Bread Crumb Amylograph Studies. II. Cause of Unique Properties[1]

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ABSTRACT

Amylograms of bread crumb have a bump in the setback stage and sometimes a minor peak before the major peak in the heating stage. In repeated amylograph cycles, bread flour alone showed a bump in both heating and cooling stages. With repeated heating and cooling cycles, bread crumb also showed a second bump in the heating stage. In a bread crumb amylogram, the minor peak temperature was superimposed on the falling edge of the bump in the second heating period, suggesting that they were caused by similar factors. Wheat starch and wheat flour polar lipids were shown to be responsible for bump formation. Viscosity changes indicated by shapes of the bumps were temperature-dependent. Differential scanning calorimetry showed endothermic and exothermic peaks, respectively, upon repeated heating and cooling. Addition of sodium stearoyl lactylate to wheat starch also caused bumps in the amylogram. Complexing of lipids, mainly polar lipids, with solubilized starch molecules, and crystallization of the complex in the cooling stage, as well as melting of the crystals and dissociation of the complex in the heating stage probably caused the changes in viscosity during bump formation.

The amylograph has been used to study the pasting characteristics of bread crumb (Yasunaga et al 1968, D'Appolonia and MacArthur 1974, Kim and D'Appolonia 1977, Morad and D'Appolonia 1980, Varriano-Marston et al 1980, Kai 1985). We previously reported the effects of storage time, shortening, flour lipids, and surfactants on bread crumb amylograph properties (Xu et al 1992). In those studies, differences were shown between amylograms of bread crumb and those of wheat starch or bread flour, including a minor peak before the major peak and a bump during the setback stage (Fig. 1). Sometimes a plateau was observed before the onset of the viscosity increase. The plateau was attributed to melting of retrograded amylopectin (Xu et al 1992). Little explanation has been provided for the other features of bread crumb amylograms. Further study is needed to determine the cause of their unique properties.

Bumplike patterns during the cooling stage of the pasting curves of starches and starch products have been reported (Paton 1979, Ali and Bhattacharya 1980, Paton 1981, Ling et al 1982, Rutenberg and Solarek 1984). Elucidation of the unique properties of bread crumb amylograms may contribute to a better understanding of this special phenomenon.

Bread crumb is different from flour in at least two aspects. First, many baking ingredients besides flour and water are present in the crumb. Second, bread has undergone heating during baking, and the starch in the crumb has been partially gelatinized (Varriano-Marston et al 1980). It is logical to relate the cause of the unique characteristics of crumb amylograms to those two differences. D'Appolonia (1972) investigated the effects of baking ingredients on starch-slurry gelatinization and reported no similarities to crumb amylograms. Therefore, the heating during baking is probably one of the factors related to the uniqueness of the bread crumb amylogram.

In this study, the amylograph heating-holding-and-cooling cycle was used to simulate the thermal processes that bread crumb has undergone, and amylograms of repeated cycles were compared to the amylogram of bread crumb. Several materials were tested in the amylograph to identify elements responsible for the unique features of the bread crumb amylogram.

MATERIALS AND METHODS

Materials

A malted commercial hard wheat flour of 10.6% protein (14% mb) was obtained from Archer Daniels Midland Co. (Abilene, KS). Unmodified wheat starch, normal maize starch, and waxy maize starch were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium stearoyl lactylate (SSL) was obtained from BREDDO Inc. (Kansas City, KS). Bread samples were prepared as described previously (Xu et al 1992).

Moisture Measurement

The moisture content of flour or starch was measured in duplicate according to AACC method 44-15A (AACC 1983) at 130°C for 60 min. The moisture content of bread crumb was measured as described previously (Xu et al 1992).

Lipid Extraction, Fractionation, and Impregnation

Lipids were extracted from the flour with petroleum ether (bp 38-52°C) using the Soxhlet apparatus over a 24-hr period (Chung et al 1977). Petroleum ether was used as the extracting solvent because free lipids were of principal interest for this study. The petroleum ether extracts were fractionated into nonpolar and polar lipids by silicic acid column chromatography (Chung et al 1977). The composition of the extracted polar lipids was examined by thin-layer chromatography (Chung et al 1977) and found to be approximately 35% phospholipids and 65% glycolipids. Lipid extraction was duplicated, and fractionation was replicated twice.

To add the extracted and fractionated flour lipids to wheat starch or defatted flour, the lipids were dissolved in petroleum ether, and the wheat starch or defatted flour was mixed thoroughly with the lipid solution. The mixtures were air-dried in a hood

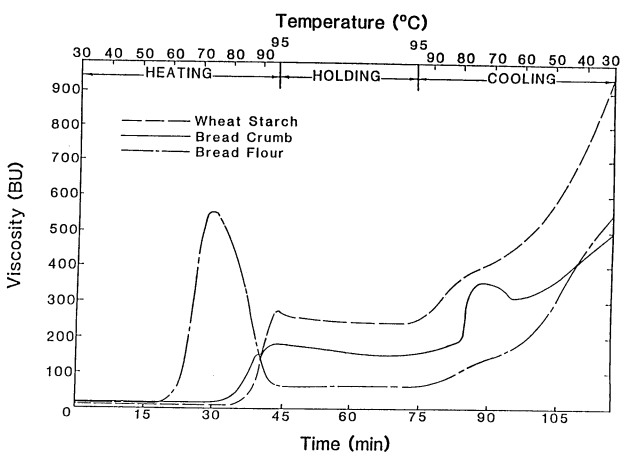

Fig. 1. Typical amylograms of bread crumb, wheat flour, and wheat starch.

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overnight at ambient temperature. Defatted flour was reconstituted to the original levels of lipids, with 1.00% flour lipids comprising 0.69% polar lipids and 0.31% nonpolar lipids (14% mb). The lipid-impregnated starch contained 1.54% flour lipids, comprising 1.06% nonpolar lipids and 0.48% polar lipids (14% mb). Each type of lipid extracted from the flour was mixed with the available starch, assuming that 65% flour weight was starch. Reconstitution and impregnation experiments were replicated twice.

Amylograph Procedure

The Brabender Viskograph-E (C. W. Brabender Instruments, Inc., South Hackensack, NJ) was used. For flours, the amylograph procedure was in accordance with AACC Method 22-10 (AACC 1983), with 100 g of flour (14% mb) in 460 ml of phosphate buffer (pH 5.30–5.35). For starches, a 40-g sample (14% mb) and 400 ml of buffer were used. For bread crumb tests, the preparation of bread crumb and the amylograph scheme were as described previously (Xu et al 1992) except that in the case of repeated cycles, the holding time after the first cycle was reduced from 30 to 10 min. All amylograph testing was replicated twice for a given sample.

Differential Scanning Calorimetry

Bread flour was tested in a differential scanning calorimeter (Perkin-Elmer DSC-2, Norwalk, CT; Flexicooler with temperature controller, FTS Systems, Stone Ridge, NY) in two replicates. Flour (2 mg, db) at a sample-water ratio of 1:4 was tested at a sensitivity of 0.5 mcal/sec. The sample was heated at 10°C/min from 7 to 127°C and held at 127°C for 30 min before it was cooled from 127 to 7°C at the same rate. This heating, holding, and cooling cycle was then repeated for the same sample four more times under the same conditions, except that the holding time was reduced from 30 to 10 min.

RESULTS AND DISCUSSION

Identification of Factors Responsible for the Uniqueness of the Bread Crumb Amylogram

Heating is likely a major factor in causing the unique features of bread crumb amylograms, so an amylograph heating-holding-and-cooling cycle was used to simulate the thermal history of bread crumb. Repeated cycling should enhance the unique features.

The amylogram for malted bread flour is shown in Figure 2. A bump similar to that found in the bread crumb amylogram appeared in each cooling period after the first amylograph cycle. A bump also appeared during each heating period and was similar in size to the bump in the cooling period of the same cycle.

To determine whether malt was essential for the bump formation, an unmalted flour was tested in the amylograph. With the standard concentration of 100 g of flour in 460 ml of buffer, the unmalted flour showed bumps in the repeated cycles on the amylogram (amylogram not shown). However, the result was not readily reproducible in every amylograph test, presumably because the viscosity was too high. When the concentration was lowered by 50%, the unmalted flour did reproducibly show repeated bumps (amylograms not shown). Therefore, malt is not a necessary factor responsible for the bumps.

Repeated cycles of bread crumb amylograms also showed repeated bumps after the first cycle in addition to the bump in the first cooling period (Fig. 3, one repeated cycle is shown). The minor peak temperature in the first cycle was the same as that of the falling edge of the bump during the heating period in the second cycle (Fig. 3). This suggested that the minor peak of the bread crumb amylogram might be due to the same phenomenon as bump formation; where the bump was concealed by a drastic viscosity increase in the main-stream curve, the falling edge of the invisible bump caused the minor peak. Starch in bread crumb was incompletely swollen and pasted during baking (Varriano-Marston et al 1980); thus it underwent further swelling and pasting in the amylograph, resulting in the sharp increase in the main-stream viscosity.

These results suggest that wheat flour alone without other baking ingredients is enough to show the unique features observed in bread crumb amylograms. It is unclear which component or components of flour, such as starch, gluten, and lipids, were responsible for these unique features. Gluten was excluded because a mixture of wheat starch and vital gluten did not show the bumps in repeated amylograph cycles (amylogram not shown).

To determine whether lipids were involved in bump formation, petroleum-ether defatted flour was tested on the amylograph with repeated cycles. The bumps were absent (Fig. 4), but when the

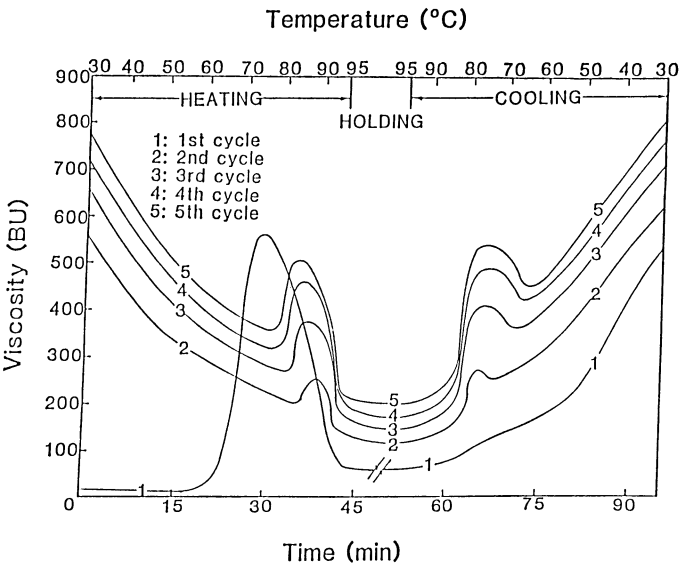

Fig. 2. Amylogram of malted bread flour with repeated cycles.

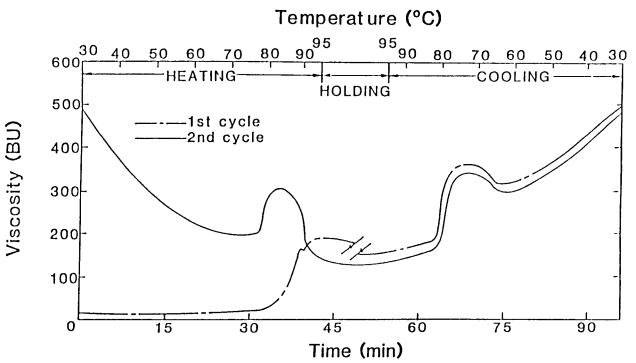

Fig. 3. Bread crumb amylogram with a repeated cycle.

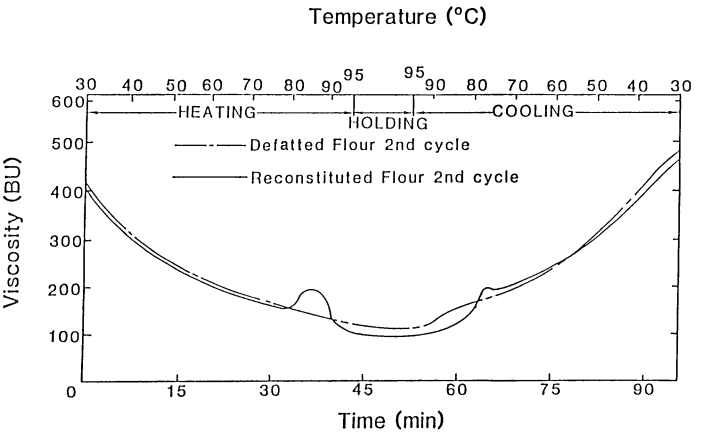

Fig. 4. Amylograms of defatted flour and reconstituted flour, showing only the second cycle .

defatted flour was reconstituted with the extracted lipids, the bumps were restored, suggesting that lipids are involved in the unique features of bread crumb amylograms.

Wheat starch alone did not exhibit a bump in the amylogram (first cycle in Fig. 1, repeated cycles not shown), whereas the addition of wheat flour lipids to wheat starch resulted in an amylogram (Fig. 5) containing the same kind of bumps as were found in the amylograms of both flour and crumb with repeated cycles.

Wheat flour lipids were further fractionated into polar and nonpolar fractions and added to defatted flour or wheat starch. The amylograms of the impregnated flour and starch showed that polar lipids were involved in bump formation (Figs. 6 and 7, respectively), whereas nonpolar lipids did not produce bumps with either defatted flour or starch (amylograms not shown). Because the bumps could form only after the first cycle in flour amylograms, it is likely that the interactions of gelatinized starch and lipids were responsible for the unique characteristics of bread crumb amylograms.

Description of Bumps in Amylogram

The bumps found in flour amylograms after the first cycle increased in size with each additional cycle (Fig. 2 and Table I). The temperature ranges over which bumps occurred were about 11–14°C lower in the cooling period than in the heating period (Table I). Within each cycle, the bumps had similar areas and similar intervals of temperature range in the heating and cooling periods. The similarity in the interval of temperature ranges raised a question as to whether the viscosity was time-dependent after the initial starting temperature in each cycle. To test this, the temperature was held constant for a time when the viscosity reached the top of the bump (Fig. 8a). The viscosity did not change during the holding period at the peak temperature of the bump, but it decreased as usual when the temperature was increased at a rate of 1.5°C/min after holding. This indicated that bump formation was temperature-dependent.

The curve in Figure 8b was obtained by decreasing the temperature after viscosity reached the top of the bump in the heating period of the third cycle of a bread flour amylogram. The bump in this amylogram, in effect, consisted of half heating bump and half cooling bump. This implied that the status of the bump-forming system (illustrated later) was the same at the tops of bumps in both the heating and cooling periods.

Amylograms of Wheat Starch with SSL

As shown previously (Xu et al 1992), shortening and various surfactants as well as the native flour lipids gave distinct shapes and sizes of bumps in the amylograms of bread crumb. To determine the effects of surfactants on wheat starch amylograph properties in the absence of flour lipids, SSL was added to wheat starch at several levels (Fig. 9). As expected, the addition of SSL increased the viscosity onset temperature and the peak viscosity. However, more dramatic effects were shown during and after the holding period. At lower levels of SSL, bumps with a temperature range similar to those of bumps found in crumb amylograms were observed. As the level of SSL increased from 0.2 to 1 g for 40 g of starch and 400 ml of buffer, the size of the bump increased and became an enormous peak that started in the holding period after the regular peak. Although this phenomenon needs further investigation, it suggests that SSL might exert an effect on bump formation in bread crumb amylograms through its direct interaction with starch in addition to its possible interaction with lipids.

Differential Scanning Calorimetry

Figure 10 shows differential scanning calorimetry curves (one set of two replicates) obtained for bread flour with repeated heating-holding-and-cooling cycles. In the first heating, besides

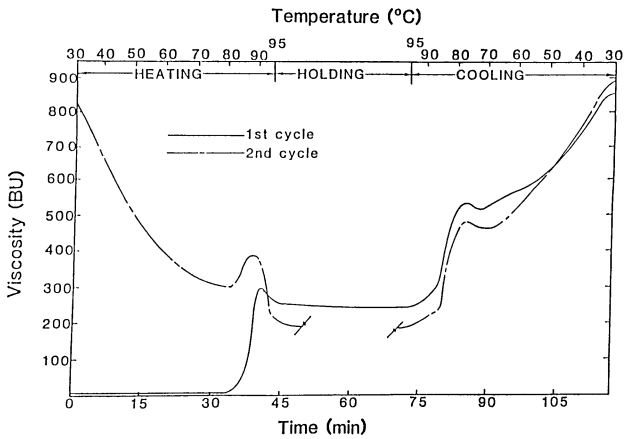

Fig. 5. Amylogram of wheat starch plus added flour lipids (1.54%) with a repeated cycle.

TABLE I
Bump Areas and Temperature Ranges of Flour Amylogram with Repeated Cycles[a]

	Bump Area, cm²		Temperature Range, °C	
Cycle	Heating	Cooling	Heating	Cooling
2nd	3.1	2.8	83–92	80–72
3rd	8.8	8.4	80–93	81–66
4th	12.6	12.5	78–93	82–64
5th	14.2	15.1	78–94	82–64

[a] Average values of two replicates.

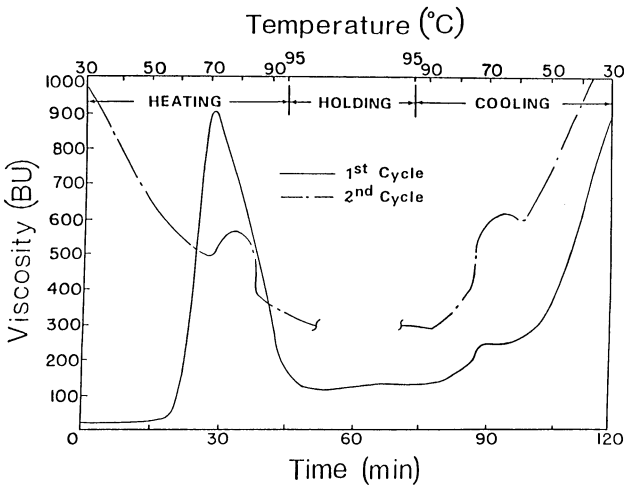

Fig. 6. Amylogram of defatted flour plus added polar flour lipids (0.31%) with a repeated cycle.

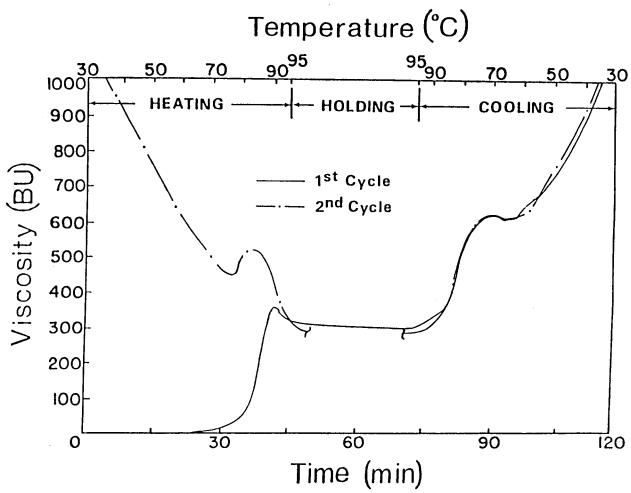

Fig. 7. Amylogram of wheat starch plus added polar flour lipids (0.48%) with a repeated cycle.

the starch gelatinization endothermic peak at 62°C, there was another broader endothermic peak around 89°C. This broad peak reappeared in the following cycles between 93 and 94°C. The enthalpy change varied from cycle to cycle and averaged 1.8 ± 0.4 J/g for two replicate tests. An exothermic peak appeared in each cooling stage with a peak temperature of 71–72°C and an average enthalpy change of −1.6 ± 0.5 J/g. The enthalpy changes appeared to have no definite relationship to the number of cycles, but the exothermic enthalpy in the cooling stage was close to the endothermic enthalpy in the next heating stage, with the correlation coefficient between them being 0.87. The shape of both the endo- and exothermic peaks was skewed to the high temperature side. The endothermic peak started between 70 and 80°C and ended at about 100°C, whereas the exothermic peak started at about 75°C and ended between 53 and 62°C.

Similar peaks have been reported by several researchers (Kugimiya et al 1980, Kugimiya and Donovan 1981, Eliasson and Karlsson 1983, Stute and Konieczny-Janda 1983, Biliaderis et al 1985, Eliasson et al 1988) with various starches and lipids or surfactants. Those peaks are generally recognized as being caused by amylose-lipid complex crystallization and melting. The peak temperature of the endotherm was often found to be around or above 100°C depending on the water ratio. The present system gave an endotherm at a relatively low temperature (93–94°C), in agreement with Schweizer et al (1986), who observed a similar melting temperature of the amylose-lipid complex with reheating of cooked wheat flour products by DSC. The temperature difference (22°C) between the endotherm and exotherm peaks falls in the range observed by Biliaderis et al (1985), who found a 16–26°C hysteresis of the exotherm peak. Bulpin et al (1982) found a 28°C hysteresis.

Comparison of the DSC thermogram (Fig. 10) of the flour-

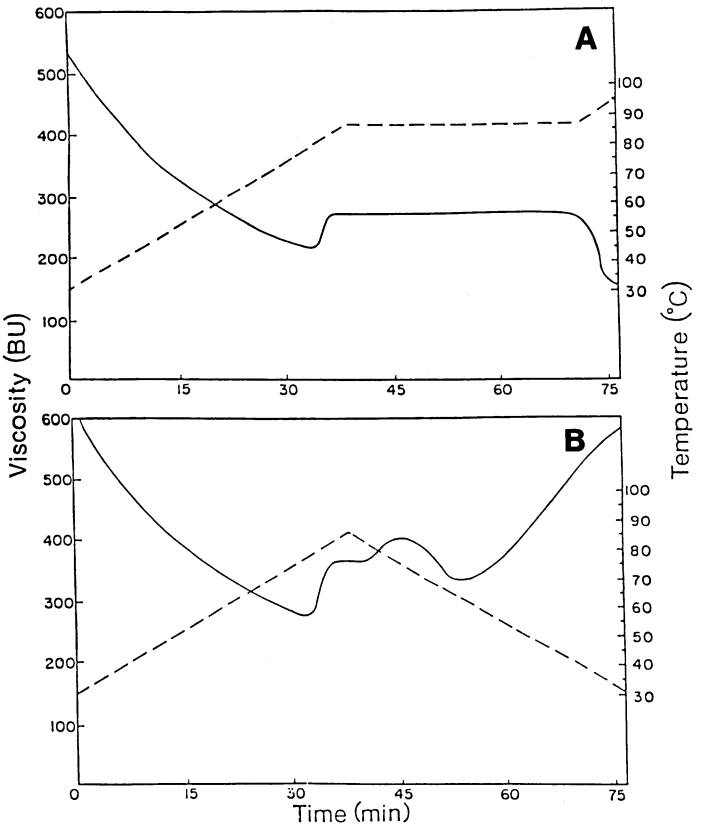

Fig. 8. Amylograph curves showing that the bump is temperature-dependent. Line = viscosity; dashed line = temperature. **A,** second heating stage of bread flour, with temperature held at the top of the bump; **B,** third cycle of bread flour, with cooling started at the top of the bump.

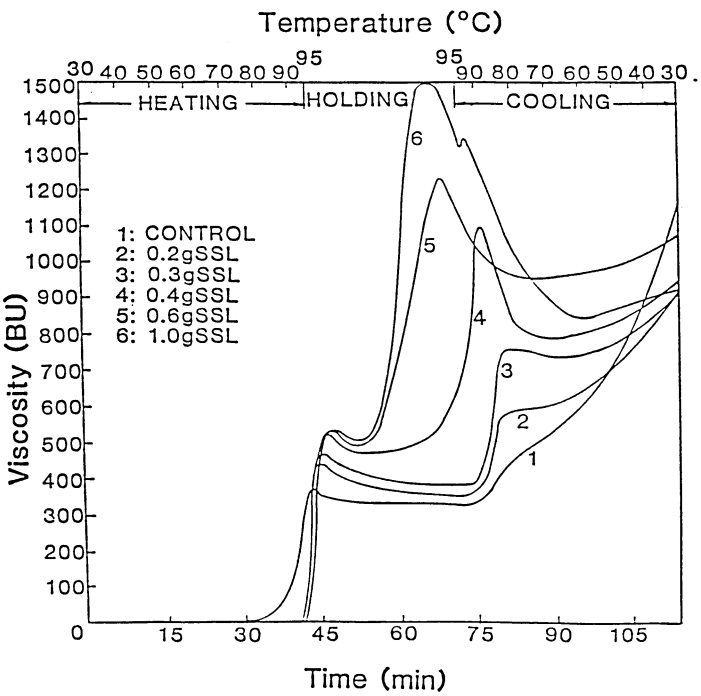

Fig. 9. Amylograms of wheat starch with different levels of sodium stearoyl lactylate (SSL).

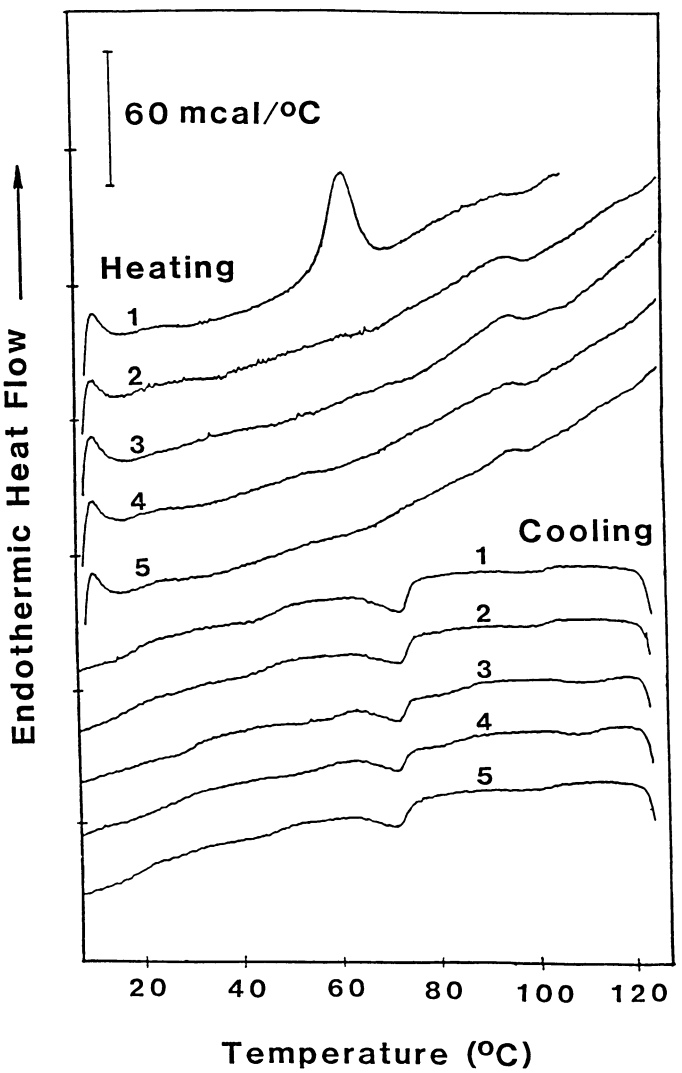

Fig. 10. Differential scanning calorimetry thermal curves of bread flour with repeated heating-holding-and-cooling cycles. 1–5 denote numbers of cycles. Heating and cooling rate: 10°C/min. Sample weight: 2.0 mg. Ratio of sample to water ratio, 1:4.

water system with the amylogram of the flour (Fig. 2) shows some similarities, including the reappearance of the peaks and bumps in each reheating and recooling, the hysteresis of the cooling peaks and bumps, and the overlapping temperature ranges of peaks and bumps. These similarities suggest that the thermal peaks are probably related to bump formation in the amylograms, although there are differences in the exact temperature ranges (6–7°C higher in heating and 6–7°C lower in cooling for DSC peaks vs. amylogram bumps), in the extent of hysteresis (22 vs. 13°C), and in the change in the size of the peaks and bumps (no change vs. increasing size). These could be attributed to differences in the conditions, e.g., mixing, heating and cooling rate, and temperature scanning range, of the samples in the two instruments.

Although interaction of amylopectin with lipids was reported, the amylopectin-lipid complex formed only after a long nucleation period (24 hr) at low temperature (4°C) (Levine and Slade 1988), unlike the conditions in the present system. In addition, when waxy maize starch (~99% amylopectin) was impregnated with flour lipids, no amylogram bumps appeared, whereas normal maize starch did show the bumps (amylograms not shown). Therefore, under the conditions of this study, it is unlikely that amylopectin complexed with lipids and caused the bumps in the bread crumb amylogram.

Proposed Explanation

Based on these observations and current knowledge about the starch-lipid system, a possible explanation for bump formation in flour amylogram is proposed (Fig. 11). At the end of the first cycle, the starch paste was cooled to 30°C, and the solubilized amylose formed single helices with lipids (State A, Fig. 11b), primarily polar lipids. The major constituents of the nonpolar lipids are triglycerides (Pomeranz and Chung 1978), which do not form an inclusion complex with amylose (Mercier et al 1980, Morrison 1988). Those amylose-lipid helices were packed in crystalline form (Sarko and Wu 1978). As the system was heated in the second cycle, the helical crystals melted at about 80°C (Transition 1, Fig. 11b) and a large number of hydroxyl groups was exposed to water molecules, absorbing water by hydrogen bonding. Thus, the amount of mobile water, i.e., the lubricant between swollen granules, would have decreased sharply. In this temperature range, the amylose-lipid inclusion complex (State B, Fig. 11b) did not dissociate, and the flexible, yet rigid, amylose molecules were easily entangled with each other and with amylopectin molecules. These conditions resulted in a sharp increase in viscosity. Shortly afterwards, the temperature reached the point at which the helical structure could no longer be maintained. Lipids dissociated from amylose (Transition 2, Fig. 11b), and the amylose was transformed to the random coil state (State C, Fig. 11b). The system with amylose at this state is more fluid and exhibits a lower viscosity (Banks and Greenwood 1968); thus, a sudden decrease in viscosity was observed. The cooling period of the second cycle involved just a reverse process of the heating cycle, except that the corresponding transitions, amylose-lipid complexing and crystallization (Transitions 3 and 4, Fig. 11b), occurred at lower temperatures. This hysteresis phenomenon is common in the crystallization and melting processes of polymeric crystals (Mandelkern 1958), as well as some other crystals (Griffin et al 1985), probably because of the necessity for nucleation before crystal growth (Biliaderis et al 1985).

In the subsequent cycles, the interactions repeated in the same manner. With each additional cycle, more soluble amylose was available as pasting of starch continued in the amylograph, resulting in a higher overall viscosity and a larger bump area. The DSC thermogram of bread flour did not show a progressive increase of amylose-lipid interaction with increased cycles (Fig. 10). This could be because the high temperature in the differential scanning calorimeter pasted the starch thoroughly in the first cycle.

In the DSC thermogram, only one endo- or exothermic peak was observed during each heating or cooling period. Crystallization or melting of the crystals was not distinguishable from the complexing or dissociation of the amylose-lipids complex.

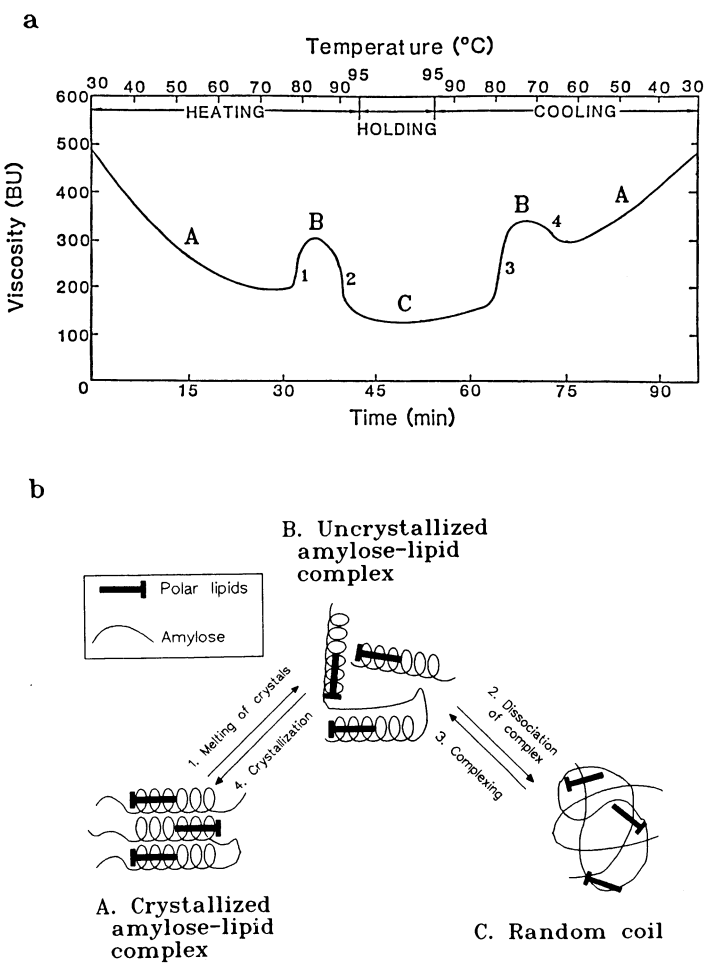

Fig. 11. A possible explanation of the bump formation phenomenon in amylograms. **a,** A typical repeated cycle of bread crumb amylogram. **b.** Possible explanation. A–C denote states; 1–4 denote transitions.

CONCLUSIONS

Interactions of solubilized amylose with flour lipids, mainly polar lipids, caused the bump in the bread crumb amylogram. The minor peak before the major peak was probably due to the same interactions. In the cooling stage, a transition of amylose from random coil to lipid-inclusion helices might be associated with the increase in viscosity; then the crystallization of the helices resulted in the decrease in viscosity, thus forming a bump. The reverse happened in the heating cycle, resulting in a minor peak of the bread crumb amylogram or in another bump if repeated amylograph cycles were used.

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