

# Enzyme-Resistant Starch in Yellow Layer Cake

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## ABSTRACT

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Enzyme-resistant starch (RS) from amylo maize VII starch replaced up to 50% of the total shortening (40 parts per 100 parts of flour) and up to 15% of the flour in a yellow layer cake. Starch gelatinization and melting of the amylose-lipid complex, as determined by differential scanning calorimetry, were delayed by a high concentration (100 parts per 100 parts of flour) of sugar in the cake batter. The melting temperature for RS was also raised by the high concentration of sugar. The endothermic peak for melting of RS was decreased by the limiting amount of water in the cake system. In cakes in which RS replaced 15% of the flour, no significant effects were recorded on physical characteristics (specific gravity of batter, cake weight and volume, water activity, moisture, and

softness [determined by a compression test]) and scanning electron microscopy pictures. Cake batters containing shortening and RS incorporated similar amounts of air, but the air cell numbers decreased as increasing levels of shortening were replaced by RS. As the level of shortening replacement increased, cake crumb became more compact. A high degree of shortening replacement also reduced the volume and firmed the cake. However, when RS was used at a low replacement level (12.5%), yellow layer cake quality was improved. RS in the cake batter or cake crumb could be detected in samples in which 50% of the shortening was replaced by RS.

The dietary fiber-like property of enzyme-resistant starch (RS) has interested many researchers. High levels of RS can be produced during heat treatment and cooling of high-amylose starch. RS escapes digestion in the small intestine of humans. The formation and physical and chemical characteristics of RS were studied by many research groups (Berry 1986; Bjorck et al 1987; Berry et al 1988; Ring et al 1988; Russell et al 1989; Sievert and Pomeranz 1989, 1990; Czuchajowska et al 1991; Sievert et al 1991; Szczodrak and Pomeranz 1991). Similarly, digestibility of RS has been studied by several researchers (Englyst and MacFarlane 1986, Englyst et al 1987, Ring et al 1988, Wyatt and Horn 1988). The microstructure of RS was described by Sievert and Pomeranz (1989) and Szczodrak and Pomeranz (1991).

Cake is a complex fat and water emulsion system containing flour, starch, sugar, fat, eggs, and baking powder. A proper combination of the ingredients can give a high-quality product with desirable flavor and texture. Shortening plays an important role in baked products. The functions of shortening in cakes were described by several researchers (Carlin 1944, Moncrieff 1970, Howard 1972, Birnbaum 1978). The major component of flour is starch; starch properties were shown to be important in making yellow layer cakes. The functionality of starch in baked goods was reported by Howard et al (1968), Hosney and Atwell (1977), and Hosney et al (1978). Differential scanning calorimetry (DSC) is a powerful tool for following changes in thermal properties during starch gelatinization. RS can give an endothermic transition in the 120–165°C temperature range with a peak around 155°C (Sievert and Pomeranz 1989). Although there are many reports about the functionality and microstructure of layer cake components, few describe the microstructure of the cake crumb. Gordon et al (1979) used scanning electron microscopy (SEM) to study the structure of cakes with different starch levels. Hsu et al (1980) used SEM to compare the crumb structure of cakes with different emulsification systems. Baker et al (1990) pointed out the differences in crumbs of cakes baked by microwave and conventional heating.

A high-fiber, low-fat, and low-caloric food is an important objective in today's food product development. From a physiological standpoint, as a natural dietary fiber, RS is still in the testing and evaluation stage. None of the published reports describe the practical uses of RS in food products. The objective of this study was to determine the effect of RS on the quality of yellow layer cake and the suitability of using RS to replace shortening in a yellow layer cake.

## MATERIALS AND METHODS

### Formation and Isolation of RS

A high (70%) amylose starch (Amylo maize VII, American Maize Products Co., Hammond, IN) was used to produce RS. The method of RS formation was adapted from Sievert and Pomeranz (1989). Amylo maize VII (200 g) was weighed into a 1,000-ml beaker and mixed with 700 ml of distilled water. The starch-water suspension was autoclaved at 20 psi (125°C) for 1 hr. After autoclaving, the sample was cooled to room temperature and stored in a refrigerator overnight at 4°C. After three autoclaving-cooling cycles, a product containing approximately 30% RS was obtained. The technique of RS isolation was based on the AOAC method for dietary fiber determination (AOAC 1985) as described by Czuchajowska et al (1991).

### Preparation and Measurement of Cake Physical Characteristics

Cakes were made from commercial soft wheat flour containing 9.2% protein (N × 5.7) on dry basis. The cake formula is listed in Table I. Sifted flour, baking powder, and RS, together with shortening, were mixed in a Kitchen Aid K5A mixer (Hobart Mfg., Troy, OH) at speed 1 (65 rpm) for 1 min. The mix of dry ingredients was scraped down; eggs and milk were added and mixed at speed 2 (97 rpm) for 1 min, scraped down and mixed at speed 6 (174 rpm) for 2 min, scraped down again, and mixed for 2 min at speed 6. At that stage, the specific gravity of the batter was measured. Portions of batter (30 g) in paper baking cups were placed in a cupcake mold and baked at 204°C for 18 min in a kitchen electric oven (Westinghouse). Cake weight and volume, by rapeseed displacement, were measured after cooling for 1 hr at room temperature. Softness was determined using an R-20 Fudoh rheometer (Fudoh Kogyo Co., Tokyo, Japan) after the cake was stored at room temperature (about 24°C) for 24 hr. Water activity ( $a_w$ ) was determined by the CX-1 water activity system (Decagon Devices, Pullman, WA) in freshly mixed

TABLE I  
Cake Formula

Ingredient	Weight, g
Flour	100
Salt	2
Shortening <sup>a</sup>	20–40
Sugar	100
Baking powder <sup>b</sup>	5
Resistant starch	0–20
Whole eggs	44
Skim milk <sup>c</sup>	71

<sup>a</sup> Crisco, hydrogenated emulsifier type.

<sup>b</sup> Double-action type.

<sup>c</sup> 7.1 g of nonfat dry milk + 63.9 g of water.

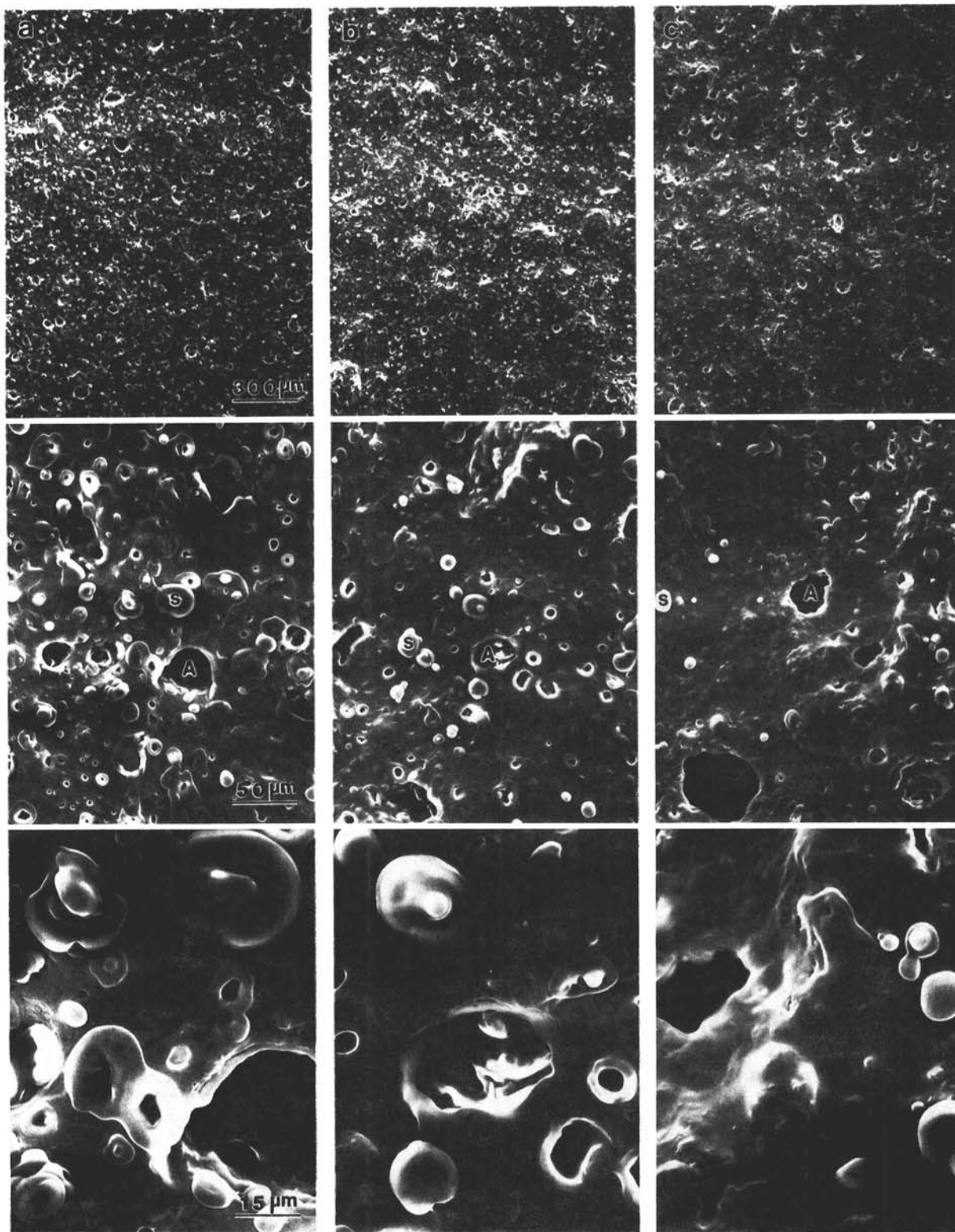
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cake batters and in cakes stored 24 hr at room temperature. Moisture content was determined by drying at 130°C for 4 hr. Water-holding capacity of cake flour and RS were determined by the method of Sollars (1972).

#### SEM Sample Preparation

A thin layer of freshly mixed batter was spread on an aluminum stub, frozen rapidly at -70°C, and lyophilized. Cake crumb was prepared for SEM by the method of Gordon et al (1979). A

8- × 8- × 2-mm crumb sample from the center of the cake was cut with a razor blade. Each sample was mounted on an aluminum stub and treated with osmium vapor overnight. Both cake batter and crumb samples were coated with gold using a Technics Hammer V sputter coater (Technics Inc., San Jose, CA) and viewed in a Hitachi S-570 scanning electron microscope operated at 20 kV. SEM micrographs were analyzed by an image analyzer equipped with Image Analysis version 1.40 (Relax Technology, Union City, CA). Shortening particles were measured by free-



**Fig. 1.** Scanning electron micrographs of cake batters. **a**, Without added enzyme-resistant starch (RS); **b**, RS replacing 25% of the shortening; **c**, RS replacing 50% of the shortening. S = shortening particles, A = air cells.

hand circling of SEM photographs on the screen. Air cell numbers and areas were determined by auto threshold setting.

### DSC

The method for DSC determination was described by Sievert and Pomeranz (1989). The samples were scanned from 20 to 180°C at a heating rate of 10°C/min. Unless stated otherwise, the total sample size was 30 mg for cake batter and 40 mg for cake crumb. For scanning by DSC, 20 mg of freeze-dried cake was tested in 20  $\mu$ l of added water; the batter was evaluated as is. Consequently, the dry matter-to-water ratio was about 1.0:0.3 for batter and 1.0:1.0 for cake crumb.

### Statistical Analysis

The least significant difference was calculated at the 5% level (SAS 1985).

## RESULTS AND DISCUSSION

Cake batters and cupcakes were prepared with RS replacement of 12.5, 25.0, 37.5, or 50.0% of shortening (40 parts per 100 parts flour in the control) or of 5, 10, or 15% of flour. Replacement of up to 15% flour had no significant effect on overall cake characteristics; 5% flour replacement produced cakes that were softer than the control cakes (data not shown). Cakes in which shortening was replaced by RS showed large and consistent effects.

### SEM of Cake Batter

Figure 1 shows the micrographs of three cake batters at three magnifications. A significant decrease in number of air cells and shortening particles as a result of replacing shortening by RS can be observed at the three magnifications. Table II summarizes shortening and air cell data measured on the micrographs by an image analyzer. The three batter samples incorporated similar amounts of air, but the number of air cells decreased with the increase in shortening replacement level. The size of the air cells was not uniform. Assuming the air cells were of uniform size, the mean diameter of an air cell in the 50% replacement sample should have been larger than that in the control batter by a factor of  $\sqrt{2}$ . The control sample incorporated a large number of small air cells, but, as the amount of shortening was reduced, the batter samples incorporated fewer air cells. Carlin (1944) used light microscopy to study the behavior of fats in cake batters and found no continuous water-in-fat emulsion in a batter system; the air cells in a layer-cake batter were surrounded by fat. During baking, as the melting fat releases its incorporated air to the flour-water system, gas produced by baking powder finds its way to the air cells and expands the cake batter. Moncrieff (1970) concluded that shortening served as a dispersion and lubrication agent in a cake system, thereby providing aeration and texture to the cake. Birnbaum (1978) stated that shortening holds all the air that is incorporated into the batter. The air provides points of origin for the cells in the cake. Shortening also provides the support for the structure of the cake batter until starch gelatinization and protein coagulation take place during baking. In this study (Fig. 1b,c and Table II), a reduction in air cell numbers indicates a decrease in efficiency of air incorporation into the batter system. This was caused by the reduction in shortening content, which decreased the capacity of shortening to surround and stabilize air cells. Large air cells in low-shortening batters coalesced during processing and could not be held in the cake batter.

### SEM of Cake Crumb

Micrographs of cake crumb at different magnifications are shown in Figure 2. As the amount of shortening replaced by RS increased, the crumb matrix became more compact. This result was consistent with the general decreases (especially at high levels of replacement) in cake volume and softness (Table III). Starch granules also showed (Fig. 2) a higher degree of swelling in the control sample than they did in the samples in which part of the shortening was replaced by RS. The degree of starch deforma-

tion is affected by heating temperature, water availability, and sugar and shortening contents (Hoseney et al 1978). The water in the cake batter in this study was constant and originated with the milk and eggs. RS absorbed much more water (water-holding capacity 2.5 ml/g) than did wheat flour (0.5 ml/g). Thus, when RS was used to replace shortening, it decreased the availability of water for the wheat starch granules. Except for the differences described above, the micrographs of the three samples were similar (Fig. 2), especially at high magnification. This is in agreement with the findings of Gordon et al (1979), who concluded, on the basis of SEM studies, that there was no significant difference in the appearance of cake crumbs containing different starch levels. Hsu et al (1980) used SEM to compare the crumb structure of model cake systems made with different emulsification systems. They reported that starch granule swelling of the cake crumb observed by SEM was not greatly different, but the characteristics of the intergranule matrix appeared to be related to the concentration of emulsifier. Cloke et al (1984) reported that, on the basis of SEM studies of cake crumb, the degree of swelling of starch granules is difficult to assess because of the limited number of granules viewed, the random orientation of the granules, the wide range of initial sizes, and the presence or absence of leached materials. Several tunnels were formed from top to bottom in the shortening-replaced cakes. This phenomenon was observed also by Bath et al (1992), who used a fat replacer in high-ratio layer cakes.

Data in Table III show an increase in specific gravity with an increase in the replacement level of shortening. This indicated a lower efficiency of air incorporation and retention and a decreased cake volume. The higher moisture content of the cake crumb and the lower  $a_w$  in the cake batter and in the crumb of the shortening-replaced samples apparently was caused by the higher water-holding capacity of RS. As the level of RS (as shortening replacement) increased, the cake surface color was lighter (data not shown). Cake in which 12.5% of shortening was replaced by RS had the highest volume and softest texture. As the replacement level increased, a smaller and firmer cake resulted. In an additional set of experiments (data not shown), the effects of shortening reduction by 12.5, 25.0, and 50.0% (without RS replacement) on cake batter specific gravity, cake volume, and cake texture were determined. The specific gravity of the cake batter increased as the level of shortening reduction was increased. Reduction of shortening caused an apparent increase in volume resulting from large tunnel formation. Reduction of 50.0% (but not of 12.5 or 25.0%) shortening reduced cake surface color.

### DSC of Cake Batter

Four endothermal transitions with onset temperatures ( $T_o$ ) at about 40, 55, 90, and 110°C were recorded in the DSC thermograms of the control cake batter before baking. DSC thermograms for four batters are shown in Figure 3. Each includes two scans (fresh batter and rescan after seven days). The results are summarized in Table IV.

*Melting of shortening.* The first two endothermic peaks (at about 44 and 56°C) were caused by melting of shortening; those two peaks were absent in testing of cake batters without shortening. This is in agreement with the Abboud and Hoseney (1984) study of cookie doughs. Shortening does not affect the starch

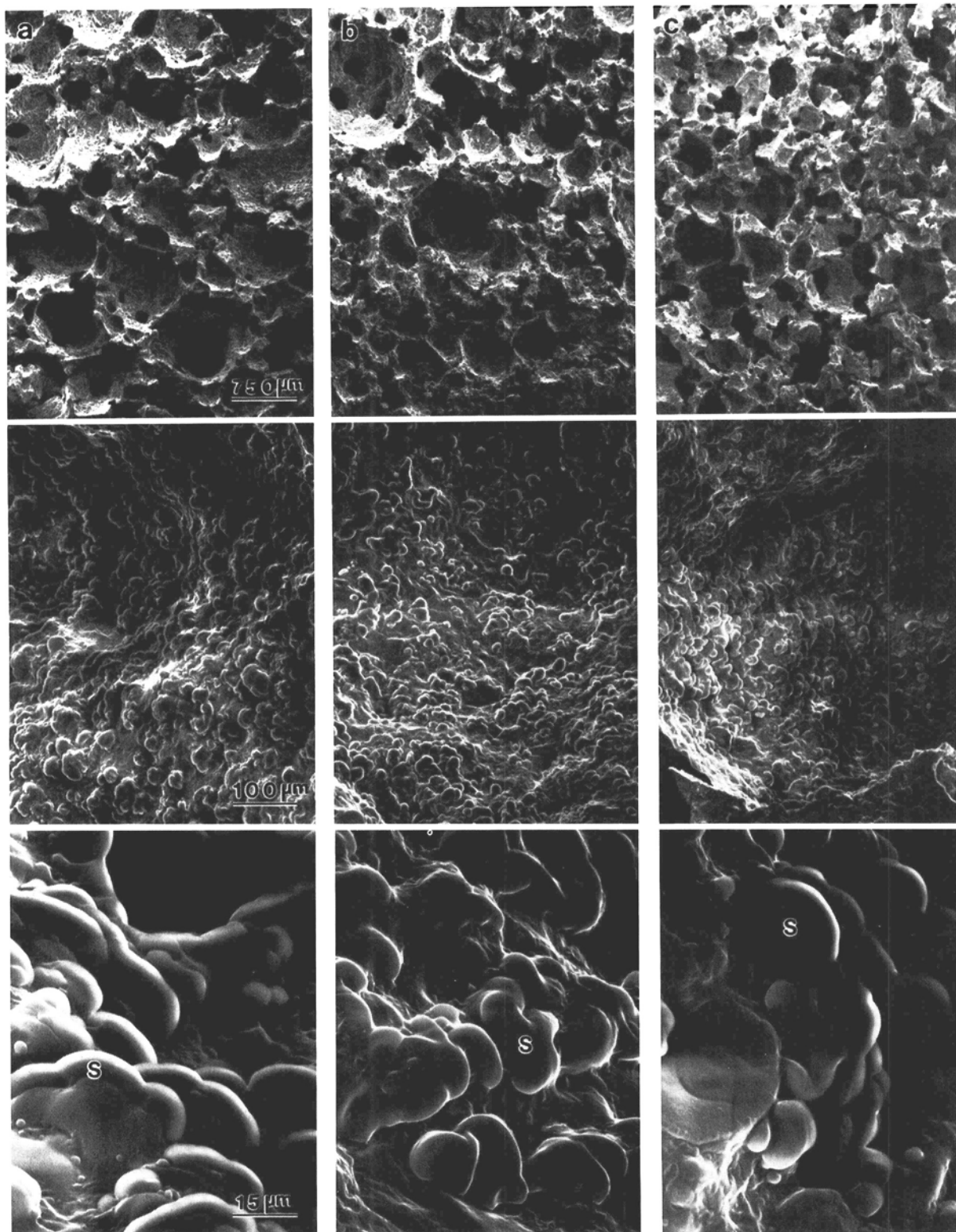
TABLE II  
Shortening Particles and Air Cells in Batter

	Shortening Replacement, %		
	Control	25	50
Shortening particles, number/mm <sup>2</sup>	629	495	390
Air cells, number/mm <sup>2</sup>	60	44	32
Air cell area % of total area	1.98	1.89	2.04



gelatinization temperature (Ghiasi et al 1983). When the samples were scanned for a second time (immediately after the first scan or after storage), only a single peak was recorded in this temperature range. The single endothermic peak from the second scan had a higher enthalpy than that of the combined first and second enthalpy peaks from the original scan. The data indicate changes in the structure of the shortening during the first scan and, probably, interaction of shortening with some other batter components.

*Starch gelatinization.* The third peak in the cake batter thermograms was caused by starch gelatinization. The endothermic transition for the complete cake batter (control) was at a much higher temperature ( $T_0 = 90.6^\circ\text{C}$ ) than it was in the batter without sugar ( $T_0 = 65.6^\circ\text{C}$ ). This was caused by a high concentration of sugar, which is known to raise the gelatinization temperature of starch. Sugar delays gelatinization by: 1) lowering the  $a_w$  of the system, and 2) interacting with starch chains to stabilize the amorphous regions of the starch granule (Spies and Hosney 1982). The starch



**Fig. 2.** Scanning electron micrographs of cake crumb. **a**, without added enzyme-resistant starch (RS); **b**, RS replacing 25% of the shortening; **c**, RS replacing 50% of the shortening. S = starch.

transition temperature range for the control was similar to that of the sample without shortening. In the thermograms of the sample without sugar, a second peak showed up at a higher temperature range. This indicated that not enough water was available for starch gelatinization. The water content in the control cake batter was 26.8%. A second scan of the sample immediately after the first scan, showed no endothermic transition in this temperature range. However, there was an endothermic peak in the thermograms from the sample after storage in the DSC capsule for seven days at room temperature. This may have been caused by retrogradation of amylopectin. Figure 4 compares the endotherms around 68°C of the fresh cake batter and after it was rescanned after storage in the DSC capsule for up to 180 days. The rate of staling (as measured by the peak around 68°C in Fig. 3) in the cake batter without sugar and shortening (curve d,  $\Delta H = 0.99$  J/g) was much faster than it was in the batter without sugar (curve b,  $\Delta H = 0.19$  J/g) or in the batter without shortening (curve c,  $\Delta H = 0.43$  J/g). The second scans of the control cake batter and cake batters with RS, each stored in

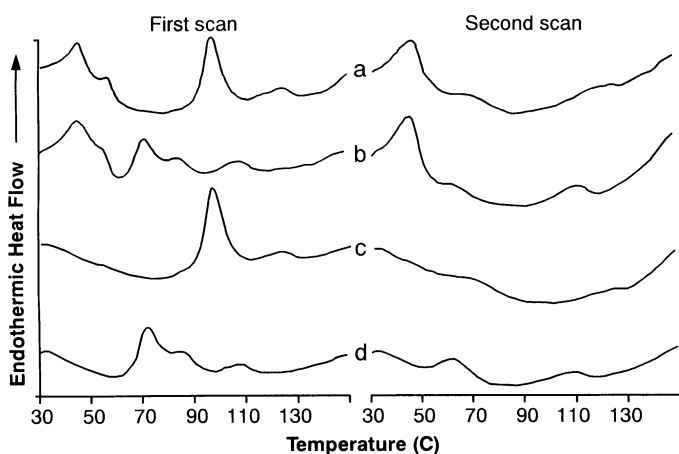


Fig. 3. Differential scanning calorimetry thermograms for: complete cake batter (a); batter without sugar (b); batter without shortening (c); and batter without sugar and shortening (d). Thermograms for the first scan (left) and thermograms for rescan after the sample was stored in the calorimetry capsule at room temperature for seven days (right).

DSC capsules at room temperature for 180 days, gave similar thermogram enthalpies in the temperature range for starch retrogradation, around 68°C (data not shown).

**Melting of the amylose-lipid complex.** The fourth peak (around 110°C) in the control (complete) cake batter thermogram was caused by the melting of the amylose-lipid complex. The  $T_o$  (above 113°C) was much higher in the control than it was in samples without sugar ( $T_o$  below 100°C), and it was similar to the  $T_o$  in samples without shortening. Thus, the endothermic transition temperature for melting of the amylose-lipid complex was delayed by a high concentration of sugar. Cake batter prepared with petroleum ether-defatted flour showed a smaller endothermic peak ( $\Delta H = 0.35$  J/g) for the melting of the amylose-lipid complex. Neither the melting temperature nor the enthalpy of the amylose-lipid complex (around 110°C) was significantly changed when the samples were stored in DSC capsules and rescanned after 180 days.

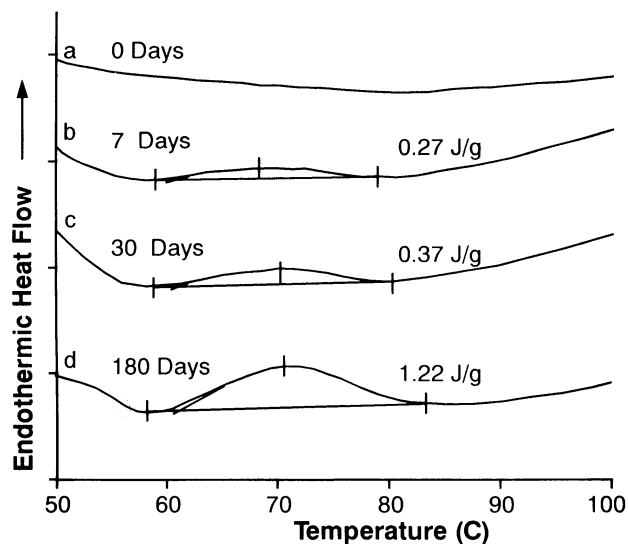


Fig. 4. Differential scanning calorimetry thermograms for complete cake batter: without storage (a); rescanned after storage at room temperature for seven days (b); rescanned after storage at room temperature for 30 days (c); and rescanned after storage at room temperature for 180 days (d).

TABLE III  
Physical Characteristics of Cake Samples<sup>a</sup>

	Control	Shortening replacement, %			
		12.5	25.0	37.5	50.0
Cake batter					
Specific gravity	0.95 c	0.96 c	1.00 b	1.04 a	1.04 a
Water activity	0.908 a	...	0.887 b	...	0.888 b
Cake crumb					
Weight, g	26.12 b	26.22 b	26.23 b	26.51 a	26.12 b
Volume, ml	89.9 ab	92.2 a	90.8 ab	88.2 b	83.7 c
Compression, g	72.5 cd	64.3 d	76.5 c	93.2 b	169.0 a
Water activity	0.845 a	0.840 a	0.844 a	0.847 a	0.829 b
Moisture, %	22.55 b	23.16 a	22.87 ab	23.25 a	23.22 a

<sup>a</sup> Values are means of at least two replications. Means with different letters in a row are significantly different at the 5% level.

TABLE IV  
Differential Scanning Calorimetry of Cake Batter<sup>a,b</sup>

Sample	Starch Gelatinization				Amylose-Lipid Complex			
	$T_i$	$T_o$	$T_c$	$\Delta H$	$T_i$	$T_o$	$T_c$	$\Delta H$
Control batter	79.2	90.6	110.6	3.07	111.1	113.8	130.7	0.48
No sugar	62.4	65.6	81.0	1.13	96.0	98.8	117.7	0.54
No shortening	80.9	90.8	111.3	3.49	112.4	113.9	130.0	0.30
No sugar or shortening	61.6	65.5	80.0	1.45	97.4	99.7	116.4	0.33

<sup>a</sup> Values are means of two determinations.

<sup>b</sup>  $T_i$ ,  $T_o$ , and  $T_c$  = initial, onset, and completion transition temperatures (°C).  $\Delta H$  = transition enthalpy (J/g).

**Melting of RS.** Thermograms of the cake batter containing RS (replacing 50% of shortening) showed a pattern similar to that of the control, except that the enthalpy for melting of shortening was reduced by about 50%, compared to that of the control (data not shown). The melting of RS in the cake system is difficult to access because of the low content of RS, high concentration of sugar, and limited amount of water. In this study, a high concentration of sugar delayed the melting of RS (Fig. 5b), and a low water content decreased the enthalpy (Fig. 5c). Figure 5b shows that adding sugar did not affect the enthalpy of the peak of RS, but it did increase its peak temperature from around 155 to 162°C. Reducing the water-to-RS ratio from 2:1 to 1:1 resulted in an additional peak-temperature increase (to 165°C) and drastically lowered the peak enthalpy (Fig. 5c).

#### DSC of Cake Crumb

Endothermic peaks for melting of shortening and amylose-lipid complex (but not for starch gelatinization) were recorded in the

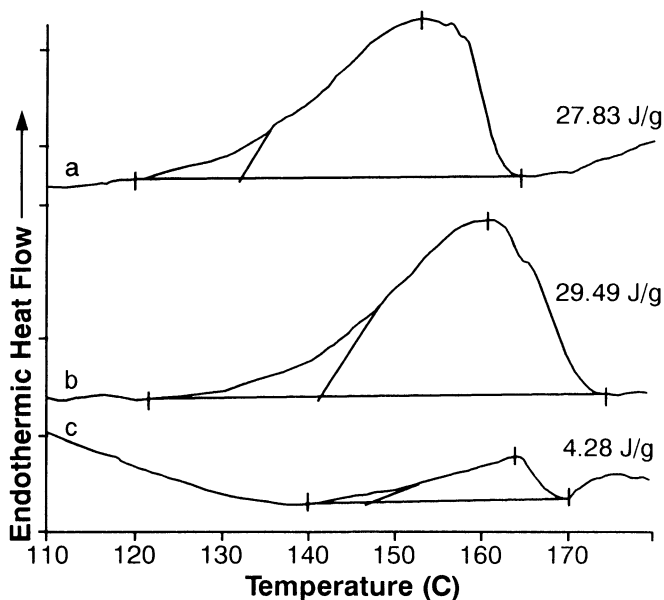


Fig. 5. Differential scanning calorimetry thermograms for samples containing various combinations of enzyme-resistant starch (RS) to water: 10 mg of RS plus 20 µl of water (a); 10 mg of RS plus 10 mg of sugar plus 20 µl of water (b); and 10 mg of RS plus 10 mg of sugar plus 10 µl of water (c).

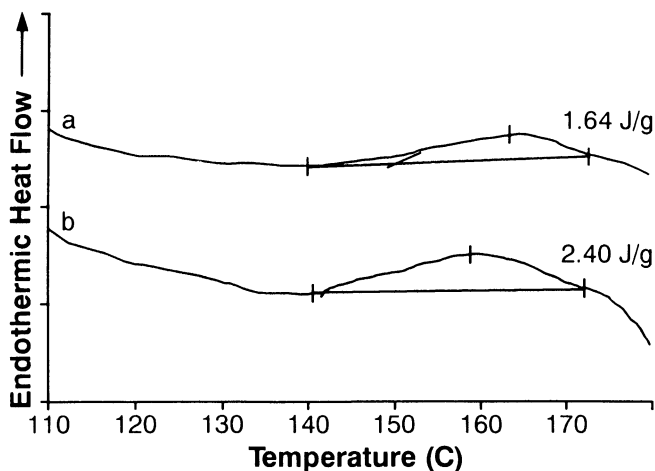


Fig. 6. Differential scanning calorimetry thermograms for cake batter and crumb containing enzyme-resistant starch (RS). a, Batter (23 mg) in which 50% of the shortening was replaced by RS plus 7 µl of water. b, Crumb (20 mg) made from batter in which 50% of the shortening was replaced by RS plus 20 µl of water.

DSC thermogram of the cake crumb (data not shown). To detect RS in the cake batter and crumb, addition of water was required (Fig. 6). No peak around 160°C was detected in cake made without RS. Batter and cake samples containing RS (Fig. 6a,b) gave an endothermal transition around 160°C, which indicated melting of RS.

#### CONCLUSIONS

When RS was used to replace 12.5% of the shortening in the control sample (40 parts per 100 parts flour), yellow layer cake quality was improved. At the replacement level of 25%, cake quality was similar to that of the control. At higher shortening replacement levels (37.5 and 50%), volume decreased and the cake firmed. Replacing 15% of flour by RS (without reducing the amount of shortening) had no consistent or significant effect on cake quality. Methods for maximizing the amount of RS without adversely affecting the quality of the cake (i.e., through the use of emulsifiers or surface-active agents) and for maintaining cake freshness need to be explored.

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