BREAD STALING STUDIES. II. EFFECT OF PROTEIN CONTENT AND STORAGE TEMPERATURE ON THE ROLE OF STARCH[1]

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ABSTRACT

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The effect of flour protein content (11.0, 13.9, and 21.6% on a 14% mb) and storage temperature (21°, 30°, and 35°C) on the role of starch during bread staling was investigated. The recovery of soluble starch extracted from bread crumb was inversely and positively related to the protein content of the flour and storage temperature, respectively. The soluble starch from fresh crumb was predominantly amylopectin, which progressively decreased as bread aged. Although the amount of amylose in the soluble starch was small, it sharply decreased during the first day of storage and thereafter the changes were minor. This implies that the amylose contributes to staling primarily during the first day of storage. The amylose was essentially absent in the soluble starch leached from the bread produced from the flour with the highest protein content, suggesting that the role of amylose in staling diminishes as the flour protein content increases. The amylose content in the soluble starch leached from the crumb after 10-min removal of the bread from the oven was small, indicating that the majority of the amylose retrograded during baking and subsequent cooling. Firmness data on refreshened bread showed that the staling of bread at 21°C was due primarily to starch crystallization. At 30° and 35°C, some other factor in addition to the starch played an important role in firming. It was calculated that starch crystallization in the crumb was about two and four times slower at 30° and 35°C, respectively, compared to the time at 21°C. Kinetic studies indicated that bread staling at 35°C was still basically characterized by the crystallization of the starch fraction in the crumb.

Although little doubt exists that the basic mechanism of bread staling involves changes analogous to crystallization of the starch component of the crumb (1), it is not unequivocally established which fraction of starch in the crumb is responsible for staling.

Schoch and French (2) theorized that amylopectin is primarily responsible for bread staling, since the water-soluble starch leached from the crumb of fresh bread at 30°C was predominantly amylopectin. However, considerable information is available to show that retrogradation of the amylose fraction is also involved in staling (3).

Little information is available on the staling of bread stored at temperatures above 30°C. Colwell *et al.* (4) reported that at 32° and 43°C, starch gels aged three to four times slower than bread at the same temperature, suggesting less starch crystallization effect at the elevated temperatures. However, no differences in aging of wheat starch gels and bread at 30°C were found in previous studies from this laboratory (5). Colwell *et al.* (4) also reported that during a storage period of 6 days at 43°C, starch crystallization contributed only about 20% to the bread crumb firmness.

It was suggested (6) that the firming of bread at constant moisture is controlled

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by crystallization of the starch and by a process having a positive temperature coefficient. Reexamining the results of Axford *et al.* (7) on the time constants for the staling of bread at temperatures from −1° to 66° C, Zobel (1) indicated that a plot of time constants against temperatures showed two slopes with the break at about 30° C, where phase transitions in water were believed to occur (8). It was reported (9) that the swelling of crumb as a function of temperature showed a change in slope above 30° C.

Willhoft (10) postulated that the protein of natural crumb undergoes certain changes during baking and subsequent storage. It is known (6) that, unlike the heat-reversible character of the retrogarded starch in bread crumb, the protein in stale crumb is not reversed by heating. Refreshening stale bread by heating to baking temperatures does not reverse the moisture redistribution phenomenon (10). If changes in protein and moisture transfer between components contribute to bread staling, refreshened bread should be firmer than the corresponding original fresh bread at a given moisture level.

The purpose of this paper is: a) to ascertain which component of starch is responsible for staling; b) to examine the effect of flour protein content on the changes in starch fractions at different storage temperatures; and c) to study the role of starch at elevated storage temperatures by refreshening the stale bread.

MATERIALS AND METHODS

Flour and Bread Samples

Flours and the bread-baking method used in this experiment were described in a previous paper (11).

Bread after baking was cooled at room temperature for 2 hr and stored at 21°, 30°, and 35° C in sealed containers to prevent moisture loss. At 0, 1, 2, and 5 days of storage, bread crumb was removed, freeze-dried, and ground on a Wiley mill to pass through a 60-mesh sieve.

Isolation of Total Water-Solubles

The total water-solubles were extracted from the bread crumb by shaking the crumb (5 g) with 50 ml of distilled water on a wrist-action shaker for 20 min. The slurry was centrifuged (2,000 × *g*, 5 min) and filtered. This procedure was repeated twice and the combined supernatant freeze-dried.

Isolation of Soluble Starch

The soluble starch was isolated according to the procedure of Schoch and French (2) from the total water-solubles prior to freeze-drying. To the total water-solubles, 3 vol of methanol was added and heated on a steam bath for 1 hr. After standing overnight at 4° C, the flocculated soluble starch was collected and dried in a vacuum oven.

Determination of Amylose and Amylopectin Content in the Soluble Starch

The amylose content in the soluble starch was determined according to the procedure of Williams *et al.* (12). A standard curve for amylose was prepared using the amylose isolated from the control flour (13.9% protein on a 14% mb).

The amylopectin content in the soluble starch was obtained by subtracting the amount of amylose content from the total amount of soluble starch.

Acetic Acid Solubles and Protein Extractability

The acetic acid solubles were extracted from the bread crumb by shaking the crumb (2 g) with 30 ml of 0.05*N* acetic acid on a wrist-action shaker for 30 min. The slurry was centrifuged (2,000 × *g*, 20 min), filtered, and freeze-dried.

Protein extractability from the crumb was expressed as a fraction of the protein content of the flour on a dry basis.

Determination of Protein Content

Protein contents (N × 5.7) of water-solubles and acetic acid solubles were determined by AACC Method 46-13 (13).

Refreshening of Stale Bread

Crumb firmness was measured with an Instron Universal Testing Instrument (Instron Corporation, Canton, Mass.) on bread during 5 days of storage at different storage temperatures. The kinetic parameters on the aging of bread stored at 35°C were determined as described previously (11). After 5 days of storage, some loaves were transferred to storage at 2°C for a further 7 days' storage followed by measurement of crumb firmness. In addition, crumb firmness measurements were made on loaves stored at 2°C for 7 days. Refreshening of stale bread was achieved by heating it to 65°C in a microwave oven. Refreshened bread was cooled at room temperature for 1 hr prior to measuring crumb firmness.

RESULTS AND DISCUSSION

The effect of staling on the quantity and composition of soluble starch extracted from bread crumb produced from flour of different protein content and stored at 21° and 30°C is given in Table I. The recovery of the soluble starch from fresh bread crumb was inversely related to the protein content of flour. The soluble starch content progressively decreased as bread aged, but the decrease was less with bread stored at 30° than it was with bread stored at 21°C.

The composition of the soluble starch (Table I) indicates that the soluble starch leached from the crumb of fresh bread was predominantly amylopectin, confirming the observations of Schoch and French (2). No differences were observed in the amount of amylose fraction between the two storage temperatures. Amylopectin content decreased slower at 30° than at 21°C with bread aging, suggesting that the differences observed in the staling rates at these two temperatures (11) were due mainly to the amylopectin fraction of starch in the crumb.

Table II shows data on the recovery of soluble starch and its composition extracted from bread crumb B stored at 35°C. The results in Tables I and II indicate that the recovery of the soluble starch and the decrease of amylopectin fraction in the soluble starch are positively related to storage temperature.

Although the amylose content in the soluble starch leached from the fresh bread crumb (Tables I and II) was small, it also progressively decreased during bread staling. The largest decrease, however, occurred during the first day of storage; thereafter, the changes were small. These results agree with those of Schoch and French (2). They considered the retrogradation of the small amount of amylose fraction to be negligible, and concluded that amylose had no effect on staling. Zobel (1) reported that when refreshened by heating at 95° C under moist conditions, the X-ray pattern of stale bread reverts to that of freshly baked bread, *i.e.*, V plus amorphous. Zobel (1) concluded that the amylopectin fraction of starch undergoes change during bread staling, since only a retrograded amylopectin gel can be reversed under these conditions. However, the results of Schoch and French (2) showed that when the soluble starch was extracted from stale bread at 50° C, the content of amylose was fully reversed to that of fresh bread crumb. In studies on the effect of pentosans on the retrogradation of wheat-starch gels (5) it was demonstrated that amylose, albeit small, retrograded

TABLE I
Effect of Staling on the Quality and Composition of Soluble Starch Extracted from Bread Crumb[a]

		Soluble Starch, %		Composition of Soluble Starch			
				Amylose, %		Amylopectin[c], %	
Bread[b]	Day	21° C	30° C	21° C	30° C	21° C	30° C
A	0	3.34	3.34	0.52	0.52	2.82	2.82
	1	2.16	2.32	0.19	0.20	1.97	2.12
	2	1.72	1.78	0.14	0.13	1.58	1.65
	5	1.22	1.74	0.10	0.13	1.12	1.61
B	0	2.36	2.36	0.39	0.39	1.97	1.97
	1	1.60	1.52	0.12	0.13	1.48	1.49
	2	1.14	1.40	0.06	0.08	1.08	1.32
	5	1.08	1.30	0.06	0.07	1.02	1.23
C	0	1.49	1.49	0.06	0.06	1.43	1.43
	1	1.35	1.42	0.04	0.04	1.31	1.38
	2	1.12	1.37	0.03	0.04	1.09	1.33
	5	1.02	1.15	0.03	0.03	0.99	1.12

[a]All results reported on a dry basis.
[b]Flour protein contents (N × 5.7) of A, B, and C were 11.0, 13.9, and 21.6%, respectively, on a 14% mb.
[c]By difference.

TABLE II
Effect of Staling on the Quantity and Composition of Soluble Starch Extracted from Bread Crumb B[a]

Day	Soluble Starch %	Composition of Soluble Starch: Amylose %	Composition of Soluble Starch: Amylopectin[b] %
0	2.20	0.31	1.89
1	1.70	0.14	1.56
2	1.52	0.12	1.40
5	1.38	0.08	1.30

[a]13.9% flour protein on a 14% mb; stored at 35° C. All results reported on a dry basis.
[b]By difference.

over the first day of storage. These results, together with those in Tables I and II (*i.e.*, a sharp decrease of the amylose fraction in the soluble starch over the first day of storage) imply that amylose contributes to bread staling, even though the amylose content in the soluble starch of fresh bread crumb was initially small.

The composition of the soluble starch extracted from bread C (Table I) indicates that the amount of amylose in the soluble starch was negligible, suggesting that for bread C the amylopectin fraction was primarily responsible for staling. These results imply that the effect of amylose on staling diminishes as flour protein content increases.

Table III shows data on the recovery of soluble starch and its composition extracted from bread crumb B during the first 12 hr of storage after baking. The amount of amylose in the soluble starch leached from bread crumb after 10-min removal of the bread from the oven was small, but a sharp decline in the amylose content occurred during the 5-hr cooling period after baking. These results support earlier observations of Schoch and French (2) that most amylose retrogradation takes place during baking and subsequent cooling of the loaf.

Table IV shows the recovery and protein content of the water-solubles extracted from bread crumb with aging. The yield of solubles decreased as the bread aged for breads A and C, but remained essentially constant for bread B. The protein content in the solubles showed a considerable increase for breads A and B stored at 30° C for 5 days. For bread C, however, the protein content in the solubles increased after 2 days of storage, and decreased thereafter.

Table V shows the effect of staling on the protein extractability from the crumb. The acetic acid solubles for bread A decreased as bread aged, while those for breads B and C remained fairly constant. The protein extractability for bread A was essentially unchanged, while those for breads B and C slightly increased after 5 and 2 days of storage, respectively.

No definite conclusion could be drawn from the results in Tables IV and V as to whether protein undergoes certain changes at higher storage temperature. Bechtel and Meisner (14), using a synthetic dough system of gluten and wheat starch, reported that changes in starch were responsible for the staling during the first 3 days and thereafter gluten affected the staling properties of bread.

TABLE III
Effect of Staling on the Quantity and Composition of Soluble Starch Extracted from Bread Crumb B (13.9% Flour Protein on a 14% mb) during 12 hr of Storage[a]

Time hr	Storage Temp. °C	Soluble Starch %	Composition of Soluble Starch: Amylose %	Amylopectin[b] %
0.1	Room temp.	2.51	0.60	1.91
2	Room temp.	2.34	0.39	1.95
5[c]	21	1.86	0.22	1.64
	30	1.92	0.25	1.67
12[c]	21	1.74	0.18	1.56
	30	1.85	0.21	1.64

[a]All results reported on a dry basis.
[b]By difference.
[c]Breads were cooled for 2 hr at room temperature and then stored at 21° and 30°C for 3 and 10 hr, respectively.

Robertson and Emami (15) showed with a liquid-jet penetrometry technique that the penetration response of bread crumb demonstrated a positive temperature coefficient, *i.e.*, greater increase of resistance at higher temperature. These results were suggested to be consistent with a response dependent on changes of gluten properties. In the study of the moisture redistribution between gluten and starch interface of baked dough, Willhoft (10) postulated that, during baking and

TABLE IV
Effect of Staling on the Water-Soluble Material Extracted from Bread Crumb[a]

Bread[b]	Day	Solubles, %		Protein (N × 5.7), %		Protein-Free Solubles, %	
		21°C	30°C	21°C	30°C	21°C	30°C
A	0	15.0	15.0	8.0	8.0	13.8	13.8
	1	14.1	14.1	8.4	8.5	12.9	12.9
	2	13.8	11.0	8.5	8.4	12.6	11.9
	5	13.1	13.1	8.5	12.6	12.0	12.0
B	0	10.5	10.5	9.2	9.2	9.6	9.6
	1	10.2	10.1	9.2	8.8	9.2	9.2
	2	10.2	10.2	9.6	9.3	9.2	9.2
	5	10.2	10.5	9.7	12.0	9.2	9.2
C	0	10.4	10.4	13.0	13.0	9.1	9.1
	1	10.5	10.2	13.4	13.6	9.1	8.8
	2	9.2	9.0	17.2	17.4	7.6	7.4
	5	9.6	9.6	15.9	14.9	8.1	8.2

[a]All results reported on a dry basis.
[b]Flour protein contents (N × 5.7) of A, B, and C were 11.0, 13.9, and 21.6%, respectively, on a 14% mb.

TABLE V
Effect of Staling on Protein Extractability from Bread Crumb[a]

Bread[b]	Day	Acid-Solubles, %		Protein (N × 5.7), %		Protein Extractability[c], %	
		21°C	30°C	21°C	30°C	21°C	30°C
A	0	13.9	13.9	6.8	6.8	6.7	6.7
	1	11.8	12.0	8.7	7.1	7.3	6.1
	2	10.9	11.1	8.5	8.0	6.6	6.3
	5	11.0	11.0	7.5	8.2	5.9	6.4
B	0	8.1	8.1	10.6	10.6	5.4	5.4
	1	8.0	7.6	10.6	10.2	5.3	4.9
	2	7.9	7.8	11.9	12.0	5.9	5.9
	5	8.3	8.3	12.2	12.3	6.3	6.4
C	0	7.5	7.5	21.1	21.1	5.9	5.9
	1	7.7	7.4	20.1	22.2	5.7	6.1
	2	6.7	6.9	29.9	26.8	7.4	6.8
	5	7.1	7.6	23.4	21.6	6.1	6.1

[a]All results reported on a dry basis.
[b]Flour protein contents (N × 5.7) of A, B, and C were 11.0, 13.9, and 21.6%, respectively, on a 14% mb.
[c]Based on % of total flour protein content (N × 5.7) on a dry basis.

subsequent storage, the hydrated protein of natural crumb undergoes a mild, first-order transformation which may be associated with an increase in the extent of denaturation of the protein and may possibly involve a change in the order of protein configuration.

TABLE VI
Data on Crumb Firmness (g/cm) of Bread Stored at 21°, 30°, and 35°C

		Bread Storage Temperature		
		21°C	30°C	35°C
Crumb firmness at 0 day		6.1	6.1	7.5
Increase in crumb firmness after 5 days' storage	A	37.4	29.9	19.1
Limiting value of crumb firmness[a]	B	69.3	69.3	63.0
Limiting value after 5 days' prior storage at storage temperature[b]	C	76.9	80.5	93.6
Crumb firmness of B after heating to 65°C	D	7.2	7.2	8.5
Crumb firmness of C after heating to 65°C	E	9.9	22.5	24.0
Difference between E and D	F	2.7	15.3	15.5
Difference between A and F	G	34.7	14.6	3.6

[a]Determined at 2°C after 7 days' storage.
[b]Determined after 5 days at storage temperature followed by 7 days' storage at 2°C.

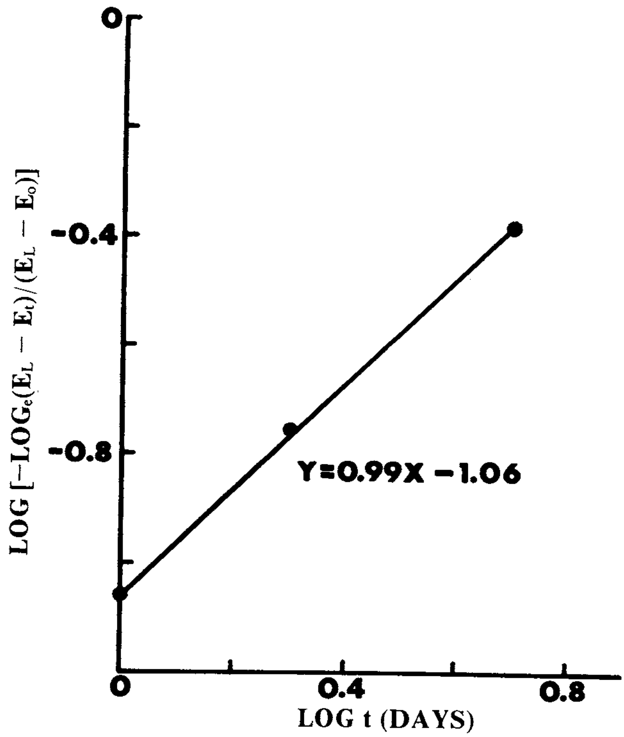

Fig. 1. Plot of log ($-\log_e \frac{E_L - E_t}{E_L - E_t}$) against log t of bread produced from flour B (13.9% protein on a 14% mb) stored at 35°C.

Data on crumb firmness of bread stored at different temperatures and of refreshened bread are presented in Table VI. The results show that when the bread stored at 2°C for 7 days was refreshened, the crumb firmness was fully reversed to that of the fresh bread (B and D). Since retrograded starch in the stale crumb is heat-reversible (6), these results indicate that at 2°C starch crystallization is primarily responsible for bread staling. Therefore, when bread was subjected to 5 days' storage at 21°, 30°, and 35°C prior to storage at 2°C for 7 days (C in Table VI) the increased values of the limiting modules were due to the uncrystallized starch in the crumb after 5 days at the different storage temperatures. These increased values for the limiting modulus increased as the storage temperature increased (C in Table VI), suggesting less crystallization of starch at higher temperatures.

When the bread stored at 21°, 30°, and 35°C for 5 days followed by 7 days' storage at 2°C was refreshened, the crumb firmness values were 9.9, 22.5, and 24.0 g/cm, respectively (E in Table VI). Although the crumb firmness of the refreshened bread at 21°C was close to that of original fresh bread, the bread stored at 30° and 35°C showed considerably higher crumb firmness values. These results imply that additional factors are involved in the firming process at these temperatures, since neither protein changes (6) nor moisture redistribution (10) are heat-reversible during bread staling.

The differences between the values of E (Table VI) and those of crumb firmness of bread stored at 2°C for 7 days followed by heating to 65°C (D in

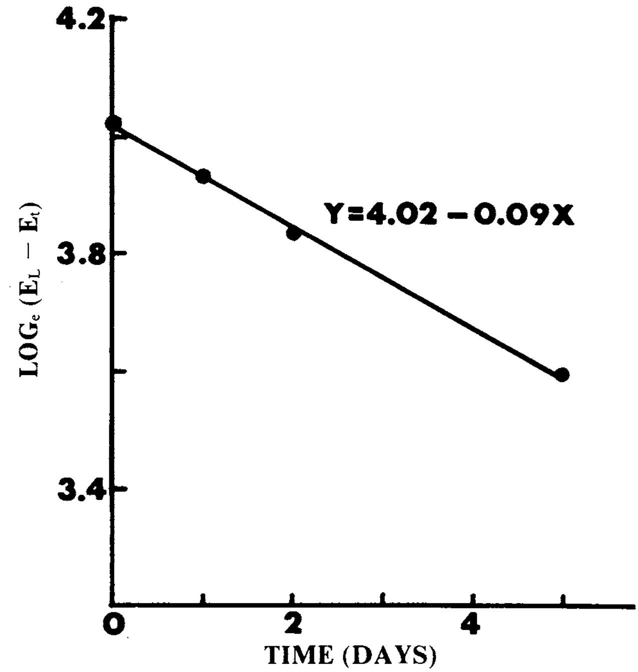

Fig. 2. Plot of $\log_e(E_L - E_t)$ against time of bread produced from flour B (13.9% protein on a 14% mb) stored at 35°C.

Table VI) were 2.7, 15.3, and 15.5 g/cm at 21°, 30°, and 35° C, respectively. These values indicate that the crumb firmness increased by some factor other than starch. Therefore, of the 37.4, 29.9, and 19.1 g/cm increase in crumb firmness after 5 days' storage at 21°, 30°, and 35° C, respectively (A in Table VI), it is possible that 34.7, 14.6, and 3.6 g/cm at the corresponding storage temperature (G in Table VI) was due to starch crystallization. This would indicate that starch crystallization contributed about 93, 50, and 20% of the total crumb firmness at 21°, 30°, and 35°C, respectively, during 5 days' storage. These results in turn imply that starch retrogradation in the crumb is about two and four times slower at 30° and 35° C, respectively, compared to 21° C.

From Fig. 1, the Avrami exponent (n) for the bread stored at 35° C was 0.99. The same value (*i.e.*, n = 1) was reported for bread stored at 21° and 30° C (11), indicating that the basic mechanism of bread staling is the same at all storage temperatures. The value of the rate constant corresponded to 0.09 reciprocal days (Fig. 2), giving a time constant of 11.83 days. This value of the time constant indicates that the bread stored at 35° C aged about 2 and about 1.5 times slower than the bread stored at 21° and 30° C, respectively (11). This also can be explained by the total increase in crumb firmness after 5 days' storage: 37.4, 29.9, and 19.1 g/cm at 21°, 30°, and 35° C, respectively (A in Table VI).

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