Changes in Amino Acids and Formation of Carbonyl Compounds during Baking

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The effect of various sugars on the formation of a number of carbonyl compounds during bread baking was investigated. In contrast to the hexoses, arabinose and xylose gave rise to marked quantities of furfural in the crumb as well as in the crust. There was a corresponding increase in both crumb and crust color. Formation of carbonyl compounds in crust was accompanied by significant decrease in all free amino acids, particularly in aspartic and glutamic acids. The importance of Maillard-type browning reactions in the production of bread flavor is discussed.

For some time, the formation of compounds responsible for bread flavor has been believed to be closely related to Maillard-type nonenzymatic browning during baking (*5*). Barnes and Kaufman (*2*) observed that a flavor similar to that of fresh bread could be obtained by reacting leucine with a carbohydrate. Kretovich and Tokareva (*9, 10*) and Lüers (*15, 16*) reported that the reactions between various amino acids and sugars, eventually leading to the formation of brown pigments, were accompanied by production of furfural and other volatile aldehydes which were responsible for the aroma. Similar observations also were made by Baker *et al.* (*1*) and later by Rotsch (*21–23*).

According to Rothe and Thomas (*20*), volatile aldehydes formed during nonenzymatic browning reactions are a major factor in bread flavor. They observed a close relationship between crust browning and the quantity of both total aldehydes and furfural. Rothe (*19*) reported the formation of a number of aldehydes through reaction of various amino acids with reducing carbohydrates during baking of rye bread, and Kiely *et al.* (*6*) found that leucine, when added to an instant bread mix, enhanced bread aroma. According to Linko *et al.* (*13*), addition of leucine and xylose in dough resulted in an increase in crust isovaleraldehyde, accompanied by an improvement in bread flavor, whereas isovaleraldehyde added in dough in great excess was largely lost during subsequent fermentation and baking. Similarly, Rothe (*19*) showed that although methylglyoxal is a natural fermentation product, that found in bread is formed during baking as a decomposition product of threonine.

This paper reports in greater detail reactions during bread baking of various carbohydrates with free amino acids of dough in relation to the formation of volatile aldehydes.

Experimental

Bread Baking. The following straight-dough formula was used for baking:

Flour (as is basis)	100	grams
Carbohydrate	6	grams
Nonfat dry milk solids	3	grams
Sodium chloride	2	grams
Malted wheat flour	0.25	gram
Yeast	2	grams
Potassium bromate	0.7	mg.

Total fermentation time was 3 hours (85° F.; 85% R.H.). The total dough was molded, proofed 45 minutes (98° F.; 95% R.H.), and baked 25 minutes at 410° F.

Crust Color. Crust color was measured with a Photovolt reflection meter by using the green filter which measures whiteness or darkness of color. The results reported are averages of three measurements.

Extraction and Determination of Carbonyl Compounds in Bread Crust. A layer of crust 2 to 3 mm. thick was carefully cut from a loaf (*13*). The crust was ground 30 seconds with a Waring Blendor, mixed well, and 50 grams ("as is" basis) of the ground material were extracted 12 hours in a Soxhlet apparatus with ethyl ether. The receiver flask was equipped with a magnetic stirrer, and contained a mixture of ether and 2,4-dinitrophenylhydrazine reagent as previously described (*14*). After the extraction, the ether was distilled away and the hydrazones taken into 100 ml. of chloroform.

Chromatographic techniques used for the separation and quantitative determination of the 2,4-dinitrophenylhydrazones of carbonyl compounds were the same as described by Linko *et al.* (*14*).

Determination of Furfural. Fifty grams of crumb or 40 grams of crust were weighed in a 1-liter flask and steam distilled without prior addition of water. Twenty-five milliliters of distillate were collected, and a 2-ml. aliquot was analyzed for furfural by a modification (*13*) of a method by Linko (*12*).

Determination of Hydroxymethylfurfural. Hydroxymethylfurfural (HMF) was determined by the method of Linko (*13*), except that only 5 grams of crumb or crust, respectively, were extracted.

Extraction and Determination of Free Amino Acids and Sugars. Two grams of crumb or crust, respectively, were extracted with five 10-ml. portions of 70% (w./v.) ethyl alcohol, using an Omnimixer. The combined extracts were passed through Amberlite IR-120 (H^+) resin (1 ml. per minute; column 6 × 100 mm.; resin 16 to 50 mesh, U.S. standard screen). The effluent was examined for sugars. The column was washed with 25 ml. of 70% (w./v.) ethyl alcohol, followed by displacement of the amino acids by 25 ml. of 1*N* ammonium hydroxide. Solutions containing sugars and amino acids were distilled to a small volume in vacuo (1 ml. for amino acids; 2 ml. for sugars).

Chromatographic techniques of Linko (*11*) were used. Extract aliquots equivalent to 10 mg. dry matter for sugars and 250 mg. dry matter for amino acids were used for chromatography.

Results and Discussion

Table I shows the effect of various carbohydrates on the formation of a number of carbonyl compounds in bread crust. The effect on the formation of furfural and hydroxymethylfurfural (HMF), as related to crust color, is shown in Table II. Generally, an increase in crust color was accompanied by an increase in furfural and/or HMF in the crust. These results agreed well with earlier observations (*13*). Thus, as expected, darkest crust color was obtained with arabinose and xylose which also gave rise to large quantities of furfural. In contrast, HMF was formed as

the primary product from the hexoses. A relatively large amount of furfural was, however, formed from fructose, but a direct formation of furfural from hexoses has been shown to take place (*21*). The color of the crumb increased visually only in loaves baked with arabinose and xylose, the increase being accompanied by formation of relatively large quantities of furfural in the crumb.

Most HMF was produced in the presence of the ketohexoses, fructose and sorbose. Methylfurfural formed from rhamnose was determined as HMF with the *p*-aminodimethylaniline reagent, and the amount thus obtained far exceeded that of HMF with other carbohydrates. Variations in other carbonyl compounds were small except for acetone, which was produced during fermentation, its production thus being independent of the browning reactions.

Table III gives the concentrations of certain amino acids in crumb and crust of bread made with different carbohydrates. Although several free amino acids were not quantitatively determined because of the minute quantities present, it was shown by paper chromatography that all decreased in crust compared with crumb regardless of the type of sugar used for baking. Although the reaction rate between amino acids and sugars is known to depend on the chemical structure of the sugar (*4*), the observed behavior obviously is due to the large excess of various carbohydrates compared with the small amounts of free amino acids. The marked decreases in free amino acids of crust, together with the formation of several aldehydes, suggest the importance of Maillard-type browning in flavor production. Similar observations regarding the participation of amino acids in nonenzymatic crust browning and in the subsequent formation of carbonyl compounds in bread have been recently reported by Kretovich and Ponomareva (*8*) and by Rothe (*19*). That amino acids act as precursors for several aldehydes

Table 1. Effect of Type of Sugar on Composition of Carbonyl Compound in Bread Crust[a]

Sugar	Formaldehyde	Acetaldehyde	Acetone,[b] Propionaldehyde	Isobutyraldehyde,[c] Methyl Ethyl Ketone	Isovaleraldehyde,[d] n-Valeraldehyde 2-Methylbutyraldehyde, n-Hexaldehyde
	Mg./100 Grams Dry Matter				
Fructose	1.3	2.1	15.1	0.3	2.8
Galactose	1.4	3.5	15.9	0.7	3.3
Glucose	1.1	2.3	8.4	0.4	2.7
Lactose	1.1	2.2	10.8	0.4	2.7
Maltose	1.1	2.2	19.4	0.3	2.4
Raffinose	1.2	2.0	18.9	0.2	2.6
Starch (wheat)	1.0	2.1	13.4	0.2	2.5
Sucrose	1.2	2.1	20.9	0.2	2.8
Xylose	2.1	2.0	7.4	0.2	3.2

[a] 2,4-Dinitrophenylhydrazones of carbonyl compounds were determined in groups as separated by paper chromatography using cyclohexane saturated with *N,N*-dimethylformamide as solvent (*14*).
[b] Determined as acetone.
[c] Determined as isobutyraldehyde.
[d] Determined as isovaleraldehyde.

Table II. Effect of Type of Sugar on Loaf Volume, Crust Color, and Formation of Furfural and Hydroxymethylfurfural

Sugar	Loaf Volume, cc.	Crust Color, % Reflectance	Mg./100 Grams Dry Matter			
			Furfural		Hydroxymethylfurfural	
			Crumb	Crust	Crumb	Crust
Arabinose	530	21	0.16	2.60	0.56	2.54
Cellobiose	775	27	0.01	0.10	0.68	1.56
Fructose	805	21	0.00	0.71	0.85	11.7
Galactose	705	23	0.01	0.10	0.60	2.84
Glucose	810	26	0.02	0.18	0.67	2.74
Lactose	760	26	0.01	0.10	0.63	1.82
Maltose	725	25	0.00	0.07	0.71	2.02
Mannose	835	24	0.00	0.03	0.61	3.73
Melibiose	735	24	0.00	0.05	0.52	1.35
Raffinose	765	24	0.00	0.08	0.50	1.79
Rhamnose	675	24	0.00	0.06	3.33[a]	23.3[a]
Sorbose	645	22	0.00	0.21	0.62	4.02
Starch (wheat)	745	34	0.00	0.03	0.50	1.02
Sucrose	875	25	0.00	0.14	0.85	3.22
Xylose	600	20	0.23	2.72	0.62	3.52

[a] Methylfurfural.

Table III. Effect of Type of Sugar on Composition of Five Amino Acids in Bread Crumb and Crust

Sugar	Aspartic Acid		Glutamic Acid		Alanine		γ-Aminobutyric Acid		Leucine(s)	
	Crumb	Crust	Crumb	Crust	Crumb	Crust	Crumb	Crust	Crumb	Crust
	Mg./100 Grams Dry Matter									
Arabinose	3.0	0.7	2.8	0.8	1.8	0.9	0.5	0.3	0.7	0.3
Cellobiose	3.0	0.2	4.0	1.0	1.5	0.6	0.2	0.1	0.3	0.1
Fructose	3.4	0.2	4.4	0.9	1.2	0.4	0.1	0.1	0.2	0.1
Galactose	3.6	1.4	3.6	1.2	1.4	0.8	0.5	0.3	0.3	0.1
Glucose	3.6	0.8	5.2	1.6	1.4	0.6	0.1	0.1	0.4	0.1
Lactose	3.6	0.6	2.8	0.7	1.6	0.8	0.3	0.2	0.4	0.1
Maltose	4.6	0.8	2.8	0.5	1.6	0.6	0.3	0.2	0.4	0.1
Mannose	4.2	0.6	4.0	1.2	1.4	0.5	0.1	0.1	0.3	0.1
Melibiose	3.4	0.5	2.8	0.4	1.5	0.8	0.3	0.2	0.2	0.1
Raffinose	4.2	0.4	4.0	0.9	1.5	0.5	0.2	0.1	0.2	0.1
Rhamnose	4.4	1.0	3.0	0.6	2.0	1.2	0.4	0.3	0.4	0.1
Sorbose	5.8	0.6	2.8	0.4	1.7	0.5	0.4	0.2	0.4	0.1
Starch (wheat)	5.8	0.6	3.0	0.9	2.2	1.2	0.2	0.1	0.2	0.1
Sucrose	3.8	0.4	4.4	0.9	1.5	0.5	0.1	0.1	0.2	0.1
Xylose	7.0	0.6	3.2	0.2	2.8	0.8	0.6	0.3	0.8	0.1

during baking has been demonstrated (*6*, *13*, *19*). Rothe (*19*) reacted 14 individual amino acids with xylose, and in each case an aliphatic aldehyde and furfural were formed. He also demonstrated that these reactions take place in the crust of rye bread during baking. The most reactive amino acids were isoleucine, leucine, valine, methionine, alanine, and phenylalanine which formed 2-methylbutyraldehyde, isovaleraldehyde, isobutyraldehyde, methional, acetaldehyde, and phenylacetaldehyde, respectively. Simultaneously, furfural was formed from pentoses, and HMF from hexoses. It has also been reported that the addition of amino acids or nonfat dry milk solids increases crust browning (*3*, *24*). Furthermore, the Amadori rearrangement has been shown to take place in bakery products (*5*, *17*).

However, as suggested by Pomeranz *et al.* (*18*), the possibility of caramelization reactions of various sugars as an additional source of brown pigments and flavor compounds cannot be overlooked. That such reactions may take place at temperatures even below those generally encountered during baking has been reported (*7*). Also, the extensive formation of methylfurfural from rhamnose does not appear to be accompanied by a corresponding decrease in amino acids, thus suggesting the formation of methylfurfural directly from the sugar.

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COFFEE AROMA

Mass Spectrometric Determination of the Volatile Components from Ground Coffee

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INTEREST in the chemical composition of coffee aroma has been increasing greatly in recent years arising mainly from the desire to improve the acceptability of soluble coffee products and also to afford a means of quality evaluation and control.

Early work on coffee aroma was carried out more than 50 years ago, by employing coffee roaster condensates as samples. The complexity of these condensates and the lack of sensitive analytical methods made complete analysis extremely difficult, if not impossible. In the past decade, several papers have appeared reporting further attempts to gain more complete information about the volatile components which comprise a coffee aroma (*2*, *3*, *8*, *9*, *12-15*, *17*, *18*). In reviewing the rather voluminous literature which has appeared on this subject, a wide divergence is found with respect to the nature of the samples taken, the methods of analysis used, and the results obtained. In one report (*6*), as many as 70 compounds are listed as identified. Consideration of the methods employed, however, raises considerable doubt as to the reliability of some of the identifications.

The answer to this complex and perplexing problem appears to lie in the utilization of more modern instrumental methods of analysis. The results reported here are based entirely on mass spectrometric analysis. In other current research (*14*, *15*, *18*), gas chromatography and mass spectrometry are also being used.

Chemical changes responsible for development of coffee flavor and aroma are generally conceded to be effected exclusively in the roasting process. The majority of previously reported studies on coffee aroma have, therefore, been made in connection with these roasting products, and the sampling procedures have employed heat, steam, water extraction, or solvent extraction, individually or in combination. The introduction of solvents and the use of steam or water might also be expected to cause chemical alteration of the volatile components. In addition, steam, water, or solvent extraction methods of obtaining a sample all suffer the disadvantage that nonvolatile but extractable materials may be collected, and the problem of subsequent separation of these impurities as well as the solvents and their associated impurities needlessly complicates the procedure. The method of sampling which employs a carrier gas to sweep out the coffee sample is apparently a satisfactory one but requires considerable extra time, and very

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