Effects of Selected Surfactants on Amylopectin Recrystallization and on Recoverability of Bread Crumb During Storage

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ABSTRACT

Types, levels, and hydrophile-lipophile balance (HLB) values of surfactants were found to have a varying effect on amylopectin recrystallization, loaf volume, cellular structure, and recoverability of bread. All surfactants inhibited starch retrogradation significantly. The retrogradation decreased with concentration but did not change with HLB number. Although 2% sucrose esters of HLB 16 gave the best loaf volume and cellular structure, they did not inhibit amylopectin crystallization after 21 days of room-temperature storage. Retardation of amylopectin crystallization by added surfactants had no effect on bread recoverable work. It was concluded that amylopectin crystallization did not contribute to the ability to collapse and the fracturability of the cells. The loss in recoverability of the bread during aging was thus contributed by changes in the amorphous components.

The effect of surfactants on retardation of bread staling has been hypothesized as being the result of 1) their interaction with starch retarding the retrogradation (recrystallization) process (Gray and Schoch 1962), 2) their blocking of moisture migration from gluten to starch, which prevents starch from taking up water and thus promotes moisture migration from crumb to crust (Pisesookbunterng and D'Appolonia 1983), and 3) other factors (reviewed by Willhoft 1973, Maga 1975, Anonymous 1983, Levine and Slade 1991).

Surfactants soften bread and retard its firming during storage (Maga 1975). Recently, Krog et al (1989) reported that increased crumb firmness during staling correlated with increasing amylopectin crystallization, both of which were inhibited by added surfactants. They concluded that the retrogradation of amylopectin was a major factor in staling and textural changes. The anti-staling function of a surfactant has been suggested to be caused by an interaction between surfactants and amylopectin that prevents amylopectin crystallization (Lagendijk and Pennings 1970). This is possibly associated with the outer linear chains (Kulp and Ponte 1981). However, Dragsdorf and Varriano-Marston (1980) argued that starch crystallization and bread firming are not synonymous. Other factors could also affect firming of bread, such as amylose-lipid complexation (Anonymous 1983), moisture migration (Willhoft 1973, Leung 1981, Pisesookbunterng and D'Appolonia 1983), or a change in gluten functionality (Krog 1977). In addition, cellular structure in a bread affects the final texture; this structure is dependent on the dough-forming characteristics, which are greatly influenced by added surfactants (Tsen and Weber 1981, Moore and Hoseney 1986). It has also been suggested that surfactants may help prevent moisture migration between starch and gluten (Maga 1975, Pisesookbunterng and D'Appolonia 1983).

In addition to increased firmness, other textural deterioration during staling includes a considerable loss in resilience ("elastic" property) that results in an increase in crumbliness (D'Appolonia and Morad 1981). This is usually not measured quantitatively and thus has not been related to any phenomena suggested to cause staling, such as amylopectin crystallization. In contrast to crumb firmness, resiliency measurement can provide information regarding bread cellular structure, such as cell wall rigidity and "elasticity," which can change the way the cells collapse upon compression.

One of the techniques to measure resiliency is the recoverability measurement (Lee et al 1983). The technique has proved useful for measuring any irreversible damage upon compression of bread (Kou and Chinachoti 1991; Nussinovitch et al 1991, 1992). Recoverability, or recoverable work, is derived from a ratio between the area under a decompression curve and that under a compression curve at a given strain level. Kou and Chinachoti (1991) reported that, as bread staled, its recoverability decreased with storage time even though the bread moisture loss was kept minimum at 2-3%. This increasing irrecoverable damage to cellular structure (upon compression) with storage time can be related to the bread's loss in "elasticity" and resiliency upon staling.

This study quantitatively related the degree of amylopectin crystallization to bread recoverability as influenced by added surfactants. If amylopectin crystallization is responsible for the development of a more rigid structure of bread cell walls, surfactant-treated bread with retarded amylopectin crystallization should result in less rigidity of the cell walls, such that recoverability would decrease less over storage time as compared to a control. Types, levels, and hydrophile-lipophile balance (HLB) numbers of selected surfactants were varied to determine the influence of various emulsifying properties of the surfactants.

MATERIALS AND METHODS

Materials

Wheat flour (12.2 protein, 13.1% moisture, 0.49% ash; King Arthur Flour Company, Norwich, CT), active dry yeast (Fleischmann's Yeast Inc., Oakland, CA), shortening (Crisco, Procter & Gamble, Cincinnati, OH), nonfat dry milk, sucrose, and salt were obtained from a local supermarket.

Food-grade surfactants included distilled monoglyceride (90% purity) made from refined, hydrogenated soybean oil (Dimodan PV, Grindsted Products, Inc., Industrial Airport, KS) and sodium

TABLE I
Bread Dough Formulation[a,b]

Ingredients	Weight in Grams (× 8)
Wheat flour	100
Water	60
Shortening	5.0
Sugar	4.7
Nonfat dry milk	2.0
Active dry yeast	1.8
Salt	1.5
Calcium propionate	0.32
Potassium sorbate	0.16

[a]Percentage flour weight basis.
[b]Mixing time = 12 min (100 rpm, Hobart mixer model D-300; mixing bowl dimensions: 42.8 cm diameter, 31.0 cm height); dough temperature = 32.2°C; flour time = 15 min; scale weight = 500 g by hand and round; intermediate proof = 55 min, 30°C, 95% rh; baked 20 min at 193.3°C (Garland electric restaurant oven, model 680-Z).

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stearoyl lactylate (SSL) (S1170 and S1670; Mitsubishi Kasei Corp., Tokyo, Japan). Sucrose esters (SEs) were those of 70% stearic acid and 30% palmitic acid with varying HLB numbers (7, 11, and 16).

Bread Preparation

A typical straight-dough bread formula was applied in this study (Table I). After baking, all loaves were cooled for 1 hr at 20°C and then cut by a slicer into slices 1 in. thick (eight slices per loaf). The slices from the end of the loaf and the center were discarded. The rest of the slices were used, since tests showed no difference in moisture content and textural properties. Each slice was cored into a cylinder 1.25 in. in diameter, using a sharp copper cork borer. Only the core center of the bread crumb of each slice was used, to eliminate the effect of moisture migration from crumb to crust.

Each cylindrical crumb sample was immediately placed in a can and hermetically sealed. All cans were stored at 25 ± 0.2°C up to 21 days. At least two duplicate samples (two cans) were used for each analysis explained below.

Surfactant Treatments

Treatments included addition to the bread dough of 0.5% (flour basis) SE, SSL, or Dimodan or 1–3% (flour basis) SEs of various HLB numbers. Breads with the various surfactant treatments and a control bread were all made on the same day. The surfactant (4 g) was mixed with 480 g of water and then heated to 50°C before it was mixed into the flour. This was to facilitate the even mixing of the surfactant. Each sample was tested for amylopectin crystallization and recoverability by an Instron Universal Testing Machine after one and seven days of storage in cans at room temperature. To observe progressive change during storage time, a control and selected SE-treated breads (with HLB 11 and 16) were studied during a 21-day period. Amylopectin crystallization and percent recoverable work were measured.

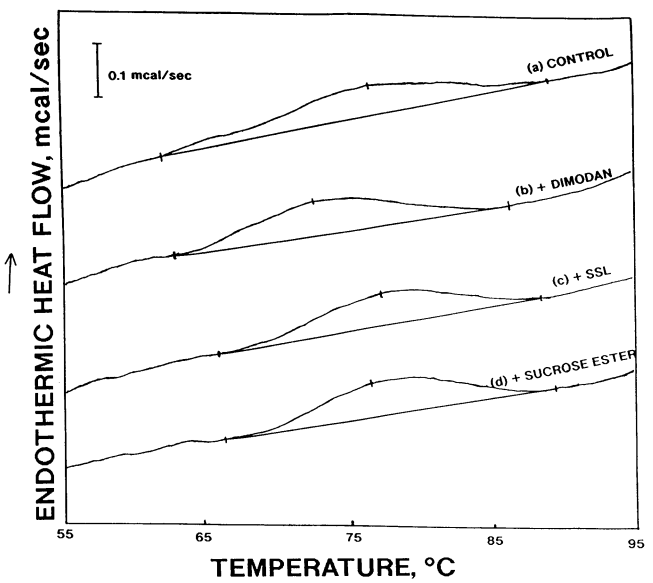

Fig. 1. Differential scanning calorimetry thermograms of bread treated with and without surfactants (0.5% flour basis) and stored at 22°C for seven days. SSL = sodium stearoyl lactylate, + = added to control.

Analyses

Recoverable work was measured by compressing each sample uniaxially between parallel lubricated plates at a cross-head speed of 1 cm/min, using an Instron Universal Testing Machine (model TM-SM; Instron Corporation, Canton, MA). Compression to 20–50% deformation and then decompression at the same speed give stress-deformation curves. The percent recoverable work was calculated as

$$\text{Recoverable work} = \frac{\text{area under decompression curve}}{\text{area under compression curve}} \times 100.$$

Amylopectin crystallization was analyzed using a differential scanning calorimeter (model DSC-2, Perkin Elmer Corp., Somerset, NJ). Bread sample (10–15 mg) was sealed in a hermetic pan (319-0218, Perkin Elmer) and heated from 27 to 107°C at a rate of 20°C/min. Each experiment was run in triplicate.

Specific loaf volume of bread was measured by the rapeseed displacement method (AACC 1983).

Microscopic observation of cellular structure was done by light microscopy of a thin section of bread. Blocks of bread (5 × 10 × 20 mm) were freeze-dried, embedded in a series of protoplast media, and then sectioned into slices of 15–20 μm thickness using a microtome (American Optical models 851C and 852C; Cryocut II, Buffalo, NY). All observation was done under a 40× light microscope. Blocks were randomly sampled, and approximately 10–15 viewing areas were observed. Representative photographs are shown here.

Moisture content was measured in duplicate by drying in a vacuum oven at 60°C under 29 in. of Hg for 24 hr. Moisture loss from the sample over storage time was within 2–3% over a period of up to 21 days of storage.

RESULTS AND DISCUSSION

Effects of Type of Surfactant

After being stored for seven days, bread samples treated with 0.5% (flour basis) SE, SSL, Dimodan (60% monoglycerides), or a control showed an endothermic peak, as demonstrated in Figure 1. These peaks occurred over the temperature range of about 64–74°C, corresponding to the typical melting peak for crystalline amylopectin (Fearn and Russell 1982). Rescanning of the samples immediately after the first scan showed no peak at all, confirming that the peak is a melting process. The broadness of the melting peak indicated that retrograded starch had a high degree of heterogeneity with a wide range of melting temperatures. Curves b–d in Figure 1 show that melting patterns for surfactant-treated breads are similar to that for the control.

Table II lists the onset temperature (T_o), peak temperature (T_p), and energy (ΔH) of melting. After seven days of storage, the ΔH value was highest in the control sample, indicating a considerable retrogradation of amylopectin. (No endothermic

TABLE II
Endothermic Onset and Peak Melting Temperature of Starch and Energies of Bread Treated with Various Surfactants (0.5% flour basis) and Stored at 22°C for Seven Days

Bread	Melting temperature, °C		Enthalpy, cal/g	
	Onset	Peak	Sample	Starch
Control				
With additive[a]	63.94 ± 1.06	73.23 ± 2.99	0.227 ± 0.015	0.600 ± 0.024
Dimodan	64.11 ± 0.64	71.66 ± 0.57	0.183 ± 0.012	0.480 ± 0.023
SSL	65.77 ± 0.32	73.70 ± 1.46	0.170 ± 0.027	0.449 ± 0.057
SE[b]	66.11 ± 0.48	73.40 ± 0.06	0.160 ± 0.028	0.443 ± 0.047

[a]SSL = sodium stearoyl lactylate, SE = sucrose ester.
[b]Hydrophile-lipophile balance = 16.

peak was found in freshly baked bread.) The three added surfactants resulted in a significantly lower ΔH, about 0.44–0.48 cal/g of starch (Table II). No significant difference ($\alpha = 0.05$) was found in ΔH among the three surfactant treatments. Although there seems to be some difference in T_o and T_p among these samples (Table II), statistical analysis ($\alpha = 0.05$) showed no significant difference. This inhibition of amylopectin recrystallization by added surfactants supports earlier findings (e.g., Krog et al 1989).

We did run a number of samples up to 150°C to observe the endotherm of the amylose-lipid complex at approximately 110°C. The data received were not consistent enough to be presented here. While, in some samples, an amylose-lipid interaction endotherm was observed, duplicates showed either erratic baseline shifting or no endotherm at all. Thus, the rest of the samples were not tested for amylose-lipid complex.

TABLE III
Effect of Hydrophile-Lipophile Balance (HLB) Values and Concentration of Sucrose Esters (SE) on Loaf Volume (cc/g) of Wheat Bread

	SE Concentration, % flour basis			
HLB	**0**	**1**	**2**	**3**
7	4.92 ± 0.10	4.76 ± 0.07	5.50 ± 0.11	3.52 ± 0.04
11	4.92 ± 0.10	5.65 ± 0.07	5.06 ± 0.80	4.92 ± 0.22
16	4.92 ± 0.10	3.72 ± 0.01	6.12 ± 0.14	5.16 ± 0.14

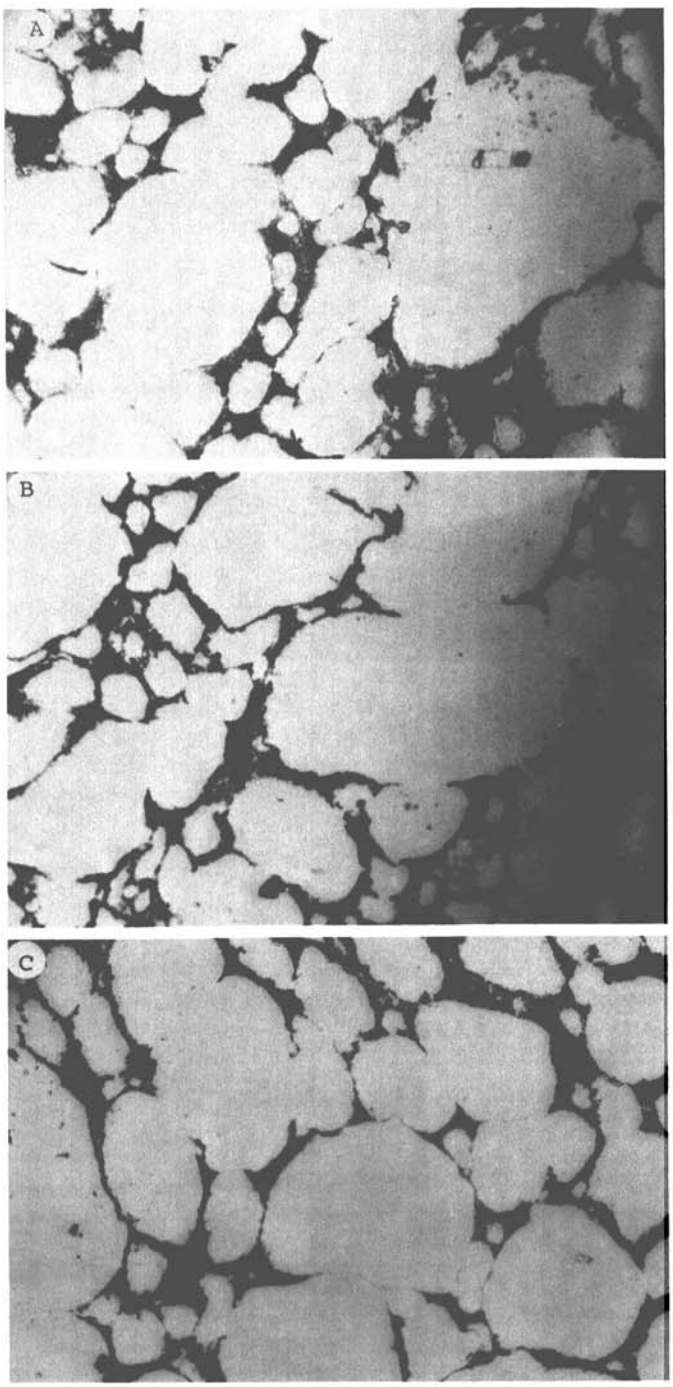

Fig. 2. Photomicrographs of bread treated with 2% (flour basis) sucrose esters of hydrophile-lipophile balance = 16 (**A**), 11 (**B**), and 7 (**C**).

Effects of HLB Values and Concentration of Sucrose Esters

SEs were further investigated to determine the best concentration and HLB value for this bread formula. Although there was no significant difference in its ability to retard starch retrogradation as compared to SSL and Dimodan, SE of HLB = 16 gave a relatively better loaf volume than other surfactants (Dimodan, 5.49; SSL, 5.65; SE 6.06; see also Bean et al 1977). Table III shows that SE of HLB = 16 gave a higher loaf volume than SEs with other HLB numbers (except for the case of 1% concentration). The 2% (flour basis) level of SE of HLB = 16 seems to be the best. Chung et al (1976) reported that SE of HLB = 16 was the best surfactant for functionally replacing lipids in bread. This effect on loaf volume agrees with the data reported by Breyer and Walker (1983). The vast difference in the concentration dependency on the HLB values shown in Table III indicates that the emulsifying ability of the SE plays a significant role in dough development and air cell formation.

The cellular structure of the bread also showed some qualitative differences among breads treated with SEs of HLB = 7, 11, and 16 (Fig. 2). The sample with HLB = 16 seems to give rounder cells and a more uniform structure than samples with HLB = 7 and 11 (Fig. 2). Figure 3 compares the microscopic cellular structures of the control bread and of breads treated with 1, 2, and 3% SE (HLB = 16). In the control samples (Fig. 3A), the cell walls were relatively thick, with evidence of breakage caused by the slicing step. As the SE content increased, this seemed to improve; there did not appear to be a significant difference between the 2 and 3% concentrations (Fig. 3C and 3D, respectively).

Amylopectin recrystallization after seven days of storage is plotted against SE concentration in Fig. 4. The endothermic energy decreased curvilinearly with concentration, leveling off at the 2% level of SE. HLB values did not affect the degree of retrogradation significantly. Surfactants may inhibit retrogradation by interacting with starch. It has also been proposed (Pisesookbunterng and D'Appolonia 1983) that surfactants become the boundaries preventing moisture migration from gluten to starch (Willhoft 1971). This migration of moisture has been described as promoting starch crystallization (Leung 1981, Levine and Slade 1991).

If SE emulsifies and thus becomes a barrier, varying its HLB number should make a significant difference in moisture migration, which promotes amylopectin crystallization. Data in Figure 4 show no difference in HLB numbers at a given SE concentration. This could mean that 1) no moisture migration occurred, 2) the moisture migration was not influenced by the emulsifying property of the SEs, or 3) moisture migration had no effect on starch crystallization.

Effect of Storage Time

The time study was done using SEs of 11 and 16 HLB values at the 2% level. The ability of SE to inhibit amylopectin recrystallization was observed from the endothermic peak enthalpy for up to 21 days of storage at room temperature. Enthalpy was plotted against storage time, as shown in Figure 5. In the control, ΔH started to increase from zero after one day of storage and then leveled off at 0.25 cal/g of sample after seven days of storage (Fig. 5). Bread treated with SE showed a much slower development of crystalline amylopectin; at day 7, ΔH values were less than one half the value of the control (Fig. 5). The crystallization level, however, eventually reached that of the control at day 21

(Fig. 5), at a ΔH level of about 0.25 cal/g of sample. The retardation of the crystallization by SEs did not vary significantly between HLB = 11 and 16.

It should be noted here that the asymptotic level of enthalpy (0.25 cal/g of sample) corresponded to 0.6–0.7 cal/g of starch and was far from the maximum possible enthalpy required to melt all amylopectin present. The reported value for that level is 2.39 cal/g of wheat starch (Chinachoti et al 1991).

At this level, SEs were effective in delaying amylopectin crystallization during the first 9 days of storage but failed to completely inhibit the process. After 21 days of storage, amylopectin retrogradation reached the value of the control (Fig. 5). This means that the interaction of SEs and amylopectin is a weak and reversible one. It has been proposed that this is a main factor affecting bread firmness during storage (e.g., Krog et al 1989). Delaying (reducing the rate) of bread firmness in surfactant-added bread has been reported (Pisesookbunterng and D'Appolonia 1983, Krog et al 1989). However, no data is available to show whether the firmness eventually reached the same level as that of a control bread, as found in the case of amylopectin crystallization (Fig. 5).

Recoverable Work

This part of the study investigated any relationship between amylopectin crystallization and changes in rigidity and suscepti-

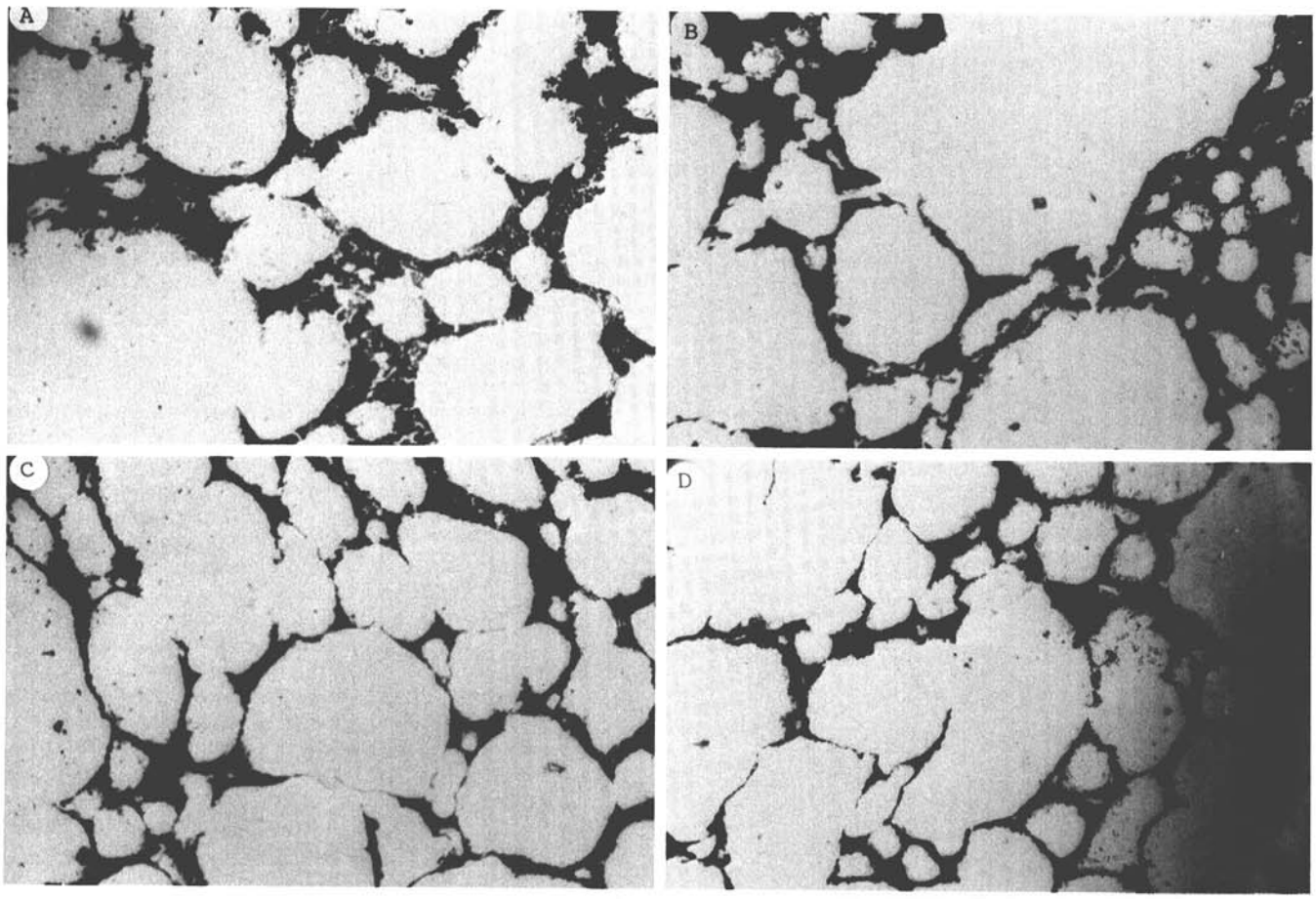

Fig. 3. Photomicrographs of bread treated with sucrose ester, hydrophile-lipophile balance = 16, at levels of 0% (**A**), 1% (**B**), 2% (**C**), and 3% (**D**) (flour basis).

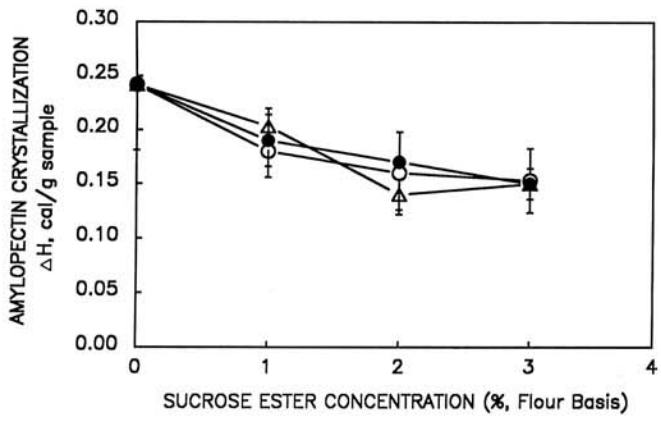

Fig. 4. Differential scanning calorimetry endothermic starch melting energies (ΔH) of bread treated with sucrose esters of hydrophile-lipophile balance [HLB] = 7 (○), 11 (●), and 16 (△) at levels of 0–3% (flour basis), stored at 22°C for seven days.

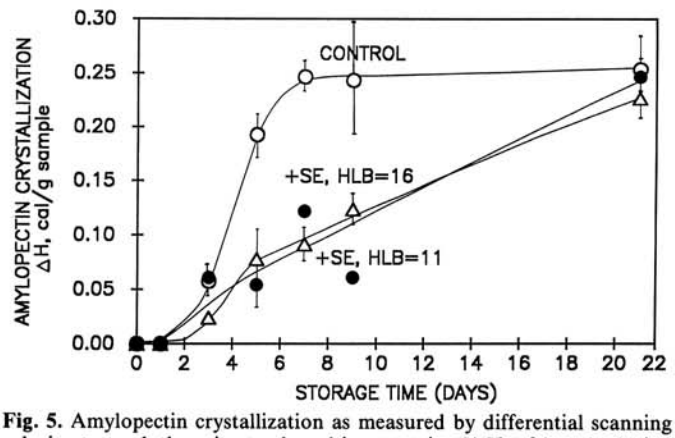

Fig. 5. Amylopectin crystallization as measured by differential scanning calorimetry endothermic starch melting energies (ΔH) of breads during storage, comparing a control bread (○) and breads treated with sucrose ester (SE) (hydrophile-lipophile balance [HLB] = 11, ●; HLB = 16, △) at a 2.0% concentration on a flour basis.

bility to damage of the cellular structure as measured by recoverable work. Table IV presents the recoverable work results comparing SSL, Dimodan, and SE treatments. At day 1, all samples recovered by approximately 45 and 22% of work after being subjected to 20 and 50% deformations, respectively (Table IV). This is as expected, since structural damage should be greater after compression of 50% as compared to 20% (Chinachoti and Nussinovitch 1990, Nussinovitch et al 1991, 1992). After seven days, all samples showed a significant decrease in recoverable work at a given compression level, as compared to their corresponding sample from day 1 (Table IV). For instance, the control sample showed at least a 10% decrease in recoverable work (from 47 to 37%) at 20% compression. At 50% compression, this decrease in recoverable work over time was very small for all samples. Microscopic observation (data not shown) indicated that the cellular structure was mostly destroyed by 50% compression and only partially destroyed by 20% compression (Rao 1991). Changes in cellular recoverability upon staling were thus more observable at the 20% level of compression.

Table IV shows no significant differences among the four surfactant treatments both for day 1 and day 7, regardless of the compression level. Thus, it can be concluded from the data in Table IV that surfactants, although inhibiting amylopectin crystallization, have no effect on recoverability. Thus, the amylopectin crystallization inhibited by these additives had no influence on the cellular mechanical properties as measured by recoverable work. This conclusion can be clearly demonstrated from the plot in Figure 6, in which the control and SE-treated breads showed a vast difference in the degree of amylopectin crystallization over about 10 days of storage (Fig. 6B) but remained close in recoverable work over time (Fig. 6A); there was no correlation between amylopectin crystallization and recoverable work.

The data above show that, although these surfactants soften bread (as reported by others), they do not prevent the development of bread cellular rigidity and susceptibility to fracture caused by staling. The effects of surfactants could be a result of other processes, such as changes in the cell wall thickness and cell size and geometry (controlled during dough development), rather than changes in the rigidity and elasticity of the cell walls (in baked bread). DeStefanis (1977) found that during dough mixing and development SSL and some other surfactants primarily associated with the protein, whereas after baking they strongly bind to the starch.

Thus recoverability, resiliency, and "elastic" property are likely to be more influenced by the gluten than the (crystalline) starch. We have found that increasing gluten content in bread results in an increase in recoverable work (*unpublished results*). Figure 7 shows micrographs of staled bread under a light microscope (Fig. 7A) and a polarized light microscope (Fig. 7B). Observation under polarized light clearly showed that starch crystals embedded themselves in a matrix of amorphous components. Thus, it is reasonable that crystallizing starch contributed to the firmness of bread but not to its recoverability, which is rather more related to the amorphous, continuous matrix. Thus the loss in recoverability over time (Fig. 6) is likely to have resulted from changes in the amorphous matrix and not from the crystallizing of starch.

TABLE IV
Recoverable Work (% of original) for Bread Treated with Various Surfactants (0.5% flour basis) After Compression to 20 and 50% Deformation

	Day 1		Day 7	
Bread	20% Compression	50% Compression	20% Compression	50% Compression
Control				
With additive[a]	47.5 ± 0.1	24.4 ± 0.7	36.8 ± 1.7	20.0 ± 2.2
Dimodan	44.8 ± 4.7	21.5 ± 1.8	31.0	18.3 ± 0.9
SSL	42.7 ± 3.6	18.9 ± 1.6	30.4 ± 4.1	17.1 ± 0.7
SE (HLB = 16)	45.6 ± 2.8	23.1 ± 1.1	32.1 ± 2.1	18.5 ± 1.5
Average among treatments	45.1	22.0	32.6	18.5

[a]SSL = sodium stearoyl lactylate, SE = sucrose ester, HLB =hydrophile-lipophile balance.

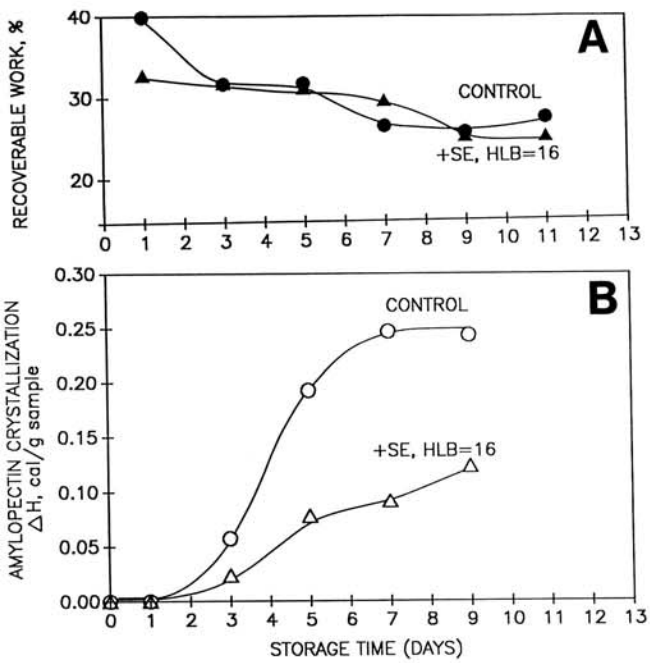

Fig. 6. Changes in recoverable work **(A)** and amylopectin crystallization **(B)** in control breads (circles) and in breads treated with sucrose esters (SE) (hydrophile-lipophile balance [HLB] = 16) (triangles). ΔH = enthalpy.

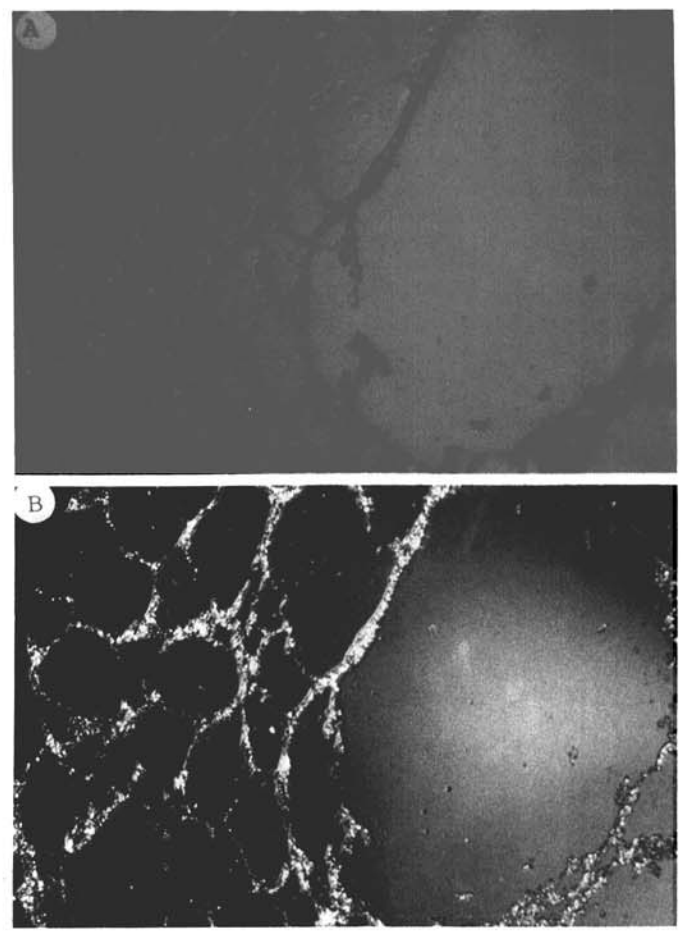

Fig. 7. Photographs of staled bread under a light microscope **(A)** and a polarized light microscope **(B)** (40×).

The presence of surfactants decreases the amount of soluble starch in bread. Indirectly, they might have some effect on the amount of amorphous material and thus might influence bread recoverability. Apparently, this was not observed in this investigation (Table IV).

The explanation above agrees with the data in Table IV showing that surfactants had no effect on the recoverable work in staled bread since their ability to inhibit retrogradation had no relationship to the bread recoverability. Recently, it has been proposed that the glass transition temperature of bread polymers changes during aging, which might be responsible for textural changes (Blanshard 1987, Levine and Slade 1991). This could be the key change related to the loss in recoverable work.

CONCLUSION

We have shown here that the types, levels, and HLB values of surfactants have varying effects on bread quality in terms of amylopectin recrystallization (retrogradation), loaf volume, cellular structure, and recoverability of bread. We found that SSL, Dimodan, and SEs of all HLB values and all levels inhibited the retrogradation process significantly. The retrogradation was dependent on the level but not on the HLB value of the SE. Bread treated with 2% SE (flour basis) inhibited the retrogradation as much as that treated with 3% SE, but it gave a higher loaf volume. The cellular uniformity was best for SE of HLB = 16. SEs only delayed (failed to totally inhibit) the amylopectin recrystallization. After 21 days of storage, the crystallization was at the same level regardless of the presence of SEs.

This retardation of amylopectin crystallization by added surfactants was found to have no effect on the bread recoverable work. This could mean that amylopectin crystallization did not contribute to the cells' ability to collapse and to their fracturability. We concluded that the loss in recoverability of the bread was contributed by changes in the amorphous components.

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