Differential Scanning Calorimetry and Wide-Angle X-ray Scattering Studies of Bread Staling

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ABSTRACT: Wide angle X-ray scattering (WAXS) was used to follow the development of crystal size and strain during the staling of bread containing different additives. In this it was observed that one can classify the good anti-staling additives as gelatin, propylene glycol, maltodextrin and anti-staling enzyme corresponding to the order of increasing crystal size for a particular Bragg reflection in all the samples and correlate using DSC (differential scanning calorimetry) studies of all the bread compositions containing different additives. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci **67:** 1597–1603, 1998

Key words: retrogradation; staling; X-ray diffraction; enthalpy change; crystal size

INTRODUCTION

Bread staling is due to starch retrogradation comprising of the following two processes: a time dependent retrogradation of amylose and time independent retrogradation of amylopectin.[1] The formation of gel structure due to retrogradation is linked to the development of crystallites, fringed miscelles.[2] The crystallites formation due to retrogradation of starch has been studied by both wide angle X-ray scattering (WAXS) and differential scanning calorimetry (DSC).[3–4] The formation of crystallites is considered to be the interchain association of the amylose and amylopectin fraction.[2] The amount of three-dimensional network formation between the amorphous (gluten) and crystallites (starch) in bread increases with time during staling.

X-ray diffraction (XRD) and DSC studies on quenched amylomaize indicate that retrogradation includes B-type crystallites with a slightly enlarged crystal lattice.[5–6] Kitamura et al.[7] used complementary X-ray data to show that the DSC thermogram at 110–120°C was caused by the melting of crystalline v-type amylose lipid complexes. Enthalpy change ΔH in DSC studies is a measure of rate of staling.[8]

Based on one-dimensional paracrystalline model of Hindeleh and Hosemann[9] and Vainshtein,[10] Somashekar and Somashekarappa[11] have developed a method by which one can uniquely determine parameters such as crystal size and strain from a given Bragg reflection. Employing this approach, we have determined these parameters and, hence, have characterized the antistaling additives like glycerol, propylene glycol, polypropylene glycol, maltodextrin, gelatin, and a commercially available anti-staling enzyme of bread in correlation with DSC enthalpy data.

MATERIALS AND METHODS

The hard wheat (Maida) flour used in the present study was obtained commercially with protein

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and moisture contents of 12.8 and 14.0%, respectively. Glycerol (GL), propylene glycol (PG), polypropylene glycol (PPG; M wt 6000), and gelatin (GE) was obtained from S. D. Fine Chemicals, Bombay. Maltodextrin (MD, dextrose equivalent 16) was procured from S. A. Chemicals, Bombay. Novamyl 1500 mg anti-staling enzyme (NE) was provided by Biochemical Industries, Bangalore. All other chemicals used were of bakery grade. GL and PG are food additives generally regarded as safe (GRAS),[12] while MD and GE are commonly used food additives.[12] PPG is used as polymeric cryoprotectents and cryostabilizers in frozen bakery products.[2] NE is a bread additive approved by WHO.[13]

Additives such as GL, PG, PPG, GE, ME, and NE were mechanically blended (dry blending) with wheat flour for 30 min, stored in polypropylene pouches, and labelled as modified flour (2–7). The modified flour was stored in walk-in cooler at −4°C until one day before use. Six and one-half per one hundred parts of flour (PPF) additives was used except NE, since this amount of glycerol was used in the meals, ready to eat (MRE; bread is a long shelf life bread, which is a speciality military ration conforming to U.S. Army specification #B-44360) bread.[14] Sixty milligrams of NE was used, as recommended by the manufacturer. The composition of dough making was 100 g of wheat/modified wheat, 3% of instant yeast, 2% salt, 3% sugar, 3% margarine, and 60% of water. Care was taken to maintain the same amount of water in all the seven compositions of dough. Doughs were prepared in triplicate. Twenty-one doughs were prepared as per the procedure described by Piazzal and Massi.[15] The dough was molded and pan proofed at 32°C at 80% RH for 45 minutes. Baking was done as per the procedure laid down in AACC method 10-09 (AACC-83). Immediately after baking, each loaf was weighed, allowed to cool for 2 h, and packed in fungistatic paper at room temperature. Slices were cut to a thickness of 25 mm.

A duPont 910 differential scanning calorimeter fitted with graphic plotter and with a thermal analyst 2100 system (TA instruments, U.S.A.) was used and the adopted procedure was as reported earlier.[16] Fungistatic paper was prepared in the laboratory by the method described by Ghosh et al.[17]

An X-ray Stoe diffractometer with curved position sensitive detector was used to collect X-ray powder data from these bread samples treated with anti-staling additives, and the Bragg reflections with the d-spacings and the diffractograms are reproduced in Figure 1. Settings of the diffractometer are as follows: 25 mA, 35 kV, 300 s exposure time. Data were collected in steps of 0.03°. The output pattern was corrected for (1) the Lorentz polarization factor and (2) instrumental broadening. It is evident from Table I that a comparison of d-spacings with the reported B-type pattern produced by amylomaize starches, which have greater amylose content, suggests that X-ray patterns are of B-type pattern. It is evident from the Figure 1 that the Bragg reflection at $2\theta = 23°$ shows some significant changes with different additives. We have carried out the profile analysis of this reflection using the method of Somashekar and Somashekarappa,[11] which is based on Hosemann's paracrystalline model. It follows that the intensity from a finite paracrystal is

$$I(s) = I_{(N-1)} + I'_N(s) \tag{1}$$

where $I'_N(s)$ is the modified intensity for the probability peak centered at D. The expression for $I'_N(s)$ is

$$I'_N(s) = \frac{2a_N}{D\pi^{1/2}} \exp(idNs) \times [1 - a_N s\{2\mathcal{D}(a_N s) + i(\pi)^{1/2}\exp(-a_N^2 s^2)\}] \tag{2}$$

where $a_N^2 = Nw^2/2$ and $\mathcal{D}(a_N s)$ is Dawson's integral or the error function with purely complex argument and can be easily computed. Using eqs. (1) and (2), one can generate intensity profiles consisting of three to four orders of reflections using the one-dimensional paracrystalline model, which is outlined.[11] The experimental profile between so and $s_o + s_o/2$ (or s_o and $s_o + B/2d$, if there is truncation of the profile $B < 1$) is matched with the corresponding simulated order of reflection between s_o and $s_o + s_o/2$ (or s_o and $s_o + B/2d$) using one-dimensional paracrystalline model for various values of N and g to minimize the difference between calculated and experimental normalized intensity values. For minimizing, we have used a SIMPLEX, a multidimensional algorithm.[18] Using this procedure, the values of N and g, and the crystal size and lattice strain obtained for the X-ray reflection at $2\theta = 23°$ of bread samples treated with various anti-staling additives are given in Table I. For a meaningful comparison, we have also estimated $\alpha^*(= N^{1/2}g)$, which

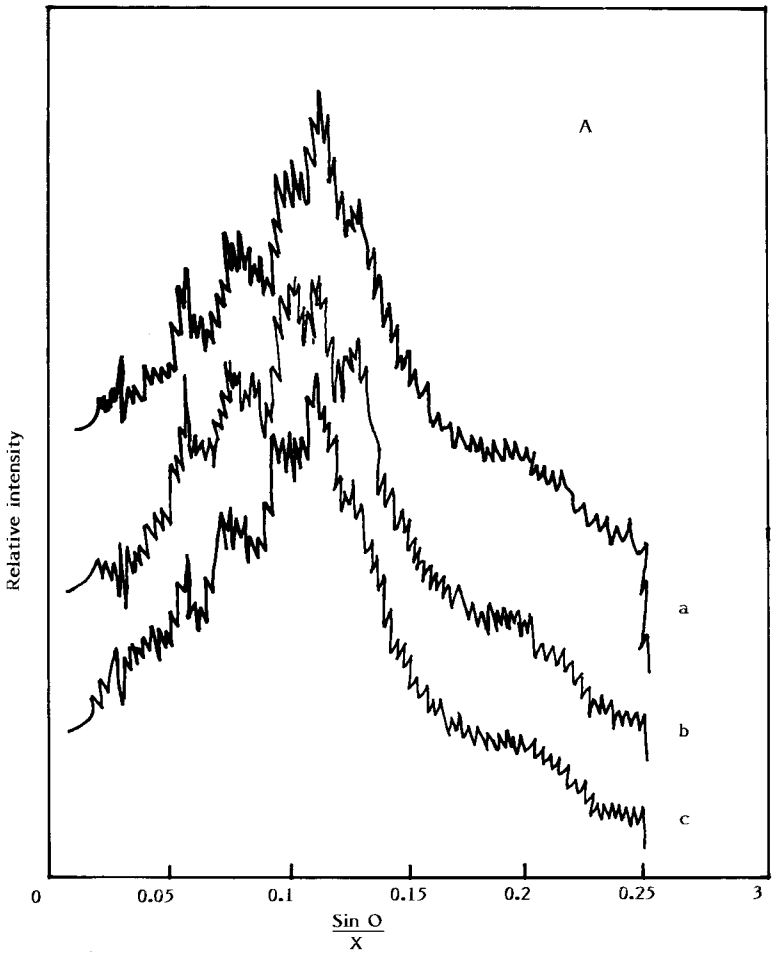

Figure 1 (A) X-ray diffraction pattern of a bread crumb stored for 15 days in (a) glycerol, (b) propylene glycol (c), and polypropylene glycol. (B) X-ray diffraction pattern of a bread crumb stored for 15 days in (d) maltodextrine, (e) gelatin, and (f) antistaling enzyme.

physically determines the equilibrium of the phase,[19] it is given in Table I.

RESULTS AND DISCUSSION

Figure 2(A) shows the thermograms of bread crumbs stored for 2 days. An endotherm peak at 110°C is observed in all the bread compositions, which may be due to the retrogradation of amylose fraction; this takes place once the bread comes out of oven and is cooled to room temperature. This endotherm peak at near about 110°C persists in all the stored bread crumbs (Fig. 2). In all the bread composition, the transition temperature, due to retrogradation of amylopectin, occurs at 60 to 65°C, except glycerol-containing bread. The small endotherm peak at 35°C is due to lipid melting, and probably the endotherm due to glycerol and lipid melting merges with each other and showed a broad peak at 51°C. Bread containing propylene glycol gives the least ΔH value followed by glycerol, maltodextrin, antistaling enzyme, gelatin, polypropylene glycol, and control bread in the order of increasing ΔH.

This result is substantiated by X-ray studies. The enthalpy (α^*), which determines the equilibrium of the phase, is almost same and lower for the additives like propylene glycol, maltodextrin, gelatin and anti-staling enzyme, whereas for additives like glycerol and polypropylene glycol, it is significantly higher (Table I). The crystal size is also more in these additives, indicating that there will not be effective retrogradation of the

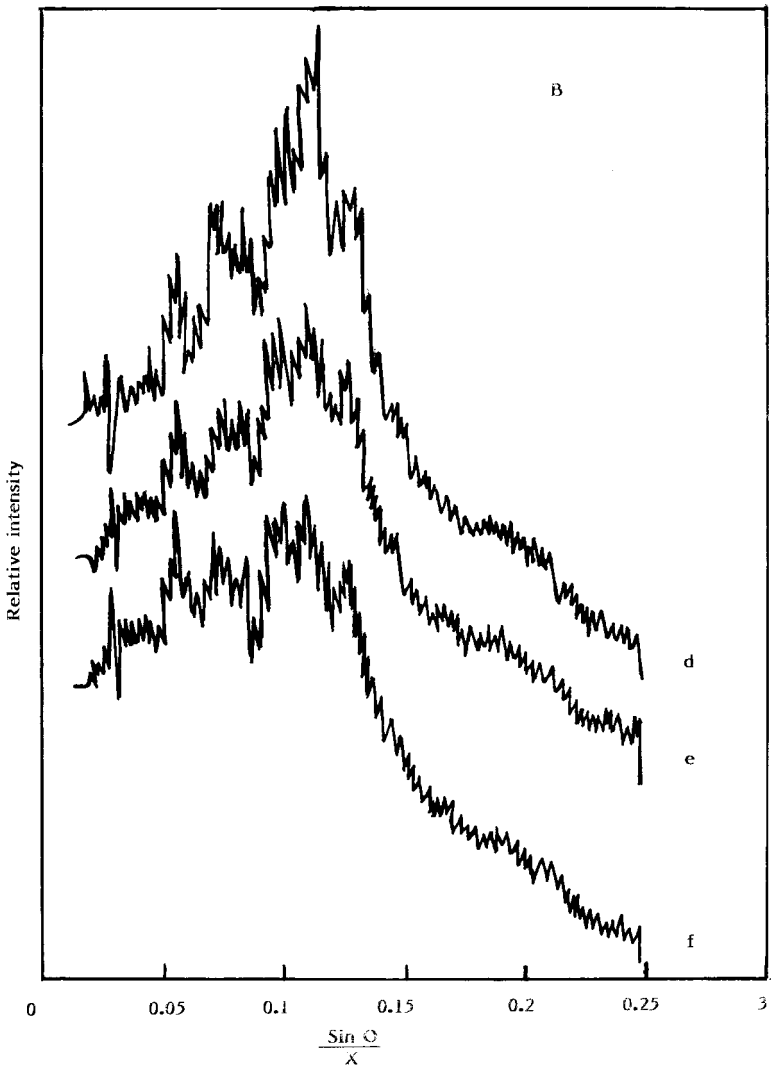

Figure 1 (*Continued from the previous page*)

staling process. The variations in crystal size distributions[11] for different additives are also given in Table II; and it is evident that crystal size distribution is quite small in case of additives like propylene glycol, maltodextrin, gelatin, and antistaling enzyme. Also, the ratio of D_{vol} to D_{surf} computed using the crystal size distributions lies within 2, which is the order usually observed for a polymer. X-ray studies show that the additives propylene glycol, maltodextrin, gelatin, and anti-

Table I Microstructural Parameters of Bread with Different Additives

Bread With	N	g (%)	p	D_{surf} (°A)	D_{vol} (°A)	Ratio	α*
Glycerol	7.53 ± 0.07	1.33 ± 0.01	0.167	19.89	31.26	1.57	0.037
Propylene glycol	3.18 ± 0.03	1.56 ± 0.02	0.448	9.91	13.39	1.35	0.028
Polypropylene glycol	5.74 ± 0.06	1.46 ± 0.02	0.260	14.95	23.60	1.58	0.035
Maltodextrin	3.43 ± 0.03	1.53 ± 0.02	0.743	10.79	14.21	1.32	0.028
Gelatin	3.03 ± 0.03	1.57 ± 0.02	0.512	9.52	12.56	1.32	0.027
Antistaling enzyme	3.08 ± 0.03	1.60 ± 0.02	0.532	9.72	12.84	1.32	0.028

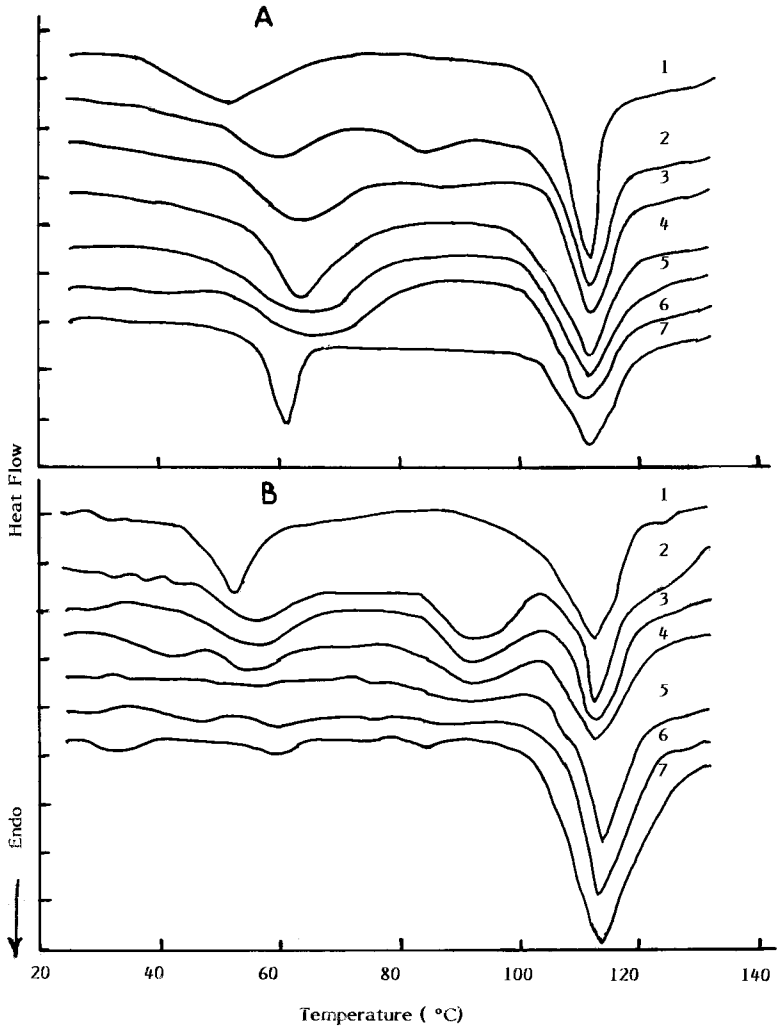

Figure 2 DSC thermogram for stored bread for (A) two days and for (B) 15 days containing the following different antistaling additives; (1) glycerol, (2) propylene glycol, (3) maltodextrine, (4) antistaling enzyme, (5) gelatin, (6) polypropylene glycol, and (7) control.

staling enzyme are on the same footing in terms of size and strain. Further classification can only be done by looking at the thermograms wherein it is clearly shown that propylene glycol has the lowest enthalpy; Hence, we can conclude that it is the best anti-staling additive.

Effect of Monomers

Monomers like glycerol and propylene glycol have a plasticizing effect on starch/gluten biopolymers. Plasticizers generally lower the amount of cross-links in retrograded starch molecules. In other words, low-molecular-weight polyols retard the rate of retrogradation of starch. The ΔH value of bread containing glycerol is higher than that of propylene glycol, which gives the lowest ΔH values compared to all other bread composition containing different additives. Slade and Lavine[2] have reported that the anomaly in the properties of glycerol and polyol in the food system is due to its vast differences in the T_g/T_m value. A progressive decrease in this value results in a decrease in local viscosity and increases in translational diffusion rate. In other words, the miscibility between the polyol and biopolymer increases. Glycerol has T_g/T_m value 1.69 and that of propylene glycol has a value of 1.25. This may be the reason for low ΔH value of PG-containing bread. After 15 days of storage of bread containing propylene

Table II Effect of Additives in Bread on Thermal Transition Temperature and Enthalpy Associated with Staling

	Thermal Condition[a]			
	Two Days		15 Days	
Bread With Additive	T_m (°C)	ΔH (J/ g)	T_m (°C)	ΔH T/ g)
Control	60.1 ± 1.5	18.5 ± 0.6	110.6 ± 2.9	28.3 ± 0.8
Glycerol	51.3 ± 1.3	10.1 ± 0.4	55.6 ± 1.4	16.9 ± 0.6
Propylene glycol	62.4 ± 1.6	5.8 ± 0.3	85.3 ± 2.5	12.4 ± 0.4
Polypropylene glycol	63.8 ± 1.6	14.3 ± 0.5	105.7 ± 2.9	23.5 ± 0.7
Maltodextrin	63.1 ± 1.6	11.2 ± 0.4	95.9 ± 2.6	18.9 ± 0.6
Gelatin	63.0 ± 1.7	12.8 ± 0.4	102.1 ± 2.9	23.6 ± 0.7
Antistaling enzyme	63.5 ± 1.7	11.8 ± 0.4	95.8 ± 2.7	19.6 ± 0.6

[a] Mean ± SD of 3 determination of enthalpy change and transition temperature.

glycol and glycerol, the ΔH value increases, corresponding with an increase in transition temperature.

Effect of Polymers

When two polymers are blended by whatever method, the most likely result is incompatibility. The reason why two polymers are not usually miscible becomes apparent from a simple thermodynamic consideration.[20] However, two polymers can be made compatible by mechanical and chemical means. In our study, the ΔH value of stored bread containing maltodextrin (DE 16), gelatin, and polypropylene glycol is 11.2, 11.8, and 14.1, respectively. Maltodextrin gives a lower ΔH value because it is soluble in water and compatible with a starch/gluten biopolymer. Gelatin, which is a protein, gives a slightly higher value compared to maltodextrin, probably, its compatibility with gluten, a wheat protein, gives more amorphous elastic structure to the bread crumb. Polypropylene glycol is a synthetic polymer that is not soluble in water and probably less compatible with starch/gluten system and, therefore, gives highest value. After 15 days of storage of bread, bread containing MD, GE, and PPG gives a correspondingly higher ΔH value; and the transition temperature at 103–105°C almost merges with that of amylose retrogradation at 110°C (Fig. 2).

Effect of Antistaling Enzyme

Novamyl is a purified maltogenic amylose produced by a genetically modified bacterial amylase. It is reported[21] that the enzyme works on the starch fraction of flour, modifying the starch in such a way that retrogradation is less likely to occur and by creating low-molecular-weight sugar and dextrins, which improve the water retention capacity of the baked goods (enzyme is active in dough but will be inactivated during baking). The ΔH value and thermogram of bread containing the enzyme is almost similar in magnitude and pattern to that of bread containing maltodextrin (Fig. 2 and Table II). The stored bread containing NE after 15 days gives similar results to that of maltodextrin.

CONCLUSION

Crystal size, strain, and entahlpy computations from X-ray studies indicate that the additives of propylene glycol, maltodextrin, gelatin, and commercially available anti-staling enzymes are the most available anti-staling additives. Further DSC study highlights that among these, propylene glycol has less transition enthalpy, emphasizing that propylene glycol is the best anti-staling agent among these additives.

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