

Effect of Trehalose on Fresh Bread and Bread Staling

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ABSTRACT

The effects of trehalose on bread properties and bread staling during storage were evaluated by sensory evaluation, bread crumb moisture retention, compression, and differential scanning calorimetry (DSC). Bread containing trehalose had higher specific volume and performed better in sensory comparisons than the control. Bread crumb hardness was reduced by adding trehalose. Kinetic study of the hardness employing the Avrami equation showed that trehalose reduced the rate of bread firming. Moreover, DSC studies showed that trehalose decreased the enthalpy of starch retrogradation and increased the glass transition temperature (T_g). All of the data indicated that trehalose could improve the quality of and retard the staling of bread effectively.

INTRODUCTION

Bread staling has been investigated for the past 150 years; however, the precise mechanism is far from understood and debate continues as to the general nature of the processes involved (6,12). Bread crumb firming is the change most widely associated with staling. Investigations into the causes of bread staling have shown that changes in starch structure, namely gelatinization and retrogradation, contribute to the texture changing from soft to firm (12).

Many efforts have been focused on the development of different additives and enzymes for retarding the staling process and extending the shelf life of bread. Different emulsifiers and hydrocolloids have been successfully used as antistaling agents. In addition, different α -amylases, hemicelluloses, and lipases are widely used for retarding bread staling (1).

Trehalose is a natural disaccharide formed by a 1,1 linkage of two D-glucose molecules. It is a nonreducing sugar that is not easily hydrolyzed by acid, and the glycosidic bond is not cleaved by α -glucosidase. Its molecular formula is $C_{12}H_{22}O_{11}$

and its molecular weight is 342.31 (10). The European Commission has approved the use of trehalose as a novel food additive in the European Union. The usage of trehalose in food was also authorized in Japan, Taiwan, and Korea.

As trehalose is not a reducing sugar, it does not undergo Maillard-type browning reactions. It does not caramelize. Trehalose is currently used in Japan to retard starch retrogradation in udon noodles (when included at 0.2% flour weight). The purpose of this study was to analyze the complicated effects of trehalose on the retrogradation enthalpies and dynamic rheological properties of bread.

MATERIALS AND METHODS

Materials

The commercial wheat flour used in this experiment contained $14 \pm 0.2\%$ protein (moisture content $13.6 \pm 0.2\%$, ash $0.5 \pm 0.02\%$). The trehalose was a gift from Shanghai Stream International Trading Co., Ltd.

Bread Samples

The basic dough recipe, on an 800-g flour basis, consisted of instant yeast (12.8 g), salt (12.0 g), sucrose (16 g), skimmed milk powder (32 g), shortening (24 g), and water (480 ml). Trehalose was added in amounts of 0%, 3%, 7%, and 11% (flour basis). The ingredients were mixed for 15 minutes, and the dough fermented at 26°C with 80% relative humidity for 45 minutes in a fermentation cabinet. Then the dough

was divided into 10 pieces, molded, and fermented at 35°C for 45 minutes. The bread dough loaves were baked at 190°C for 15 minutes. Baked loaves were allowed to cool at room temperature for 60 minutes. Cooled bread samples were packaged in polypropylene bags and stored at $22 \pm 1^\circ\text{C}$. The storage time varied between one and five days, as discussed below.

Specific Volume of a Loaf and Sensory Evaluation of Bread

Bread loaf volume and weight were measured on three replicates after being stored at 25°C for one hour. Bread loaf volume was measured by millet seed displacement. The volume of a container (V1) was measured by filling it with millet seed and then measuring the volume of millet seed. We then put the bread loaf in the container and filled it with millet seed until it was full. The volume of millet seed used in the second measurement was measured as V2. Thus, the volume of bread loaf was $V1 - V2$. The specific volumes of breads were calculated as volume/weight (8,12).

Sensory evaluation was performed by a group of 15 students who evaluated overall acceptance of fresh and 20-hour-old bread samples (3,4,11,12). The attributes evaluated were specific volume, visual appearance, aroma, taste, color, etc. The average response from all of the judges was calculated for each attribute. Overall acceptability was calculated by weighted arithmetic mean, given the following weight to each attribute: specific volume, 15 (regarding to 5 mL/g); crust, 15 (color and thickness); texture, 15 (elasticity, stickiness); crumb color, 10 (cream white as better); crumb grain, 10 (alveolus size and shape); aroma, 15 (fresh bread like); and taste, 20 (flavor and mouth feeling), according to the influence of each attribute on acceptance of the product by consumers (9).

Water Content and Water Activity of Breads

The moisture contents of the bread crumbs were measured by drying them at 103°C for five hours. Water activity was determined with a digital water activity

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probe (ROTRONIC AG, Shanghai, China). About 2.0 g of crumbs were cut into cubes ($4 \times 4 \times 4 \text{ mm}^3$), which comprised approximately two-thirds of the probe pan's volume. The water activity value was measured automatically at 25°C for 15–20 minutes (6).

Hardness of Bread Crumbs

The texture of the bread crumb was determined using a texture analyzer (LFRA 4500, Brookfield Engineering Laboratories, Inc., Middleboro, MA). The machine's parameters were: mode, compression; plot, peak; trigger, automatic, 4.5 g; test speed, 0.5 mm/s; distance, 5 mm; probe, TA 41 (4mm \varnothing Perspex Cylinder); option, cycle; temperature, 22°C. After discarding the highest and lowest readings, the mean values and their standard deviations were calculated (5). Bread samples were cut into 15 mm thick slices. Two slices were taken from each loaf and four measurements were taken at different points on each slice.

Avrami Model

Hardness and retrogradation enthalpy were fitted to the Avrami equation:

$$\theta = \frac{F_{\infty} - F_t}{F_{\infty} - F_0} = e^{-kt^n}$$

$$\log \left(-\ln \frac{F_{\infty} - F_t}{F_{\infty} - F_0} \right) = \log k + n \log t$$

where θ was the fraction of retrogradation that occurred, F_0 , F_t , and F_{∞} were the hardness of bread at the time of zero, t , and infinity, respectively, k was a rate coefficient, and n was the Avrami exponent.

Differential Scanning Calorimetry

Amylopectin retrogradation was evaluated by differential scanning calorimetry (DSC) (DSC-7, Perkin-Elmer, Waltham, MA). Samples from the central portions of the loaves were cut into rectangles (30 \times 20 mm) and compressed at room temperature. Samples (8–10 mg) were shaped into pieces of 3.0 mm diameter and placed in a hermetically sealed aluminum DSC pan to avoid moisture loss. After being stored at 4°C for 4 days, each sample was heated from 30°C to 130°C at a rate of 10°C/min-

ute, then cooled down to ambient temperature at the rate of 2°C/minute, and then chilled down to -10°C by liquid nitrogen. This cooling is a command for DSC measure (see, for example, Mohamed [7]). An empty aluminum pan was used as the reference.

RESULTS AND DISCUSSION

Influence of Trehalose Addition on Bread Quality

As shown in Table I, adding trehalose to bread resulted in quality improvement and better acceptability by the panel after 20 hours of storage at 22°C. The specific volume of bread in groups B, C, and D increased by 2.09%, 6.70%, and 5.65%, respectively, compared with the control sample. Sensory evaluations indicated a distinct preference for samples B, C, and D (especially C and D) over the control sample.

Influence of Trehalose Addition on Moisture Retention and Water Activity of Bread Crumbs

As illustrated in Figure 1, trehalose had an obvious effect on moisture retention during bread storage, and the more trehalose that was added, the less moisture was lost. Water activity remained almost the same after 10 days at 22°C.

Hardness Evolution of Bread During Storage

A progressive increase in hardening occurred during bread storage, and the rate of hardening (slope of the hardening curve) can be calculated. As shown in Ta-

ble II, the hardening rate of the bread slowed in proportion to the increase of trehalose content (bread was stored at 22°C for 5 days).

The Avrami equation, which was originally derived to describe the equilibrium crystallization of high polymer melts, has been extensively used to study the kinetics of these processes. The firmness data were analyzed by means of a restricted model with a fixed value of the Avrami exponent, $n = 1$, giving an adequate fit.

As shown in Table III, the evaluated Avrami equation coefficient k for crumb hardness of B, C, and D samples decreased by 20.92%, 28.52%, and 32.74%, respectively, compared with the control. The reduction coefficient k indicated a lower firming rate in the presence of trehalose.

The Trehalose Effect on the Enthalpy of Retrogradation

Prior to baking, starch crystalline regions are primarily composed of the linearly aligned nonreducing ends of amylopectin molecules. During baking, the starch is gelatinized and the crystalline order is largely lost. Retrograded amylose forms a strong hydrogen bond between molecules and forms a cement-like bond in amorphous regions (6). This may be the main reason why bread or cooked rice becomes hard upon staling. In this experiment, recrystallization of amylopectin occurred gradually, followed by gathering of branched chain molecules. DSC was applied to measure the structural changes of starch in the bread during its aging process. When staled bread was heated in the DSC, a prominent endothermic peak

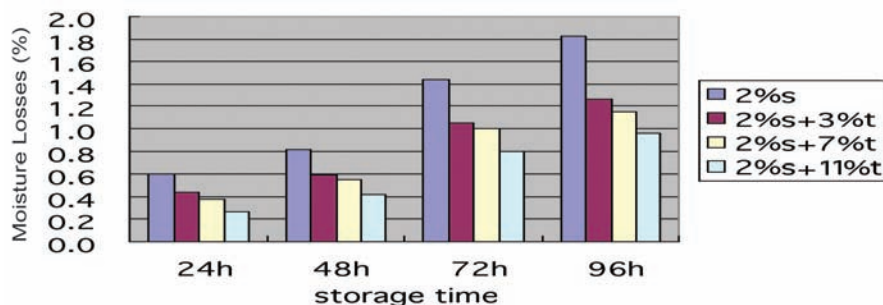


Fig. 1. The influence of trehalose on the moisture retention of bread crumb when stored at 22°C. t—trehalose; s—sucrose.

Table I. Bread quality evaluated by the panel

Bread	Specific volume (cm ³ /g)	Sensory evaluation (score out of 100)
A (basic, as control)	4.78 \pm 0.10	67
B (A + 3% trehalose)	4.88 \pm 0.48	78
C (A + 7% trehalose)	5.10 \pm 0.44	97
D (A + 11% trehalose)	5.05 \pm 0.24	98

Table II. Effects of sugars on hardening rate of bread

Bread	Hardening rate (g/d)	R ²
A (basic, as control)	9.0343	0.9924
B (A + 3% trehalose)	7.3286	0.9939
C (A + 7% trehalose)	5.9229	0.9899
D (A + 11% trehalose)	5.5914	0.9958

emerged around 50°C, which was not observed in the fresh bread, and notably increased with storage time. The endothermic peak was due to the melting of retrograded amylopectin.

As shown in Table IV, the degree of retrogradation of starch in bread crumb was

calculated from the endothermic enthalpy (ΔH) of retrograded amylopectin using DSC. The value of ΔH had significant differences between the controls and samples. The ΔH of samples containing 11% trehalose, 7% trehalose, and 3% trehalose decreased by 87%, 48%, and 43%, respec-

tively, which indicated that trehalose had a strong effect on anti-aging.

The Change of T_g During Retrogradation

There was a strong correlation between the glass transition temperature of food products and their stability (shelf life). When the storage temperature is lower than T_g , foods tend to be stable. T_g can be used to predict the extent of staling.

The T_g of our stored bread (4°C, 4 days) is shown in Table IV. The highest T_g was observed in 11% trehalose, which rose by 21.6°C compared with the control. The T_g of bread containing 7% trehalose rose 18.6°C, which also proved that trehalose could enhance the T_g of bread. The onset temperature (T_O), peak temperature (T_P), and conclusion temperature (T_C) were also determined. The retrogradation temperature was determined by the equation $\Delta T_r = T_C - T_O$. Trehalose (11%) enhanced the onset temperature (T_O) and retrogradation temperature (ΔT_r) compared with the control dough.

Correlation Analysis of Major Indices of Bread During Storage

As shown in Table V, trehalose had high correlation with the rate of moisture loss, hardness of bread crumb, the rate of hardening, k , and glass transition temperature of breads. Trehalose had negative correlation with the moisture loss of bread crumb and hardness, which indicated that trehalose promoted preservation of water in bread and reduced the hardness of crumb bread. Trehalose had negative correlation with k and ΔH , showing that trehalose could reduce the rate of bread firming and retard retrogradation. Trehalose had positive correlation with T_g , which indicated that trehalose can increase the T_g of bread.

CONCLUSIONS

Adding trehalose to bread can improve quality and effectively retard retrogradation. Trehalose can decrease the hardness of bread crumb and the rate of bread firming; enhance glass transition temperature

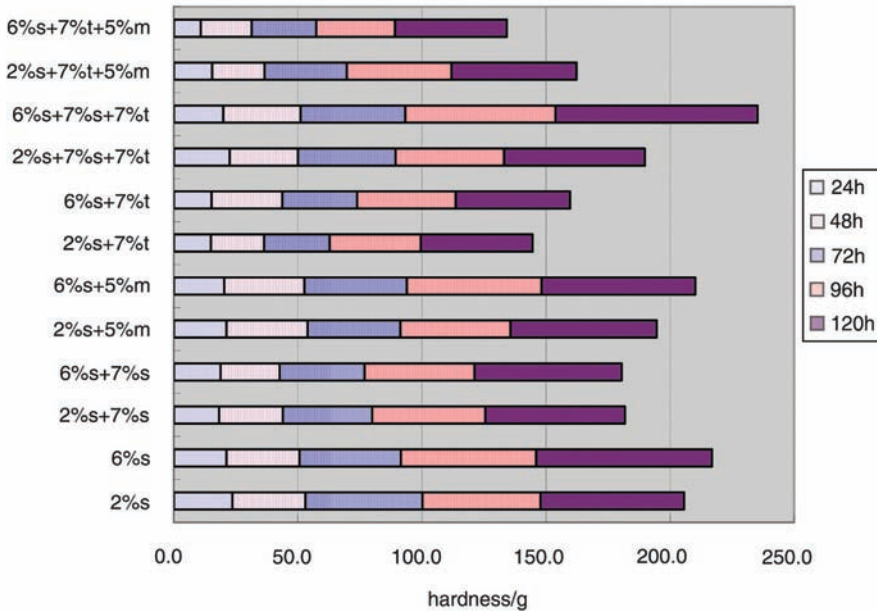


Fig. 2. The effects of the sugars on bread crumb hardness when stored at 22°C. m—maltose; t—trehalose; s—sucrose.

Table III. Avrami parameters for crumb hardness increase curves ($22 \pm 1^\circ\text{C}$)

Bread	F_a (g)	F_∞ (g)	$F_\infty F_0$ (g)	K (day ⁻¹)	r^2
A (basic, as control)	11.1	56.3	45.2	0.4432	0.9475
B (A + 3% trehalose)	9.6	47.6	38.0	0.3505	0.9365
C (A + 7% trehalose)	8.7	39.8	31.1	0.3168	0.9263
D (A + 11% trehalose)	7.9	37.0	29.1	0.2981	0.9964

Table IV. Effects of trehalose on thermal properties of bread (stored for 4 days at 4°C)

Samples	T_O (°C)	T_P (°C)	T_C (°C)	ΔT_r (°C)	ΔH (J/g)	T_g (°C)
A (basic, as control)	43.5	54.8	68.9	25.4	4.135	29.2
B (A + 3% trehalose)	37.8	52.8	65.9	28.1	2.359	30.7
C (A + 7% trehalose)	41.9	55.8	69.4	27.5	2.163	47.8
D (A + 11% trehalose)	47.7	54.8	65.6	17.9	0.522	50.8

Table V. Correlation analysis of major indices of bread during storage

Item	Trehalose	Moisture retention of bread crumb	Rate of moisture retention	A_w	Hardness	Rate of hardening	k	ΔH (J/g)	T_g (°C)
Trehalose	1.0000								
Moisture retention of bread crumb	-0.5957	1.0000							
Rate of moisture retention	-0.9325	0.6663	1.0000						
A_w	0.2415	0.6124	-0.0169	1.0000					
Hardness	-0.8356	0.9336	0.8827	0.3294	1.0000				
Rate of hardening	-0.6021	1.0000	0.6715	0.6064	0.9363	1.0000			
k	-0.8338	0.9391	0.8658	0.3260	0.9988	0.9418	1.0000		
ΔH	-0.9459	0.5771	0.9926	-0.1363	0.8275	0.5830	0.8101	1.0000	
T_g	0.9410	-0.7478	-0.8277	0.0653	-0.8917	-0.7527	-0.9039	-0.8146	1.0000

(T_g), moisture retention, and the specific volume of breads; and improve bread sensory quality.

Fructose, glucose, sucrose, maltose, and complex sugars (See Fig. 2. A variety of sucrose, maltose, and trehalose mixtures were used) were also inspected in the same way; however, none of these exhibited advantages over trehalose. The interference of the other sugars can be seen in Figure 2, where the mixture of maltose (m) and trehalose (t) was not as effective as that of trehalose alone (with 2% sucrose as a basic component).

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