Studies of the Carbonyl Compounds Produced by Sugar-Amino Acid Reactions. I. Model Systems[1]

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ABSTRACT

The kind and quantity of carbonyl compounds produced by the reaction of amino acids with reducing sugars in an aqueous buffered solution and in a starch paste were studied. For the aqueous system, the solutions were buffered with acetate at pH 5.5 and heated for 12 hr. at 95°C. For the starch pastes, the amino acids and sugars were mixed with wheat starch to give a doughlike consistency, and the mixtures were baked in an oven for 30 min. at 430°F. The carbonyl compounds produced were extracted and quantitatively determined by chromatographic methods. The coloration and production of carbonyl compounds were attributed to the Maillard-type reaction in both model systems. Alanine, valine, leucine, isoleucine, phenylalanine, and methionine produced mainly acetaldehyde, isobutyraldehyde, isovaleraldehyde, 2-methylbutanal, phenylacetaldehyde, and methional, respectively. In addition, smaller quantities of acetone, formaldehyde, propionaldehyde, and other carbonyl compounds were produced. Lysine, arginine, histidine, and tryptophan caused rapid and intense browning but did not produce significant quantities of specific carbonyl compounds. Glutamatic acid and proline caused relatively little browning and production of carbonyl compounds. The rate of browning and carbonyl compound formation changed with the kind of sugar. Xylose was most reactive, followed by glucose and maltose. Lysine, leucine, and isoleucine produced acceptable and pleasing aromas; phenylalanine and methionine produced unpleasant aromas.

At least 70 different organic compounds thought to be involved in bread flavor have been isolated from pre-ferments, doughs, oven vapors, and breads (1). Both fermentation and baking are required to produce acceptable bread flavor. However, baking appears to be the more important process, since most of the flavor compounds that are retained by the bread are formed during baking (2). Linko *et al.* (3, 4) and Rothe and Thomas (5, 6) found that certain compounds present in the fermented doughs were lost during baking but were replaced by compounds formed during crust formation. Considerable evidence is available indicating that the Maillard type of nonenzymatic browning functions significantly in the formation of crust and in the production of flavor compounds in bread (2, 5, 7).

Several excellent reviews of the literature on nonenzymatic browning are available (8, 9, 10). The complex reaction between free amino groups and reducing sugars involves condensation, dehydration, rearrangement, fission, and polymerization reactions that produce complex brown pigments. In addition, various by-products and intermediates are produced which may

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contribute to flavor and aroma. Reynolds (10) emphasized the importance of Strecker degradation in producing certain aldehydes during the browning reaction. During the Strecker degradation reaction, the amino acid loses the carboxyl and amino groups and becomes an aldehyde with one less carbon atom. Aldehydes that are formed via Strecker degradation of certain amino acids have been found in bread crust and crumb (1, 3). Hodge (9) integrated the various theories and facts into a general reaction scheme that accounted for the origin of many compounds found in bread. This scheme emphasized that several different pathways could be followed which depend on reaction conditions including temperature, moisture content, pH, reactants, reaction time, and other variables.

Model reaction systems have been used to study the browning reaction (8,9). Barnes and Kaufman (11) observed that an aroma similar to that of fresh bread was obtained by reacting leucine or isoleucine with a sugar. Kiely *et al.* (12) found that a distinct breadlike aroma was produced by the reaction of leucine, histidine, or arginine with glucose. Addition of leucine, combined with either arginine or histidine in the formula, enhanced the flavor of chemically leavened bread. Generally, the aromas were thought to correspond to the aldehyde produced by Strecker degradation of the amino acid. Type of sugar did not appear to affect the aroma. Wiseblatt and Zoumut (13) thought a component produced by proline and dihydroxyacetone was important in producing bread flavor.

Many investigators have studied the Maillard reaction in model systems, but only a few investigators have analyzed the carbonyl compounds produced as by-products or intermediates of the browning reaction between amino acids and sugars (8, 9, 10). Information is particularly lacking on analyses of the carbonyl compounds produced by reaction of amino acids and sugars which may affect bread flavor. Therefore, a study was initiated to gain more information on the kind and quantity of carbonyl compounds formed as by-products or intermediates of the browning reaction. A liquid buffered solution, a starch-paste system, and finally a modified bread formula were the reaction media. This paper describes the results obtained with the aqueous, buffered reaction medium and a starch paste system which had a consistency similar to that of bread dough.

MATERIALS AND METHODS

Model Browning Systems

The liquid model system consisted of reacting the known sugar (0.2*M*) and known amino acid (0.2*M*) in an aqueous acetate buffer system (0.4*M*, pH 5.5) for 12 hr. at 95° ± 1°C. The reaction flask was fitted with a reflux condenser, and the volatile compounds formed were swept from the reaction flask by a stream of nitrogen gas. The carbonyl compounds were isolated as the 2,4–dinitrophenylhydrazine (2,4–DNPH) derivatives by bubbling the nitrogen gas stream through an adsorption tower filled with 400 ml. of warm (50°C.) 1% 2,4–DNPH reagent solution. An additional adsorption tower failed to remove additional quantities of carbonyl compounds.

At the end of 12 hr. of reflux, the reaction liquid was extracted three

times with carbonyl-free chloroform and the combined extracts were refluxed for 1 hr. with 400 ml. of 2,4–DNPH reagent. The chloroform layer was separated and concentrated under vacuum to 50 ml. This extract is referred to as the reaction liquid in the presentation and discussion of results. The 2,4–DNPH derivatives of the volatile carbonyl compounds which were removed by the gas stream were extracted with 100 ml. of chloroform and concentrated to 50 ml. This extract is referred to as the vapor fraction.

Aliquots of the concentrated chloroform extracts were used for analysis by paper, gas-liquid (GLC), and thin-layer chromatography (TLC). The 2,4–DNPH reagent for isolating the carbonyl compounds was prepared by dissolving 4 g. of 2,4–DNPH (Eastman Kodak) in 55 ml. of concentrated sulfuric acid. The solution was added to 345 ml. of distilled water to give a 1% 2,4–DNPH solution.

In some experiments, the intensity of the color was followed by light absorption at 500 mμ with a Beckman model DU spectrophotometer.

For the starch paste system, 20 g. pregelatinized wheat starch and 80 g. of native wheat starch were mixed with 65 ml. of a solution containing 0.02*M* each of amino acid and sugar and adjusted to pH 5.5. The paste was rolled into a layer ½ to ¾ in. thick, 8 in. square, which was baked at 425°F. for 30 min.

The surface color of the baked starch paste was determined with a Photovolt reflectometer with a green tristimulus filter (3). The carbonyl compounds were extracted from the baked starch with successive 100-, 75-, and 50-ml. portions of carbonyl-free chloroform, in a Waring Blendor. The 2,4–DNPH derivatives were formed by refluxing the combined chloroform extracts with 400 ml. of 2,4–DNPH solution for 1 hr. The chloroform layer was removed, the 2,4–DNPH reagent was extracted with two aliquots of chloroform, and the combined extracts were concentrated and adjusted to 50 ml.

Paper Chromatography of 2,4-DNPH Derivatives of Carbonyl Compounds

The method of Linko *et al.* (4) was found most suitable for separation of the 2,4–DNPH derivatives. Suitable amounts of the chloroform extracts containing the 2,4–DNPH derivatives were spotted in a 1-in. streak on Whatman No. 4 paper. The paper was impregnated with a 1:1 (v./v.) mixture of N,N–dimethylformamide (N,N–DMFA) and absolute ethyl alcohol; dried for 20 min.; equilibrated for 5 hr. inside the chromatography chamber; and developed for 4.5 hr. with the solvent, cyclohexane saturated with N,N–DMFA. In some experiments, larger quantities of unknown 2,4–DNPH's were separated. Another paper-chromatography solvent system described by Linko *et al.* (4) was employed to aid in the separation and identification of the 2,4–DNPH derivatives. Whatman No. 1 paper spotted with 2,4-DNPH extracts was impregnated with a 10% solution of 2-phenoxyethanol in acetone and irrigated 30 hr. with n-heptane saturated with 2-phenoxyethanol. Where separation of the compounds was still incomplete, the mixture of 2,4–DNPH derivatives of the carbonyl compounds was extracted from the paper with absolute methanol and concentrated, and 2,4–DNPH deriva-

tives were further resolved by GLC or TLC. Identification was by comparison with known 2,4–DNPH derivatives.

Gas-Liquid Chromatography of Carbonyl Compounds

An Aerograph, A-90-P instrument, equipped with a model A-500-B hydrogen flame ionization detector and a 5-mv. Speedomax (model S600) recorder, was used. Two stainless-steel columns (10 ft. × ⅛–in. o.d.) were used. One was packed with 60/80–mesh Chromosorb–P coated with 20% Carbowax 20-M; the other was packed with 60/80-mesh Chromosorb-W coated with 20% dinonyl phthalate.

The flash-exchange technique of Stephen and Teszler (14) was used. A mixture of the unknown 2,4–DNPH derivatives of the carbonyl compounds was heated with a mixture of alpha-ketoglutaric acid and the 2,4–DNPH derivative of formaldehyde in a capillary tube for 30 sec. at 250°C. (4). Thus, the free aldehydes and ketones were released and separated.

Thin-layer Chromatography of 2,4-DNPH Derivatives

Thin-layer chromatography techniques were used as an aid in identifying the 2,4–DNPH derivatives of the carbonyl compounds. A modification of the method of Onoe (15) gave the best resolution. Silica gel G plates and a water-saturated benzene solvent system were used. A slurry of silica gel G (30 g.) in water (60 ml.) was poured into a commercial applicator (G. A. Brinkman & Co., Great Neck, N. Y.) and five clean, dry glass plates, 20 by 20 cm., were covered with the slurry. The covered plates were dried at room temperature for 20 min. and then dried for 1 hr. in an air oven at 130°C. The silica gel G plates were spotted with five 50-μl. aliquots of the chloroform extracts and developed for 45 min. in a chromatography chamber which was previously equilibrated with the solvent. Resolution was best when the plates were removed from the chamber, air-dried, and developed two additional times in the water-saturated benzene solvent.

Quantitative Determination of Carbonyl Compounds

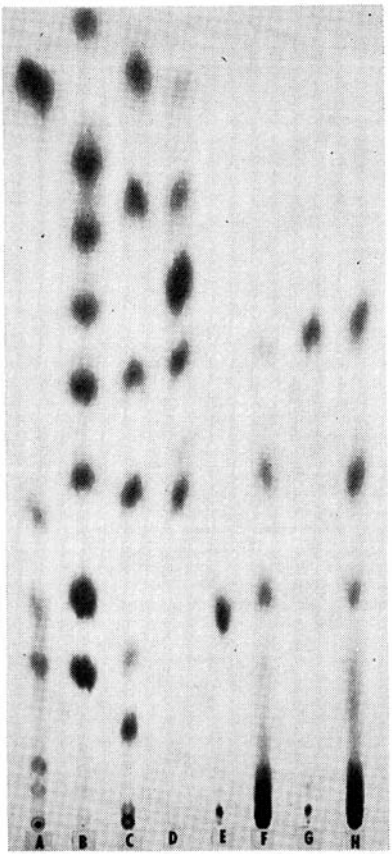
The quantity of the carbonyl compounds was estimated by extracting the 2,4-DNPH zones with ethanol and determining the ultraviolet absorption at the optimum absorption peak for each carbonyl compound. When several compounds were in a zone, the maximum wave length of the 2,4-DNPH derivative present in greatest quantity was used.

RESULTS AND DISCUSSION

Separation and Identification of Carbonyl Compounds

A paper chromatogram (Fig. 1) illustrates the separation of carbonyl compounds, as 2,4-DNPH derivatives, resulting from the reaction of xylose with alanine (E&F) and valine (G&H). The separation with known 2,4-DNPH derivatives is shown in the first four spaces. The second solvent system for paper chromatography, 2-phenoxyethanol-saturated n-heptane, was used to separate propionaldehyde and acetone, 2-butanone and n-butyraldehyde and isobutyraldehyde.

Figure 2 illustrates the separation of the 2,4-DNPH derivatives resulting from the reaction of glucose with phenylalanine, histidine, arginine, and



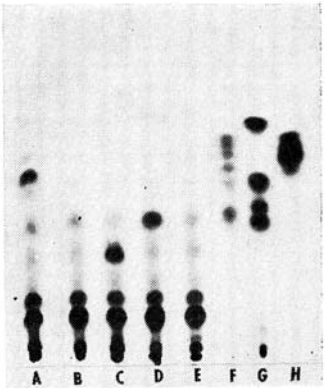


Fig. 1 (left). Paper chromatogram of 2,4-DNPH derivatives. Solvent: cyclohexane saturated with N,N-dimethylformamide. A–D, known carbonyl compounds, reading from bottom to top:

A: 1, pyruvaldehyde; 2, diacetyl; 3, unknown; 4, benzaldehyde; 5, crotonaldehyde; 6, 4-heptanone.

B: 1, formaldehyde; 2, acetaldehyde; 3, propionaldehyde; 4, n-butyraldehyde; 5, n-valeraldehyde; 6, n-hexaldehyde; 7, n-heptaldehyde; 8, n-decylaldehyde.

C: 1, HMF; 2 and 3, isomers of 2,4-DNPH derivatives of furfural; 4, acetone; 5, 2-butanone; 6, 2-hexanone; 7, 3-heptanone.

D: 1, phenylacetaldehyde; 2, isobutyraldehyde; 3, isovaleraldehyde and 2-methylbutanal; 4, 2-methylpentanal; 5, 2-ethylhexanal.

E, F: carbonyl compounds of alanine-xylose: E, vapor fraction; F, reaction liquid.

G, H: carbonyl compounds of valine-xylose: G, vapor fraction; H, reaction liquid.

Fig. 2 (right). Thin-layer chromatogram of 2,4-DNPH derivatives. Solvent: water-saturated benzene, multiple ascent technique. A to E: carbonyl compounds of (reaction liquid in each case): A, glucose-phenylalanine; B, glucose-histidine; C, glucose-methionine; D, glucose-arginine; E, glucose-tryptophan.

F, G, H: known carbonyl compounds, reading from bottom to top. F: 1, formaldehyde and acetaldehyde; 2, propionaldehyde; 3, n-butyraldehyde; 4, n-valeraldehyde; 5, n-hexaldehyde; 6, n-heptaldehyde.

G: 1, HMF; 2, acetone; 3, furfural; 4, 2-butanone; 5, 3-heptanone.

H: 1, phenylacetaldehyde; 2, isobutyraldehyde; 3, isovaleraldehyde and 2-methylbutanal; 4, 2-methylpentanal.

tryptophan by TLC. Although the separation of compounds was not as distinct as desired, this technique permitted confirmation of the presence of phenylacetaldehyde, acetone, and propionaldehyde. In addition, it assisted in identifying the other carbonyl compounds that were isolated by paper chromatography.

Figures 3, 4, and 5 are gas chromatograms of the vapor fraction of the carbonyl compounds formed during reaction of amino acids with glucose. The chromatograms indicate the great predominance of certain carbonyl compounds in the vapor. In most cases only the major compounds were detected

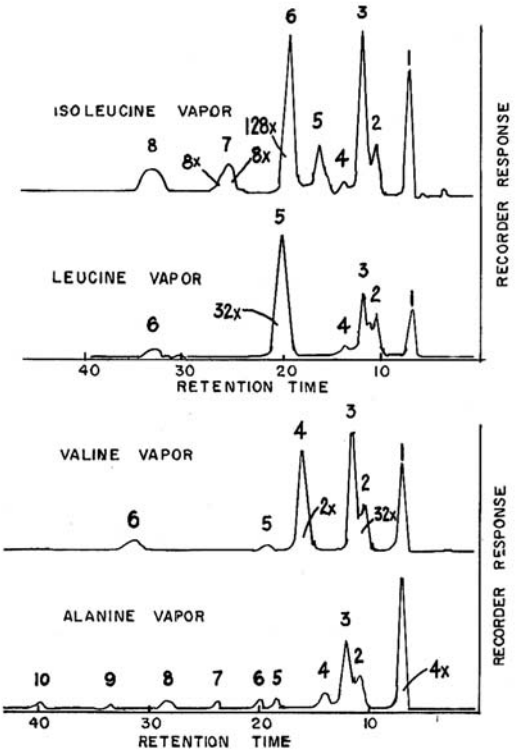


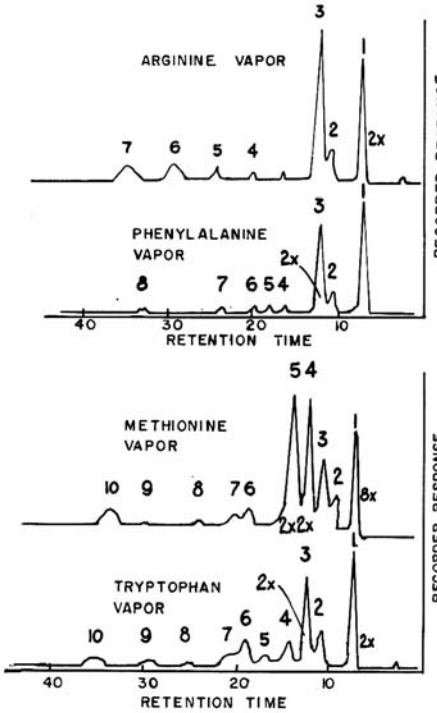
Fig. 3. Gas chromatograms of 2,4-DNPH derivatives. Conditions: column temperature, 100°C.; helium flow rate. 50 ml./min.; column, carbowax 20 M. A to D: vapor from reactions as indicated.

A, isoleucine and glucose: 1, formaldehyde and acetaldehyde; 2, propionaldehyde; 3, acetone and isobutyraldehyde; 4, unknown; 5, n-butyraldehyde; 6, 2-methylbutanal and isovaleraldehyde; 7, n-valeraldehyde; 8, unknown.

B, leucine and glucose: 1, formaldehyde and acetaldehyde; 2, propionaldehyde; 3, acetone and isobutyraldehyde; 4, unknown; 5, 2-methylbutanal and isovaleraldehyde; 6, unknown.

C, valine and glucose: 1, formaldehyde and acetaldehyde; 2, propionaldehyde; 3, acetone and isobutyraldehyde; 4, n-butyraldehyde; 5, 2-methylbutanal and isovaleraldehyde; 6, unknown.

D, Alanine and glucose: 1, formaldehyde and acetaldehyde; 2, propionaldehyde; 3, acetone and isobutyraldehyde; 4, unknown; 5, 2-methylbutanal and isovaleraldehyde; 6, unknown; 7, n-valeraldehyde; 8, 9, 10, unknown.



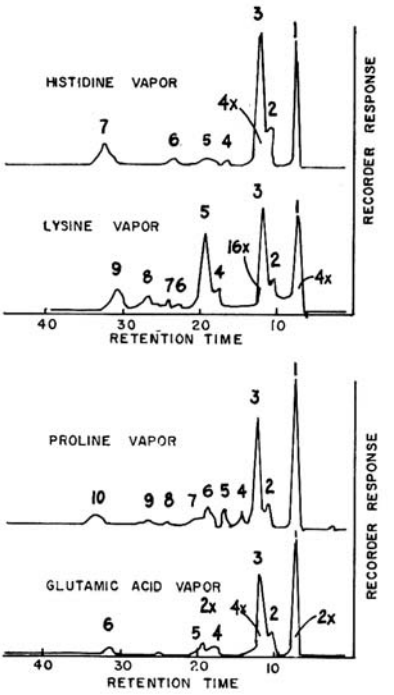


Fig. 4 (left). Gas-liquid chromatograms of 2,4-DNPH derivatives. Conditions: column temperature, 100°C.; helium flow rate, 50 ml./min.; column, carbowax 20 M. A to D: vapor from reactions as indicated.

A, arginine-glucose: 1, formaldehyde and acetaldehyde; 2, propionaldehyde; 3, acetone; 4, isovaleraldehyde; 5, n-valeraldehyde; 6 and 7, unknown.

B, phenylalanine-glucose: 1, formaldehyde and acetaldehyde; 2, propionaldehyde; 3, acetone; 4, n-butyraldehyde; 5, phenylacetaldehyde; 6, isovaleraldehyde; 7, n-valeraldehyde; 8, unknown.

C, methionine-glucose: 1, formaldehyde and acetaldehyde; 2, unknown; 3, propionaldehyde; 4, acetone; 5, methional; 6, unknown; 7, isovaleraldehyde; 8, n-valeraldehyde; 9 and 10, unknown.

D, tryptophan-glucose: 1, formaldehyde and acetaldehyde; 2, propionaldehyde; 3, acetone; 4, unknown; 5, n-butyraldehyde; 6, unknown; 7, isovaleraldehyde; 8, n-valeraldehyde; 9 and 10, unknown.

Fig. 5 (right). Gas-liquid chromatograms of 2,4-DNPH derivatives. Conditions: column temperature, 100°C.; helium flow rate, 50 ml./min.; column, carbowax 20 M. A to D: vapor from reactions as indicated.

A, histidine-glucose: 1, formaldehyde and acetaldehyde; 2, propionaldehyde; 3, acetone; 4, n-butyraldehyde; 5, isovaleraldehyde; 6, n-valeraldehyde; 7, unknown.

B, lysine-glucose: 1, formaldehyde and acetaldehyde; 2, propionaldehyde; 3, acetone; 4, n-butyraldehyde; 5, isovaleraldehyde; 6, unknown; 7, n-valeraldehyde; 8 and 9, unknown.

C, proline-glucose: 1, formaldehyde and acetaldehyde; 2, propionaldehyde; 3, acetone; 4, unknown; 5, n-butyraldehyde; 6, unknown; 7, isovaleraldehyde; 8, n-valeraldehyde; 9 and 10, unknown.

D, glutamic acid-glucose: 1, formaldehyde and acetaldehyde; 2, propionaldehyde; 3, acetone; 4, n-butyraldehyde; 5, isovaleraldehyde; 6, unknown.

by paper chromatography. Gas chromatography was most useful for separation of acetone and propionaldehyde, n-valeraldehyde and isovaleraldehyde, and n-butyraldehyde and isobutyraldehyde, none of which could be separated readily by other techniques. However, neither formaldehyde and acetaldehyde nor 2-methylbutanal and isovaleraldehyde, when present in mixtures, could be resolved by gas chromatography. By combining gas-liquid, thin-layer, and paper chromatographic techniques, it was possible to identify most of the carbonyl compounds.

Reactivity of Amino Acids and Sugars in the Aqueous System

Tables I, II, and III show the color intensity of the reacted solution of three different sugars with different amino acids. Generally, when the sugars were heated without amino acid, they produced relatively few compounds absorbing light at 500 mμ. Addition of amino acid increased the color intensity. Comparison of the color-intensity values for each amino acid with each of the sugars indicates that xylose produced the most intense brown color, followed by glucose and then maltose. There were large differences in color intensity owing to the type of amino acid. Generally, the basic and monoaminomonocarboxylic acids were most reactive; glutamic acid and proline were least reactive as measured by absorption at 500 mμ. The color intensity data suggested that Maillard-type browning was mainly responsible for the brown color produced.

Quantities of the major carbonyl compounds produced by the reaction of sugars with different amino acids are expressed in total mg. (Tables I–III). The results were obtained by using the cyclohexane solvent system, which does not separate all the carbonyl compounds completely; therefore, the results represent five distinct mixtures of carbonyl compounds. The groups

TABLE I

AMOUNT OF CARBONYL COMPOUNDS IN AN AQUEOUS SYSTEM OF GLUCOSE AND AMINO ACIDS[a]

AMINO ACID	COLOR INTENSITY[b]	FORMALDEHYDE	ACETALDEHYDE	ACETONE	ISOBUTYRALDEHYDE	ISOVALERALDEHYDE
		mg.	*mg.*	*mg.*	*mg.*	*mg.*
No amino acid	0.3	0.4	0.16	0.08	.....	.....
Glycine	3.9	.60	2.06	0.56	0.34	0.30
Alanine	1.5	.48	13.72	0.95	1.26	0.29
Valine	3.2	.82	3.16	4.93	40.1	4.08
Leucine	3.8	.79	2.13	2.00	1.32	82.3
Isoleucine	4.0	.73	2.64	1.75	2.64	79.0[c]
Glutamic acid	1.2	.15	1.41	0.45	0.37	.....
Histidine	7.1	.18	0.59	0.47	0.29	.....
Lysine	8.2	.30	1.88	0.98	0.57	0.40
Arginine	2.5	.30	0.88	1.24	0.36	.....
Phenylalanine	6.6	.56	2.19	0.85	.....	6.28[d]
Tryptophan	5.5	.39	2.11	6.08	1.32	0.3
Proline	0.2	.09	0.68	0.34	0.23	.....
Methionine	11.6	0.59	7.60[e]	2.09	0.63	0.59

[a] All values are averages of duplicates.
[b] Absorbance of solution at 500 mμ.
[c] 2-Methylbutanal.
[d] Phenylacetaldehyde.
[e] Methional and acetaldehyde.

TABLE II

AMOUNT OF CARBONYL COMPOUNDS IN AN AQUEOUS SYSTEM OF XYLOSE AND AMINO ACIDS[a]

AMINO ACID	COLOR INTENSITY[b]	FORMALDEHYDE	ACETALDEHYDE	ACETONE	ISOBUTYRALDEHYDE	ISOVALERALDEHYDE
		mg.	*mg.*	*mg.*	*mg.*	*mg.*
No amino acid	0.6	0.16	0.52	0.27	0.34	.....
Glycine	19.6	0.85	5.23	7.30	1.57	1.61
Alanine	9.7	1.99	82.5	6.76	5.08	1.95
Valine	12.0	2.50	7.41	13.77	2.76	1.78
Leucine	17.8	2.62	8.06	7.49	0.95	215.
Isoleucine	17.4	2.80	9.66	11.4	7.75	343.[c]
Glutamic acid	21.0	0.74	1.90	1.51	1.02	1.20
Histidine	24.0	1.68	5.55	7.68	0.90	0.73
Lysine	24.0	0.44	3.20	2.52	1.80	0.81
Phenylalanine	13.0	1.36	3.29	4.96	.....	51.6[d]
Proline	1.6	0.81	1.97	1.45	1.13	0.79

[a] All values are averages of duplicates.
[b] Absorbance of solution at 500 mμ.
[c] 2-Methylbutanal.
[d] Phenylacetaldehyde.

TABLE III

AMOUNT OF CARBONYL COMPOUNDS IN AN AQUEOUS SYSTEM OF MALTOSE AND AMINO ACIDS[a]

AMINO ACID	COLOR INTENSITY[b]	FORMALDEHYDE	ACETALDEHYDE	ACETONE	ISOBUTYRALDEHYDE	ISOVALERALDEHYDE
		mg.	*mg.*	*mg.*	*mg.*	*mg.*
No amino acid	0.07	0.24	0.22	0.38	.....	.....
Glycine	0.55	.54	0.67	0.30	0.64	0.36
Alanine	0.15	.40	1.19	0.50	1.04	0.24
Valine	1.00	.70	2.60	1.75	9.07	.....
Leucine	0.85	.81	2.33	1.58	1.00	5.50
Isoleucine	0.15	.44	0.90	0.57	0.40	3.62[c]
Glutamic acid	0.15	.21	0.53	0.18	0.20	.....
Histidine	0.27	.10	0.64	0.33	0.57	0.19
Lysine	1.02	.07	0.41	0.36	0.75	.....
Phenylalanine	0.19	.15	0.79	0.23	.....	1.46[d]
Proline	0.11	0.20	0.58	0.30	0.75	.....

[a] All values are averages of duplicates.
[b] Absorbance of solution at 500 mμ.
[c] 2-Methylbutanal.
[d] Phenylacetaldehyde.

were composed of 1) formaldehyde and furfural; 2) acetaldehyde; 3) acetone and propionaldehyde; 4) isobutyraldehyde and n-butyraldehyde; 5) isovaleraldehyde, n-valeraldehyde, and 2-methylbutanal. The mixtures in every case were composed predominantly of one aldehyde or ketone which was used to express the total quantity of carbonyl compounds. These were 1) formaldehyde, 2) acetaldehyde, 3) acetone, 4) isobutyraldehyde, and 5) isovaleraldehyde. Certain amino acids produced other carbonyl compounds, which are indicated in the tables.

Comparison of the data for the three sugars—glucose, xylose, and maltose—reacted with several amino acids (Tables I–III) indicates that the same carbonyl compounds were produced for each sugar, but that the quan-

tities of the carbonyl compounds were affected by the type of sugar. Xylose reacted with amino acids produced the greatest quantity of carbonyl compounds, followed by glucose and then maltose. For example, isoleucine produced 379.0, 34.0, and 3.6 mg. of 2-methylbutanal when reacted with xylose, glucose, and maltose, respectively; phenylalanine produced 51.6, 6.3, and 1.5 mg. of phenylacetaldehyde when reacted with xylose, glucose, and maltose, respectively. Pentose and hexose sugars formed furfural and hydroxymethylfurfural, respectively. Otherwise, type of sugar did not affect the kind of carbonyl compounds formed.

The addition of amino acid to the sugar markedly increased the total quantity of carbonyl compounds formed. The type of amino acid affected the kind and the total amount of carbonyl compounds produced. Generally, leucine, isoleucine, valine, alanine, and phenylalanine produced the largest quantities of carbonyl compounds, whereas glutamic acid and proline produced the smallest amount. The amino acids which produced the most carbonyl compounds were those that formed aldehydes via the Strecker degradation. Alanine, valine, isoleucine, leucine, phenylalanine, and methionine formed mainly acetaldehyde, isovaleraldehyde, 2-methylbutanal, phenylacetaldehyde, and methional, respectively. All these compounds were tentatively identified by a combination of chromatographic techniques and were the aldehydes expected to result from the Strecker degradation which occurs during Maillard-type browning.

Reactivity of Amino Acids and Sugars in the Starch Paste System

Since the reaction conditions used in the liquid system were far removed from those occurring in bread-baking, a model system employing conditions more analogous to baking was developed. Tables IV, V, and VI show the

TABLE IV

AMOUNT OF CARBONYL COMPOUNDS PRODUCED BY GLUCOSE AND AMINO ACIDS REACTED IN A STARCH PASTE SYSTEM[a]

AMINO ACID	REFLECTANCE	FORMALDEHYDE	ACETALDEHYDE	ACETONE	ISOBUTYRALDEHYDE	ISOVALERALDEHYDE
	%	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*
No amino acid	67.5	0.03	0.01	0.05	0.09	.....
Glycine	27.3	.06	.33	.14	0.28	0.16
Alanine	32.0	.06	.87	.25	0.34	0.23
Valine	32.2	.08	.45	.26	3.15	0.32
Leucine	34.6	.07	.27	.18	0.43	1.36
Isoleucine	28.2	.04	.32	.14	0.26	5.29[b]
Glutamic acid	34.3	.06	.28	.16	0.24	0.29
Histidine	9.6	.06	.29	.12	0.26	0.06
Lysine	31.0	.05	.44	.15	0.31	0.21
Phenylalanine	23.6	.06	.43[c]	.18	0.14	0.15
Proline	50.8	.03	.33	.14	0.25	0.16
Arginine	28.0	.04	.29	.14	0.26	0.10
Methionine	23.0	0.09	0.06[d]	0.21	0.27	0.19

[a] All values are averages of duplicates.
[b] 2-Methylbutanal.
[c] Phenylacetaldehyde and acetaldehyde.
[d] Methional and acetaldehyde.

TABLE V

AMOUNT OF CARBONYL COMPOUNDS PRODUCED BY XYLOSE AND AMINO ACIDS REACTED IN A STARCH PASTE SYSTEM[a]

AMINO ACID	REFLECTANCE	FORMALDEHYDE	ACETALDEHYDE	ACETONE	ISOBUTYRALDEHYDE	ISOVALERALDEHYDE
	%	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*
No amino acid	52.0	0.03	0.06	0.04	0.09	.....
Glycine	23.4	.08	.39	.16	0.36	0.20
Alanine	22.8	.06	.71	.20	0.29	0.23
Valine	21.2	.07	.26	.18	3.15	0.26
Leucine	28.4	.07	.26	.15	0.29	1.90
Isoleucine	18.2	.08	.33	.22	0.40	11.5[b]
Glutamic acid	18.6	.05	.22	.12	0.14	0.13
Histidine	9.2	.06	.29	.16	0.26	0.09
Lysine	23.2	.08	.49	.18	0.34	0.21
Phenylalanine	16.6	.08	.30[c]	.15	0.10	0.17
Proline	39.0	0.04	0.46	0.16	0.30	0.16

[a] All values are averages of duplicates.
[b] 2-Methylbutanal.
[c] Phenylacetaldehyde and acetaldehyde.

TABLE VI

AMOUNT OF CARBONYL COMPOUNDS PRODUCED BY MALTOSE AND AMINO ACIDS REACTED IN A STARCH PASTE SYSTEM[a]

AMINO ACID	REFLECTANCE	FORMALDEHYDE	ACETALDEHYDE	ACETONE	ISOBUTYRALDEHYDE	ISOVALERALDEHYDE
	%	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*
No amino acid	71.0	0.04	0.03	0.03	.....	.....
Glycine	34.2	.09	.20	.14	0.26	0.15
Alanine	40.8	.05	.87	.25	0.31	0.22
Valine	38.8	.10	.43	.26	3.63	0.32
Leucine	48.8	.07	.26	.20	0.40	3.00
Isoleucine	41.2	.07	.36	.22	0.31	6.23[b]
Glutamic acid	47.0	.06	.43	.08	0.07	0.10
Histidine	31.2	.06	.39	.18	0.32	0.12
Lysine	36.8	.08	.16	.17	0.34	0.23
Phenylalanine	23.6	.05	.46[c]	.18	0.19	0.16
Proline	65.0	0.15	0.49	0.16	0.31	0.21

[a] All values are averages of duplicates.
[b] 2-Methylbutanal.
[c] Phenylacetaldehyde and acetaldehyde.

surface color of the baked starch layer and the amount of certain carbonyl compounds formed by the reaction of three different sugars with several amino acids. The addition of amino acids increased the brown color of the baked starch. The reflectance data, in addition to the amount and type of carbonyl compounds produced, generally parallel the results obtained for the aqueous model system. It is impossible to compare the relative quantities of carbonyl compounds reported in the starch paste with the carbonyl compounds produced in the liquid system because of the losses occurring during baking of the starch paste. The aldehydes corresponding to the Strecker degradation aldehydes were present in relatively large quantities in the baked starch.

Aroma of the Model Reaction Systems

Subjective evaluation of the volatiles formed during the reactions indicated that no breadlike aromas were formed, possibly because of the large quantities of specific carbonyl compounds formed. Lower concentrations of volatile components might produce the breadlike aromas described by other investigators who reacted amino acids with sugars (11,12). The aromas of phenylacetaldehyde and methional were strongly objectionable, but those of lysine, leucine, and isoleucine were appealing. Otherwise, none of the aromas were deemed acceptable.

The results for both model systems support the concept that Maillard-type browning functions significantly in the production of aldehydes and ketones as by-products or intermediates of the reaction. Moreover, the kind of aldehyde produced is controlled mainly by the amino acid, whereas amount is determined mainly by the sugar type.

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