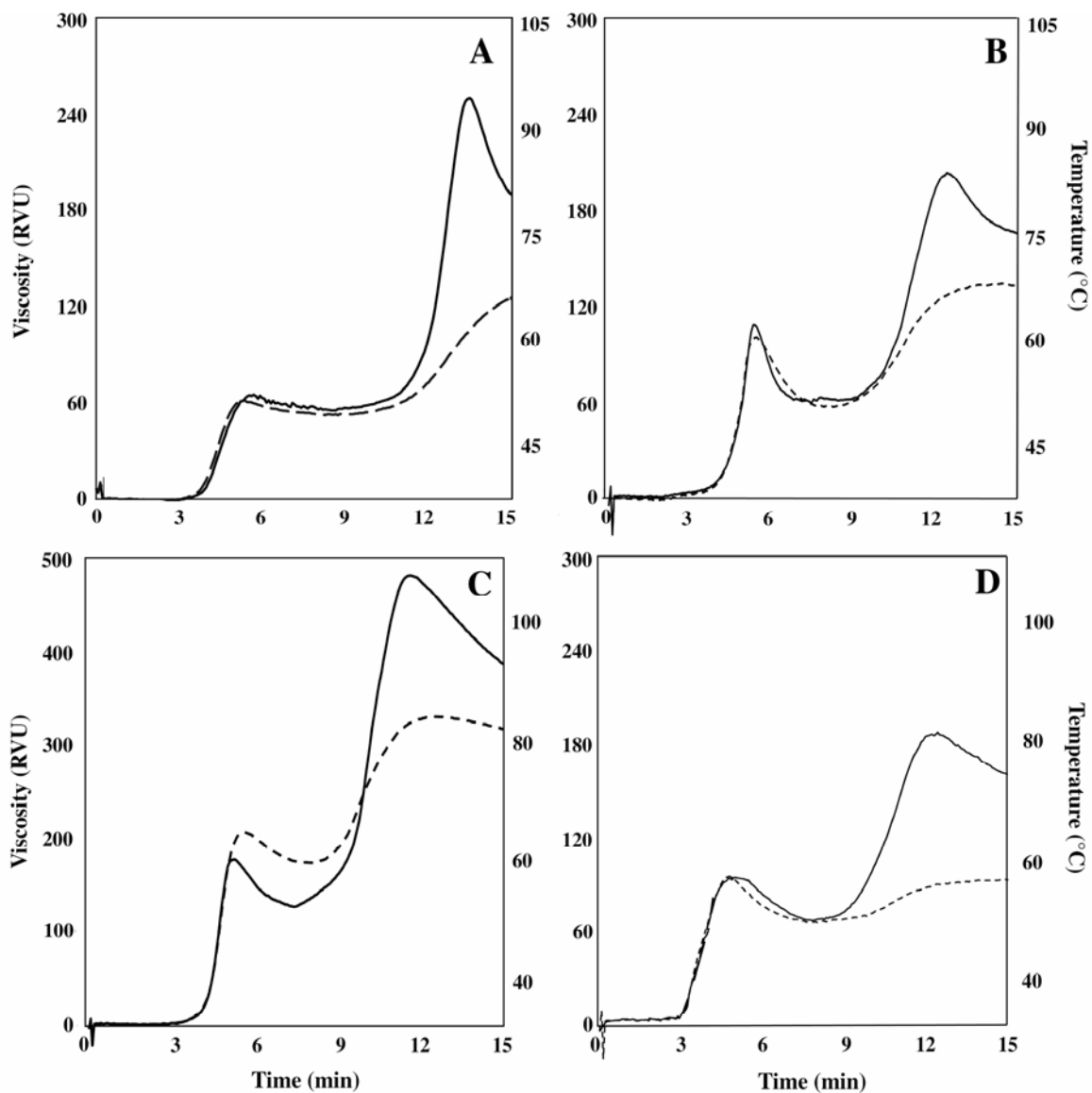


Fig. 2. RVA profiles of several cereal flours in the presence of 200 mg of FFA mixture with corn (A), soft wheat (B), rice (C), and millet (D). Dotted line is the control (without added FFA) and solid line is with added FFA.

typical with the presence of one peak in the 95°C holding period representing starch gelatinization under a constant shear, a breakdown of viscosity at the holding period, and a typical increase in setback viscosity on cooling caused by the reassociation of constituent starch molecules, mainly amylose, into a more ordered state (Tharanathan 2002). As the storage time increased, gelatinization peak viscosity and viscosity at the holding period increased slightly, while the most notable effect was a large increase in setback viscosity and, at two months of storage, a second viscosity peak in the cooling stage. The conclusion that FFA caused the viscosity increase and peak formation was supported by the appearance of the second high viscosity peak when a laboratory-prepared mixture of FFA was added to freshly ground whole grain flour (data not shown). When higher amounts of FFA were added, the cooling stage peak appeared earlier but without further increased height of the viscosity at the peak. The same phenomenon was observed when FFA were added to freshly ground flour of corn, wheat, rice, and millet (Fig. 2) and thus is not unique to sorghum.

RVA profiles were similarly affected when pure palmitic, oleic, and linoleic acids were individually added to defatted sorghum flour (Fig. 3). Increased viscosity and height of second peak were more pronounced from palmitic to oleic to linoleic acids. Therefore,

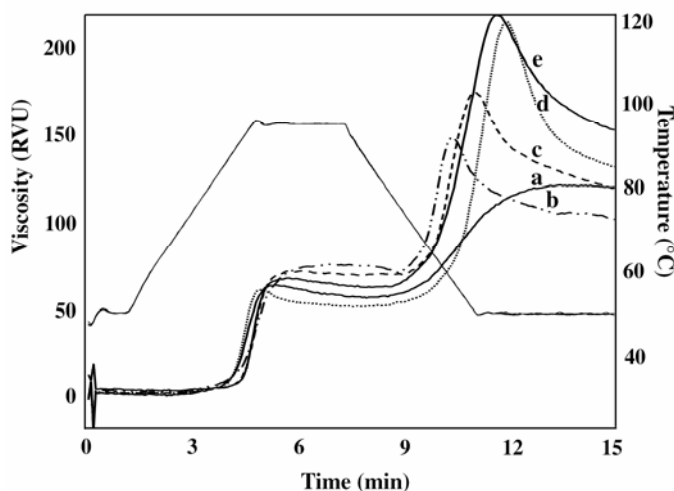


Fig. 3. Effect of addition of 100 mg of FFA on RVA profiles of defatted sorghum flour; defatted flour control (a), control + palmitic acid (b), control + oleic acid (c), control + linoleic acid (d), normal whole grain flour (containing FFA) stored for six months (e).

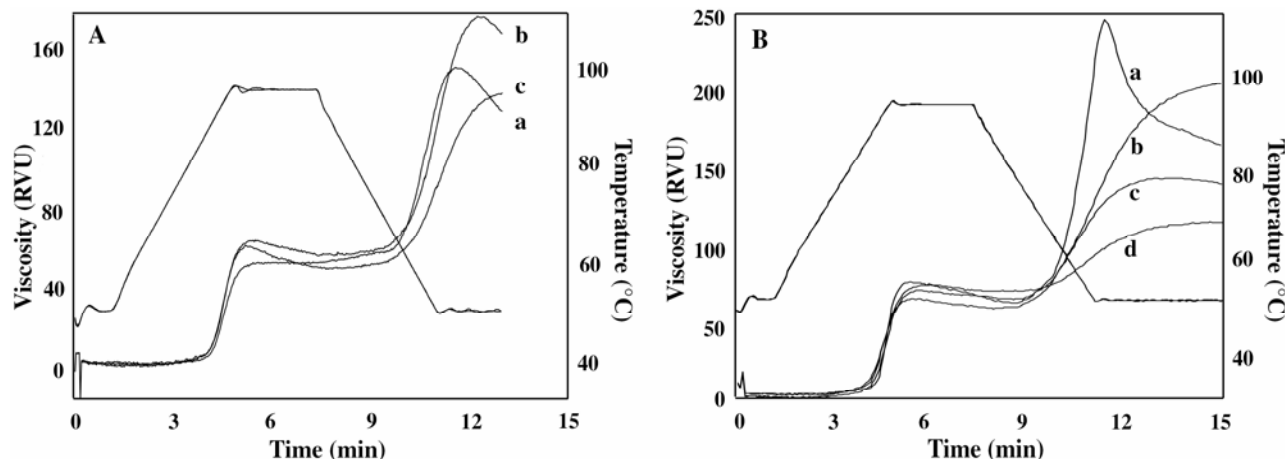


Fig. 4. A, Effect of thermolysin (2.5 mg) pretreatment on RVA profiles of stored flour samples. Control (a), without preincubation (b), incubation for 30 min (c). **B,** Effect of pepsin pretreatment (40 min) of defatted sorghum flour on RVA profile. Flour + 0.1g FFA (a), flour + pepsin + 0.1g FFA (b), flour control (c), flour + pepsin control (d).

length of the aliphatic chain and degree of unsaturation determine the magnitude of the response. These data further confirmed that, in stored sorghum flour, FFA were responsible for the increase in viscosity and appearance of the second peak at the cooling stage of RVA profiles of stored whole grain sorghum flour (Fig. 1).

Effect of Treatments on RVA Profiles of Flour Samples

Our previous data from a model system showed that a cooling stage viscosity peak only appears when starch, whey protein, and FFA were present together in the system, while only a mild increase of setback viscosity was shown in the RVA profiles when solely purified sorghum starch and FFA (palmitic, oleic, and linoleic acid) were combined (Zhang and Hamaker 2003). This result suggested that protein is needed to produce the high cooling stage viscosity peak. Thus, in the flour system used here, proteases and the reducing agent DTT were used to test the role of protein in the cooling stage viscosity peak formation.

Thermolysin is a thermostable protease with an optimum reaction temperature at 70°C. When thermolysin (2.5 mg) was added to stored flour and analyzed by RVA, the appearance of the cooling stage viscosity peak was delayed until complete disappearance after incubation of the sample with protease (Fig. 4A). This is a first indication that flour protein, as one of the flour components, is responsible for the appearance of the cooling stage viscosity peak. Further evidence that protein has a role in the cooling stage viscosity peak formation was shown by the effect of pepsin treatment on the RVA profiles of defatted sorghum flour (Fig. 4B). In this experiment, the cooling stage viscosity peak disappeared when the flour was first treated with pepsin and FFA were added (Fig. 4B, b). The RVA profile of pepsin-treated defatted sorghum flour (Fig 4B, d) showed that the starch itself was not significantly affected by pepsin treatment and that the protein hydrolysis was the reason for the disappearance of the cooling stage viscosity peak.

DTT is a commonly used reducing agent to break disulfide bonds formed between or within proteins. The addition of DTT to the system delayed the appearance of the cooling stage viscosity peak in the RVA profiles of stored whole grain flour samples until its disappearance at an increasing concentration of 100 mM (Fig 5). The necessity for high concentration of DTT to eliminate the cooling peak might be due to a limiting accessibility of DTT to disulfide bonds in the paste system because there was no incubation time for the chemical reaction to complete before RVA operation. In addition to the changes in the second viscosity peak, the height of the first viscosity peak increased after addition of DTT. Thus, the flour protein that is associated with the second viscosity peak formation is likely disulfide-linked and the disulfide-

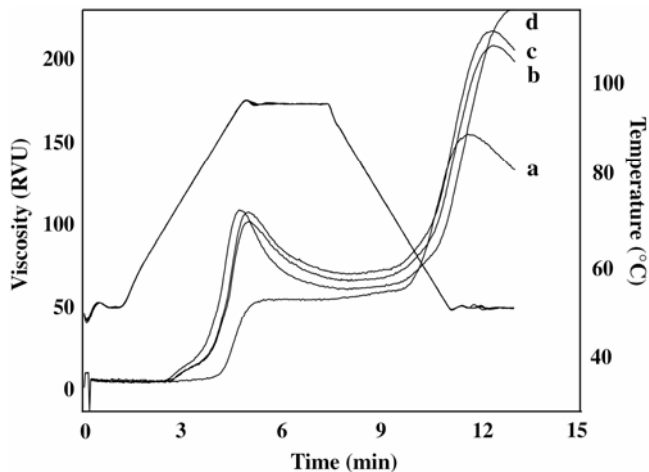


Fig. 5. Effect of reducing agent, dithiothrietol (DTT), addition on RVA profiles of stored flour. Control (a), 5 mM DTT (b), 20 mM DTT (c), 100 mM DTT (d).

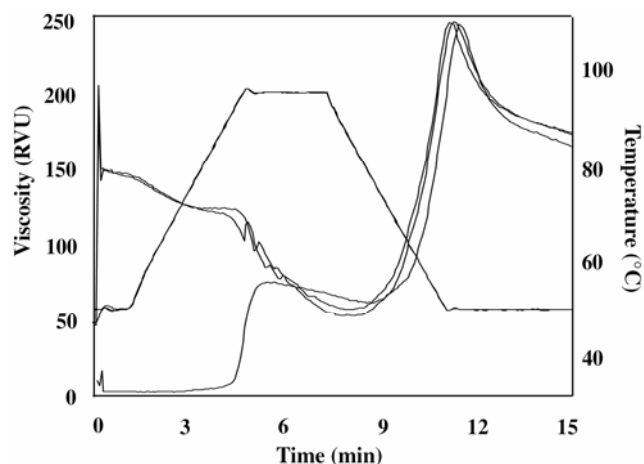


Fig. 6. RVA profiles of normal whole grain sorghum flour (stored for 6 months) run in three consecutive RVA cycles.

linked protein is the functional form involved in the cooling peak formation. In our model system, a disulfide-linked protein polymer was similarly implicated in the three-component complex formation (Zhang et al 2003).

Cooling Peak Properties

The increase in cooling stage viscosity for cereal flour pastes in the presence of FFA began at $\approx 70^\circ\text{C}$ during the RVA cooling period, and the peak, when present, formed during the 50°C holding period. Formation of the high paste viscosity second peak was reversible, as repeated passes of stored sorghum flour paste through the RVA produced the same cooling cycle viscosity profile (Fig. 6). This suggests a dissociation of components occurred during the heating cycle followed by a reassociation to form the high viscosity cooling stage peak. The reason for paste viscosity reduction after peaking may be due to the increasing order of the starch-lipid complex as shown in our previous report (Zhang and Hamaker 2004). Thermal cycling history of sorghum flour had no effect on the appearance and the shape of the second viscosity peak due to the finding that FFA could be added to defatted flour after one RVA cycle with the same results (Fig. 7).

Effect of pH and Salt on RVA Profiles in the Presence of FFA

The cooling stage paste viscosity peak of stored sorghum flour containing FFA was sensitive to pH. While above pH 7 there was no effect on the high viscosity peak (not shown), the viscosity of

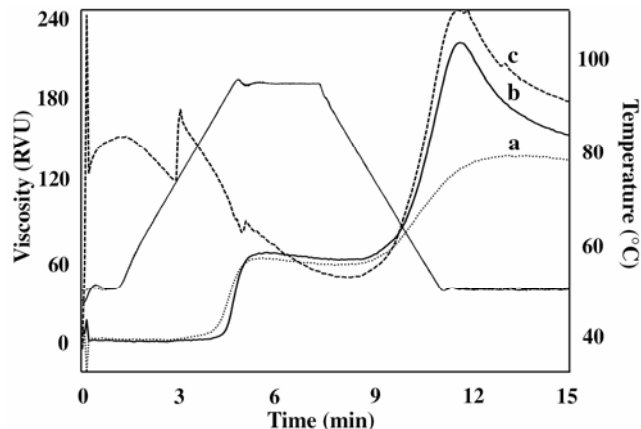


Fig. 7. Thermal cycling of sorghum flour has no effect on RVA profiles in the presence of FFA. Defatted flour control (a), stored flour control (b), 2nd cycle of control with addition of 100 mg of FFA (c).

the cooling peak decreased as the pH decreased below pH 7 and the peak was eliminated around pH 5 (Fig. 8). In a control experiment (not shown), there was no significant effect on cooling stage viscosity of isolated sorghum starch plus FFA (but with no added protein) in the range of pH 5.0–7.0. These findings suggest that ionic forces contributed by either the carboxylate group of FFA or protein charged groups are involved in the cooling stage viscosity increase and peak formation. In our view, the interaction is dependent on the negatively charged FFA carboxylate ($\text{pK}_a \approx 4.8$) interacting with a positive charge likely coming from the protein component.

Salt concentration also had a significant effect on cooling peak viscosity. Using stored sorghum flour (six months), the cooling peak viscosity decreased as the salt concentration increased (Fig. 9). This data supports the above hypothesis that ionic forces are involved in this phenomenon.

Possible Mechanism for Formation of Cooling Stage Viscosity Peak

It is well known that starch is the major component controlling pasting properties of cereal flour but these properties are also affected by other components in the system. In this study, the unusual high viscosity peak in the cooling stage of the RVA profile of stored whole grain sorghum flour was the result of starch interacting with liberated FFA and flour protein. Such a three-component interaction was described in two recent reports from our laboratory using a model system (Zhang and Hamaker 2003; Zhang et al 2003), however the formation mechanism resulting in the viscosity peak remains unclear.

Researchers have shown before that polar lipids (mainly phospholipids) mixed with starch can produce a second minor visco-graph or RVA viscosity peak. Takahashi and Seib (1988) reported a comparably small cooling stage viscosity bump in a viscograph pasting curve when isolated wheat and maize starch had been impregnated with polar lipids extracted from wheat starch granules. They attributed the second peak to amylose-polar lipid complexation. Likewise, others have noted a small peak in the cooling stage part of viscograph or RVA profiles (Xu et al 1992; Kim and Seib 1993; Bhattacharya and Corke 1996). Nelles et al (2000) also reported a second viscosity peak in maize starch mixed with phospholipids, though it appeared in an extended 90°C holding period of RVA pasting curves rather than in the cooling stage period. The common property of these peaks is that they are thought to be caused by starch-polar lipid interaction. Nierle and Elbaya (1990) reported a small cooling stage viscosity peak caused by stearic acid addition to wheat starch but no peaks when oleic acids were added. In our study, the amount of stearic

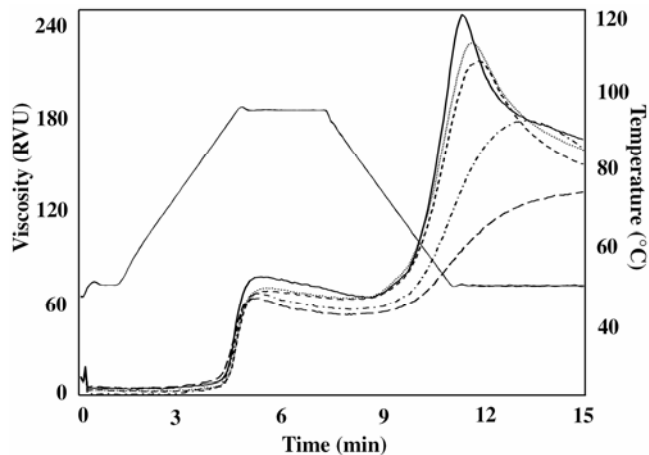


Fig. 8. RVA profiles of stored sorghum flour showing effect of pH on cooling stage high viscosity peak. pH Values (from top to bottom at the cooling peak) are 7.0, 6.5, 6.3, 6.1, and 4.9.

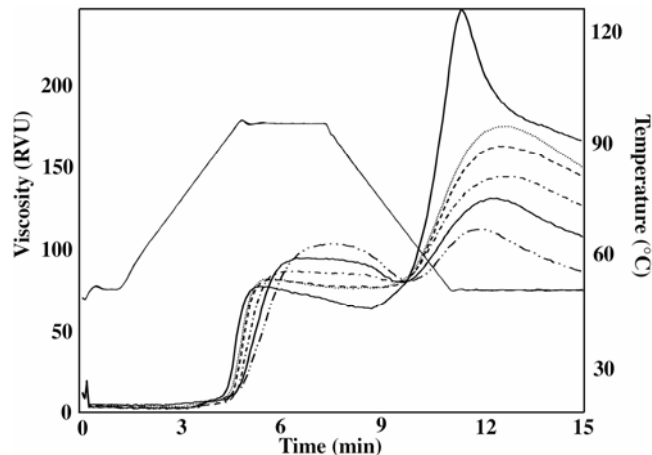


Fig. 9. RVA profiles of stored sorghum flour showing the effect of NaCl on the cooling stage high viscosity peak. NaCl concentration (from top to bottom) is 0, 0.1, 0.2, 0.4, 0.8, and 1M at the cooling stage.

acid in stored sorghum flour was only 2.5% of the liberated FFA (Table I) and we found such a low net amount of stearic acid was not enough to cause noticeable changes of the RVA profile (data not shown).

Results from protease (pepsin and thermolysin) treatments showed that both FFA and protein are required to produce the actual peak in the cooling stage viscosity profile, although FFA alone increased cooling stage viscosity with no peak. This is consistent with the starch-FFA-protein three-component interaction reported previously by our laboratory (Zhang and Hamaker 2003). In a model system composed of isolated starch, whey protein, and FFA (palmitic, oleic, and linoleic acid), RVA analysis showed a very similar cooling stage viscosity peak when all three components were combined. Lauric acid alone did cause formation of a viscosity peak in the cooling stage, however, if a very small amount of protein was added into the system, the height of the cooling stage viscosity peak doubled (Zhang and Hamaker 2004). Theoretically, it seems reasonable to claim that both starch-lipid complexation and starch-protein-lipid complexation could cause the formation of the cooling stage viscosity peak. The key is the structure of lipid components. From the above literature, both lauric and stearic acids alone caused a cooling stage viscosity peak. However, the amounts in a natural cereal flour system are negligible. Fitzgerald et al (2003) found that rice proteins can increase the setback viscosity of rice flour pastes and offset the breakdown at holding period. Comparatively, the protein effect on the viscosity was much greater than lipids. Teo et al (2000) also found that modification of the protein component, rather than starch, was primarily responsible for the observed changes of rheological properties of aged rice flour. Perhaps the observed effect of protein in these two studies actually reflected the three-component interaction observed here.

These data present evidence that a three-component interaction among starch, lipids, and protein likely occurs in real foods and has a profound effect on food quality. In regard to sorghum, the data indicate that stored sorghum flour, at least in whole grain flour, rather quickly (two months) produces sufficient FFA to dramatically change pasting properties during cooling at 50–70°C. In sorghum-growing regions in Africa, the grain is often consumed in the form of thick porridges similar to those studied here. Presence of FFA due to lipolysis of triacylglycerols from whole or imperfectly decorticated sorghum flours could cause large variations in porridge quality at temperatures similar to that at which food is eaten. Additionally, these findings suggest a new way to manipulate starch pasting properties during cooling that could have practical applications.

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LITERATURE CITED

- AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. The Association: St. Paul, MN.
- Aboubacar, A., Kirleis, A. W., and Oumarou, M. 1999. Important sensory attributes affecting consumer acceptance of sorghum porridge in West Africa as related to quality tests. *J. Cereal Sci.* 30:217-225.
- Azudin, M. N., and Morrison, W. R. 1986. Nonstarch lipids and starch lipids in milled rice. *J. Cereal Sci.* 4:23-31.
- Badenhuizen, N. P. 1964. General method for starch isolation. Pages 14-15 in: *Methods in Carbohydrate Chemistry*. IV. Starch. R. L. Whistler, ed. Academic Press: New York.
- Bhattacharya, M., and Corke, H. 1996. Selection of desirable starch pasting properties in wheat for use in white salted or yellow alkaline noodles. *Cereal Chem.* 73:721-728.
- Biliaderis, C. G., and Tonogai, J. R. 1991. Influence of lipids on the thermal and mechanical properties of concentrated starch gel. *J. Agric. Food Chem.* 39:833-840.
- Cornell, D. G., and Patterson, D. L. 1989. Interactions of phospholipids in monolayers with lactoglobulin adsorbed from solution. *J. Agric. Food Chem.* 37:1455-1459.
- Fitzgerald, M. A., Martin, M., Ward, R. M., Park, W. D., and Shead, H. J. 2003. Viscosity of rice flour: A rheological and biological study. *J. Agric. Food Chem.* 51:2295-2299.
- Grinberg, V. Y., and Tolstoguzov, V. B. 1997. Thermodynamic incompatibility of proteins and polysaccharides in solutions. *Food Hydrocolloids* 11:145-158.
- Guraya, H. S., Kadan, R. S., and Champagne, E. T. 1997. Effect of rice starch-lipid complexes on in vitro digestibility, complexing index, and viscosity. *Cereal Chem.* 74:561-565.
- Kim, W. S., and Seib, P. A. 1993. Apparent restriction of starch swelling in cooked noodles by lipids in some commercial wheat flours. *Cereal Chem.* 70:367-372.
- Li, Y., and Watkins, B. A. 1998. Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo prostaglandin E2 biosynthesis in rats fed *n-6* or *n-3* fatty acids. *Lipids* 33:417-425.
- Liu, H., Arntfield, S. D., Holley, R. A., and Aime, D. B. 1997. Amylose-lipid complex formation in acetylated pea starch-lipid system. *Cereal Chem.* 74:159-162.
- Matsunaga, A., and Kainuma, K. 1986. Studies on the retrogradation of starch in starchy foods. 3. Effect of the addition of sucrose fatty acid ester on the retrogradation of corn starch. *Starch* 38S:1-6.
- Murty, D. S., and Kumar, K. A. 1995. Traditional uses of sorghum and millets. Pages 185-221 in: *Sorghum and Millets: Chemistry and Technology*. D. A. V. Deny, ed. AACC International: St. Paul, MN.

- Nelles, E. M., Dewar, J., Bason, M. L., and Taylor, J. R. N. 2000. Maize starch biphasic pasting curves. *J. Cereal Sci.* 31:287-294.
- Nierle, W., and Elbaya, A. W. 1990. Lipids and rheological properties of starch. I. The effect of fatty-acids, monoglycerides and monoglyceride ethers on pasting temperature and viscosity of wheat starch. *Starch* 42:268-270.
- Rahmatallah, M. S. K. S., Shukla, V. K. S., and Mukherjee, K. D. 1994. Enrichment of γ -linolenic acid from evening promrose oil and borage oil via lipase-catalyzed hydrolysis. *J. Am. Oil Chem. Soc.* 71:569-573.
- Shibanuma, Y., Takeda, Y., and Hizukuri, S. 1996. Molecular and pasting properties of some wheat starches. *Carbohydr. Polym.* 29:253-261.
- Takahashi, S., and Seib, P. A. 1988. Paste and gel properties of prime corn and wheat starches with and without native lipids. *Cereal Chem.* 65:474-483.
- Tan, S. L., and Morrison, W. R. 1979. The distribution of lipids in the germ, endosperm, pericarp, and tip cap of amylomaize, LG-11 hybrid maize and waxy maize. *J. Am. Oil Chem. Soc.* 56:531-539.
- Teo, C. H., Karim, A. A., Cheah, P. B., Norziah, M. H., and Seow, C. C. 2000. On the role of protein and starch in the aging of non-waxy rice flour. *Food Chem.* 69:229-236.
- Tharanathan, R. N. 2002. Food-derived carbohydrate—Structural complexity and functional diversity. *Crit. Rev. Biotechnol.* 22:65-84.
- Tufvesson, F., Wahlgren, M., and Ellasson, A. C. 2003. Formation of amylose-lipid complexes and effects of temperature treatment. I. Monoglycerides *Starch* 55:61-71.
- Xu, A., Ponte, J. G., and Chung, O. K. 1992. Bread crumb amylograph studies. II. Cause of unique properties. *Cereal Chem.* 69:502-507.
- Zhang, G., and Hamaker, B. R. 1998. Low-amylase starch digestibility of cooked sorghum flours and the effect of protein. *Cereal Chem.* 75:710-713.
- Zhang, G., and Hamaker, B. R. 2003. A three component interaction among starch, protein, and free fatty acids revealed by pasting profiles. *J. Agric. Food Chem.* 51:2797-2800.
- Zhang, G., and Hamaker, B. R. 2004. Starch-free fatty acid complexation in the presence of whey protein. *Carbohydr. Polym.* 55:419-424.
- Zhang, G., Maladen, M. D., and Hamaker, B. R. 2003. Detection of a novel three component complex consisting of starch, protein, and free fatty acids. *J. Agric. Food Chem.* 51:2800-2805.

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