Staling of White Bread: Changes in Carbonyl Composition and Glc Headspace Profiles

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A direct relationship was found between total glc headspace area, total carbonyl content, and the score of an organoleptic evaluation of white bread. Panel scores increased, indicating a less desirable product, as total carbonyl content and total glc headspace area increased during the staling of bread. The initial decrease in total carbonyl content and total glc headspace area between the oven-fresh bread and the 1-day-old bread has to be attributed to the continued volatilization of certain flavor compounds from the warm loaf of bread. The percentage of aldehydes in the mixture of carbonyl compounds in fresh bread was 72.9, decreasing to 15.1 in 5-day-old bread, while the percentage of ketones increased from 27.1 to 84.9. The decrease in the aldehyde content contributes to the decreased flavor and taste acceptability of stale bread.

The research on bread flavor has been published in the form of comprehensive reviews (Otterbacher, 1959; Wiseblatt, 1961; Johnson, 1963, 1967; Collyer, 1964; de Figueiredo, 1964; Coffman, 1967). Carbonyl compounds have been found to be the principal flavor components in all types of bread. A correlation between the taste of bread and the total volatile aldehyde content has been established (Wiseblatt, 1961; Carlin, 1959). Flavor is derived from the ingredients of the formulation (McWilliams and Makey, 1969; Jackel, 1969) modified through the formation of new compounds formed under the constantly changing conditions of moisture, pH, and temperature of the baking process (Niederauer, 1969). The amounts of carbonyl compounds were found to depend on baking time. Doubling the baking time caused a fourfold increase in carbonyl compounds. This explains why dark breads such as rye and Pumpernickel have the highest total carbonyl content while white bread has the lowest (Niederauer, 1969).

The flavor of bread, however, is lost quite rapidly upon cooling and standing, which has been explained in a number of ways. The level of carbonyl compounds in a fresh loaf of bread is almost ten times as high in the crust as it is in the crumb (Jackel, 1969), but there seems to be a partial diffusion of aldehydes from the crust into the crumb upon aging (Wiseblatt, 1961), while other carbonyl compounds are lost by volatilization or possibly by chemical reactions such as oxidation (Schoch, 1965; Niederauer, 1969).

Bread aroma, however, may be regenerated by simply heating a stale slice or a complete loaf of bread. This phenomenon indicated to Schoch (1965) that bread flavor substances are not lost through volatilization or chemical reaction, but that they might be locked within the linear fraction of the starch through formation of helical complexes, making these flavor compounds insoluble and thereby imperceptible to the taste. He stated that reheating a stale loaf of bread would break such helical complexes, releasing the flavor components.

Gas chromatograms of 6-day-old bread were quite similar to those of freshly baked bread (Carlin, 1959). Only two compounds, ethanol and acetone, were identified.

This study was undertaken to devise a rapid and simple technique for the flavor evaluation of bread and to study the change in the principal flavor components, the carbonyl compounds, as bread becomes stale.

EXPERIMENTAL METHODS

Baking Procedure. White bread was baked by the straight dough procedure using a baker's patent flour (12.4% protein, 0.44% ash), 6% sugar, 4% NFDM, 3% shortening, 2.5% yeast, 2% salt, 0.5% yeast food, and 0.3% calcium propionate. To avoid batch to batch variation in bread production, one large batch of bread was baked, sufficient to provide enough loaves for the entire study. The absorption was determined with the mixograph. Fermentation time was 120 min at 30°C. The loaves were scaled 500 g each. They were mechanically molded, proofed to height at 35°C, and were baked at 218°C for 20 min. Two hours after baking the loaves were wrapped and sealed in polyethylene bags and stored at room temperature until needed.

Panel Evaluation. Sensory evaluation of the bread was conducted by the rating scale method as described by Bechtel and Meisner (1954) using 20 untrained college students. An eight-point scale ranging from "like extremely" (1) to "dislike extremely" (8) was selected to differentiate between samples on the basis of aroma and taste. The panel test was conducted 2 hr after baking and after 2 and 4 days of storage at room temperature. A control sample, baked each day a panel test was given, was included in each test.

The test samples consisted of a half-slice of bread, 0.5-in. thick. Samples were packaged immediately after slicing in moisture-proof bags. Each sample was coded with a randomly selected two-digit number. The judgments of the 20 panel members were averaged.

Total Carbonyl Content. Carbonyl compounds were extracted with chloroform from 50 g of bread (1½ center slices) and determined by the method of Lappin and Clark (1951). The optical density of the hydrazones was measured at 480 mμ using a Bausch & Lomb Model 20 spectrophotometer. A standard curve was prepared using butanal as the standard and results were expressed as ppm relative to the color of that standard. Total carbonyl content was determined each day for 6 days from a freshly cut stored loaf of bread. The initial determination was made 2 hr after baking.

Aroma Profiles. For glc headspace analyses 50 g of bread (1½ center slices) were placed in 1-pt screw-top glass jars and heated in a 60°C water bath for 30 min. Two milliliter vapor samples were taken through a septum in the lid of the jar and were injected into the gas chromatograph. The

Table I. The Effect of Bread Storage on Flavor Score, Carbonyl Content, and glc Headspace Area

Bread storage, days	Average panel flavor score	Total carbonyl compounds, ppm	Total glc headspace area, cm²
0	1.95	224	64.2
1		136	61.9
2	3.55	176	63.7
3		280	66.6
4	4.50	280	67.8
5		328	69.5

Table II. Bread Carbonyl Composition

	Freshly baked bread		5-day-old bread	
	glc area, cm²	%	glc area, cm²	%
Formaldehyde	1.2	2.5	0.1	0.2
Acetaldehyde	2.0	4.0	0.4	0.9
Acetone	2.2	4.5	0.8	1.7
Propanal	6.7	13.8	0.6	1.3
Butanal	11.1	22.7	0.3	0.8
2-Butanone	5.4	11.0	6.4	14.7
2-Hexanone	4.0	8.2	29.4	67.4
Hexanal	4.3	8.9	1.0	2.4
2-Heptanone	1.7	3.4	0.5	1.1
Heptanal	0.6	1.3		
Nonanal	6.9	14.2	4.1	9.5
Unknown	2.7	5.5		
	48.6	100.0	43.6	100.0

above procedure was compared with one in which the loaf of bread was simply cut in half and a 2-ml vapor sample taken immediately out of the center of the loaf. Resolution and identification was accomplished using a Hewlett-Packard Model 5750 gas chromatograph equipped with a dual column hydrogen flame ionization detector. The 8-ft × ⅛-in. o.d. stainless steel columns were packed with 20% Carbowax 20M on 60/80 mesh HMDS Chromosorb P. The injection port was 190°C, the column temperature 85°C isothermal, and the flame detector 250°C. The carrier gas was nitrogen with a flow rate of 65 ml/min. The sensitivity was 2 × 10, with a chart speed of 0.5 in./min.

Tentative identification was accomplished by measuring relative retention times of known compounds reported to be present in bread (Wiseblatt, 1961). These compounds were separated under the same gas chromatographic conditions. Headspace analyses were made each day for 6 days from a freshly cut, stored loaf of bread.

Carbonyl Composition. Carbonyl compounds were extracted as described above and were converted to their 2,4-dinitrophenylhydrazones. The mixture of hydrazones was separated by glc using the flash-exchange technique of Stephan and Teszler (1960). Resolution was accomplished using the same glc conditions described above. To identify the carbonyl compounds, hydrazones of known carbonyl compounds were prepared, and their retention times were measured and compared with the retention times of compounds in the unknown mixture. Additionally, they were compared with retention times reported by Lorenz and Johnson (1971) and Maga (1970), who separated carbonyl compounds under similar conditions. The total area under the peaks was calculated by multiplying the height of each peak by its width at one-half height and adding the individual peak areas.

RESULTS AND DISCUSSION

Since the carbonyl compounds were found to be the principal flavor component of bread, it was of interest to see if there is a relationship between total carbonyl content and results of a sensory evaluation by a panel. Average flavor scores and total carbonyl content of bread stored for up to 5 days at room temperature are given in Table I. The panel evaluation showed that bread aroma and taste becomes less desirable as bread ages. Four-day-old bread was given a score of 4.50 or "dislike slightly." The total carbonyl content of the 1-day-old bread was lower than that of the freshly baked bread, but then increased gradually with time, exceeding the original total carbonyl content after 3 days of storage.

The initial decrease in total carbonyl content has to be attributed to volatilization of certain flavor compounds from the still warm loaf of bread.

Even though the total carbonyl content of the 2-day-old bread was lower than the original one, the panel score was higher than the initial score of the aroma and taste evaluation by the panel. On day 4, total carbonyl content was slightly higher than the initial one, but the panel score had more than doubled.

Generally, however, aroma and taste acceptability, as determined by the panel, decreased as total carbonyl content increased after the initial decrease in carbonyl content due to volatilization during the first 24 hr of storage. The gradual
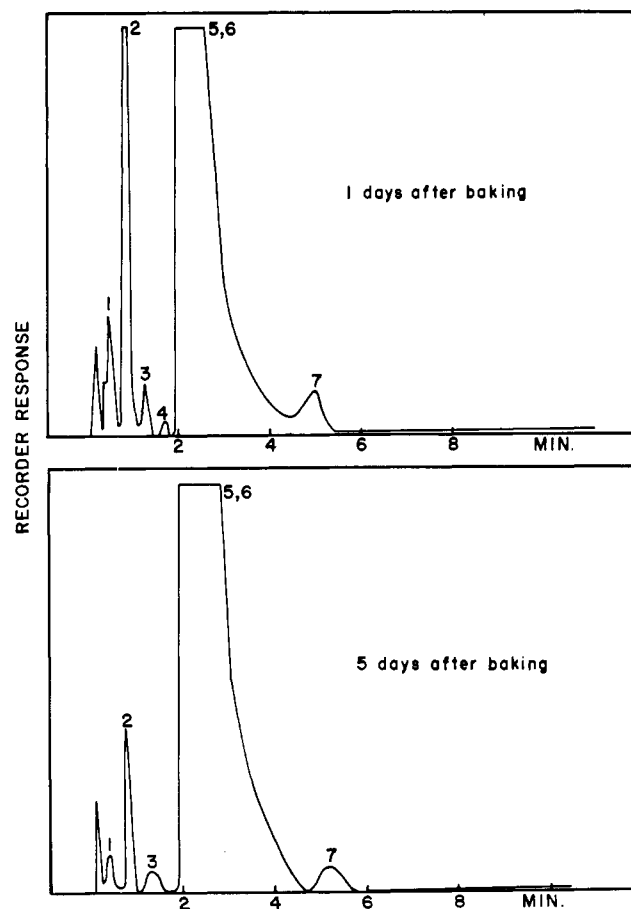
Figure 1. Glc headspace profiles of bread stored 1 and 5 days, respectively. Tentative identification: 1, acetaldehyde; 2, acetone; 3, 2-butanone; 4, methanol; 5, ethanol; 6, 2-hexanone; 7, butanol; 8, pentanol

increase in total carbonyl content, which coincides with an increase in the panel score, can be attributed to a diffusion of aldehydes from the crust into the crumb, as reported by Wiseblatt (1961) and to chemical reactions such as oxidation in the crumb (Schoch, 1965; Niederauer, 1969). The percentage of ketones in the carbonyl mixture increased from 27.1% in the freshly baked bread to 84.9% in the 5-day-old bread, as seen in Table II.

If the flavor changes during the staling of bread are due to the complexing of flavor compounds with the linear starch fraction as postulated by Schoch (1965), there should be a gradual decrease in total carbonyl content with time of storage, rather than an increase as observed in this investigation unless the carbonyl extraction procedure caused such helical complexes to break.

Glc headspace profiles of bread stored for 1 and 5 days, respectively, are given in Figure 1. Tentatively identified were acetaldehyde, acetone, 2-butanone, methanol, ethanol, 2-hexanone, butanol, and pentanol. The general profile changed very little as the bread staled. Changes included an increase in the ethanol and 2-hexanone peaks, a slight decrease of the 2-butanone, methanol, and butanol peaks, and the disappearance of the pentanol peak. Furfural, the compound reported to be the major constituent of bread volatiles (Rotsch, 1956), was not separated by this technique.

The total glc headspace areas are presented in Table I. Total area decreased during the first 24 hr of storage at room temperature but then increased gradually with time following the same trend as observed for the total carbonyl content. Simplification of this headspace technique by merely taking a vapor sample out of the center of the loaf produced a profile identical to the one presented in Figure 1, except for the size of the peaks, which decreased somewhat.

The data in Table I indicated that there is a direct relationship between total glc headspace area, total carbonyl content, and the score of an organoleptic evaluation in white bread stored between 1 and 5 days at room temperature. Panel scores increased, indicating a less desirable product, as total carbonyl content and total glc headspace area increased. The initial decrease in total carbonyl content and total glc headspace area between the oven-fresh bread and the 1-day-old bread has to be attributed to the continued volatilization of certain flavor compounds from the warm loaf of bread.

A carbonyl compound separation of fresh and 5-day-old bread is presented in Figures 2 and 3. Tentatively identified were formaldehyde, acetaldehyde, acetone, propanal, butanal, 2-butanone, 2-hexanone, hexanal, 2-heptanone, heptanal, and nonanal. The individual peak areas and the percentage of the total composition for each compound are given in Table II. Total peak area decreased slightly as the bread became stale due to the disappearance or decreased amounts of some of the longer chain carbonyl compounds with storage time. The 5-day-old bread contained a smaller percentage of formaldehyde, acetaldehyde, acetone, propanal, butanal, hexanal, 2-heptanone, and nonanal than the freshly baked bread but larger percentages of 2-butanone and 2-hexanone. The heptanal peak and the unknown peak had completely disappeared in the 5-day-old bread.

The total volatile aldehyde content was reported to have a direct bearing on bread flavor and taste (Wiseblatt, 1961). The percentage of aldehydes in the fresh bread was 72.9, decreasing to 15.1 in the 5-day-old bread, while the percentage of ketones increased from 27.1 to 84.9 during the same storage time. The decrease in the aldehyde content contributes to the decreased flavor and taste acceptability of stale bread.
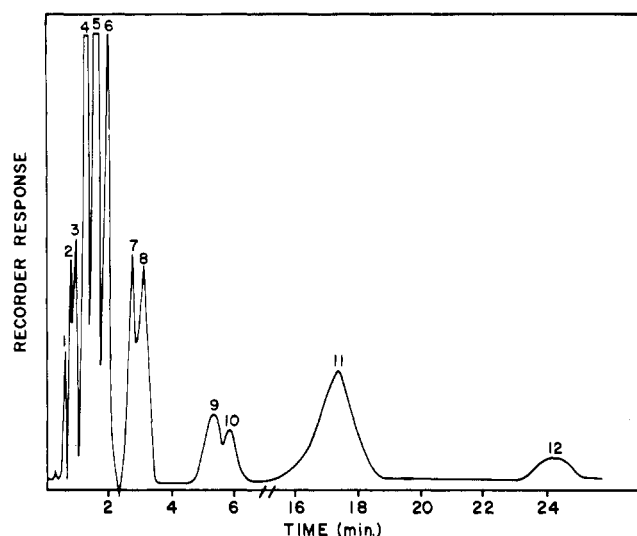

Figure 2. Glc separation of carbonyl compounds in fresh bread. Tentative identification: 1, formaldehyde; 2, acetaldehyde; 3, acetone; 4, propanal; 5, butanal; 6, 2-butanone; 7, 2-hexanone; 8, hexanal; 9, 2-heptanone; 10, heptanal; 11, nonanal; 12, unknown
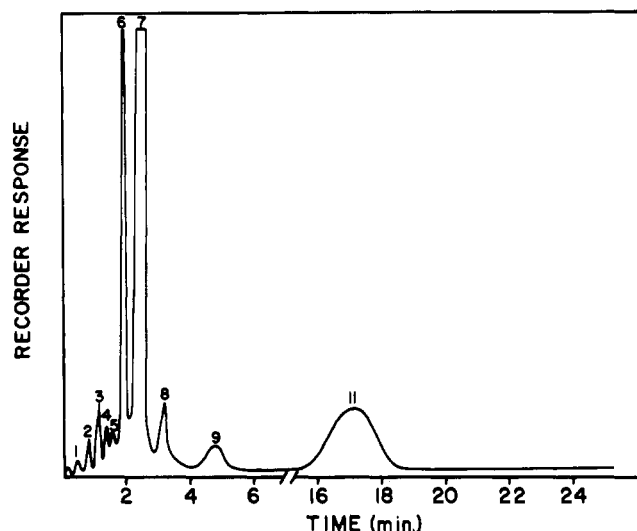

Figure 3. Glc separation of carbonyl compounds in 5-day-old bread. Tentative identification: same as in Figure 2

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