Impact of Maltodextrins and Antistaling Enzymes on the Differential Scanning Calorimetry Staling Endotherm of Baked Bread Doughs

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Different concentrations (1.2−3.6%) of maltodextrin preparations with average degrees of polymerization (DP) varying between 4 and 66 reduced the differential scanning calorimetry (DSC) staling endotherm in baked and stored (7 days, 23 °C) bread doughs from 3.4 mJ/mg to values within a 3.0−1.9 mJ/mg range. Commercial enzymes used in industrial practice as antistaling agents for bread also reduced amylopectin retrogradation. This suggested that the maltodextrins used are promising antistaling components and that the staling of bread and amylopectin retrogradation are related phenomena. In addition, the results obtained suggest that starch hydrolysis products resulting from enzymic attack may well be responsible for the antistaling effect induced by antistaling enzymes.

Keywords: *Amylopectin retrogradation; differential scanning calorimetry; maltodextrin*

INTRODUCTION

It has long been recognized that some α-amylases effectively reduce the staling rate of bread (Conn et al., 1950; Dragsdorf and Varriano-Marston, 1980; Martin and Hoseney, 1991), although the way they act is not fully understood.

Dragsdorf and Varriano-Marston (1980) confirmed that the staling of bread (in terms of firming) is more than a mere retrogradation process. In their experiments, the authors used bacterial, barley malt, and fungal α-amylases. Although the breads prepared with bacterial α-amylase showed the highest degree of retrogradation (measured in terms of crystallinity by X-ray diffraction), they also showed the highest softness. The authors postulated a possible role of the retrograded starch crystal structure in bread firming, as the breads supplemented with bacterial α-amylase showed V_h and A crystal structures instead of the more typical V_h and B structures of retrograded starch. In their view, a tighter structure of the A-type crystal would inhibit the transfer of water from the gluten to starch and thus allow for a softer bread.

More recently, Martin and Hoseney (1991) and Martin et al. (1991) proposed a model that maintains that the firming of bread results from cross-links between the gluten matrix and starch granule remnants. Such cross-link formation would be influenced by the presence of maltodextrins. The authors correlated the positive impact of some α-amylases on the retardation of bread staling (measured as bread firming) with the size of the maltodextrins formed, the antifirming effect being related to the presence of maltodextrins of degree of polymerization (DP) 3−9.

Every et al. (1992) reported a correlation between increased levels of maltodextrins especially of DP 3−10 and a decreased firming of bread supplemented with bacterial α-amylase. Reconstitution experiments with starch, gluten, and corn maltodextrins, however, did not support the above results. Indeed, whereas bread firming was unaffected by the addition of maltodextrins of DP 3−10, only a slight increase in firming rate resulted from the addition of maltodextrins of DP 2 to DP ≥ 200 or DP 11 to DP ≥ 200. In their reconstitution experiments, the authors added 1.8−3.6% maltodextrins on fresh crumb weight. However, the authors were not able to deduce from their data whether the maltodextrins directly caused the observed effect on bread firming or if they merely indicated a specific enzymic modification of the starch resulting in a reduced retrogradation.

High-performance anion-exchange chromatography (HPAEC) analyses of the water-soluble extract of aged bread crumb by Akers and Hoseney (1994) confirmed that the functionality of α-amylases in retarding bread firming is related to the size and ratio of the maltodextrins produced.

In an attempt to gain more insight into the role of starch−gluten interactions in bread firming, Morgan et al. (1997) developed a gluten-free starch bread. As these starch breads firmed at the same rate as regular wheat breads, the authors concluded that bread firming essentially can be explained by changes in starch−starch interactions. Solid-state ^{13}C cross-polarization/magic-angle spinning nuclear magnetic resonance (CP/MAS NMR) spectroscopy revealed a correlation between reduced bread firmness and reduced starch retrogradation.

Gerrard et al. (1997) reformulated the model proposed by Martin and Hoseney (1991) and Martin et al. (1991). Indeed, they concluded that the antistaling effect of α-amylases is not due to the production of dextrins but to a modification of the starch, interfering with the starch−gluten interactions. Finally, Min et al. (1998) suggested that maltotriose and maltotetraose produced by amylases such as Novamyl and the amylase from

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Streptomyces albus KSM-35 are responsible for retarding bread retrogradation.

The present work aimed to better understand the relationship between the structure of maltodextrins and their activity in retarding bread firming. To that end, maltodextrins were added to flour. Doughs were baked in sealed DSC pans, and the aging of the baked doughs was evaluated by calorimetric investigation of amylopectin retrogradation. To evaluate any possible relationship between the impact of a component on the staling of bread and the retrogradation of amylopectin as recorded for doughs baked in sealed differential scanning calorimetry (DSC) pans, industrial enzymes known to retard bread staling were added to the doughs and submitted to the described DSC experiment as well.

MATERIALS AND METHODS

Minaret (European wheat, harvest 1996) was conditioned overnight at room temperature to 14.0% moisture. Additional water was added 30 min before milling to reach a final moisture content of 14.5%. A Bühler MLU-202 laboratory roller mill adjusted for bread-making wheats (AACC method 26-31, 1995) was used. The milling yield was 67%.

A farinograph (Brabender, Duisburg, Germany) with a 50 g mixer was used to evaluate the impact of sucrose and maltodextrins on the water absorption of wheat flour. Dough consistency was adjusted to 600 Brabender units (BU). Sucrose and maltodextrins were added at a 1.2, 2.3 or 3.6% level (on flour dry matter) in a 10% solution.

A mixograph (TMCO-National Manufacturing, Lincoln, NE) with a 10 g mixer was used to evaluate the impact of maltodextrins on the mixing time of a wheat flour dough. Samples were run at the 600 BU water absorption level determined by farinography. Maltodextrins were added as described above.

Maltodextrins. Paselli MD6 (Pas, potato starch-derived maltodextrin) and Star-Dri 5 (Star, waxy maize starch-derived maltodextrin) were from Avebe (Antwerpen, Belgium) and Amylum (Aalst, Belgium), respectively. The maltodextrins had a DE (dextrose equivalent) of 6 and 5, respectively, as stated in the technical product information sheets. The ratios of (1→6)-α-linkages to (1→4)-α-linkages were 0.03 and 0.056, respectively (Defloor et al., 1998). Fractionation by ethanol precipitation (Defloor et al., 1998) of 300 g of maltodextrins in 1500 mL of water yielded precipitates (P) at 50 and 75% ethanol and a supernatant (SN) at 75% ethanol (P/50/Pas, P/75/Pas, SN/Pas, P/50/Star, P/75/Star, and SN/Star for Paselli MD6 and Star-Dri 5, respectively.) These fractions were of different size, as evidenced by their gel permeation profiles and their degrees of polymerization (Defloor et al., 1998) given in Table 1.

Maltodextrins were added to the recipe as solutions in water (10.0 g/100 mL).

Enzymes. Novamyl 1500 MG (Novo Nordisk, Bagsvaerd, Denmark) is a bacterial maltogenic amylase used to extend the shelf life of baked goods and is believed to have no effect on loaf volume.

Veron ANTI (Röhm Enzyme GmbH, Darmstadt, Germany) and Biobake 2000 and Biobake Fresh (both from Quest International, Naarden, The Netherlands) are fungal amylases containing bacterial amylase. These enzyme preparations improve the freshness of baked goods and increase loaf volume. Their amylase activities were determined by the amylazyme method (McCleary and Sheehan, 1989), and when expressed as the absorbance of the sample filtrate at 590 nm against a control filtrate, whereby the sample filtrate represents a 1.0 mL aliquot containing enzyme extract from 0.02 mg of enzyme, they were 0.026, 0.049, 0.422, and 0.068, respectively.

All enzymes were added to the flour at the maximum recommended dosage as stated in the respective technical product information sheets (i.e. 500, 50, 300, and 100 ppm on flour basis). The added activities (i.e. the product of the maximum recommended dosage per 10.0 g of flour and the amylase activity) of this were therefore 6.5, 1.2, 63.3, and 3.4, respectively.

Table 1. Degrees of Polymerization of Low Molecular Weight Carbohydrates and Enthalpy Values of the Staling Endotherm of Doughs Prepared with Minaret Flour with and without 1.2, 2.3, or 3.6% Sucrose or Maltodextrin with Doughs "Baked" in Sealed DSC Aluminum Pans in an Oven at 105 °C for 30 min and Pans Stored at 23 °C for 7 Days

carbohydrate	DP[a]	addition[b]	enthalpy[c]			av
none		none	3.4	3.5	3.4	3.4
sucrose	2	1.2	3.4	3.7	d	3.5
		2.3	3.2	3.3	d	3.2
		3.6	3.2	3.3	d	3.2
Paselli MD6[e]	17	1.2	3.0	2.9	3.0	3.0
		2.3	2.6	2.9	2.9	2.8
		3.6	2.5	2.3	2.5	2.4
P/50/300/Pas	66	1.2	2.2	2.0	2.3	2.2
		2.3	1.9	1.7	2.0	1.9
		3.6	1.8	1.7	2.3	1.9
P/75/300/Pas	17	1.2	3.0	3.0	3.1	3.0
		2.3	2.7	3.1	3.1	3.0
		3.6	2.8	2.9	3.1	2.9
SN/Pas	5	1.2	2.3	2.4	2.3	2.3
		2.3	2.0	2.2	2.1	2.1
		3.6	2.4	2.2	2.4	2.3
Star-Dri 5[e]	20	1.2	2.2	2.3	2.5	2.3
		2.3	2.5	2.4	2.4	2.4
		3.6	2.5	2.3	2.6	2.5
P/50/300/Star	61	1.2	2.2	2.1	2.4	2.2
		2.3	2.1	2.6	2.2	2.3
		3.6	1.9	1.9	2.1	2.0
P/75/300/Star	19	1.2	2.1	2.2	2.2	2.2
		2.3	2.0	2.0	2.0	2.0
		3.6	2.0	1.8	1.9	1.9
SN/300/Star	4	1.2	2.3	2.0	2.3	2.2
		2.3	2.1	2.3	2.3	2.2
		3.6	2.3	2.3	2.2	2.3

[a] DP: degree of polymerization. [b] % on flour dry matter. [c] In mJ/mg, measurement at least in duplicate. [d] Measurement only in duplicate. [e] The enthalpy values obtained for doughs supplemented with commercial maltodextrins or with their fractions proved significantly different from the values for the control doughs, as determined by a two-sided *t*-test ($P < 0.05$).

Gelatinization Properties of Doughs. Doughs were prepared with and without sucrose or maltodextrin (1.2, 2.3, or 3.6% on flour dry matter, in a 10% solution) or with and without enzyme, in a 10 g mixer (TMCO-National Manufacturing). Water absorption was determined with the farinograph as outlined above, while mixing time was determined at the farinograph water absorption level with the mixograph (see above).

DSC (Seiko DSC 120, Kawasaki Kanagawa, Japan) was used to investigate the gelatinization properties. Indium and tin were used as standards (Defloor et al., 1993). Dough samples (16−25 mg) were accurately weighed in aluminum pans (Seiko, P/N 50-023) and heated in the DSC at 4 °C/min from 5 to 150 °C. An empty pan was used as reference.

"Baked Dough" Aging. The effects of maltodextrin solutions or enzymes on the aging of "baked dough" were quantified by DSC evaluation of amylopectin retrogradation. The progress of amylopectin retrogradation can indeed be evaluated by this technique (Eliasson, 1985).

Dough samples were weighed in four DSC aluminum pans as described above. The sealed pans were placed in an oven at 105 °C for 30 min to bake. For the enzyme supplemented doughs, this baking was preceded by a 30 min incubation at 70 °C. After being cooled to room-temperature, one aluminum pan was scanned by DSC to confirm that the starch had been gelatinized. Three aluminum pans were stored at 23 °C for 7 days. Amylopectin retrogradation was evaluated by DSC (4 °C/min, 5 to 150 °C).

The results obtained were analyzed using a two-sided *t*-test (Wonnacott and Wonnacott, 1977).

RESULTS AND DISCUSSION

Impact of Maltodextrins on Dough Rheology. The 600 BU farinograph water absorption of Minaret wheat flour was 50.9% (on 14.0% moisture basis). Addition of maltodextrins (1.2, 2.3, or 3.6% on flour dry matter) changed the water absorption by no more than 3.0%.

Minaret flour had a mixing time of 110 s as determined by mixography at the optimal water absorption level (determined by farinography). Addition of maltodextrins as described above had very little influence on the dough mixing time.

Gelatinization Properties of Doughs. Thermograms of the doughs were not affected by the addition of the maltodextrins. Three endothermic peaks were registered, with onset, peak, and conclusion temperatures of about 51, 65, 75, about 65, 90, 115, and 105, 115, and 126 °C, respectively. The first peaks with peak temperatures of 65 and 90 °C are attributed to the gelatinization of starch in low water systems (Donovan, 1979; Eliasson, 1980; Evans and Haisman, 1982; Biliaderis, 1990) and have previously been observed for wheat doughs (Defloor et al., 1993). The third endothermic peak is attributed to the melting of amylose−lipid complexes (Kugimiya et al., 1980).

"Baked Dough" Aging. After 7 days of storage, the samples aged "baked doughs" with and without sucrose or maltodextrin showed onset and conclusion temperatures of the staling endotherm at about 41 and 72 °C, respectively. Enthalpy values of this staling endotherm are listed in Table 1. None of the sucrose additions had a statistically significant impact on the amylopectin retrogradation. Sugars have indeed been reported (Slade and Levine, 1991) to only noticeably alter the mobility of aqueous solutions at concentrations exceeding 30% and to only have an impact on starch retrogradation at high concentrations (Eerlingen et al., 1994).

The addition of 1.2, 2.3, or 3.6% maltodextrins (on a flour dry matter basis) reduced the staling endotherm enthalpy value. Indeed, a two-sided *t*-test ($P < 0.05$) showed that the enthalpy values obtained for doughs supplemented with commercial maltodextrins or with their fractions proved significantly different from the values for the control doughs. For the Star-Dri 5 sample, the effect at low levels was more pronounced than for the Paselli MD6. An addition of the fractions of Star-Dri 5 resulted in a similar or even larger reduction of the enthalpy value. The P/75/Pas was less effective in reducing the staling endotherm even at 3.6% addition. Results in Table 1 suggest that this fraction contained the population making Paselli MD6 less effective than Star-Dri 5. Indeed, the P/50/Pas, as well as the SN/Pas fraction, caused an effect similar to that of the corresponding Star-Dri 5 fractions.

It is of note that the P/75/Pas needed to be heated to about 85 °C to obtain a clear solution and showed retrogradation after about 3 h at room temperature. This retrogradation explained the limited effect of the fraction, as illustrated in Table 1. Although the P/50/Pas was also insoluble at room temperature, the solution was stable for several hours.

It is unfortunate that no straightforward relationship between the DP values of the fractionated dextrins and their ability to reduce the staling endotherm was observed because it would have made speculations about the exact mechanism of their antistaling effect possible.

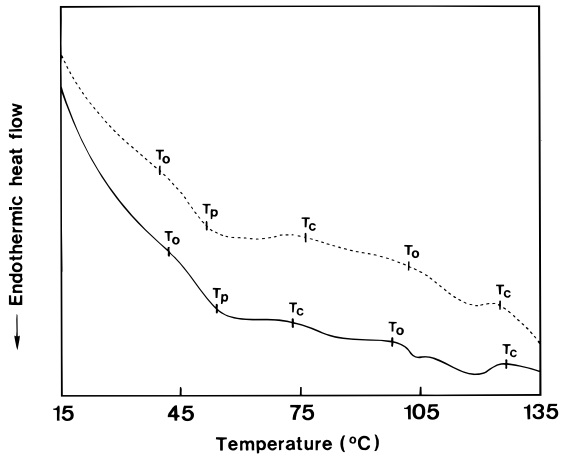

Figure 1. DSC thermograms of aged "breads" prepared with (full line) and without (dotted line) 3% Star-Dri 5 and stored for 7 days at 23 °C. Onset (T_o), peak (T_p), and conclusion (T_c) temperatures are indicated.

To ensure that the reduced retrogradation after storage could not be attributed to the reduced farinograph water absorption of the maltodextrin-supplemented doughs, the aging of a "baked dough" supplemented with 3.6% Star-Dri 5 was evaluated at the same water absorption (i.e. 50.9%) as that of the control dough. Although the addition of more water increased the enthalpy of the retrogradation endotherm by 27%, the reduction of the amylopectin retrogradation enthalpy was still clearly observed.

Krüsi and Neukom (1984) reported that glucose oligosaccharides of DP 3−8 inhibit starch recrystallization in a 50% wheat starch gel. The authors related the antistaling effect to the fact that the oligosaccharides would slow the starch gelatinization and/or that they would interfere with starch polymer interactions. DSC thermograms of doughs with and without maltodextrins, however, did not reveal significant differences in gelatinization properties. Also, the absence of a gelatinization endotherm immediately after "baking" of the doughs suggested that all starch had been gelatinized.

The antistaling effect of glucose oligosaccharides of DP 3−8 at relatively high sugar concentrations (1:1 sugar/water) has been attributed (Slade and Levine, 1987; Levine and Slade, 1990) to their impact on T_g (glass transition temperature), resulting in a smaller ΔT above T_g, eventually retarding the starch recrystallization process.

While glucose oligosaccharides of DP 3−8 were identified in Paselli MD6 and Star-Dri 5, as well as in their respective fractions (Defloor et al., 1998), their natural abundance in a normal bread-making recipe is probably too low to account for antistaling effects (Table 1).

Thermograms of the aged "baked doughs" are shown in Figure 1. The first peak was identified as the "staling endotherm" (Eliasson, 1985). It is not clear, however, whether another thermal transition occurs between ca. 74 and 100 °C. Finally, the transition occurring above 100 °C has been attributed to the melting of amylose−lipid complexes (Kugimiya et al., 1980). The double peaks noticed at temperatures above 100 °C (Figure 1) were attributed to the occurrence of different polymorphs of amylose−lipid complexes, as reported earlier

Table 2. Enthalpy Values of the Staling Endotherm of Doughs Prepared with Minaret Flour with and without Star-Dri 5 or Commercial Antistaling Enzymes with Doughs "Baked" in Sealed DSC Aluminum Pans in an Oven at 105 °C for 30 min and Pans Stored at 23 °C for 7 Days

addition	enthalpy[a]				av
none	2.3	2.3	2.2	2.4	2.3
Star-Dri 5 (3.6%)[b]	1.5	1.6	1.5	1.5	1.5
Novamyl[b]	1.4	1.3	1.5	1.4	
Veron ANTI[b]	2.1	2.2	2.1	2.1	
Biobake 2000[b]	1.6	1.6	1.5	1.6	1.6
Biobake Fresh[b]	1.9	1.6	1.6	1.8	1.7

[a] In mJ/mg, measurement at least in triplicate. [b] The enthalpy values obtained for doughs with commercial enzymes proved significantly different from the values for the control doughs, as determined by a two-sided *t*-test ($P < 0.05$).

(Raphaelides and Karakalas, 1988; Biliaderis and Galloway, 1989; Seneviratne and Biliaderis, 1991).

Maltodextrins reduced starch recrystallization (Table 1). To evaluate the possible impact of maltodextrins on the staling of "real" bread, enzymes known in the baking industry for their antistaling effects were used in the DSC experiment. Enthalpy values of the staling endotherm (amylopectin retrogradation) are listed in Table 2. Dough with 3.6% addition of Star-Dri 5 was included for comparison.

Linear regression analysis (omitting Biobake 2000 and including the control dough) of the effect of the amylase activity of the added dosage on the enthalpy value of the staling endotherm of the "baked" doughs (Table 2) showed an R^2 value of 0.98.

As was the case with maltodextrin addition, all added enzymes induced a significant (5% probability level) reduction of the staling endotherm, in comparison with the control (Table 2). However, these results do not prove that there is a causal relationship between the impact of a component on the staling of bread and the retrogradation of amylopectin as recorded for doughs baked in sealed DSC pans. They nevertheless suggest that staling of bread and amylopectin retrogradation are related, as also stated recently by Morgan et al. (1997). Although the effectiveness of maltodextrins in a practical environment needs to be studied, the above results suggest that the DSC experiment described can be used as a valuable screening method to evaluate the antistaling potential of components. Table 1 clearly shows that the addition of maltodextrins caused an important reduction of the staling endotherm. Consequently, maltodextrins are promising components with regard to antistaling of baked products. This, however, is inconsistent with the conclusions of Gerrard et al. (1997) that the antistaling effect of α-amylase is not due to the presence of dextrins but to a modification of the starch.

One final remark concerns the above-described DSC experimental approach. It has the advantage that the retrogradation enthalpy can be measured for a small and therefore homogeneously baked sample. Apart from this, the slightly higher water level in both the control and the experimental samples than in a real bread making system, where some water evaporation occurs, leads to higher retrogradation enthalpy values and therefore increases the sensitivity of the method.

ABBREVIATIONS USED

DP, degree of polymerization; DSC, differential scanning calorimetry; HPAEC, high-performance anion-exchange chromatography; CP/MAS NMR, cross-polarization/magic-angle spinning nuclear magnetic resonance; BU, Brabender units; Pas, Paselli MD6; Star, Star-Dri 5; DE, dextrose equivalent; P/50/, precipitate at 50% ethanol concentration; P/75/, precipitate at 75% ethanol concentration; SN/, supernatant; T_g, glass transition temperature.

LITERATURE CITED

Akers, A. A.; Hoseney, R. C. Water-soluble dextrins from α-amylase-treated bread and their relationship to bread firming. *Cereal Chem.* **1994**, *71*, 1, 223−226.

American Association of Cereal Chemists. *Approved Methods of the AACC*, 9th ed: AACC: St. Paul, MN, 1995; Method 26-31, approved Oct 1994.

Biliaderis, C. G.; Galloway, G. Crystallization behavior of amylose-V complexes: structure-property relationships. *Carbohydr. Res.* **1989**, *189*, 31−48.

Biliaderis, C. G. Thermal analysis of food carbohydrates. In *Thermal Analysis of Foods*; Harwalkar, V. R., Ma, C. Y., Eds.; Elsevier Applied Science: London, 1990; pp 168−220.

Conn, J. F.; Johnson, J. A.; Miller, B. S. An investigation of commercial fungal and bacterial α-amylase preparations in baking. *Cereal Chem.* **1950**, *27*, 191−205.

Defloor, I.; Nys, M.; Delcour, J. A. Wheat starch, cassava starch, and cassava flour impairment of the breadmaking potential of wheat flour. *Cereal Chem.* **1993**, *70*, 526−530.

Defloor, I.; Vandenreyken, V.; Grobet, P. J.; Delcour, J. A. Fractionation of maltodextrins by ethanol. *J. Chromatogr. A* **1998**, *803*, 103−109.

Donovan, J. W. Phase transitions of the starch-water system. *Biopolymers* **1979**, *18*, 263−275.

Dragsdorf, R. D., Varriano-Marston, E. Bread staling: X-ray diffraction studies on bread supplemented with α-amylases from different sources. *Cereal Chem.* **1980**, *57*, 310−314.

Eerlingen, R. C.; Van Den Broeck, I.; Delcour, J. A.; Slade, L.; Levine, H. Enzyme-resistant starch. VI. Influence of sugars on resistant starch formation. *Cereal Chem.* **1994**, *71*, 472−476.

Eliasson, A. C. Effect of water content on the gelatinisation of wheat starch. *Starch* **1980**, *32*, 270−272.

Eliasson, A. C. Retrogradation of starch as measured by differential scanning calorimetry. In *New Approaches to Research on Cereal Carbohydrates*; Hill, R. D., Munck, M., Eds.; Elsevier Science Publishers: Amsterdam, 1985; pp 93−98.

Evans, I. D.; Haisman, D. R. The effect of solutes on the gelatinisation temperature range of potato starch. *Starch* **1982**, *34*, 224−231.

Every, D.; Mann, J. D.; Ross, M. The relationship of dextrins in bread to staling and crumb gumminess. In *Proceedings of the forty-second Australian cereal chemistry conference;* Humphrey-Taylor, V. J., Ed.; Cereal Chemistry Division, Royal Australian Chemical Institute: Sydney, 1992.

Gerrard, J. A.; Every, D.; Sutton, K. H.; Gilpin, M. J. The role of maltodextrins in the staling of bread. *J. Cereal Sci.* **1997**, *26*, 201−209.

Kugimiya, M.; Donovan, J. W.; Wong, R. Y. Phase transitions of amylose-lipid complexes in starches: a calorimetric study. *Starch* **1980**, *32*, 265−270.

Krüsi, H.; Neukom, H. Untersuchungen über die Retrogradation der Stärke in konzentrierten Weizenstärkegelen (Research on retrogradation in concentrated wheat starch gels). *Starch* **1984**, *36*, 300−305.

Levine, H.; Slade, L. Influences of the glassy and rubbery states on the thermal, mechanical, and structural properties of doughs and baked products. In *Dough Rheology and Baked Product Texture*; Faridi, H., Faubion, J. M., Eds.; Van Nostrand Reinhold: New York, 1990; pp 157−330.

Martin, M. L.; Hoseney, R. C. A mechanism of bread firming. II. Role of starch hydrolyzing enzymes. *Cereal Chem.* **1991**, *68*, 503−507.

Martin, M. L.; Zeleznak, K. J.; Hoseney, R. C. A mechanism of bread firming. I. Role of starch swelling. *Cereal Chem*. **1991**, *68*, 498−503.

McCleary, B. V.; Sheehan, H. Measurement of cereal alpha-amylase: a new assay procedure. *J. Cereal Sci.* **1989**, *6*, 237−251.

Min, B.-C.; Yoon, S.-H.; Kim, J.-W.; Lee, Y.-W.; Kim, Y.-B.; Park, H. A. Cloning of novel maltooligosaccharide-producing amylases as antistaling agents for bread. *J. Agric. Food Chem*. **1998**, *46*, 779−782.

Morgan, K. R.; Gerrard, J.; Every, D.; Ross, M.; Gilpin, M. Staling in starch breads: the effect of antistaling α-amylase. *Starch* **1997**, *49*, S. 54−59.

Raphaelides, S.; Karakalas, J. Thermal dissociation of amylose-fatty acid complexes. *Carbohydr. Res.* **1988**, *172*, 65−82.

Seneviratne, H. D.; Biliaderis, C. G. Action of α-amylases on amylose-lipid complex superstructures. *J. Cereal Sci.* **1991**, *13*, 129−143.

Slade, L.; Levine, H. Recent advances in starch retrogradation. In *Industrial Polysaccharides-The Impact of Biotechnology and Advanced Methodologies*; Stivala, S. S., Crescenzi, V., Dea, I. C. M., Eds.; Gordon and Breach: New York, 1987; pp 387−430.

Slade, L.; Levine, H. Beyond water activity: recent advances on an alternative approach to the assessment of food quality and safety. *Crit. Rev. Food Sci. Nutr.* **1991**, *30*, 115−360.

Wonnacott, T. H.; Wonnacott, R. J. *Introductory Statistics*; Wiley and Sons: New York, 1977.

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