Studies of the Carbonyl Compounds Produced by Sugar-Amino Acid Reactions. II. In Bread Systems[1]

ALI SALEM, LLOYD W. ROONEY, and **JOHN A. JOHNSON,** Department of Flour and Feed Milling Industries, Kansas State University, Manhattan

ABSTRACT

The effect of reactions between amino acids and glucose or xylose on production of carbonyl compounds, color, and aroma in bread was studied. The carbonyl compounds were isolated from both the crust and crumb. The 2,4-dinitrophenylhydrazone derivatives were prepared and quantitatively determined. Furfural and hydroxymethylfurfural were determined with specific color reactions. Reaction of amino acids and sugar increased the color intensity of bread crust. Xylose produced a darker crust color than glucose. The bread crust color was influenced by the kind of amino acid when the amino acids were reacted with the same sugar. The amount of carbonyl compounds increased in the bread crust when amino acids were added. However, furfural and hydroxymethylfurfural content decreased slightly. The carbonyl content in the bread crumb did not increase. Formaldehyde, acetaldehyde, isobutyraldehyde, isovaleraldehyde, 2-methylbutanal, phenylacetaldehyde, and methional were produced mainly from glycine, alanine, valine, leucine, isoleucine, phenylalanine, and methionine, respectively. The other amino acids produced no predominant aldehyde, presumably because of reactivity of the Strecker degradation products. Type of sugar had no effect on the kind of carbonyl compounds formed. Since various amino acids produced different aldehydes, aroma of the bread changed accordingly.

Some of the pertinent research on the importance of the Maillard reaction in the production of aromatic compounds was summarized by Rooney *et al.* (1). Rothe (2) indicated that the aroma of rye bread was influenced by reaction of xylose with certain amino acids. The reaction of proline with reducing sugars has been shown to produce compounds which have a distinct breadlike or crackerlike aroma (3,4,5). Wick *et al.* (6) indicated that flavor appeared to be increased when proline was added to the bread formula. Hunter *et al.* (4) tentatively identified one of the compounds from the reaction of proline and glycerol as 1-azabicyclo (3. 3. 0) oct-4-one.

Rooney *et al.* (1) studied sugar-amino acid reactions in both an aqueous buffered model system and a starch paste model system, and concluded that Maillard-type browning was mainly responsible for the production of carbonyl compounds and brown color. The type of amino acid influenced the kind of carbonyl compounds formed in both model reaction systems, whereas sugar type influenced the amount of carbonyl compounds formed.

This paper summarizes a study of the carbonyl compounds produced when the individual amino acid and sugar were added to a modified bread formula.

---

[1] Contribution No. 562, Department of Flour and Feed Milling Industries, Kansas Agricultural Experimental Station, Manhattan. The work presented constitutes portions of theses of Lloyd W. Rooney and Ali Salem submitted to the Graduate Faculty of Kansas State University in partial fulfillment of the requirements for the Doctor of Philosophy degree.

Present address of Salem: Agronomy Department, Seed Laboratory, University of Nebraska, Lincoln.

Present address of Rooney: Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas.

MATERIALS AND METHODS

Bread-Baking

One-pound loaves of bread were baked by use of a straight dough procedure with the following formula:

Ingredients	*Weight* g.
Flour	700.0
Arkady	2.0
Yeast	14.0
Sugar	21.0
Salt	14.0
Shortening	21.0
Water	343.0

A hard red winter wheat flour containing 11.9% protein was used. The amino acid and sugar (0.02*M* of each) were dissolved in the water and added to the formula prior to mixing. The dough was mixed to optimum consistency, and fermented at 85°F. and 90% r.h. for 110 min. The dough was punched; 50 min. later it was divided, was rested for 20 min., and was moulded and proofed for 50 min. The bread was baked for 30 min. at 425°F.

Crust Color

The color was measured with a Photovolt reflectometer with a green filter (7). The data are reported as the average of six readings on each of the two loaves.

Extraction of Bread Crust and Crumb

A 50-g. portion of ground crust (a 2–3-mm.-thick layer), or the crumb, was extracted three times with carbonyl-free chloroform. The extract was added to 400 ml. of 1% 2,4-dinitrophenylhydrazine reagent (2,4-DNPH) and the mixture was refluxed at 60°C. for 1 hr. to form the hydrazones. The chloroform layer containing the hydrazones was separated and concentrated to 50-ml. volume.

Qualitative and Quantitative Determination of Carbonyl Compounds

Paper, thin-layer, and gas-liquid chromatography methods were used to separate and identify the carbonyl compounds as the 2,4-DNPH derivatives according to the methods described by Rooney *et al.* (1). The quantity of carbonyl compounds was estimated by extraction of the 2,4-DNPH derivatives and measurement of the ultraviolet absorption at the wave length of maximum absorption. Data are expressed as the number of mg. of free carbonyl compounds present in 100 g. of bread crumb or crust on an as-is moisture basis.

Determination of Furfural

Furfural content of the crust and crumb was determined on a 2-ml. aliquot of the steam distillate of the ground crust (50 g.) or crumb (100 g.) as described by Linko (8).

Determination of Hydroxymethylfurfural (HMF)

A 10-g. sample of the ground crust or crumb was extracted five times in an Omnimixer with a total volume of 25 ml. benzene. The combined benzene extract was centrifuged for 15 min. The combined concentration of HMF and furfural was determined from a 5-ml. aliquot of the benzene extract of the crust or crumb according to the method of Linko (8).

RESULTS AND DISCUSSION

The effect of adding various amino acids and sugars on the production of carbonyl compounds, color, and aroma in white bread was studied. Since bread dough is a complex system, a modified formula was employed in which the nonfat dry milk and malted wheat flour were omitted and the sugar content was reduced to 3%. Because of the lean formula, differences in crust color were obtained that a high sugar formula would have masked.

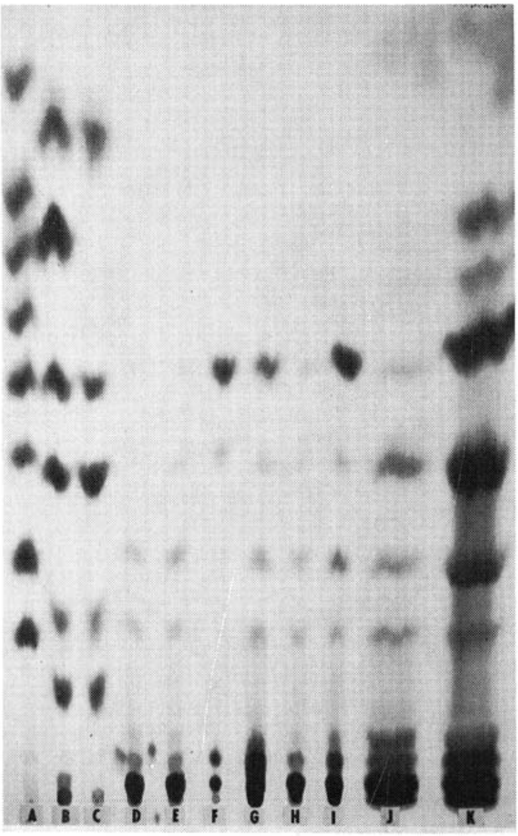

Fig. 1. Paper chromatogram of 2,4-DNPH derivatives of carbonyl compounds produced in various systems including bread crust.

A, B, C, known carbonyl compounds, from bottom to top: A: 1, formaldehyde; 2, acetaldehyde; 3, propionaldehyde; 4, butyraldehyde; 5, valeraldehyde; 6, hexaldehyde; 7, heptaldehyde; 8, decylaldehyde.

B: 1, HMF; 2 and 3, isomeric furfural derivatives; 4, acetone; 5, 2-butanone; 6, 2-hexanone; 7, 3-heptanone.

C: 1, HMF; 2 and 3, isomeric furfural derivatives; 4, acetone; 5, isobutyraldehyde; 6, 3-heptanone.

D to K: carbonyl compounds; D, of vapor fraction from heating of glucose (100 μl.); E, of reaction liquid from heating of glucose (100 μl.); F, of vapor fraction from reaction of valine with glucose (5 μl.); G, of reaction liquid of valine and glucose (25 μl.); H, extracted from starch paste with glucose (100 μl.); I, extracted from starch paste with glucose and valine (25 μl.); J, of bread crust control (250 μl.); K, of bread crust with valine (250 μl.).

Carbonyl Compounds Produced in Bread Crust and Model Systems

The production of carbonyl compounds from amino acid-sugar reactions in model systems indicated that many of these compounds result from Strecker degradation of the amino acids (1,9). In bread dough the system is somewhat different, because there are naturally present many free amino acids as well as proteins. An example of the kind of carbonyl compounds produced in three different systems when glucose was reacted with and without valine is shown in Fig. 1. It is evident that addition of valine to each model system increased the amount of certain carbonyl compounds formed. The reaction products of the amino acids and sugars in bread were similar to those of the reactions observed in model systems (1). The paper chromatogram illustrates how the 2,4-DNPH derivatives of the carbonyl compounds were separated. The major compound appearing in Fig. 1 from reaction of valine was tested further by use of another solvent system for paper chromatography and by thin-layer and gas-liquid chromatographic techniques. The major compound was identified as isobutyraldehyde.

Crust Color Intensity

Table I shows the average reflectance values on the top and bottom crust of bread to which various amino acids and glucose (or xylose) were added. Addition of amino acids increased the intensity of the crust color. Methionine and arginine, reacting with glucose, produced the darkest crust color. Proline had little effect on the crust color, as was expected from study of model systems (1). Generally, the crust of bread made with xylose and amino acids was darker than that of bread made with glucose-amino acids, probably because xylose is nonfermentable and more reactive in browning (1).

TABLE I

REFLECTANCE OF BREAD CRUST BAKED WITH ADDED AMINO ACIDS

AMINO ACID	GLUCOSE		XYLOSE	
	Top	Bottom	Top	Bottom
	%	%	%	%
No amino acid	30.3	51.0	21.8	41.5
Glycine	18.5	40.2	12.2	23.9
Alanine	19.5	43.6	14.5	29.1
Valine	22.2	41.6	15.0	25.5
Leucine	20.0	41.0	13.8	30.0
Isoleucine	21.8	41.0	18.0	33.4
Glutamic acid	19.0	43.9	17.5	37.8
Histidine	17.0	29.8	10.6	22.8
Lysine	17.6	36.1	12.2	33.3
Phenylalanine	18.3	38.8	12.0	25.0
Proline	23.5	45.5	18.5	35.0
Arginine	8.5	38.6	....	....
Methionine	7.0	28.5	....	....

Quantitative Determination of Carbonyl Compounds

The recovery of isovaleraldehyde with the chloroform extraction technique was determined. Isovaleraldehyde (0.1 ml.) was added to ground crust (50 g.) and the crust was extracted and chromatographed in the normal manner. The isovaleraldehyde zone from the paper chromatogram of bread crust extract prepared without adding isovaleraldehyde was used as a blank to correct for isovaleraldehyde originally present in bread. It was found that three extractions of the crust with chloroform produced a maximum recovery of 75 ± 2%. Additional extraction did not improve recovery. Other carbonyl compounds could be similarly recovered.

TABLE II

EFFECT OF ADDING VARIOUS AMINO ACIDS AND GLUCOSE ON THE AMOUNT OF SEVERAL CARBONYL COMPOUNDS IN BREAD CRUST[a]

AMINO ACID	FORMALDEHYDE	ACETALDEHYDE	ACETONE	ISOBUTYRALDEHYDE	ISOVALERALDEHYDE	FURFURAL	HMF
	mg./100 g.[b]	*mg./100 g.*[b]	*mg./100 g.*[b]	*mg./100 g.*[b]	*mg./100 g.*[b]	*mg./100 g.*[b]	*mg./100 g.*[b]
Control	0.49	1.06	1.40	1.10	1.33	0.08	1.11
Glycine	1.88	1.73	3.62	1.56	1.41	.03	0.83
Alanine	0.90	3.44	6.60	3.26	1.27	.10	0.80
Valine	1.30	2.38	6.25	8.57	2.61	.02	0.95
Leucine	0.51	1.18	2.10	1.22	2.78	.02	0.47
Isoleucine	0.91	1.90	3.18	2.27	3.94[c]	.01	0.46
Glutamic acid	1.12	2.03	2.88	1.83	1.68	.04	1.05
Histidine	1.43	3.59	5.40	2.72	2.62	.04	1.09
Lysine	1.74	3.75	4.73	3.68	3.33	.02	1.04
Phenylalanine	0.61	1.67	3.33[d]	1.22	1.68	.06	1.03
Proline	0.56	0.78	2.20	1.13	1.08	.04	0.91
Arginine	0.36	1.31	1.44	0.97	1.96	.15	0.79
Methionine	0.49	1.43	1.98[e]	1.43	1.22	0.11	1.65

[a] Values are averages of duplicates.
[b] Fresh weight.
[c] 2-Methylbutanal predominated in the mixture.
[d] Phenylacetaldehyde comprised 33% of the mixture.
[e] Methional and acetone.

TABLE III

EFFECT OF ADDING VARIOUS AMINO ACIDS AND GLUCOSE ON THE AMOUNT OF SEVERAL CARBONYL COMPOUNDS IN BREAD CRUMB[a]

AMINO ACID	FORMALDEHYDE	ACETALDEHYDE	ACETONE	ISOBUTYRALDEHYDE	ISOVALERALDEHYDE
	mg./100 g.[b]	*mg./100 g.*[b]	*mg./100 g.*[b]	*mg./100 g.*[b]	*mg./100 g.*[b]
Control	0.29	0.50	0.79	0.56	0.85
Glycine	.25	0.65	0.98	0.70	0.86
Alanine	.41	0.90	1.80	0.95	0.77
Valine	.52	0.84	1.80	1.03	0.80
Leucine	.29	0.78	0.88	0.70	0.72
Isoleucine	.44	0.80	1.06	0.71	0.79
Glutamic acid	.33	0.80	1.07	0.84	1.05
Histidine	.35	0.67	1.15	0.60	0.92
Lysine	.64	1.09	2.26	1.09	1.40
Phenylalanine	.77	0.69	1.10	0.57	0.97
Proline	.38	0.47	1.20	0.70	0.75
Arginine	.31	0.65	0.87	0.52	0.79
Methionine	0.52	1.02	1.49	0.75	0.87

[a] Values are averages of duplicates.
[b] Fresh weight.

Data in Tables II and III indicate that the composition of carbonyl compounds in white bread crust and crumb depended on the type of amino acid available for reaction. Addition of amino acids to the formula increased the content of total carbonyl compounds in bread crust, but they were increased only slightly in bread crumb. The content of furfural and HMF in bread crust decreased in comparison with the bread crust of the control, except in the case of methionine, where the HMF content was increased. Furfural and HMF are known to be produced during the Maillard reaction (9). However, they serve mainly as intermediates and undergo further condensation with free amino groups to form polymers. Therefore, lower content of HMF and furfural would be expected when amino groups were present to react further with HMF and furfural. In addition, the concentration of furfural and HMF appeared to be lowest in bread crust containing the largest amounts of the other carbonyl compounds.

Glycine increased acetone and formaldehyde somewhat in the crust. Formaldehyde, the Strecker degradation product of glycine, is highly volatile and hence was not recovered in large quantities. Addition of alanine or valine to the formula increased acetone content as well as the respective Strecker degradation aldehydes in the crust. No corresponding increases in acetone content was found in the model systems. Addition of leucine doubled the isovaleraldehyde content of the bread crust; added isoleucine increased the 2-methyl-

TABLE IV

EFFECT OF ADDING VARIOUS AMINO ACIDS AND XYLOSE ON THE AMOUNT OF SEVERAL CARBONYL COMPOUNDS IN BREAD CRUST[a]

AMINO ACID	FORMALDEHYDE	ACETALDEHYDE	ACETONE	ISOBUTYRALDEHYDE	ISOVALERALDEHYDE	FURFURAL	HMF
	mg./100 g.[b]	*mg./100 g.*[b]	*mg./100 g.*[b]	*mg./100 g.*[b]	*mg./100 g.*[b]	*mg./100 g.*[b]	*mg./100 g.*[b]
Control	0.61	0.78	1.19	0.70	0.92	0.45	1.05
Glycine	0.82	1.33	2.20	1.20	1.35	.12	0.95
Alanine	0.77	1.99	2.44	1.34	1.30	.06	0.99
Valine	0.74	1.29	2.16	3.89	1.35	.08	1.09
Leucine	0.56	1.24	2.38	0.93	3.56	.06	0.60
Isoleucine	0.66	1.27	1.89	0.84	4.68[c]	.10	0.64
Glutamic acid	1.14	2.55	4.32	2.18	2.28	.09	0.92
Histidine	0.49	1.22	2.52	0.95	1.18	.07	0.66
Lysine	0.63	1.45	3.38	1.47	1.41	.08	0.76
Phenylalanine	0.57	1.41	2.78[d]	1.12	1.17	.14	0.76
Proline	0.69	1.70	3.87	1.45	1.40	0.12	0.66

[a] Values are averages of duplicates.
[b] Fresh weight.
[c] 2-Methylbutanal predominantly.
[d] Phenylacetaldehyde accounted for 30% of the mixture.

TABLE V

EFFECT OF ADDING VARIOUS AMINO ACIDS AND XYLOSE ON THE AMOUNT OF SEVERAL CARBONYL COMPOUNDS IN BREAD CRUMB[a]

AMINO ACID	FORMALDEHYDE	ACETALDEHYDE	ACETONE	ISOBUTYRALDEHYDE	ISOVALERALDEHYDE
	mg./100 g.[b]	*mg./100 g.*[b]	*mg./100 g.*[b]	*mg./100 g.*[b]	*mg./100 g.*[b]
Control	0.23	0.41	0.67	0.35	0.52
Glycine	.20	.33	0.54	.22	.36
Alanine	.20	.32	0.49	.33	.62
Valine	.24	.40	0.56	.43	.50
Leucine	.27	.58	1.10	.48	.75
Isoleucine	.25	.61	0.96	.50	.79
Glutamic acid	.20	.61	0.72	.33	.53
Histidine	.26	.37	0.45	.30	.43
Lysine	.27	.33	0.67	.33	.37
Phenylalanine	.26	.58	0.90	.33	.62
Proline	0.33	0.51	1.04	0.35	0.54

[a] Values are averages of duplicates.
[b] Fresh weight.

butanal content threefold. Phenylacetaldehyde and methional were found in the crust of bread made with phenylalanine and methionine added. Lysine increased the concentration of all carbonyl compounds in bread crust approximately three- to fourfold over the control. Histidine increased the content of acetone and acetaldehyde three to four times in the crust, and isovaleraldehyde and isobutyraldehyde concentrations were enhanced twofold over that of the control crust.

Tables IV and V show the carbonyl compounds present in crust and crumb of bread made with different amino acids and xylose added. The concentration of the respective aliphatic aldehydes from each amino acid was enhanced only in the crust, which illustrates the importance of the Maillard reaction in crust browning and formation of carbonyl compounds. However, the concentration of the aldehydes produced with xylose was lower than with glucose, despite the fact that xylose is a nonfermentable sugar and more reactive in the aqueous model reaction conditions. The explanations are: 1) that xylose was so reactive that most of the carbonyl compounds formed in the early stages of baking were volatilized, or 2) that xylose was less reactive than glucose under baking conditions. The first explanation appears more plausible, because crust color of the bread was more intense and furfural content decreased with amino acids added in the presence of xylose. Moreover, glutamic acid and proline, which were shown to be less reactive in the model systems, produced relatively large quantities of certain carbonyl compounds. Linko and Johnson (7) found that the total of carbonyl compounds isolated from bread to which xylose alone was added was less than that from bread made with glucose.

From the results of the increase in the respective aliphatic aldehydes with one carbon atom less than the amino acid, and the increase in crust color when amino acids were added to the formula, it is evident that the Maillard reaction occurs in the crust during baking. Although aldehydes are produced during fermentation (10), there is evidence that the fermentation products are volatilized during the later stages of fermentation and baking (11). Addition of 100 mg. of isovaleraldehyde to the dough did not increase significantly the isovaleraldehyde content of the crumb or the crust. However, addition of 0.02*M* leucine to the dough produced a two- to threefold increase in the isovaleraldehyde content of the crust, suggesting that aldehydes formed during fermentation are volatilized and that fresh aldehydes are produced by nonenzymatic browning during baking. While the type of sugar had no effect on the kind of aldehyde or aroma, the type of amino acid significantly affected the aroma of bread. Leucine and isoleucine produced a cheeselike aroma; phenylalanine, an aroma like that of rose oil; methionine, an obnoxious odor; the other amino acids produced more subtle, indescribable aromas. Addition of lysine produced bread with a richer aroma than that of the control bread.

During Maillard-type browning many compounds are produced, including Strecker degradation products of the amino acids, furfural, HMF, reductones, and others. Although large quantities of the compounds are lost in the vapor, the amount retained in the crust influences the aroma of the bread. It appears possible to alter bread aroma and flavor by the addition of amino acids to bread formulas. However, more research is needed to determine the proper ratio of various amino acids required for acceptable bread aroma and flavor. Possibilities for enhancing the aroma of toasted bread, in particular, appear promising.

Literature Cited

1. ROONEY, L. W., SALEM, A., and JOHNSON J. A. Studies of the carbonyl compounds produced by sugar-amino acid reactions. I. Model systems. Cereal Chem. **44:** 539–550 (1967).
2. ROTHE, M. Über Fluchtige Aromastoffe des Roggenbrotes. Ernaehrungsforschung **5:** 131–147 (1960).
3. MORIMOTO, T., and JOHNSON, J. A. Studies on the flavor fraction of bread crust adsorbed by cation exchange resin. Cereal Chem. **43:** 627–637 (1966).
4. HUNTER, I. R., WALDEN, M. K., MCFADDEN, W. H., and PENCE, J. W. Production of breadlike aromas from proline and glycerol. Cereal Sci. Today **11:** 493, 494, 496, 500, 501 (1966).
5. WISEBLATT, L., and ZOUMUT, H. F. Isolation, origin, and synthesis of a bread flavor constituent. Cereal Chem. **40:** 162–169 (1963).
6. WICK, EMILY L., DE FIGUEIREDO, M., and WALLACE, D. H. The volatile components of white bread prepared by a pre-ferment method. Cereal Chem. **41:** 300–315 (1964).
7. LINKO, YU-YEN, and JOHNSON, J. A. Changes in amino acids and formation of carbonyl compounds during baking. J. Agr. Food Chem. **11:** 150–152 (1963).
8. LINKO, P. Spectrophotometric determination of 2-furaldehyde, 5-(hydroxymethyl) 2-furaldehyde, cinnamaldehyde and citral with *p*-aminodimethylaniline and *m*-phenylenediamine. Anal. Chem. **33:** 1400–1403 (1961).
9. HODGE, J. E. Chemistry of browning reactions in model systems. J. Agr. Food Chem. **1:** 928–943 (1953).
10. LINKO, YU-YEN, MILLER, B. S., and JOHNSON, J. A. Quantitative determination of certain carbonyl compounds in pre-ferments. Cereal Chem. **39:** 263–272 (1962).
11. ROTHE, M., and THOMAS, B. Über Bildung, Zusammensetzung and Bestimmung von Aromastoffen des Brotes. Die Nahrung **3:** 1–17 (1959).

[Received April 7, 1966. Accepted May 12, 1967]