

THE ORIGIN AND FATE OF CERTAIN CARBONYL COMPOUNDS IN WHITE BREAD¹

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ABSTRACT

The effects of baking techniques and storage conditions on development and retention of carbonyl compounds in bread were investigated. The "total" carbonyl content varied with baking techniques and length of storage. Acetone and hydroxymethyl furfural were the major components. Several others were present in minor quantities. With increasing increments of sucrose in the formula, furfural increased slightly and hydroxymethyl furfural increased greatly in the crust but not in the crumb. The "total" carbonyl content of crust increased as the crust color became darker. Adding leucine and xylose to dough increased the iso-valeraldehyde in the crust, whereas iso-valeraldehyde added to dough was largely lost during fermentation and baking. The formation of certain carbonyl compounds in crust would appear to originate mainly from the reaction of certain amino acids with carbohydrates during baking.

In both wrapped and unwrapped bread little decrease occurred in the "total" carbonyl content of crust up to 5 days of storage; thereafter, a rapid decline occurred in the unwrapped bread. In the crumb, "total" carbonyl compounds first increased up to 3 days of storage, then declined rapidly.

Qualitative aspects of compounds found in bread that might be associated with flavor have been reported by several investigators. In 1952, Pence (12) showed that approximately 1% of the baking oven vapor condensate consisted of organic compounds, such as acids, alcohols, esters, and carbonyl compounds. Independently, Baker *et al.* (1) identified pyruvic aldehyde, diacetyl, furfural, isoaldehydes, ketones, and esters in oven vapors, while Wiseblatt (20) later demonstrated the presence of ethyl alcohol, acetaldehyde, and acetic acid. Ng, Reed, and Pence (11), and Wiseblatt and Kohn (21) examined

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bread for compounds responsible for flavor and identified acetaldehyde, acetone, crotonaldehyde, diacetyl, formaldehyde, furfural, hexanone-2, heptanone-3, isobutyraldehyde, 2-methylbutanal, methylglyoxal, methylethylketone, n-valeraldehyde, and pyruvic acid. Kohn, Wiseblatt, and Fosdick (5) demonstrated that acetone, acetaldehyde, n-butyraldehyde, n-hexanal, 2-hexanone, pyruvaldehyde, and isovaleraldehyde are produced during normal dough fermentation, independent of the bacterial count. In addition Rothe and Thomas (14) have reported methional and phenylacetaldehyde in rye bread. Simultaneously, furfural was formed from pentoses and hydroxymethylfurfural (HMF) from hexoses.

Rothe and Thomas (14) stated that volatile aldehydes formed by nonenzymatic browning reaction during baking are a major factor in bread flavor. They observed that both total aldehydes and furfural increased with increased baking time. Rothe (13) reported the formation of a number of aldehydes through reaction of various amino acids with reducing carbohydrates during baking of rye bread. The most reactive amino acids (with corresponding aldehydes in parentheses) were, in decreasing order of reactivity, iso-leucine (2-methylbutanol), leucine (iso-valderaldehyde), valine (iso-butyraldehyde), methionine (methional), alanine (acetaldehyde), and phenylalanine (phenylacetaldehyde).

However, quantitative estimations of bread flavorants have been limited. Visser't Hooft and de Leeuw (19) demonstrated in fresh bread the presence of acetylmethyl carbinol which slowly oxidized to diacetyl. Little diacetyl accumulated in bread crumb, however, because of its high volatility. Thomas and Rothe (17,18) indicated that acetylmethyl carbinol and diacetyl were not likely responsible for bread flavor. Komm and Lehmann (6) found methyl ketones to be more abundant in white than in sour rye bread. Johnson *et al.* (3) measured acetic and lactic acids and ethyl acetate in pre-ferments used in breadmaking. Linko *et al.* (10) have examined pre-ferments for the development of carbonyl compounds.

This paper describes the formation of certain carbonyl compounds during bread baking and changes which occur during subsequent storage of the bread. Techniques to identify and quantitatively determine certain carbonyl compounds in white bread are discussed.

Materials and Methods

Bread Baking. A sponge-dough formula was used as standard for all experiments unless otherwise mentioned. The formulas for baking experiments were as follows:

	<i>Sponge-Dough</i>		<i>Pre-ferment</i>		<i>Straight Dough</i>	<i>No-time</i>
	<i>Sponge</i>	<i>Dough</i>	<i>Pre-ferment</i>	<i>Dough</i>		
Flour, g.	490	210	...	700	700	700
Arkady, g.	3.5	...	2.0	1.5	3.5	3.5
Malted wheat flour, g.	3.5	...	3.5	...	3.5	3.5
Compressed yeast, g.	14	...	14	7	14	35
Water, ml.	294	140	300	134	434	434
Sugar, g.	...	35	14	21	35	35
Salt, g.	...	14	7	7	14	14
Nonfat dry milk, g.	...	21	35	...	21	21
Shortening, g.	...	21	...	21	21	21

Doughs were mixed to optimum consistency. All fermentations were at 86°F. (30°C.) and 90% r.h. A 4-hour sponge time was followed by 30 minutes of floor time and 15 minutes of dry proof time with the sponge-dough method. For the pre-ferment method, a 3-hour liquid ferment was mixed with other ingredients into a normal dough, and was given 40-minute floor time and 20-minute dry proof time. In the straight-dough method, a total primary fermentation time of 180 minutes and two intermediate punches were used. The optimally mixed dough, in the no-time dough method, was given 30 minutes of fermentation time, punched, and allowed 10 minutes for recovery before moulding. All doughs were divided into two equal 510-g. (18-oz.) portions prior to moulding.

Doughs from the sponge-dough and pre-ferment methods were proofed for 50 minutes, while the doughs from the straight-dough method required 60 minutes. No-time doughs were proofed for 35 minutes. All bread was baked 25 minutes at 425°F. (218°C.).

In some experiments, either 0.5, 0.75, or 1.0 g. of leucine together with 1.0 g. of xylose, or 100 mg. of iso-valeraldehyde, were added at the dough stage. In other experiments valine and leucine were added (2.0 g. per 700 g. flour) to the bread formula using dextrose only.

For storage experiments, some of the loaves were immediately wrapped and heat-sealed in waxed paper; an equal number from the same lot were left unwrapped. Bread was stored at room temperature (23°–25°C.).

Extraction of Carbonyl Compounds of Bread Crust and Crumb. A layer of crust 2 to 3 mm. thick was cut from a loaf of bread, ground for 30 seconds in a Waring Blendor⁴, and mixed well. A 50-g. ("as is" basis) portion was extracted for 12 hours in a Soxhlet apparatus with ethyl ether. The receiver flask was equipped with a magnetic

⁴The mention in this publication of a trade product, equipment, or a commercial company does not imply its endorsement by the U.S. Department of Agriculture over similar products not named.

stirrer and contained a mixture of ethyl ether and 2,4-dinitrophenylhydrazine reagent (10). At the end of extraction, the ether was distilled away and the hydrazones dissolved in 100 ml. of chloroform.

The crumb from two loaves, approximately 500 g., was divided in four equal portions and homogenized in a Waring Blendor with a total of 1,800 ml. of sodium chloride-saturated water. The carbonyl compounds were extracted from the homogenate with ethyl ether in a liquid-liquid extractor (10).

*Determination of the Carbonyl Compounds.*⁵ Chromatographic techniques used to separate and quantitatively determine the carbonyl compounds were the same as described by Linko *et al.* (10). Standard deviations ranging from 4 to 7% for the determination of carbonyl compounds in pre-ferments were reported previously (10).

Determination of Furfural. The crumb or crust (100 g. on "as is" basis) was weighed into a 1-liter flask and steam-distilled without prior addition of water. Twenty-five milliliters of distillate were collected. It was shown that furfural distilled quantitatively under these conditions. A 2-ml. aliquot of the 25 ml. of distillate collected was analyzed for furfural as described by Linko (9).

Determination of Hydroxymethylfurfural (HMF). Ten grams of crumb, or of crust, were extracted several times with a total volume of 25 ml. of benzene using an Omni-Mixer. The combined concentration of HMF and furfural was determined from a 5-ml. extract aliquot by Linko's (9) method. The amount of HMF could be calculated by subtracting the value of furfural obtained from the steam distillate.

Results and Discussion

Composition of Carbonyl Compounds in White Bread. The data in Table I show the composition of carbonyl compounds in white bread in relation to the baking procedure. Since acetoin, diacetyl, and methylglyoxal have been shown to be of minor importance for bread flavor (16), they were not determined. The amount of methylketones in yeast-leavened white bread has been reported to be around 8 mg. per 100 g. (6). A number of unidentified carbonyl compounds were chromatographically resolved but were not included in the "total" carbonyl compounds reported. The value for "total" carbonyl compounds was obtained as the sum of the individual carbonyl compounds determined, singly or in groups, and thus represents a minimal value. Ng, Reed, and Pence (11) and Wiseblatt and Kohn (21) have reported several carbonyl compounds in bread which, because of the trace

⁵All results are reported on a dry-matter basis.

TABLE I
EFFECT OF BAKING TECHNIQUE ON THE QUANTITY OF CARBONYL
COMPOUNDS IN BREAD

METHOD OF BAKING (Dough)	FORM- ALDEHYDE	ACET- ALDEHYDE	ACETONE AND PROPION- ALDEHYDE ^a	ISO-BUTYR- ALDEHYDE AND METHYLETHYL- KETONE ^b	ISO-VALERALD., N-VALERALD., 2-ME-BUTANAL, AND N-HEXALDEHYDE ^c	FURFURAL	HMF ^d	TOTAL CARBONYL COMPOUNDS
Straight	0.99	2.20	12.8	0.82	2.02	0.16	3.19	22.2
Sponge	0.98	2.17	17.1	0.97	1.60	0.34	6.65	29.8
No-time	0.86	1.65	10.7	1.47	1.18	0.31	5.43	21.7
Pre-fmt.	1.02	1.82	15.6	0.70	3.23	0.04	3.29	25.7
Crumb: mg. per 100 g.								
Straight	0.20	0.32	0.75	0.14	0.51	...	0.65	2.6
Sponge	0.20	0.35	0.85	0.15	0.76	...	0.72	3.0
No-time	0.17	0.29	0.81	...	0.86	...	0.59	2.8
Pre-fmt.	0.14	0.35	2.11	0.23	0.62	...	0.56	4.0

^a Expressed as acetone.

^b Expressed as iso-butyraldehyde.

^c Expressed as iso-valeraldehyde.

^d Hydroxymethylfurfural.

quantities involved, were not determined in this study. Acetone and propionaldehyde (as acetone), iso-butyraldehyde and methylethylketone (as iso-butyraldehyde), and iso-valeraldehyde, n-valeraldehyde, 2-methylbutanal, and n-hexaldehyde (as iso-valeraldehyde), respectively, were determined quantitatively as groups.

The crust of the bread made with the sponge-dough method had the highest "total" carbonyl content, whereas the crumb of bread made by the pre-ferment method had the highest value among comparable samples, largely because of its high acetone content. In general, of the carbonyl compounds measured, acetone (10-17 mg. per 100 g.) and HMF (3-7 mg. per 100 g.) were present in greatest amounts. Although HMF has been reported in rye bread (7,8) it has not been reported previously in white bread. This is surprising in view of the large quantities of hexoses present and the conditions during baking which are favorable for the formation of HMF. The quantity of HMF in the crust was always much higher than that of furfural. No detectable amount of furfural was found in the crumb, confirming the observation of Rotsch (15) with yeast-fermented rye and wheat breads.

Origin of Carbonyl Compounds during Baking. The data in Table II illustrate the effect of sugar concentration in the dough on the quantity of HMF and furfural formed during baking. HMF in-

TABLE II
EFFECT OF SUCROSE CONCENTRATION ON THE QUANTITIES OF FURFURAL AND
HYDROXYMETHYLFURFURAL IN BREAD MADE BY THE SPONGE PROCESS

SUCROSE	CRUST		CRUMB HMF
	Furfural	HMF ^a	
%	mg/100 g	mg/100 g	mg/100 g
4	0.325	4.05	0.675
5	0.338	6.65	0.723
6	0.350	8.31	0.614
7	0.386	8.37	0.677
8	0.435	9.09	0.638

^a Hydroxymethylfurfural.

creased in bread crust roughly in proportion to sugar concentration, as might be expected if HMF were an intermediate in browning. No increase in HMF could be observed in the crumb. The slight increase in furfural in the crust may be attributed to a small amount of pentoses present as impurities in the sucrose. Small quantities of furfural may also arise directly from hexoses during oven baking (15). Although the relationship with crust color was most obvious with furfural and HMF, all other carbonyl compounds measured also exhibited a similar trend. Rothe and Thomas (14) have also observed with rye bread that the carbonyl content and crust color increased with extended baking time.

Barnes and Kaufman (2) observed that a flavor similar to that of fresh bread could be obtained by reacting leucine with a carbohydrate. Kiely *et al.* (4) reported that leucine, when added to an instant bread mix, enhanced bread aroma. In our study, adding 1 g. of leucine and 1 g. of xylose increased iso-valeraldehyde from 3.1 to 17.4 mg. per 100 g. in the bread crust. No increase was observed in the crumb. When valine was added (2.0 g. per 700 g. flour) with dextrose, the isobutyraldehyde in the crust increased from 1.0 to 7.0 mg. per 100 g.; and similarly, when leucine was added (2.0 g. per 700 g. flour), the iso-valeraldehyde increased from 0.6 to 1.6 mg. per 100 g. of crust.

The importance of crust browning to total bread flavor was evident since the bread made with added leucine or valine and xylose or dextrose possessed improved flavor compared with the control. There was little evidence to support the prediction of Baker *et al.* (1) that volatile aldehydes produced during browning of the crust would permeate the crumb during cooling. The present data show that little transfer of carbonyl compounds from crust to crumb takes place during baking or shortly thereafter. However, considerable diffusion of carbonyl compounds into the crumb may take place during extended storage.

TABLE III
 QUANTITATIVE CHANGES IN CARBONYL COMPOUNDS AS BREAD AGES
 (A, unwrapped; B, wrapped)

DAYS	MOISTURE		FORM-ALDEHYDE		ACET-ALDEHYDE		ACETONE AND PROPION-ALDEHYDE ^a		ISO-BUTYR-ALDEHYDE AND METHYLETHYL-KETONE ^b		ISO-VALERALD., N-VALERALD., 2-ME-BUTANAL, AND N-HEXALDEHYDE ^c		FURFURAL		HMF ^d		TOTAL CARBONYL COMPOUNDS		
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
Crust: mg. per 100 g.																			
0	19.5		0.91		2.01		15.8		0.90		2.62		0.34		6.65		29.2		
1	19.2	25.2	0.96	1.41	2.25	2.22	11.3	10.2	0.84	0.75	2.64	4.23	0.13	0.23	7.18	7.62	25.3	26.7	
2	18.1	25.0	0.97	1.28	2.08	2.32	12.6	9.12	1.18	1.13	2.46	3.66	0.04	0.11	6.99	6.26	26.3	23.9	
3	16.5	29.7	1.04	1.34	2.14	1.81	13.1	6.30	1.23	0.45	2.60	2.63	0.10	0.17	7.58	5.98	27.8	18.7	
5	14.5	27.5	1.00	1.18	2.02	1.40	12.7	4.56	0.71	1.01	2.35	3.60	0.07	0.17	5.27	6.46	24.1	18.4	
7	11.9	26.9	0.85	1.29	1.54	2.22	7.02	3.38	0.65	0.51	2.11	3.75	0.04	0.14	1.87	6.10	13.3	17.4	
Crumb: mg. per 100 g.																			
0	42.4		0.20		0.36		0.88		0.15		0.45		0.72		2.77		
1	39.0	43.2	0.30	0.29	0.47	0.62	1.27	1.47	0.25	0.27	0.80	1.12	0.77	0.78	3.85	4.54	
2	37.5	42.6	0.27	0.30	0.63	0.61	2.79	1.43	0.29	0.23	0.83	1.09	0.68	0.66	5.98	4.31	
3	35.4	42.2	0.29	0.26	0.72	0.74	2.85	2.31	0.24	0.35	0.95	0.78	0.65	0.78	5.70	5.21	
5	32.6	41.4	0.25	0.14	0.30	0.33	0.92	0.56	0.15	0.10	0.53	0.39	0.58	0.72	2.72	2.24	
7	25.7	40.4	0.19	0.16	0.49	0.41	0.78	0.88	0.16	0.06	0.46	0.46	0.46	0.74	2.54	2.71	

^a Expressed as acetone.

^b Expressed as iso-butyraldehyde.

^c Expressed as iso-valeraldehyde.

^d Hydroxymethylfurfural.

Adding 100 mg. of iso-valeraldehyde at the dough stage resulted in no increase in this compound in the crumb (1.0 mg. per 100 g., compared with 1.2 mg. per 100 g. in the control) and only a slight increase in the crust (3.8 mg. per 100 g., compared with 3.1 mg. per 100 g. in the control). This suggests that a major portion of the carbonyl compounds formed during early stages of fermentation may be lost during subsequent fermentation and baking. Similarly, Rothe (13) reported that although methylglyoxal is formed during fermentation, methylglyoxal found in the finished loaf is entirely formed as a breakdown product of threonine during baking, which further emphasizes the importance of the oven-baking process for carbonyl formation.

Changes in Carbonyl Compounds during Aging of Bread. The changes observed in carbonyl compounds as bread ages are summarized in Table III. In general, "total" carbonyl compounds decreased in the crust with increasing storage time of both wrapped and unwrapped bread. A sharp drop occurred after 5 days of storage. Minor deviations in these data were due to slight differences in crust color of different loaves of bread from the same batch. The carbonyl compounds were retained best in the bread wrapped immediately after removal from the oven, indicating that the loss of carbonyl compounds from the crust of unwrapped bread was exceedingly rapid during the first hour or so after baking.

In the crumb, "total" carbonyls increased during the first 3 days of storage, then declined rapidly. The quantity of HMF was little affected during storage of wrapped bread, but decreased slightly in the unwrapped loaves. Rothe and Thomas (14) found an increase up to 9 days of storage in total carbonyls in crumb of rye bread distilled with steam. The decrease during the latter part of storage likely results mainly from oxidation of reactive carbonyl compounds (14) and from volatilization. The latter losses are perhaps minimum because the decrease in carbonyl compounds occurs mainly after extended storage rather than when the bread is fresh.

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