Influence of Yeast Fermentation and Baking on the Content of Free Amino Acids and Primary Amino Groups and Their Effect on Bread Aroma Stimuli[1]

AHMED A. EL-DASH[2] and JOHN A. JOHNSON[3], Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan

ABSTRACT

Yeast is a potential source of primary amino groups in dough. When yeast was added to dough, approximately a 400% increase in total free amino acids was observed. Lysine, alanine, proline, cystine, and dicarboxylic acids showed the highest percentage increase of all amino acids analyzed. Although fermentation reduced the dough content of free amino acids, about twice as much remained in the dough after fermentation as was originally present in flour. The marked decrease in free amino acid content in the bread crust demonstrated their importance in the nonenzymatic browning reaction during baking. Amino acids in the crust were reduced for all free amino acids except the aromatics. Basic and sulfur-containing amino acids, proline, and glutamic acid were the most reactive in browning. The concentration of intermediate compounds and brown melanoidin pigments produced by the nonenzymatic browning reaction was considerably increased in bread crust as a result of fermentation which improved bread flavor. The fermentation process had no effect on the type of carbonyl compounds produced in bread crust, but changed the quantity of carbonyl compounds. Furfural and 5-(hydroxymethyl)-2-furaldehyde (hydroxymethylfurfural), 2-propanone, 2-methylpropanal, butanal, 2-methylpentanal, and an unknown carbonyl compound were increased slightly in bread crust by fermentation.

The contribution of amino acids to the nonenzymatic browning reaction has been studied with the use of model systems and bread. Rothe and Thomas (1) showed that the separate addition of several amino acids to rye-bread doughs resulted in an increase in the corresponding aldehydes formed from the amino acids through the loss of one carbon atom and an amino group. The reaction of various amino acids with sugar caused different color intensities and different aromas in a model system and in bread (2,3). Kretovich and Ponomareva (4), studying the changes of free amino acids in rye and white breads by paper chromatography, found a significant change in the quantity of free amino acids in rye dough, but only a slight change in white-bread doughs. Morimoto (5) found decreases in free amino acid content of crackers and French bread caused by fermentation.

No previous study has been made concerning the effect of fermentation on the role of free amino acids. The present study was made to investigate changes in the content of free amino acids and primary amino groups in bread doughs as a result of fermentation and baking and to study their effect on carbonyl compounds produced in bread crust.

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[2]Postdoctoral Research Associate, Northern Utilization Research and Development Division, USDA, Peoria, Illinois 61604.

[3]Professor, Kansas State University, Manhattan, Kansas 66502.

MATERIALS AND METHODS

Bread-Baking

One-pound loaves of bread were baked with the following formula, by the sponge dough procedure:

Ingredients	*Sponge* g.	*Dough* g.
Flour	490	210
Arkady	3.5	....
Yeast	21	....
Water	318	137
Dextrose	....	28
Salt	....	14
Shortening	....	21

A hard red winter wheat flour containing 14% protein was used. The sponge was mixed for 3 min. and fermented at 86°F. and 90% r.h. for 4 hr. The sponge was remixed with the remaining ingredients for 5 min.; this was followed by 30 min. of fermentation and a 10-min. rest before moulding. The dough was then proofed 50 min. at 100°F. and 96% r.h. and baked 28 min. at 428°F. The procedure was used to produce bread by regular fermentation. For comparison, unfermented doughs were prepared with the same ingredients mixed as a straight dough. The mixed dough was immediately extracted with 70% ethanol for analysis of primary amino groups and free amino acids. To analyze unfermented dough baked into bread, the dough mass was baked without the customary fermentation or proof.

Sample Preparation

A 200-g. portion of ground crust, or dough, was extracted with 70% ethanol and made to 500-ml. volume. The extract was centrifuged at 4,500 r.p.m. and 3° to 5°C. for 50 min. to remove insoluble materials. The clear ethanol extract was designated as fraction A and analyzed for the presence of primary amino groups. Fraction A contained 70% ethanol-soluble proteins, peptides, and free amino acids.

Proteins were removed from the ethanol extracts before analysis for amino acid content by precipitation with picric acid (6). Deproteinization was done in a glass-stoppered flask by adding 400 ml. of 1% picric acid solution per 100 ml. of the ethanol extract. After a few sec. of shaking, the suspension was centrifuged for 20 min. at 6,000 r.p.m. Excess picric acid was removed by a strong basic anion exchanger, the chloride form of Dowex 2-x8 (200- to 400-mesh). Sufficient resin was used to give a bed of 1 X 1½ in. in a 1 X 10-in. chromatograph column. A circle of soft filter paper was placed on the resin surface. The resin was washed with 15 ml. 1N HCl followed by water until the effluent was neutral. A known volume of the clear, centrifuged, deproteinized solution was passed through the resin bed under a pressure of 1½ to 2 in. of mercury. The resin was washed five times with 3 ml. of 0.02N HCl. The clear, colorless effluent and washings were collected and made up to volume. The fraction was designated as fraction B and contained free amino acids and low-molecular-weight peptides.

The content of cysteine could not be determined directly by use of the amino

acid auto-analyzer, so it was necessary to convert it to cystine. The deproteinized effluent was concentrated on a rotary evaporator to a volume of about 3 ml. If any material remained suspended in the solution, a few mg. of Celite was added, and the suspension was filtered through a paper previously washed with 1N HCl and water. The concentrate was transferred into a 10-ml. volumetric flask; the total volume was kept to about 5 to 6 ml. The solution was brought to pH 7 to 8 (Hydrion paper) by dropwise addition of 1N NaOH and allowed to stand at room temperature for 4 hr. After this conversion of cysteine to cystine, the solution was adjusted to pH 2.0 by addition of 1N HCl. The volume was made up to 10 ml. with the use of citrate buffer pH 2.2 solution. The samples were frozen until analyzed.

Amino Acid Analysis

The deproteinized samples, after conversion of cysteine to cystine, were analyzed for amino acid content with a Beckman amino acid analyzer, model 120 B. The areas under the curves of each amino acid chromatogram were calculated and compared with those of the corresponding amino acid in a standard chromatogram of known quantity. The results were expressed as μmoles of amino acid per 100 g. of sample on a dry basis. The total amino acid content, however, was expressed in mg. per 100 g. dry weight.

Determination of Primary Amino Groups

The primary amino groups were determined quantitatively according to the procedure of Satake et al. (7). One milliliter of sample solution was mixed well with 2 ml. of 4% sodium bicarbonate to bring the pH to 8.0; 2 ml. of freshly prepared 0.1% 2,4,6-trinitrobenzene-1-sulfonic acid was added; and the mixture was placed in a dark cabinet at 40°C. After 2 hr., 2 ml. of 1N HCl was added, and absorbance was measured at 340 mμ with a Beckman model DU spectrophotometer. A standard curve was established for the reaction with glycine, and results were expressed in terms of μmoles of primary aminogroups equivalent to μmoles of glycine.

Measurements of the Intensity of Maillard Reaction Intermediate Compounds and Brown Pigments in Bread Crust

The absorbance at 490 or 500 mμ indicated progress of the formation of brown pigments in bread crust; the absorption of light at 278 mμ measured the intermediate compounds formed. A 10-g. portion of ground crust was extracted three times with acetate buffer (pH 5.4) in an Omni-mixer at 3° to 5°C. for 25 min. After filtering, the clear aliquot was collected and the volume was adjusted to 50 ml. Absorption at 278 and 500 mμ was determined.

Formation and Extraction of 2,4-Dinitrophenylhydrazine (2,4-DNPH) Derivatives of Carbonyl Compounds from Bread Crust

A thin layer of crust from the three 1-lb. loaves of fresh bread was carefully removed and ground for 45 sec. in a Waring Blendor. A 50-g. portion of the ground crust was extracted three times with carbonyl-free chloroform. Chloroform extract was added to 500 ml. of 1% 2,4-DNPH reagent and reacted for 2 hr. at 50°C. The chloroform extract of the hydrazones was mixed with 1 g. sodium sulfate to remove water and concentrated in a vacuum. The concentrated extract was filtered and adjusted to 50 ml.

Gas-Liquid Partition Chromatography of the Carbonyl Compound 2,4-DNPH Derivatives

The gas-liquid partition chromatography of the carbonyl compounds was performed in an Aerograph A-90-P equipped with a model A-500-B hydrogen flame ionization detector. A 10-ft. stainless-steel column 1/8-in. o.d., packed with 60- to 80-mesh Chromosorb-P coated with 20% Carbowax 20-M was used. A modified technique of the flash exchange of Stephens and Teszler (8) was used to release the carbonyl compounds from their 2,4-DNPH derivatives. For quantitative analysis, 25 mg. of Celite was mixed with 1 ml. of chloroform containing the hydrazones and the mixture was dried. Alpha-ketoglutaric acid (45 mg.) was mixed with the dry Celite and hydrazones. Ten milligrams of this mixture was then placed in a capillary tube which was inserted into the gas-liquid chromatography. The capillary tube was heated 30 sec. at 250°C. The areas under the peaks were measured and compared to known samples.

Quantitative Determination of Furfural and Hydroxymethylfurfural (HMF)

Furfural and HMF were determined by extraction from the crust with benzene, and spectrophotometric measurement of the color was developed by reaction with *p*-aminodimethylaniline stannous chloride double salt (P-ADA) (9).

RESULTS AND DISCUSSION

Changes in Concentrations of Primary Amino Groups and Free Amino Acids during Fermentation and Baking

Primary Amino Groups and Total Free Amino Acids. The effect of fermentation on the primary amino group content of the ethanol extract of flour, both unfermented and fermented dough, is shown in Table I. As shown by the difference in the primary amino group content of flour and unfermented dough, the yeast contributed a 376 and a 489% increase over the primary amino group content originally present in the flour in fractions A and B, respectively. (Dough contains only 88.9% flour.) That increase was due mainly to 70% ethanol-soluble, low-molecular-weight peptides and free amino acids, as indicated by results obtained from fraction B. Fermentation reduced the content of primary amino groups, but the concentration of amino groups remaining in dough after fermentation was twice as high as that contributed by the flour.

TABLE I. PRIMARY AMINO GROUP CONTENT OF ETHANOL EXTRACTS OF FLOUR AND DOUGH

	Fraction A[a] μmoles/100 g.[c]	Fraction B[b] μmoles/100 g.[c]
Flour	242.1	160.3
Unfermented dough	1,025.3	840.0
Fermented dough	451.6	329.1

[a]Fraction A contained ethanol-soluble proteins, peptides, and free amino acids.

[b]Fraction B contained ethanol-soluble, low-molecular-weight peptides and free amino acids.

[c]Dry basis.

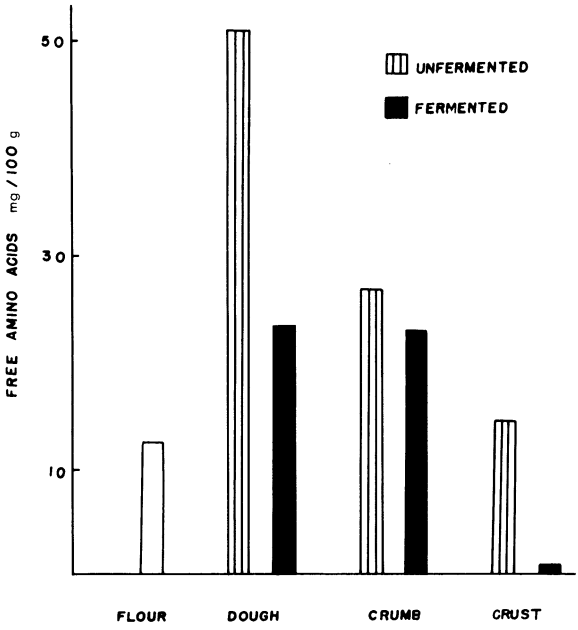

Fig. 1. Total free amino acid content in flour, fermented and unfermented doughs, and bread crust and crumb.

Approximately a 400% increase in total free amino acid content was also observed on addition of yeast to flour, as shown in Fig. 1. This suggests that yeast is a primary source of free amino acids in dough, although Morimoto (5) found that yeast contributed only a small quantity of free amino acids to cracker sponge, whereas flour supplied the major portion. The increase in primary amino groups due to yeast must be attributed mainly to the increase in free amino acids. Fermentation resulted in considerable reduction in the total free amino acid content of the dough. However, approximately twice the concentration of free amino acids contributed by the flour remained in dough after fermentation. The baking process was accompanied by a marked reduction of total free amino acids in the crumb of bread made from unfermented, but not in that made from fermented dough. The observed behavior may be due to yeast fermentation during the first few minutes of baking of the unfermented dough until the loaf temperature reaches the point of inactivating the yeast.

An even greater decrease in the free amino acid content was seen in the bread crust after baking, suggesting that free amino acids played a significant role in bread-crust formation and bread-flavor development. Similar observations concerning the participation of amino acids in the nonenzymatic browning reaction and the subsequent formation of bread flavor were reported by Kretovich and Ponomareva (4). A considerable reduction in the quantity of free amino acids occurred in fermented dough during crust formation although changes were slight in unfermented dough. The results suggest that the fermentation process affects the participation of amino acids in the nonenzymatic browning reaction during baking.

Changes in Free Amino Acid Content Attributed to Yeast. The concentration of various free amino acids in flour, fermented and unfermented doughs, and bread crumb and crust is summarized in Table II. The percent of changes in the content of free amino acids as a result of the addition of yeast to dough and of fermentation and baking are shown in Table III. Seventeen amino acids were detected in most cases. Serine and threonine, however, were not separated completely and were expressed as threonine, and when the separation of histidine and ammonia was incomplete they were expressed as ammonia. The free amino acids were divided into seven groups based on similarity of chemical structure. As shown in Table II, columns 1, 2, and 5, serine and threonine, alanine, aspartic and glutamic acids were the predominant free amino acids in flour, and along with lysine were present in comparatively large concentrations in unfermented dough. In fermented dough, glutamic acid, lysine, alanine, and serine and threonine predominated.

As a result of adding yeast to flour, the concentration of all free amino acids detected in the unfermented dough increased. On the basis of the percentage change in the quantity of free amino acids present from flour to unfermented dough (Table III, column 2), lysine increased the most, followed by glutamic acid, alanine, cystine, proline, and aspartic acid. Therefore, yeast may be considered a primary source of lysine, alanine, dicarboxylic amino acids, proline, and cystine in dough.

Changes in Free Amino Acid Content during Fermentation. All free amino acids except arginine diminished considerably during dough fermentation (Table III, column 1). Aromatic, hydroxy, and sulfur-containing amino acids, aspartic acid, and monoamino-monocarboxylic acids, with the exception of glycine, were markedly reduced in quantity by fermentation. That reduction was likely due to the free amino acids utilized by yeast as metabolites. Arginine, however, may have been metabolized at a slower rate than it was being formed from the wheat proteins by proteolytic action. Similar findings were reported by other investigators (5,10). The dehydrogenation of some amino acids by baker's yeast was studied by Krauze et al. (11), who found that an unpurified extract of baker's yeast catalyzed dehydrogenation of glutamic acid, alanine, dl-alpha-aminobutyric acid, threonine, and serine. The dehydrogenase character of the conversion of those amino acids was indicated by the identification of alpha-ketobutyric acid, alpha-keto-beta-hydroxybutyric acid, and hydroxypyruvic acid from mixtures of baker's yeast extract and dl-alpha-aminobutyric acid, threonine, and serine, respectively.

The combined effect of adding yeast to dough and fermentation on the amino acid content is seen by studying the percentage change of the free amino acid content from that in flour to that in fermented dough (Table III, column 5). Aromatic and hydroxy amino acids, as well as leucine, isoleucine, and valine, were markedly reduced, suggesting that they were utilized as metabolites to a greater extent. All other free amino acids were present in considerably higher concentrations in fermented dough than in flour. Ponomareva et al. (10) found that during the fermentation process, the concentration of alpha-aminobutyric acid was greatly increased, although there was a significant drop in the content of glutamine, valine, leucine, and isoleucine.

TABLE II. FREE AMINO ACID CONCENTRATIONS IN FLOUR, DOUGH, AND BREAD CRUMB AND CRUST

Amino Acid	Flour	Unfermented			Fermented		
		Dough	Crumb	Crust	Dough	Crumb	Crust
		μmoles/100 g. (dry basis)					
Monoamino-monocarboxylic acids							
Glycine	4.08	11.74	12.81	7.66	10.43	12.43	0.84
Alanine	14.34	86.98	31.18	31.89	22.94	29.05	2.12
Valine	6.83	16.15	5.87	4.75	4.60	6.41	0.62
Leucine	5.54	8.66	3.92	2.74	1.73	4.79	0.27
Isoleucine	4.13	6.60	2.51	2.04	1.58	3.07	0.52
Dicarboxylic acids							
Aspartic acid	14.17	60.53	14.55	12.34	14.91	10.92	0.83
Glutamic acid	8.85	71.85	53.88	9.75	46.83	51.39	1.13
Secondary: Proline	4.66	21.65	19.52	8.69	17.11	11.21	0.44
Basic							
Lysine	2.39	32.31	21.27	9.88	25.53	12.76	trace
Arginine	2.11	6.66	16.27	0.53	15.30	11.43	trace
Histidine	0.95	...	...	...	...	...	...
"Ammonia"	50.50	353.35[a]	51.94	154.29	70.88 [a]	39.47	60.88
Aromatic							
Tyrosine	3.12	5.99	0.92	3.11	0.49	1.09	
Phenylalanine	3.31	,5.86	1.62	12.17	0.18	1.95	1.64[b]
Sulfur-containing							
Cystine	0.56	3.25	0.62	0.10	1.16	1.01	trace
Methionine	0.69	2.11	1.12	1.39	0.95	1.83	trace
Hydroxy							
Serine and threonine	27.50	68.14	22.04	13.17	19.22	22.88	1.24

[a]Histidine and ammonia. [b]Tyrosine and phenylalanine.

TABLE III. CHANGES IN THE CONTENT OF FREE AMINO ACIDS IN DOUGH AND BREAD CRUMB AND CRUST

Amino Acid	Free Amino Acid Change Due to Fermentation[a] %	Unfermented[b] Dough from Flour[c] %	Unfermented[b] Crumb from Dough %	Unfermented[b] Crust from Dough %	Fermented[b] Dough from Flour[c] %	Fermented[b] Crumb from Dough %	Fermented[b] Crust from Dough %
Monoamino-monocarboxylic acids							
Glycine	− 11.16	+ 223.42	+ 9.11	− 34.75	+ 187.33	+ 19.18	− 91.90
Alanine	− 73.63	+ 582.20	− 64.15	− 63.34	+ 79.92	+ 26.63	− 90.76
Valine	− 71.52	+ 166.06	− 63.65	− 70.59	− 24.22	+ 39.35	− 86.52
Leucine	− 80.02	+ 75.66	− 54.73	− 68.36	− 64.91	+176.87	− 84.39
Isoleucine	− 76.06	+ 79.84	− 61.97	− 69.09	− 56.94	+ 94.30	− 67.09
Dicarboxylic acids							
Aspartic acid	− 75.37	+ 380.40	− 75.96	− 79.61	+ 18.33	− 26.76	− 94.43
Glutamic acid	− 34.82	+ 812.96	− 25.01	− 86.43	+ 495.04	+ 9.74	− 97.59
Secondary: Proline	− 20.97	+ 422.95	− 9.84	− 59.86	+ 313.29	− 34.48	− 97.43
Basic							
Lysine	− 20.98	+1424.06	− 34.17	− 69.42	+1104.25	− 50.02	−100.00
Arginine	+129.73	+ 256.15	+144.30	− 92.04	+ 718.18	− 25.94	−100.00
Histidine	...	...		...		...	...
"Ammonia"	− 79.94[d]	+ 672.69[d]	− 85.30	− 56.34	+ 54.99[d]	− 44.31	− 14.11
Aromatic							
Tyrosine	− 91.82	+116.24	− 84.64	− 48.08	− 82.31	+122.45	...
Phenylalanine	− 96.93	+ 99.32	− 72.35	+107.67	− 93.88	+983.33	+144.78[e]
Sulfur-containing							
Cystine	− 64.31	+ 550.00	− 80.92	− 96.92	+ 132.00	− 12.93	−100.00
Methionine	− 54.97	+ 245.90	− 46.92	− 34.12	+ 55.74	+ 92.63	−100.00
Hydroxy							
Serine and threonine	− 71.79	+ 178.69	− 67.65	− 80.67	− 21.39	+ 19.04	− 93.55

[a,b]Percent change was calculated from the following equation: (Amino acid content of fermented dough) − (amino acid content of unfermented dough)/(amino acid content of unfermented dough) X 100.

[c]The amount of amino acids contributed by flour was calculated by multiplying the figure in Table II, column 1 by 0.889. (Dough contains only 88.9% flour.) [d]Histidine and ammonia. [e]Tyrosine and phenylalanine.

Changes in Free Amino Acid Content during Baking. The crumb of bread made from unfermented dough underwent a sharp decline in content of all free amino acids except glycine and arginine (column 3, Tables II and III). That may be due to the sharp increase in metabolic rate of yeast during the first few minutes of baking and before the crumb temperature reached that of deactivation of the yeast enzyme system. On the other hand, the crumb of bread made from fermented dough showed a lesser degree of amino acid depletion (column 6, Tables II and III). However, monoamino-monocarboxylic acids, aromatic and hydroxy amino acids, glutamic acid, and methionine were relatively more abundant in the crumb than in the fermented dough.

The formation of the bread crust during baking resulted in a reduction in free amino acid content (columns 4 and 7, Tables II and III). Reduction occurred for all free amino acids except the aromatics, which were found in higher concentration in the crust than in the fermented dough.

The percentage change of free amino acid content from dough to crust was a measurement of the reactivity of the amino acid in crust formation. The more negative this value, the more reactive the amino acid. Monoamino-monocarboxylic acids were the least reactive of all the amino acids assayed and the basic and sulfur-containing amino acids were the most reactive. Proline and glutamic acid showed the same trend, with a percent change of −97%. Hydroxy amino acids and aspartic acid were also reactive. The aromatic amino acids probably played a negligible role in the nonenzymatic browning reaction during crust formation. This appears in contrast to other investigations (2,3).

The reactivity of free amino acids, except tyrosine, in crust formation was generally greater in fermented dough than in unfermented dough. Methionine, glycine, lysine, and proline reaction increased sharply upon fermentation. Cystine and arginine, however, were reactive in both unfermented and fermented dough, and isoleucine reactivity showed no response to fermentation. It appears that yeast fermentation induces a marked reactivity increase of most free amino acids in the browning reaction.

Role of Free Amino Acids in Nonenzymatic Browning Reaction. Formation of a brown crust is essential for full bread flavor. Bread baked without forming a brown crust possesses little flavor (12). Formation of a brown crust takes place by Maillard-type nonenzymatic browning reactions.

Effects of fermentation on the intermediate and end products of the nonenzymatic browning reaction are given in Table IV. Fermentation was accompanied by a marked increase in concentration of intermediate compounds of the nonenzymatic browning reaction. Brown melanoidin pigments were

TABLE IV. EFFECT OF FERMENTATION ON INTERMEDIATE AND END PRODUCTS OF NONENZYMATIC BROWNING REACTION IN BREAD CRUST

Dough	Absorbance at 287 mμ	Absorbance at 500 mμ
Unfermented	0.180	0.169
Fermented	0.320	0.195

considerably more abundant in bread crust made from fermented dough than from unfermented dough. A similar trend was observed when crust color was measured by a Photovolt reflectance meter. Also, in the absence of fermentation, the basic mild flavor identified with all baked products was detected but the aroma was not recognizable as that of bread and was judged to be inferior and unacceptable. Observations indicate that fermentation is essential for developing intermediate compounds and forming melanoidin pigments during crust formation.

In spite of the high concentration of primary amino groups and free amino acids in unfermented dough (Table I and Fig. 1), the brown pigments and nonenzymatic reaction intermediate compounds in the crust were lower in concentration than those in bread crust from fermented dough. Moreover, only 45.35% of the total free amino acid content was consumed[4] during crust formation in unfermented dough although 94.87% was consumed in fermented dough. The results indicate clearly that the rate of reaction of amino groups with reducing sugar was increased significantly as a result of fermentation. The aroma of bread made from fermented dough was superior to that of bread made from unfermented dough. It appears that the fermentation by-products are intimately involved in development of the bread flavor complex by accelerating the rate of the browning reaction during the baking process.

Fermentation is known to produce significant quantities of acetic and lactic acids and is believed to produce more flavorful bread. Johnson et al. (13) quantitatively analyzed the acids in pre-ferments. It was found that acetic acid developed during the first hours of fermentation whereas lactic acid continued to develop slowly for an extended period of time. Total acid production reached maximum values within 3 to 5 hr. of fermentation (14). However, the significance of organic acids in flavor is not known (15).

Thomas and Rothe (16) stated, rather broadly, that fermentation merely creates the preconditions necessary for the formation of bread flavor stimuli under the influence of oven heat. The effect of acid-base catalysts on the rate of the nonenzymatic browning reaction was studied by Rosen et al. (17,18) and Reynolds (19). Acetic acid was found to be a good catalyst for both the formation and decomposition of fructose-*p*-toluidine (17). Rosen et al. (18) studied the effect of carboxylic acids and their salts on the rearrangement of glucosyl-*p*-toluidine. They also found that the yield of glucosylamine was affected by an acid-catalyzed reaction. Also, the formation of D-allosamine and D-altrosamine from D-psicose was catalyzed strongly by organic acids according to Heyns et al. (20). Such results suggest that the Amadori rearrangement for condensation products of the amino groups with the reducing sugars in the Maillard-type reaction is subject to general acid-base catalysis.

Results obtained from the present investigation and others (13,17,18,19,20) support the theory that organic acids formed during fermentation, particularly acetic and lactic acid, act as catalysts for rearranging the condensation products of amines and reducing sugars during the baking process, thus increasing the production of Maillard-type-reaction intermediate compounds and melanoidin

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[4]Percent consumption of amino acids = (amino acids in crumb − amino acids in crust)/(amino acids in crumb) X 100.

TABLE V. CARBONYL COMPOUND CONTENT OF CRUST OF BREAD MADE FROM FERMENTED AND UNFERMENTED DOUGH

	Unfermented μmoles/100 g.[a]	Fermented μmoles/100 g.[a]
Ethanal	37.4	27.0
Propanal	21.6	21.6
2-Propanone, 2-methylpropanal	26.9	30.0
Unknown No. 3A	15.6	17.4
Butanal	trace	0.5
2-Butanone	53.2	29.1
2-Methylbutanal, 3-methylbutanal	1.4	1.1
Unknown No. 6A	1.1	1.3
Pentanal	0.7	0.8
2-Methylpentanal	8.4	9.9
Hexanal	4.6	4.8
Furfural and 5-(hydroxymethyl)-2-furaldehyde (HMF)	14.2	16.11

[a]Dry basis.

brown pigments. That consequently could result in development of a darker crust color and more flavorful bread.

Role of Free Amino Acids in Production of Carbonyl Compounds in Bread Crust

Quantitative determination of individual carbonyl compounds is needed to determine their respective roles in bread aroma. Thomas and Rothe (16) stated that concentration of a substance above the threshold of human perception must be established before it may be assumed to be a component of bread flavor. Ethanal, for instance, was detected at a threshold level of 1.3 p.p.m. and the threshold for 2-propanone was 500 p.p.m. (21). Lea and Swoboda (22) found that the aroma threshold for alkanals decreased with increasing chain length from C-3 to C-12 but rose sharply at C-14. The threshold values ranged from 0.0012 p.p.m. for propanal to 0.0001 p.p.m. for decanal, 0.525 p.p.m. for 2-butanal and 0.0008 p.p.m. for 2-nonenal.

The carbonyl compound content of the crust of bread made from fermented and unfermented dough is shown in Table V. Those compounds isolated from the bread crust made from unfermented dough were essentially the same ones isolated from fermented dough crust. 2-Butanone and ethanal, however, were the predominant carbonyl compounds in the former, and 2-propanone and 2-methylpropanal in the latter. As shown in Figs. 2 and 3, ethanal and 2-butanone decreased as a result of fermentation. Propanal, 2-methylbutanal and 3-methylbutanal, pentanal, hexanal, and unknown 6A remained essentially constant, whereas 2-propanone and 2-methylpropanal, unknown 3A, furfural and HMF, and 2-methylpentanal increased slightly. Such a slight increase in the concentration of some of these may be considered significant, however, since the threshold value for 2-methylpropanal is extremely low (0.0009 p.p.m.) (22) and furfural and HMF have a special importance in bread flavor (1,23). It thus appears that the fermentation process had no effect on the type of carbonyl compounds formed in bread crust during baking.

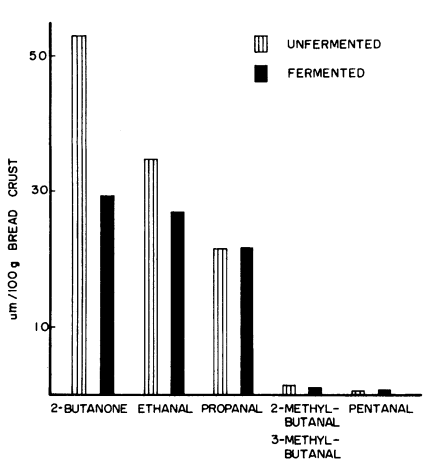

Fig. 2 (left). Carbonyl compound content of the crust of bread made from fermented and unfermented dough.

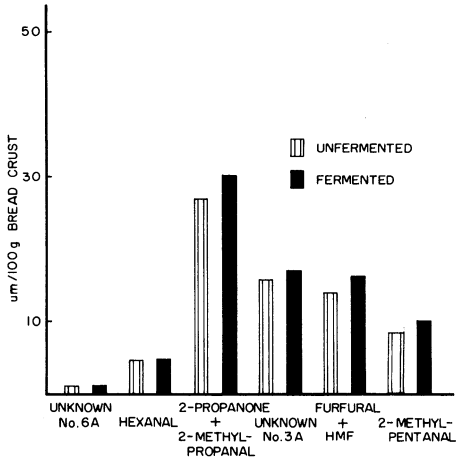

Fig. 3 (right). Carbonyl compound content of the crust of bread made from fermented and unfermented dough.

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