EFFECTS OF SUGARS AND FREE AMINO ACIDS ON BREAD CHARACTERISTICS

by

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B. S., Kansas State University, 1960

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Flour and Feed Milling Industries

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1964

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INTRODUCTION

At the present time many believe that the color in crust formation of baked products is closely related to the Maillard reaction, a non-enzymatic reaction between amino acids and reducing sugars. Until recent years the mechanism of crust browning has been controversial. Nevertheless, the research reported to date has been concerned with a relatively small number of amino acids and sugars. Accordingly, the studies reported in this thesis were concerned with the effects on bread crust color formation of certain of the previously reported amino acids and sugars, together with a number of additional sugars and amino acids not previously reported in the literature, particularly when employing fermented wheat flour doughs that were baked into bread.

Bread is not an ideal system for studying the browning reaction because of the presence of small or trace amounts of many flour components including amino acids, soluble proteins, sugars, lipids, and pentosans. Thus, a second objective of the work reported in this thesis was to study the use of a blend of 20% pregelatinized wheat starch and 80% raw wheat starch in place of wheat flour, thereby affording a semi-synthetic model system for studying the effects on crust color formation of sugars and amino acids, alone and in combination, independent of interfering wheat flour components.

Additional studies were concerned with the increase in loaf volume resulting from certain concentrations of glutamic acid to wheat flour bread doughs.

REVIEW OF LITERATURE

The chemistry and significance for the non-enzymatic browning of baked products was reviewed by Hodge (9), and Johnson and Miller (12). This rather complex reaction produces the desirable flavor and aroma in bread. Baker et al. (1) reported the flavor of the bread crust was carried into the crumb in the form of volatile aldehydes and alcohols which were produced by the reduction of amino acids in browning. Linko and Johnson (18) studied the effects of various sugars on the formation of carbonyl compounds during bread baking. They found that 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 and the formation of carbonyl compounds in the crust was accompanied by significant decreases in all free amino acids, and particularly aspartic and glutamic acids. Kretovich and Panomaryova (14) also found a significant decrease of free amino acids in wheat and rye bread crust compared with that in dough and crumb. Amino acids in the crust decreased with increase of sugar content in the bread. These changes were attributed to the participation of free amino acids in the reaction of melanodin formation.

Zenter (31) reported that adding amino acids to wheat flour increased browning of the baked bread crust, and that adding glycine and lysine lowered gas production. Pomeranz et al. (24) followed the rate of browning of 20 sugars or sugar derivatives by reflectance measurements of cookies and spectrophotometric measurements of browning of dilute buffered solutions of sugars with glycine or lysine heated at 114°C. in an autoclave. Pentoses were most reactive. They were followed by reducing hexoses and disaccharides. Sugars without a reducing group failed to show browning.

Ehle et al. (4) have reported that lysine at levels recommended for bread enrichment did not affect the loaf volume of standard white bread, but that browning increased with increasing lysine concentration. Menger (20) studied the relation between the soluble carbohydrates of durum wheat and the non-enzymatic browning of wheat pastes. It has been suggested that browning colorations in pastes prepared from durum wheats were the result of condensation products involving the soluble carbohydrates.

Burton (2) found that phosphatides in wheat flour might accelerate or inhibit browning. Most of the model systems of sugars and amino acids described in literature were studied in an aqueous medium. Several reports deal with browning of dry mixtures of proteins and reducing sugars (15,16,22) or of amino acids and reducing sugars (25,29). Studies of browning in buffered starch suspensions containing various bread ingredients, and stored for up to 25 days at 75°C. were made by Stenberg and Geddes (28). Rothe and Voigt (27) attempted to duplicate baking conditions by heating sand coated with amino acids and xylose at 130°C.

MATERIAL AND METHODS

Flour

Two untreated flours for experimental baking tests were experimentally milled from two composite grists of several hard winter wheat varieties grown at a number of locations throughout the Great Plains in 1961 and 1962, respectively. Certain chemical and baking properties (14% moisture basis) of the two flour composites were as follow:

Flour Composite	Ash	Protein	Bromate Requirement	Water Absorption	Mixing time	Loaf Volume
	%	%	mg	%	Min	cc
RBS-61A	0.39	12.6	3	62.5	3	947
RBS-62	0.37	12.9	3	61.5	3½	949

The flour designated as RBS-62 was used after depletion of RBS-61A. The two flours were essentially identical in their overall bread baking properties.

Amino Acids and Sugars

The sugars and amino acids used were of analytical grade. The sugars chosen for the first three phases of study were xylose, sucrose, and galactose. These sugars represent the nonfermentable, highly reactive (with amino acids) pentoses, the readily fermentable sugars, and the practically nonfermentable hexoses, respectively. The amino acids selected were the monoamino, monocarboxylic glycine, the monoamino, dicarboxylic glutamic acid, and the diamino, monocarboxylic lysine.

The fourth phase of study involved fourteen additional sugars giving a total of seventeen. These included the pentoses arabinose, ribose, and xylose, the hexoses galactose, glucose, levulose, mannose, sorbose, and rhamnose, the disaccharides cellobiose, lactose, melibiose, sucrose, maltose, and trehalose, and the trisaccharides melezitose and raffinose. The amino acids were the same three studied in the first three phases.

The last phase of study involved nineteen additional amino acids or related compounds. Included in this group were glutamic acid and its derivatives glutamine, L-pyroglutamic acid, mono-sodium glutamate, α-keto glutaric acid, acetyl-L-glutamic acid, benzoyl-L-glutamic acid, and glutamic acid-γ-ethyl ester; aspartic acid and its derivatives asparagine, glycyl-L-aspartic acid, glycyl-L-asparagine, DL-alanyl-DL-asparagine; the butyric acid derivatives DL-α-amino-N-butyric acid, γ-amino butyric acid, 2,4-diaminobutyric acid, α-amino isobutyric acid, glycyl-γ-amino butyric acid; and the non-amino compounds tartaric acid and ammonium chloride.

Baking Method

Baking properties of the two composite flours were determined by employing a formula which included, in addition to the basic formula, 4 g. sucrose, 0.25 g. 120° Lintner malt syrup, 4 g. nonfat dry milk solids, and 1.5 mg. potassium bromate (5). The basic formula included 100 g. flour, 2 g. sucrose, 1.5 g. salt, 3 g. shortening, 2 g. yeast, 1.5 mg. potassium bromate (optimum), and water as needed. An optimum mixing time with the straight dough procedure (6) and a three hour fermentation at 30°C. was employed. The basic formula was employed in all the sugar and amino acid studies.

The basic starch dough of the semi-synthetic system contained a blend of 20 g. pregelatinized and 80 g. raw wheat starch, 2 g. sucrose, 1.5 g. salt, 3 g. shortening, 2 g. yeast and optimum water (about 72 ml.) to permit handling.

Punching and panning were performed mechanically. Bake time was 24 minutes at 218°C. Bakings were replicated at least twice. A third replicate was made when loaf volumes differed more than 25 cc. Data for all replicates were averaged. Average loaf volume differences of 20 cc. were significant at the 0.5 level.

Crumb and Crust Color Determinations

Crumb and crust colors were measured with a Photovolt Reflectometer Model 610, equipped with a green filter. The higher the reading the less color. Results reported are averages of three measurements rounded off to the nearest 0.5 units.

EXPERIMENTAL RESULTS AND CONCLUSIONS

Various Concentrations of Amino Acids and a Fixed Concentration of Sugars

Varying the concentrations of each of three amino acids in combination with a fixed concentration of each of three sugars in the basic bread formula had no significant effect on bromate requirement, mixing time, or water absorption of the wheat flour doughs. The Data in Table 1 show the results for bread containing various levels of amino acids with xylose, sucrose, and galactose. Adding glycine generally reduced loaf volume much more than did an equal or even equimolar amount of lysine. Actually, loaves containing additional lysine even at a level of 0.8 g., which is far above the levels suggested for supplementation of flour (26), were comparable in loaf volume to breads con-

Table 1. Loaf volume and crust color of bread containing the basic formula and levels of each of 3 amino acids with 2 g. each of 3 sugars.

Sugar[1]	Amino Acid	GLYCINE Loaf Volume	GLYCINE Crust Color Bottom	GLYCINE Crust Color Top	LYSINE Loaf Volume	LYSINE Crust Color Bottom	LYSINE Crust Color Top	GLUTAMIC ACID Loaf Volume	GLUTAMIC ACID Crust Color Bottom	GLUTAMIC ACID Crust Color Top
	g	cc			cc			cc		
None	0.2	650	53.0	31.0	685	60.0	44.0	740	61.5	41.5
Xylose	0.0	700	32.0	15.5	700	32.0	15.5	700	32.0	15.5
Xylose	0.2	645	21.5	12.5	720	26.0	13.5	730	29.5	13.5
Xylose	0.4	650	19.0	11.0	685	22.0	12.0	705	26.0	12.0
Xylose	0.8	625	14.5	10.0	720	17.0	10.0	670	22.0	10.5
Sucrose	0.0	780	41.5	21.5	780	41.5	21.5	780	41.5	21.5
Sucrose	0.2	750	38.0	18.5	785	42.5	21.0	860	42.5	19.5
Sucrose	0.4	775	33.5	15.0	790	38.5	18.0	795	39.0	16.5
Sucrose	0.8	750	26.0	13.0	740	33.0	16.0	755	38.5	18.5
Galactose	0.0	715	33.5	17.5	715	33.5	17.5	715	33.5	17.5
Galactose	0.2	640	25.5	14.0	750	31.5	16.0	760	36.0	15.5
Galactose	0.4	665	19.5	11.5	745	27.5	13.5	725	32.0	15.0
Galactose	0.8	630	17.0	9.5	695	23.0	11.5	685	29.0	13.5

1/ In addition to 2% sucrose in the basic formula.

taining no additional amino acids. On a weight basis, glycine imparted a deeper color to bread crust than did lysine. The molecular weights of lysine and glutamic acid are approximately twice that of glycine. Therefore, on an equimolar basis, lysine was essentially equal in crust browning to glycine. Glutamic acid had the least effect on crust browning. In practically all cases, adding as little as 0.2 g. glutamic acid gave loaf volumes higher than those without added free amino acids and sugars. High concentrations (0.8 g.) of added free amino acids resulted in darkening of crust color accompanied by a decrease in loaf volume. No clear-cut differences in crumb color could be attributed to added amino acids or sugars.

Various Concentration of Sugars and a Fixed Concentration of Amino Acids

Varying the concentration of the three types of sugars and employing a uniform addition of each of three amino acids (0.2 g.) at a 1.5 mg. bromate level gave the results shown in Table 2. Adding 0.2 g. amino acid to the basic formula, which as stated previously contained only 2% of sucrose, caused loaf volume with lysine to decrease from 711 to 687 cc.; that with glycine from 711 to 650 cc.; but those with glutamic acid increased from 711 to 740 cc.

Changes resulting from increased sugar levels depended on the type of sugar added. Sucrose improved the quality and size of the loaf because of its contribution as a readily fermentable sugar; xylose was most detrimental; galactose gave intermediate results. The results of crumb and crust color determinations paralleled those recorded in Table 1.

Sugars and Amino Acids Independent of Each Other

Effect of each of three sugars and each of three amino acids on volume and crust color of bread were investigated. The results summarized in Table 3 show that under conditions of panary fermentation, the effects of free amino acids and sugars on loaf volume or bread crust might not be causatively re-

Table 2. Loaf volume and crust color of bread baked with the basic formula and various levels of each of 3 sugars with 0.2 g. level of 3 amino acids.

Sugar	Sugar Level	GLYCINE Loaf Volume	GLYCINE Crust Color Bottom	GLYCINE Crust Color Top	LYSINE Loaf Volume	LYSINE Crust Color Bottom	LYSINE Crust Color Top	GLUTAMIC ACID Loaf Volume	GLUTAMIC ACID Crust Color Bottom	GLUTAMIC ACID Crust Color Top
	g	cc			cc			cc		
None	0.0[1]	711	62.5	41.0	711	62.5	41.0	711	62.5	41.0
Xylose	0.0	650	53.0	31.0	687	60.0	44.0	740	61.5	42.0
Xylose	0.5	635	37.5	21.5	705	46.0	25.5	780	43.5	26.0
Xylose	1.0	645	26.5	16.0	695	34.0	18.0	760	37.0	20.0
Xylose	2.0	625	20.0	11.5	695	24.0	12.5	715	27.0	12.5
Xylose	4.0	615	16.0	9.0	685	18.0	8.5	640	19.0	9.0
Sucrose	0.0	650	53.0	31.0	687	60.0	44.0	740	61.5	42.0
Sucrose	0.5	635	47.0	26.5	705	52.5	34.0	785	58.0	35.0
Sucrose	1.0	665	46.0	23.5	700	50.5	27.0	825	57.0	29.0
Sucrose	2.0	750	40.0	18.0	750	43.5	21.5	850	40.5	18.0
Sucrose	4.0	800	25.0	10.5	815	34.0	12.5	875	29.0	11.0
Galactose	0.0	650	53.0	31.0	687	60.0	44.0	740	61.5	42.0
Galactose	0.5	645	35.0	22.5	705	45.5	27.0	755	46.5	27.5
Galactose	1.0	650	30.0	17.0	705	40.5	19.5	750	40.5	20.5
Galactose	2.0	655	25.0	13.0	730	30.5	14.5	765	30.5	14.0
Galactose	4.0	705	24.5	10.5	700	24.0	11.5	715	26.5	11.0

1/ No amino acid.

Table 3. Effect of 2 g. each of 3 sugars or 0.2 g. each of 3 amino acids on loaf volume and bread crust color.

Sugar	Sugar Level	Amino Acid	Amino Acid Level	Loaf Volume	Crust Color Bottom	Crust Color Top
	g		g	cc		
Basic formula	0	none	0	711	62.5	41.0
Arabinose	2	none	0	700	31.5	13.5
Sucrose	2	none	0	782	47.5	23.0
Galactose	2	none	0	725	36.0	15.0
None	0	Glycine	0.2	650	53.0	31.0
None	0	Lysine	0.2	685	60.5	44.0
None	0	Glutamic acid	0.2	740	61.5	42.0

lated, but might be two separate, independent reactions. Although adding sugars, at the levels employed and in the absence of added free amino acids, resulted in a pronounced darkening of the crust color, it did not adversely affect the loaf volume. The increase in loaf volume from adding sucrose results from the contribution of additional fermentable sugar. Adding glycine enhanced crust coloration, while lysine and glutamic acid had no effect when added at 0.2 g. level without sugar supplementation. Adding lysine had a small effect (decrease) on loaf volume, adding glycine gave the smallest loaf, and glutamic acid again had a beneficial effect. These results show that whereas amino acids added with sugars might enhance bread crust coloration, the extent of crust browning is primarily a result of the contribution of the sugars added or originally present in the dough.

Equimolar Concentration of Sugars and Amino Acids

Effect of equimolar concentrations of 17 sugars and a fixed concentration of each of three amino acids on volume and crust color of bread (Table 4) were investigated after considering the results of experiments summarized in Table 1-3. In this work the bromate level was 1.5 mg. per 100 g. of flour; the level of amino acids added was kept equimolar (0.00266 moles/100 g. flour), and the level of sugars added was kept equimolar relative to 2 g. of the hexoses employed. The levels of sucrose and maltose added were equal to those of the hexoses, assuming that under conditions of the experiment these two sugars are enzymically hydrolyzed to glucose and levulose and to two moles of glucose, respectively, during panary fermentation.

The apparent bread-crumb color depends on both crumb texture and crumb color. A properly developed bread has a fine crumb, in which the fine cell walls surrounding the individual gas cells tend to reflect the light and look much whiter than an improperly fermented bread baked from the same flour. Differences in loaf volume would, therefore, be expected to affect the color of the

Table 4. Loaf volume and crust color of breads baked with equimolar concentrations of each of three amino acids and each of seventeen sugars.[1]

Type & Name of Sugar	Control			Glycine			Lysine			Glutamic Acid		
	Loaf Vol.	Crust Color Bottom	Crust Color Top	Loaf Vol.	Crust Color Bottom	Crust Color Top	Loaf Vol.	Crust Color Bottom	Crust Color Top	Loaf Vol.	Crust Color Bottom	Crust Color Top
	cc			cc			cc			cc		
None	711	62.5	41.0	650	53.5	27.0	710	54.5	34.5	705	63.0	44.5
PENTOSES												
Arabinose	720	32.0	15.5	650	24.0	13.0	730	23.5	11.5	715	30.0	14.0
Ribose	715	32.0	15.0	655	21.5	11.0	730	23.5	10.5	750	31.5	12.5
Xylose	700	32.0	15.5	635	21.0	11.0	705	21.0	10.0	705	29.5	13.0
HEXOSES												
Galactose	715	33.5	17.5	645	21.5	10.5	730	26.0	11.5	715	33.0	13.0
Glucose	750	42.0	21.5	725	33.0	14.5	760	34.0	15.0	795	50.5	23.0
Levulose	780	42.0	20.0	750	31.5	14.0	755	32.0	14.0	795	51.5	21.0
Mannose	775	39.0	19.5	720	28.0	14.5	760	31.5	14.5	785	40.0	19.5
Sorbose	700	33.0	16.0	615	21.0	11.0	650	24.5	11.0	695	32.0	14.0
Rhamnose	720	42.0	23.0	650	29.0	15.0	720	29.0	14.0	720	40.0	18.5
DISACCHARIDES												
Cellobiose	765	38.0	20.0	735	32.0	14.0	735	29.0	10.5	770	39.0	17.0
Lactose	725	37.0	17.0	675	24.0	11.0	745	27.0	10.5	735	35.0	15.5
Melibiose	715	33.0	15.5	685	20.0	11.0	735	24.5	10.0	710	31.5	15.0
Sucrose	780	41.5	21.5	745	29.0	15.5	760	32.5	14.0	790	44.5	17.0
Maltose	745	47.5	25.0	685	35.5	18.0	735	37.5	16.0	755	49.5	21.0
Trehalose	710	55.0	31.0	665	45.0	22.5	735	47.5	25.5	710	57.0	35.0
TRISACCHARIDES												
Melezitose	725	57.0	26.0	670	41.5	20.5	745	50.0	18.0	735	56.5	21.0
Raffinose	765	30.0	12.5	735	25.0	10.0	780	20.0	9.0	790	25.0	10.5

1/ Equimolar concentrations were based on 0.2 g. of glycine for the amino acids and on 2 g. of the hexoses for the sugars.

crumb as measured by the reflectance method. No consistent differences in crumb color, as related to loaf volume, could be noted. Loaves containing ribose were distinctly yellow and their crumb color was consistently the poorest among all the tested sugars and amino acids. The effect of adding sugars on browning of the crust is similar to that observed in cookies and dilute solutions (24). Some differences, however, were noted. Raffinose, which in cookies and dilute sugar solutions produced little browning, imparted a deep crust color to bread. This seems to result from the cleavage of the trisaccharide by yeast to fructose and melibiose, both of which darken the crust color.

All three pentoses tested imparted a dark brown color to the bread crust. Lactose and melibiose which both contain galactose, produced a darker crust color than did any of the other disaccharides. Here the picture is more complicated because the enzymatic hydrolysis of sucrose and maltose, subsequent fermentation of the cleavage products, and the amount of residual sugars present at the baking stage may influence the extent of browning. None of the sugars had a detrimental effect on loaf volume or associated with it crumb texture in the absence of added free amino acids. Adding sugars fermentable by yeast consistently increased loaf volume. Under conditions of the experiment, adding sucrose gave the best loaf volumes. Raffinose and sucrose produced comparable increases in loaf volume; loaves containing added maltose were slightly lower in volume than those containing sucrose. Only sorbose of the sugars added in the presence of amino acids reduced loaf volume. Adding glycine resulted in a consistent and pronounced decrease in loaf volume accompanied by enhanced crust coloration. Glutamic acid, in general, slightly increased loaf volume and had little effect on crust color. Adding lysine had little or no effect on loaf volume but imparted a brown color to the crust.

Starch Doughs

The results of studies of the effects of sugars and free amino acids, alone and in combinations, on browning of a dough prepared and baked under conditions simulating breadmaking but substituting a starch mixture for the wheat flour used previously are summarized in Table 5. Crust colors of the starch doughs baked with arabinose alone, arabinose-amino acid mixture, galactose and lysine, or galactose and glycine were significantly darker than crust colors of starch doughs baked with sucrose alone, amino acids alone, sucrose-amino acid mixtures, or without amino acids and sugars. The least significant difference at the 5% level was 3.51, at the 1% level 4.92 and at the 0.1% level 6.95.

Starch Doughs vs. Wheat Flour Doughs

Figure 1 compares top crust color of baked starch doughs with bread crust color. The crust color of bread was consistently darker than the color of baked starch doughs. The three amino acids did not differ in their effect on crust color of baked starch doughs but differed materially in their effect on crust color of baked wheat flour doughs. This difference seems to result not from the browning effect of the amino acids alone, but from the interaction between amino acids added and available sugars or other compounds present in flour but not in starch.

Sucrose, galactose, and arabinose differed materially more in their effects on the top crust color of starch bread than of wheat flour bread. The effects of mixtures of amino acids and sugars on crust color are similar to those of the sugars alone, and both are better differentiated in starch bread than in wheat flour bread.

It would seem, therefore, that the starch dough system enables one to evaluate the effects of amino acids on crust browning, independent of sugars or other compounds present in wheat flour.

Table 5. Crust color of starch-doughs baked with equimolar concentrations of amino acids and sugars added to the basic formula.

Sugar	Sugar level	Amino acid	Amino acid level	Top crust color
	g		g	
Basic formula	0	None	0	58.0
Arabinose	1.67	None	0	43.3 ***
Sucrose	2.00	None	0	60.0
Galactose	2.00	None	0	58.0
Arabinose	1.67	Glycine	0.200	48.0 ***
Arabinose	1.67	Lysine	0.486	45.0 ***
Arabinose	1.67	Glutamic acid	0.489	50.0 ***
Sucrose	2.00	Glycine	0.200	57.5
Sucrose	2.00	Lysine	0.486	57.5
Sucrose	2.00	Glutamic acid	0.489	61.0
Galactose	2.00	Glycine	0.200	53.5 *
Galactose	2.00	Lysine	0.486	53.0 **
Galactose	2.00	Glutamic acid	0.489	56.5
None	0	Glycine	0.200	59.0
None	0	Lysine	0.486	58.0
None	0	Glutamic acid	0.489	60.0

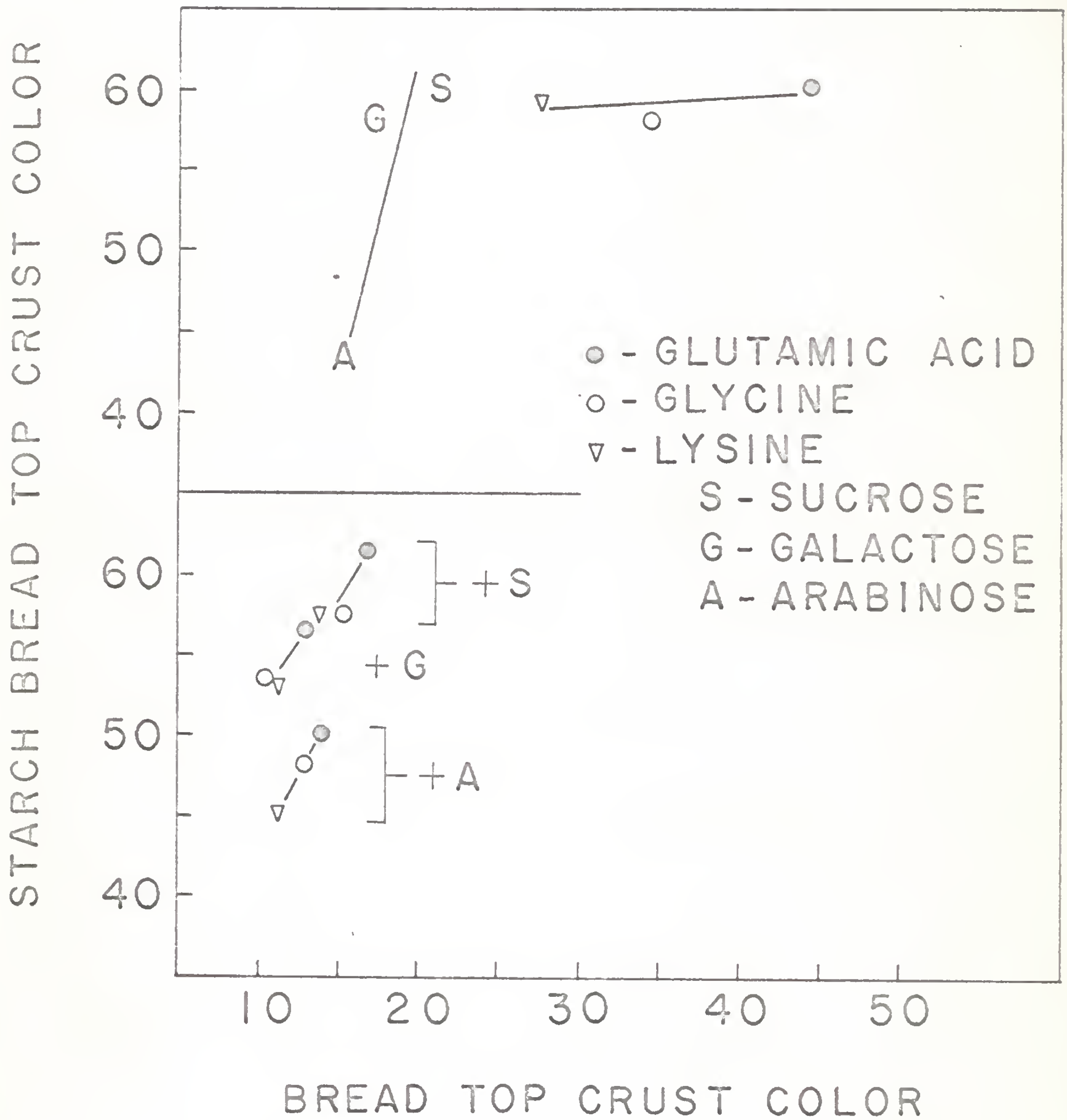

Fig. 1. Comparison of top crust color of wheat flour bread and starch bread baked with equimolar concentrations of amino acids and sugars added to the basic formula.

Glutamic Acid and Volume of Bread

Glutamic acid consistently increased loaf volume (0 vs. 0.2 g. levels, Table 1 and the first two lines of Table 2). This seemed to warrant further investigating, particularly since glutamic acid constitutes almost one third of the amino acids in wheat proteins (8). Ungerminated wheat and fermented bread doughs contain, however, only microgram quantities of the free glutamic acid (10,17). It appears that glutamic acid either increased gas retention of the gluten proteins, or increased gas production by increasing yeast metabolism.

Various levels of glutamic acid and 2% of the three different sugars were added to the basic formula given previously in materials and methods to test the potentialities of bread improvement by glutamic acid. A level of 0.10-0.20 g. of glutamic acid per 100 g. of flour gave the greatest increase in loaf volume as shown in Table 6. The increase depended on the sugar added; glucose gave better relative volume responses than arabinose or galactose, as would be expected because of its fermentability. These findings were confirmed for a number of compounds related to glutamic acid (Table 7) at each of three sugar levels. The higher the level of sugar in the formula, the higher was the loaf volume response from glutamic acid or related compounds.

This is in agreement with reports in the literature that yeasts are strongly stimulated by aspartic and glutamic acids and their amides, asparagine and glutamine (7,11,13). Bakers' yeast also grows well on γ-amino butyric acid as a sole source of nitrogen (23). The utilization of amino acids depends on their ease of deamination to the corresponding keto-acid. Glycine and lysine resist deamination, whereas aspartic and glutamic acids are readily available for nitrogen assimilation (7,11). There appears to be agreement that the group $-CH \cdot NH_2$ in amino acids is essential for N-utilization by yeasts.

Table 6. Loaf volume of bread baked with various levels of glutamic acid and three different types of sugars.[1]

Glutamic acid	Loaf volume in the presence of 2 g. of		
	Arabinose	Glucose	Galactose
g.	cc	cc	cc
0	725	790	725
0.05	750	805	750
0.10	780	830	775
0.15	-	835	765
0.20	770	860	760
0.25	735	835	730
0.30	745	835	715
0.40	710	795	720

1/ Basic formula containing 2 g. sucrose.

Table 7. Effects of 0.15 g. glutamic acid and equimolar concentrations of related amino acids on loaf volume.

Amino Acid	Loaf Volume		
	2% sucrose[1/]	4% sucrose	6% sucrose
	cc	cc	cc
None	710	790	805
Glutamic acid	775	865	875
Aspartic acid	695	775	750
Asparagine	745	865	875
Glutamine	780	845	865

1/ Basic formula.

To determine the relation, if any, of the functional groups of glutamic acid and related compounds to variations in loaf volume, 20 compounds related to glutamic acid were tested. Each was added on an equimolar basis to 0.15 g. glutamic acid. They were baked using the basic formula plus 4.0 g. sucrose and 0.25 g. of 120° Linter malt syrup. For gassing power determinations, Sandstedt-Blish pressuremeters (28) were used, and a synthetic nitrogen base medium for carbon assimilation tests with yeast was prepared in accordance with the formula in the Difco Manual (3). Six g. of sucrose, 10 ml. of the nitrogen base medium, 5 ml. of a yeast suspension (7.5 g. cake bakers' yeast in 95 ml. of a 2% NaCl solution) and 15 ml. water were added and mixed to 10 g. of pregelatinized wheat starch. The results (Table 8) show that differences in loaf volume, in general, are comparable with changes in gassing power. A deleterious effect to loaf volume generally was accompanied by a decrease in fermentation rate or gassing power.

Maw (19) has shown in his study on metabolism of sulphur compounds by a brewers' yeast that the conditions under which growth or metabolism are studied may influence utilization of nutrients. He found only a small number of the compounds tested supported growth under conditions of inadequate aeration. When aerations was adequate, a number of additional compounds became available as sulphur sources. Differences in utilization of certain amino acids and related compounds in a synthetic nutrient solution and in bread were observed. These differences may have resulted from variations in aeration, use of a semi-solid medium, and interaction between the added amino acids and flour components during the baking process.

It appears obvious that glutamic acid increased loaf volume as a result of yeast stimulation, and that the increase was greatest at the higher sugar levels (Table 7). This indicates that yeast action was enhanced under conditions of

Table 8. Effects of glutamic acid and related compounds (equimolar basis of 0.15 g. glutamic acid) on loaf volume and on gassing power of a synthetic medium.

Compound	$KBrO_3$ Requirement	Loaf Volume	Loaf Volume Change	Gassing Power Change
	mg	cc	%	%
None	1.5	889	-	-
GLUTAMIC ACID DERIVATIVES				
Glutamic acid	1.5	963	+8.3	+0.2
Glutamine	1.5	973	+9.4	0
L-pyroglutamic acid	1.0	862	-3.0	-7.3
Mono-sodium glutamate	1.5	948	+6.6	+0.2
α-Keto glutaric acid	0.5	808	-9.1	-
Acetyl-L-glutamic acid	0.5-1.0	825	-7.2	+0.7
Benzoyl-L-glutamic acid	0.5-1.0	794	-10.7	-8.1
Glutamic acid-γ-ethyl ester	1.0-2.0	855	-3.8	-3.7
ASPARTIC ACID DERIVATIVES				
Aspartic	1.0	912	+2.6	+4.6
Asparagine	2.0	978	+10.0	+9.1
Glycyl-L-aspartic acid	1.5	925	+4.0	+1.7
Glycyl-L-asparagine	2.0	948	+6.6	+2.4
DL-alanyl-DL-asparagine	1.5	883	-0.7	-4.8
BUTYRIC ACID DERIVATIVES				
DL-α-amino-N-butyric acid	2.5	880	-1.1	-2.4
γ-Amino butyric acid	1.5	860	-3.3	+2.0
2,4-Diamino butyric acid	1.0-1.5	818	-7.9	-
α-Amino isobutyric acid	1.5-2.0	845	-4.9	-
Glycyl-γ-amino butyric acid	1.5-2.0	855	-3.8	-0.2
OTHER AMINO ACIDS				
Glycine	2.5	875	-1.6	+4.4
L-lysine	2.0	868	-2.4	+3.2
NON-AMINO COMPOUND				
NH_4Cl	1.0	960	+8.0	-
Tartaric acid	0.5	843	-5.2	-

adequate sugar supply, and is in agreement with reports on enhanced utilization of certain amino acids provided the medium contains sugar (21).

The oxidation requirement of the bread varied from 0.5-2.5 mg. of $KBrO_3$, depending on the added amino acid (Table 8). Glutamic acid, glutamine, monosodium glutamate, asparagine, and glycyl-L-asparagine increased the loaf volume to an extent comparable to an equimolar concentration of ammonium chloride; aspartic acid and glycyl-L-aspartic acid were less effective; DL-alanyl-DL-asparagine had no measurable effect. Glycine and lysine, four of the five amino butyric acids, and five derivatives of glutamic acid decreased the loaf volume to varying extents irrespective of the presence or absence of a free amino group. It appears that the amino acids that increased loaf volume possessed an α-amino group as well as a carboxyl or amide group at the other end of the chain.

SUMMARY

Studies reported were concerned with the effect on bread crust color formation of certain of the previously reported amino acids and sugars, together with a number of additional important sugars and amino acids not previously reported in the literature, particularly when employing fermented wheat flour doughs that were baked into bread.

A second objective of the study was to replace wheat flour with a semi-synthetic model system containing wheat starch. Bread is not an ideal system to study the browning reaction in because of the presence of many interfering components of the flour.

Additional studies were concerned with the consistent increase in loaf volume when adding 0.2 g. of glutamic acid to wheat flour bread doughs.

Varying the amount and kind of sugars and amino acids had no measurable effects on bromate requirements, mixing time, or water absorption of wheat flour doughs. Glycine generally reduced loaf volume much more than did lysine. On an equimolar basis lysine was essentially equal to glycine in crust browning. Glutamic acid had the least effect on crust browning of all the amino acids studied. Under conditions of panary fermentation the effects of free amino acids and sugars on loaf volume and on bread crust color might not be causatively related, but might be two separate independent reactions. Amino acids added with sugars might enhance bread crust browning, but the extent of crust browning is primarily a result of the contribution of the sugars, added or originally present in the dough.

The effects of adding glycine, lysine, and glutamic acid alone or in combination with each of 17 sugars on bread-baking potentialities and crust coloration of bread were investigated. Glycine had the most adverse effect on loaf volume and it caused pronounced browning of the crust. The effect of lysine on

loaf volume was insignificant despite increased crust browning. Glutamic acid, generally, improved loaf volume and slightly enhanced browning. None of the amino acids and only ribose among the sugars affected the crumb color. Added on an equimolar basis (0.011 moles/100 g. of flour) raffinose and the pentoses imparted the deepest color to the crust; melibiose, sorbose, lactose, and galactose followed in order. The hexoses glucose, levulose, and mannose had little effect and were followed by the disaccharides cellobiose, sucrose, and maltose; the smallest effect was exerted by melezitose and trehalose.

The effect of saccharides on loaf volume, except those contributing as fermentable sugars, was small. Adding certain amino acids (0.0026 moles/100 g. of flour) along with sugars augment the effects of each separately on loaf volume or crust color.

The effect of sugars and free amino acids on browning of dough prepared and baked under conditions simulating breadmaking but substituting a starch mixture for the wheat flour was studied. The sugars differed materially more in their effect on the top crust color of starch than of wheat flour bread. The effects of mixtures of amino acids and sugars on crust color were similar to those of sugar alone, and both are better differentiated in starch bread than in wheat flour. The starch dough system enables one to evaluate the effects of browning components without interference from sugars or other compound present in the complex wheat flour.

Adding 0.15 g. of glutamic acid to 100 g. of flour increased significantly the loaf volume of bread baked from hard winter wheat. A number of compounds related to glutamic acid had similar improving effects; the largest effects were exerted by glutamine and asparagine.

Twenty compounds related to glutamic acid were baked on an equimolar basis to determine the relation, if any, of the functional groups. Gas production was also determined for all doughs containing these compounds and was found to be very comparable with changes in loaf volume. The amino acids that increase loaf volume possess an α-amino group as well as a carboxyl or amide group at the other end of the chain.

ADKNOWLEDGMENTS

The author is greatly indebted to Professor Karl F. Finney and Dr. Yeshajahu Pomeranz for their supervision of the study and their guidance in the preparation of this manuscript.

He also extends sincere thanks to Dr. Majel MacMasters, and Dr. D. B. Parrish for their cooperation and guidance as members of his advisory committee, and to Dr. J. A. Shellenberger, Head of the Department of Flour and Feed Milling Industries.

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EFFECTS OF SUGARS AND FREE AMINO ACIDS ON BREAD CHARACTERISTICS

by

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B. S., Kansas State University, 1960

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

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Manhattan, Kansas

1964

At the present time many believe that the color in crust formation of baked products is closely related to the Maillard reaction, a non-enzymatic reaction between amino acids and particular sugars. Until recent years the mechanism of crust browning has been controversial. Studies reported were concerned with the effect on bread crust color formation of certain of the previously reported amino acids and sugars, together with a number of additional sugars and amino acids when baked with fermented wheat flour doughs.

A second objective of the study was to replace wheat flour with a semi-synthetic model system containing wheat starch. Studying the browning reaction in bread is not an ideal system because of the presence of many interfering components of the flour. A dough was prepared and baked under conditions simulating bread making only a starch mixture (20% pregelatinized wheat starch and 80% raw wheat starch) was substituted for wheat flour. Most of the model systems described were studied in an aqueous medium or deal with dry mixtures of protein and reducing sugars. The closest attempt to duplicate baking conditions used sand coated with amino acids and xylose and then heated.

Additional studies were concerned with the consistant increase in loaf volume when adding 0.2 g. of glutamic acid to wheat flour bread doughs.

Varying the amount and kind of sugars and amino acids had no measurable effects on bromate requirements, mixing time, or water absorption of wheat flour doughs. Glycine generally reduced loaf volume much more than did lysine. On an equimolar basis lysine was essentially equal to glycine in crust browning. Glutamic acid had the least effect on crust browning.

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