BROWNING REACTION IN BAKED PRODUCTS

by

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INTRODUCTION

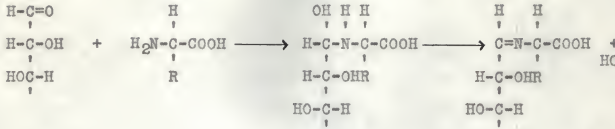
The non-enzymic browning or Maillard reaction has been recognized by the food industries for many years. It has been considered seriously only within the last few years and has come to be viewed as advantageous in some foods, but detrimental in others. The detrimental effects are considered mainly psychological since no toxic effects have been attributed to the products of the browning reaction. Prior to World War II, the reaction had been little more than a laboratory curiosity. The increased demand for dried and frozen foods, in which the browning reaction was undesirable, brought forth serious considerations of the reaction. Due to the intense research for the cause, result, and control of the reaction, the literature on the subject has been voluminous during the past five years. Most of the literature treating of the browning reaction has to do with foods other than baked products. A few investigators have mentioned the possible occurrence of the reaction in baked goods (5, 35).

Schmiedeberg, according to Pigman and Goepp (32), was the first worker to suggest that the initial reaction was a condensation of carbohydrates with available groups on the protein molecules. Actually, the first fundamental approach dates back to the work of Maillard (24), who, in an effort to elucidate the biological synthesis, studied the condensation of amino acids in the presence of polyhydric alcohols.

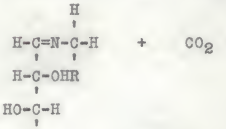
Theories of Browning

Actually there are no accepted theories concerning the reaction. There are, however, three which are generally recognized: 1. Maillard (or melanoidin) condensation theory, 2. the ascorbic acid theory, and 3. the active aldehyde theory. The lack of an accepted theory is probably due to inadequate research, obvious complexity of the reaction, and lack of uniform approach to the problem; i.e., industrial and fundamental.

The Maillard (or melanoidin) condensation theory is the oldest for or against which there is little evidence. Maillard postulated that the active group of the sugar (carbonyl or aldehyde group) reacts with the amino group to form first a Schiff base. Decarboxylation and dehydration of this product gives the dark materials known as melanoidins. According to Maillard, 12 moles of water are given off per mole of carbon dioxide. All the dark-colored materials are soluble in water until the completion of the evolution of the carbon dioxide. The formation of water-insoluble products may start as low as 34° C., and increase rapidly as the temperature is raised to 100° C. Maillard's reaction conditions were concentrated solutions, wide temperature ranges, long periods of time, and pH values between 3.2 and 6.1. The following scheme would represent the condensation theory:



Decarboxylation of this condensation product presumably gives:



Danehy and Pigman (10) reported that there are four general types of condensation interacting systems. These are given in the following data:

1. Sugars and amino compounds other than amino acids.
2. Sugars and amino acids.
3. Sugars and proteins or polypeptides.
4. Polysaccharides and proteins.

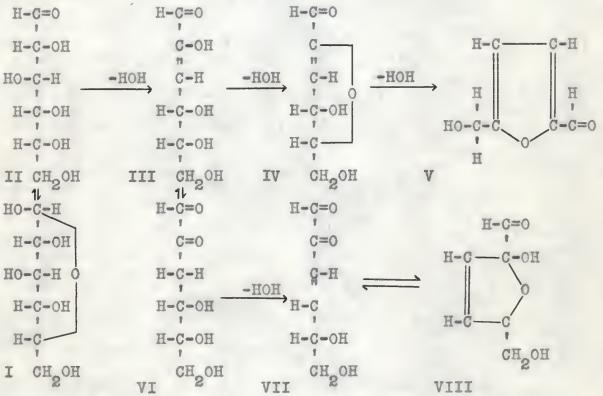
The alpha amino group is said to be largely responsible for the reaction in the case of amino acids, but the beta group may also be responsible (10). Other amino groups are not involved.

Ascorbic acid, if present, is destroyed to the extent of 85 percent. The disappearance of ascorbic acid roughly parallels color production and carbon dioxide production in dilute solutions. According to this, the most important pre-

cursors to browning are ascorbic acid and related compounds, which oxidize to yield reactive products that polymerize or react with nitrogenous constituents of the products to form brown pigments (28). Ascorbic acid is involved in the browning reaction in one of two ways: 1. It may act as an antioxidant, being oxidized in preference to other substances present, which, upon oxidation yield dark compounds or precursors of dark compounds; or 2. the oxidation products of ascorbic acid may themselves be the actual precursors of dark compounds. Moore et al. (27) and Beattie et al. (7) have demonstrated by the addition of ascorbic acid to fruit juices that ascorbic acid is effective, not as an antioxidant, but rather as an intermediate in the browning reaction. Hamburger and Joslyn (20) have implied that ascorbic acid is involved as an antioxidant in browning. Esselen et al. (12) have reported a decrease in the rate of browning upon addition of ascorbic acid to apple, cranberry, and grape juices. This is contrary to the common belief that ascorbic acid is involved in the browning of fruit juices. The fruit products industries have been confronted chiefly with this problem. The ascorbic acid theory seems more evident in this field than does the condensation theory. Ascorbic acid and uronic acid readily decompose to form furfuraldehyde. Addition of small amounts of furfuraldehyde greatly accelerates the reaction with the evolution of carbon dioxide. No exact mechanism has been suggested for this theory. Experience has shown

that the ascorbic acid theory is probably not involved in non-enzymatic browning of baked products.

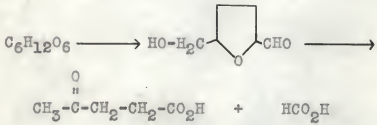
Wolfrom et al. (43) have advanced the active aldehyde theory. They have studied the ultraviolet absorption spectrum given when glucose is refluxed in distilled water. The characteristic absorption curve for 5(hydroxymethyl)-2 furfural exhibited its maxima at 228 and 285 mμ. The mechanism of this reaction is considered as a process of dehydration as follows:



I is transformed to II or its aldehydrol by dehydration. III is then produced from II by loss of water. Evidence for III is found in both absorption regions. Other intermediates and products are formed by a process of dehydration and development

of double bonds as indicated above.

Wolfrom et al. (44) studied the interaction of amino acids and reducing sugars. They found that xylose was in part transformed to 2-furfuraldehyde, while the hexose molecule was transformed to 5(hydroxymethyl)-2-furaldehyde, and finally to levulinic acid. The transformation of the hexose molecule was enhanced by acidity. It was noted that the presence of significant amounts of glycine had a promoting effect upon the furan bodies formed. Proof that 5(hydroxymethyl)-2-furaldehyde was formed from D-glucose in the presence of glycine was established by isolation of this compound from such a mixture. The overall reaction proceeds as follows:



The products were extracted with ethyl acetate. The solvent was removed and the residue identified by ultraviolet absorption (43) and by preparation of two derivatives. They concluded that the carbonyl-amino reaction could occur to only a slight extent, if at all, in dilute aqueous solutions of D-glucose and glycine.

Singh et al. (34) are in agreement with Wolfrom et al. They point out that 5(hydroxymethyl) furfural upon polymerization could give rise to colored products because it would

possess the requisite number of conjugated double bonds for selective absorption in the visible region. In their studies on starch hydrolysates with ultraviolet absorption, they found absorption peaks at approximately the same wavelengths as those found by Wolfrom and his co-workers (43). They conclude that the glucose-amino acid condensation is rather a remote possibility at 100° C. and pH 5-6 for heating periods of three hours.

Pacsu and Hiller (29) have reported on the ultraviolet absorption of sugar solutions in highly acid media. Their results are comparable to those of Wolfrom et al. (43) and Singh et al. (34) as to the wavelength of the absorption peaks.

According to this theory, nitrogen is not a constituent of the colored products formed in the browning reaction. Some furan bodies are probably formed when sugars are reacted with amino acids, especially in acid media. Convincing evidence that nitrogen is a component of the browning reaction products appears in the literature (10).

Causes of Browning in Baked Products

There are two possible causes of the brown coloration in baked goods; namely, caramelization and Maillard products. According to Zerban (45), caramelization products of sucrose and glucose are a complex mixture of sugar anhydrides, the

composition depending on the pH, time, and temperature of heating. The products contain carboxyl groups and phenolic hydroxyls. These humins also contain hydroxymethyl furfural. The colored products of glucose are rapidly destroyed at a pH of 13 or above if heating is continued in this range. Both glucose and fructose in high acid media are converted into anhydrides, so-called reversion products. These reversion products consist of polysaccharides containing from two to eight glucose units. At high concentrations these reversion products are formed, but in dilute solutions they are quickly hydrolyzed.

Physical Changes Associated with the Browning Reaction

The physical changes occurring during brown pigment formation are convincing evidence that a chemical reaction is taking place. Many of these physical changes have been used to determine the extent of the reaction.

The browning products fluoresce strongly under ultraviolet light, so fluorescence has been used as a measurement of the extent of browning. Tarassuk and Simonson (35) and Jenness and Coulter (13) have shown conclusively that browning and fluorescence develop simultaneously, and their formation proceeds at a parallel rate. Other investigators (11, 17, 21, 36) have shown good correlation between browning and

fluorescence.

As the browning reaction proceeds, the pH decreases. Frankel and Katchalsky (15) have demonstrated that pH depressions may be used successfully to determine the extent of interaction between aldoses and alpha-amino acids. They concluded that the differences in reactivity of amino acids and peptides are mainly due to differences in the well-known dissociation constants of the acids.

Spectrophotometric absorption studies have been useful also in following the rate of color development in the browning reaction. Mohammad et al. (26) have obtained data with the Coleman spectrophotometer at 500 mμ. Barnes and Kaufman (5) describe a technique for measuring "half transmission time" at 400 mμ.

Maillard (24) used carbon dioxide evolution as a measure of degree of the browning reaction. He postulated that decarboxylation of the amino acid occurred as a secondary reaction. Color formation and carbon dioxide evolution proceeded at a parallel rate.

Several other methods of following the rate of browning have been mentioned in the literature. Cryoscopy was successfully used by Von Euler et al. (37). Von Euler and Josephson (38) also have attacked the problem by observing changes in optical rotation.

Reaction Mechanism

Several investigators have expressed different opinions as to the actual mechanism of the browning reaction. Grunhut and Weber (18) suggested that there are three distinguishable stages of reaction: 1. interaction to furnish a product containing a primary amino group, 2. loss of the amino group, either by substitution or condensation, and 3. a final stable state which is reached by decarboxylation. Mohammad et al. (26) have suggested that the hydroxyl ion is a catalyst for the reaction, since the browning is accelerated by increased alkalinity. According to Danehy and Pigman (10), Enders is in agreement regarding the reactivity being governed by the basisity of the nitrogenous substance present. Frankel and Katchalsky (16) concluded that reactibilities of amino acids and peptides vary mainly through the difference in the well-known dissociation constants.

By ultra-microscopic study of a weakly basic solution, Weast and Mackinney (39) found considerable light scattering by the particles which were of colloidal size. They concluded that the dark materials probably do not represent a single chemical entity, but a mixture of closely-related entities. Borsook and Wasteneys (8) concluded that the reduction of amino nitrogen is not accompanied by a synthesis of lower fragments into more complex derivatives. Zerban (45) and Weast and Mackinney (39) agree that the condensation

products of reducing sugar and amino acids are N-glycosides which are readily hydrolyzed by acid to the original components. Upon gentle heating at high concentrations, the products turn yellow, then turn brown with the evolution of carbon dioxide and form dark brown to black amorphous, insoluble, and non-hydrolyzable material.

Simmich (33) has advanced the idea that melanoidins are simply caramel substances on which nitrogenous products are adsorbed or held to form molecular aggregates. No evidence of this appears in the literature.

Using ion exchange resins Duolite A-3 and Duolite C-3, undarkened apricot juice was separated into three distinctly different fractions (19). Browning occurred only in the cationic portion which contained 81 percent of the organic nitrogen in addition to all the inorganic cations. From this study, conclusions were drawn that at least three types of browning were involved: 1. reactions involving nitrogenous compounds and sugars, 2. reactions involving organic acids and sugars, and 3. reactions involving nitrogenous compounds and organic acids.

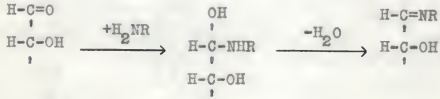
To determine the mechanism of the reaction, the concept of blocking reactive groups has been applied by some investigators. Wolfrom et al. (42) have suggested that sugar enolization is involved when aqueous solutions of glucose and glycine are heated at a slightly alkaline reaction. They have shown that at an acid reaction, both the hydrogen of the amino

group and the carbon adjacent to the carbonyl group are involved in the decomposition. They have shown the effect of various reactive groups on browning by using sugars and amino acids with the reactive centers appropriately blocked by methylation.

Bate-Smith and Hawthorne (6) applied the concept of blocking reactive groups of the glucose to show that browning and solubility of dried eggs were due to a reaction involving the sugar present in the egg. They concluded that the reducing group of glucose is the reactive group in the sugar-amine condensation. The protein becomes insoluble after condensation of the glucose and amino groups of the protein.

The use of acetylated glycosyl halides and amino acids or derivatives has been shown to be a more certain method of condensation than the direct action of amino acids and sugars (10). Compounds of glucose and sarcosine ethyl esters have been prepared by this method.

Other attempts to determine the mechanism of the browning reaction have been through the study of systems containing sugars and amines other than amino acids. In a review of the literature by Danehy and Pigman (10) the reaction is shown as follows:



Many of these reactions occur under mild conditions. The combinations formed from sugars with amines, hydrazines, and oximes exhibit mutarotation when dissolved in water or many other solvents.

The literature does not indicate that systems containing aldehydes other than sugars with amino acids have been extensively studied. According to Anson and Edsall (1) many different reactions may occur between formaldehyde and glycine, the reaction becoming very complex as higher molecular weight aldehydes are used. For this reason the study of these products would not be satisfactory as a simplification of the browning reaction between amino acids and sugars. Other interacting systems have been mentioned previously.

Relative Reaction Rates of Sugars and Amino Acids

There are some conflicting reports as to which of the amino acids and sugars react the faster. Of the sugars, the pentoses react the most rapidly (10). According to Danehy and Pigman (10), Enders reported that under standard conditions, the rate of development of color in aqueous solutions of sugars and nitrogenous substances increases as the basisity of the nitrogenous substances increases. Grunhut and Weber (18) concluded that of the three monobasic amino acids, glycine is more reactive than alanine and leucine is completely unreactive. Glutamic acid was found to be the most reactive of

these amino acids. Maillard's original work (24) showed that the following sugars reacted with glycine in order of decreasing activity: xylose, arabinose, fructose, galactose, mannose, glucose, lactose, and maltose. In all cases it was reported that sucrose and amino acids produced no color. This order of reactivity is not in agreement with Grunhut and Weber (18). They found the sugars had the following order of reactivity: arabinose, glucose, galactose, and fructose. Other reports differ in many respects regarding the activity of the amino acids and sugars (10).

Lea (23) recently has reported on the reaction between casein and glucose. He found that during the first stage of the reaction there was extensive combination of the sugar with lysine side chains. As the reaction proceeded, arginine, histidine, tyrosine, and methionine became involved. When this product was subjected to acid hydrolysis, all the tyrosine and methionine, and part of the lysine were regenerated. The remainder of the lysine and all of the arginine and histidine seemed to be in stable combination and thus did not regenerate. Patton et al. (31) have found that the essential amino acids that apparently are destroyed in part by refluxing casein with 5 percent glucose solution for 24 hours were lysine, arginine, and tryptophan. Mohammad et al. (26) found that the indole groups of tryptophan were affected in the reaction with glucose.

Methods of Inhibiting Browning

Many methods of prevention or retardation of the browning reaction are practiced by industry. Lea (23) has mentioned some of these: 1. removal of one of the reactants which may be present only in small quantities, 2. storing at low temperatures, 3. drying to a very low moisture content, 4. control of the pH, 5. packing in an inert gas such as nitrogen or carbon dioxide, and 6. use of chemical inhibitors. Of the inhibitors, sulfur dioxide is widely used in fruit and vegetable products. Other inhibitors mentioned are formic acid, urea, thiourea, benzaldehyde, benzenephosphoric acid, sodium thiosulfate, and alpha-naphthol (22).

Those conditions affecting the formation of browning reaction products have been described by Tarassuk and Simonson (35) in connection with the evaporation of milk. The pH of the milk, the lactose concentration, and the time and temperature of heating are of major importance; minor factors are copper concentration and amount of stabilizing salts. In addition, the protein concentration affects the degree of browning (26).

The complete characterization of the browning reaction is undertain at present and its connection with bakery products has not been previously investigated. The present investigation, therefore, has involved the attempt to devise a method by which browning reaction products may be identified and to

determine to what extent this reaction is involved in baked goods.

MATERIALS AND METHODS

The chemicals used in these experiments, and their manufacturers, are listed below:

l-Glutamic acid	International Minerals and Chemical Supply
dl-Valine	GBI*
dl-Leucine	GBI
dl-Isoleucine	GBI
l(+) Histidine monohydrochloride	GBI
l(+) Arginine	GBI
l(+) Lysine monohydrochloride	GBI
Glycine	GBI
dl-Phenylalanine	GBI
Tryptophan	Merck & Co., Rahway, N.J.
Tryosine	GBI
l(-) Cystine	GBI
dl-Threonine	Nutritional Biochemical Corporation
dl-Methionine	Eastman Kadak Company
l-Proline	GBI
Methyl sulfate (Practical)	Eastman Kodak Company
Dextrose (anhydrous)	Eastman Kodak Company
Dextrose (cerelose brand)	Corn Products Inc.

* General Biochemicals Inc., Chargin Falls, Ohio.

All chemicals used were of C. P. grade or its equivalent.

Fluorometric

Fluorescence studies were made on 15 amino acids: glutamic acid, leucine, isoleucine, valine, lysine, arginine, histidine, tryptophan, phenylalanine, glycine, tyrosine, threonine, methionine, proline, and cystine with dextrose (cerelose brand). Two-tenths gram of each amino acid and one gram of dextrose were dissolved in 0.4N hydrochloric acid. The mixtures were diluted to 50 ml and fluorescence measurements were made immediately. The fluorometer used was a Coleman electronic photofluorometer, the modified model 12. The solutions were incubated at 70° C. and fluorescence measurements made each 24 hours for 7 days. A solution of sodium fluorescein, the concentration of which was 1 gamma per ml, was used as a standard. The B set of filters was used with adjustment of the fluorometer to 50 with the sodium fluorescein.

Spectrophotometric

The 15 amino acids were separately reacted with dextrose (cerelose brand). Two-tenths gram of each and one gram of dextrose were dissolved in dilute hydrochloric acid and the solutions adjusted to a pH of about 3 by use of dilute base. Tyrosine was dissolved in dilute sodium hydroxide and the pH adjusted to 9.8. The solutions were diluted to 50 ml and

light absorption curves made between 220 and 1000 mμ. The solutions were incubated at 70° C. and absorption curves made each 24 hours for 7 days.

Confirmation of the above results was obtained by reacting the same amino acids with dextrose in a similar manner. The concentration of the reactants was increased to amplify small differences not detected in the previous experiment. One percent of each amino acid and 4.0 percent of dextrose were dissolved in acetic acid and the pH adjusted to 3.0 with sodium acetate. Dilutions of 1 to 100 were necessary to make the light absorption curves between 220 and 350 mμ. Higher concentrations of dextrose and amino acid were not feasible as the dilutions became exceedingly large. It was evident that the reaction did not follow Beer's law. It was necessary to use a dilution of 1 to 500 with the tryptophan reaction mixture, and a dilution of 1 to 400 with the tyrosine reaction mixture. This same procedure was used where formaldehyde, acetaldehyde, and acetone were substituted for the dextrose, using only glycine as the nitrogen component.

Preparation of Special Compounds

Acetaldoxime and butyraldoxime were prepared and purified by the pyridine method according to McElvain (25). One hundred ninety mg of the acetaldoxime and 490 mg of the butyraldoxime were dissolved in 25 cc of water. An absorption spectrum was

run on each solution between 220 and 350 mμ.

Tetramethylmethylglucoside and tetramethylglucose were prepared by the method described by West and Halden (40), with a few modifications. A stainless steel propeller-type stirrer was used instead of the monel stirrer, and the seal around the drive shaft was omitted. Where large amounts of product were needed, the crude product of several reactions was combined before purification. In the case of tetramethylglucose, several recrystallizations were necessary to obtain a product of sufficient purity. The yield was about 17.3 percent.

The alpha methylglucoside was prepared by the method of Palterson and Robertson (30). The reaction period of three hours was extended to six hours.

The reactions of the glucose derivatives and glycine were followed spectrophotometrically. The solutions were made up and the spectrophotometric data taken in the same manner as was used for the buffered systems of amino acids and dextrose. The dilution of the mixtures varied as the reaction time proceeded. The coloration of glucose derivatives alone also was followed in a similar manner, omitting the use of the 1 percent glycine. Since the reaction did not follow Beer's law, it was necessary to plot the logarithms of the optical density against the logarithms of the reciprocal of the dilutions in order to get an estimate of the optical density values. This plot produced a straight line with a negative slope. The antilogarithm of the intercept was used as the true optical density.

This made it possible to put the results on a comparable basis. It was necessary to assume that if a dilution curve had been made each day, a family of parallel lines would have been obtained, since the basis of these data was from dilutions made on the final day of reaction.

Dialysis of the resulting reaction mixtures was made using a cellophane osmosis membrane. Dialysis was made against tap water.

Production and Treatment of Bakery Products

For bread production, the following formula was employed:

Ingredient	Sponge, percent	Dough, percent
Flour	70.0	30.0
Mineral yeast food	0.25	
Malted wheat flour	0.25	
Water	Variable	
Yeast	2.0	
Dry milk solids*		4.0
Shortening		3.0
Salt		2.0
Sugar*		3.0
Paniplus		0.5

* The use of dry milk solids and the type of sugar were varied.

Bread crusts, extracted with 95 percent ethanol followed by water, were filtered and diluted for optical density measurements.

Bread, with different amino acids substituted for the milk solids normall used, was baked and extracts of the crust were made with water. Optical density measurements of bread curve extracts were made at 340 mμ in an attempt to correlate optical density with visual colored appearance.

In order to determine if nitrogen was present in the water-soluble brown products, and the amount of nitrogen present in other extractable constituents, 6 g of crust were extracted with 150 ml of water for 3 minutes in the Waring blender. The extract was filtered, the precipitate discarded, and the filtrate allowed to stand at room temperature for several days. The extract was filtered again and Kjeldahl determinations made on the filtrate and precipitate. The precipitate was not colored to any degree.

In an attempt to decrease the solubility of the protein and large carbohydrate molecules, the following extraction was made: Five grams of the crust from bread containing 0.2 percent of threonine as substituted for milk, and 3.0 percent dextrose, was dried over concentrated sulfuric acid at 30.0 mm Hg pressure for 5 hours. The crust then was dried over phosphorus pentoxide under 30.0 mm Hg for 12 hours. Extraction of the crust with 210 cc of absolute ethanol in a Soxlet extractor was completed. Only molecules of small dimensions were soluble

under these conditions. Optical density measurements were made from 220 to 350 mμ on the extract.

To obtain a solution of the colored products in a non-polar solvent, water extracts of bread crust were treated with about 50 percent acetone and the volume approximately doubled with diethyl ether. The solutions then were run several times through a scrubber tube into an excess of water. The ether layer was withdrawn and dried over calcium chloride, and optical density measurements were made throughout the ultraviolet spectrum.

Cookies were produced from methylated derivatives on a micro scale, as described under Micro Method III by Finney et al. (14), with a few modifications. Dry milk solids were omitted from the formula. Creaming of the sugar, shortening, and sodium bicarbonate was done in a Hobart Model N-50 using number 2 speed for two and one-half minutes and number 3 speed for one and one-half minutes. This mixture was made up with 55.0 percent sugar, the remaining 5.0 percent being added after the creamed mass had been divided into portions containing 34.6 g each. The 5.0 percent being added was varied as to type of sugar and was creamed in with the micro mixer for 2 minutes, stirring down each half minute.

The pH determinations were made according to the procedure described in AOAC (2). The pH measurements were made with the Beckman glass-electrode pH meter, model H-2. External color measurements were made with the Photovolt reflectometer with the three filters, tri-green, tri-blue, and tri-amber.

Cookies prepared for storage tests were prepared according to the following formula:

Ingredient	Percent	Quantity, g
Flour	100.0	1120.0
Sugar*	60.0	672.5
Shortening	30.0	335.0
Ammonium bicarbonate**	0.75	8.4
Sodium bicarbonate**	1.0	11.2
Salt	1.0	11.2
Water	26.79	300.0

* Sucrose was used as the control, and a part of it was substituted for by dextrose (cerelose brand) in the following quantities: 0, 0.5, 1.0, 3.0, 5.0, 8.0, 10.0, 15.0, 25.0, and 50.0 percent.

** The kind or combination of leavening agents was varied in the following manner: 1 percent sodium bicarbonate with .75 percent ammonium bicarbonate, 1.75 percent sodium bicarbonate, and 1.75 percent ammonium bicarbonate with each of the following concentration of dextrose: 0, 1.5, 3.0, and 5.0 percent.

The sugar and shortening were creamed, the ammonium bicarbonate and/or sodium bicarbonate and water were then blended in, and the flour was added. Mixing was minimized after addition of flour. The cookies were rolled out 6 mm in thickness and 59 mm in diameter. Baking was at 425° F. for 9 minutes. Four dozen cookies were baked with each combination of leavening agent and dextrose mentioned above.

Color, pH, and fat acidity determinations were made on one dozen of each mixture immediately, and at 30-day intervals

for 90 days. Sealright ice cream containers, sealed with parafin wax, were used for storage of the cookies. They were stored at room temperature.

The cookies, after color determinations were made, were ground in a Wiley mill and blended together. The fat acidity determinations were made in duplicate according to the standard method for wheat and wheat flour described in AOAC (3). The pH determinations were made from this same ground mass, according to the method of AOAC (2).

EXPERIMENTAL RESULTS

Fluorometric

The fluorometric studies indicate that tryptophan and tyrosine are very active in producing materials which fluoresce. The other amino acids, in combination with dextrose, showed an increase in fluorescence with increased reaction time; however, the increase was only a fraction of that increase displayed by tryptophan and tyrosine. Tryptophan was by far the most reactive. All of the solutions became quite darkened. The pH in these experiments was not controlled. These results do tend to indicate, however, that of the amino acids present in gluten, tryptophan and tyrosine would be the most likely ones to cause the formation of dark browning products. Table 1 indicates these results.

Table 1. Fluorescence measurements of glucose-amino acid mixtures for various lengths of time.

No.	Amino acid	pH	Time heated (hrs.) at 70° C. 0	24	70	94	118	142	166
1	Glutamic acid	0.7	5.5	13.8	21.0	21.9	19.8	19.3	16.1
2	Valine	2.7	5.1	9.0	18.4	22.4	28.7	40.4	50.1
3	Leucine	2.6	6.5	11.4	20.8	24.0	25.6	27.2	35.0
4	Isoleucine	2.5	7.2	13.8	25.0	30.8	38.5	46.2	57.1
5	Histidine	2.4	6.2	12.8	23.7	30.0	38.9	45.2	56.3
6	Arginine	2.4	5.5	8.5	11.5	17.3	20.3	23.4	30.0
7	Lysine	2.5	7.7	10.2	16.9	21.8	27.4	32.1	40.2
8	Glycine	3.1	7.3	35.5	43.7	49.7	40.0	20.9	11.9
9	Phenylalanine	2.2	7.0	12.8	20.5	26.0	32.1	36.4	50.1
10	Tryptophan	1.5	16.5	2,200.0	7,420.0	13,800.0	18,640.0	30,850.0	46,000.0
11	Tyrosine	10.3	11.8	2,950.0	2,525.0	2,200.0	2,075.0	2,005.0	1,740.0
12	Cystine	0.7	59.0	73.3	56.5	48.9	36.8	31.2	24.1
13	Threonine	2.3	5.4	25.8	65.2	69.8	84.5	95.9	181.6
14	Methionine	2.6	4.9	10.4	26.5	35.4	46.1	53.0	62.5
15	Proline	2.4	6.8	13.5	24.2	32.1	39.6	54.6	80.4
16	None (Control)	1.8	5.0	22.3	33.7	38.8	41.5	50.0	55.6

Spectrophotometric

Light absorption curves between 220 and 1000 mμ were run on the reaction mixtures of glucose and each of the 15 amino acids found in flour gluten. In all cases the only definite points of maximum absorption were found to be in the ultraviolet region. The optical density measurements were high in the low ultraviolet region (200-240 mμ), but dropped abruptly between 240 and 250 mμ. The absorption then increased gradually to a peak between 275 and 285 mμ. Beyond this wave length, only a gradual decrease in absorption was observed through the rest of the ultraviolet region (except in the case of glycine) and throughout the visible region. An absorption peak was observed at 350 mμ when glycine and glucose were reacted. However, as will be pointed out later, this peak could not be located when a buffered system was employed.

In these studies tryptophan and tyrosine again exhibited more activity with time than did the other amino acids. Other amino acids which were quite active were valine, threonine, glycine, histidine, and phenylalanine. Proline, an imino acid, with dextrose exhibited very little light absorption other than that characteristic for dextrose alone. Dextrose with cystine produced no maximum absorption peak. Absorption in this case was high in the lower ultraviolet region, and decreased throughout this spectrum and throughout the visible range.

In the buffered systems much of the previous work was con-

firmed; however, a few major differences were found. Only one light absorption peak was found in each reaction mixture. Phenylalanine developed a minor peak at 258 mμ and tryptophan developed one at 356 mμ during the primary stages of the reaction. These minor absorption bands disappeared as the reaction proceeded. Insoluble precipitates and odors formed after varying lengths of reaction time, depending on the amino acid used. Methionine developed a very definite odor of sauerkraut within 23 hours reaction time. Characterization of other odors was not attempted. A slight increase in point of maximum light absorption was observed in these reaction mixtures, consisting of 4.0 percent dextrose and 1.0 percent amino acid. The reactivity is illustrated by plotting optical density of the absorption peaks against reaction time in Figs. 1, 2, and 3. Table 2 lists the wave lengths at which the maximum absorption for each mixture was observed and the final pH of the mixture.

In a similar manner, absorption studies were made of buffered systems containing tetramethylmethylglucoside, tetramethylglucose, and alpha methylglucoside, each reacted with glycine. As the reaction time proceeded, it was necessary to make dilutions of the reaction mixtures in order to keep the optical density within the scale limit of the instrument. Plotting the optical densities of the absorption peaks against reaction time produced the curves illustrated in Fig. 4. Dextrose and glycine reacted much faster than did any of the

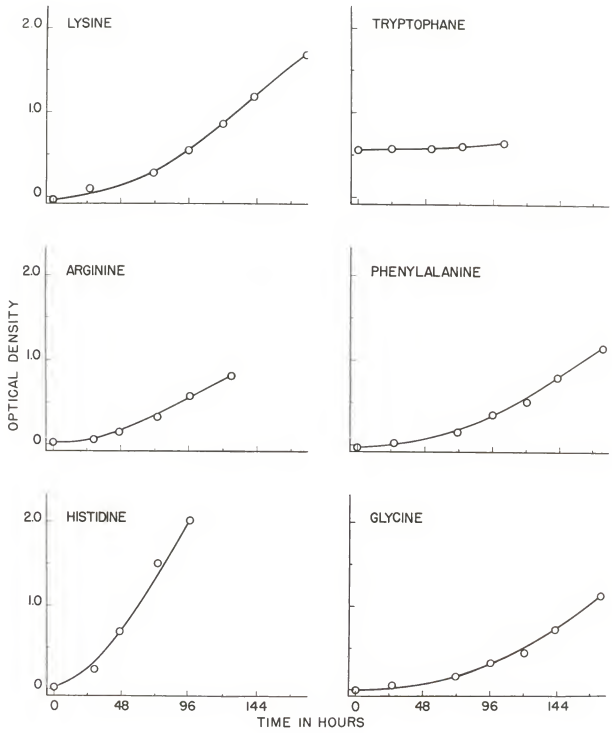


Fig. 1. The interaction of the amino acid and dextrose at 70° C., measured by light absorption in the ultraviolet spectrum.

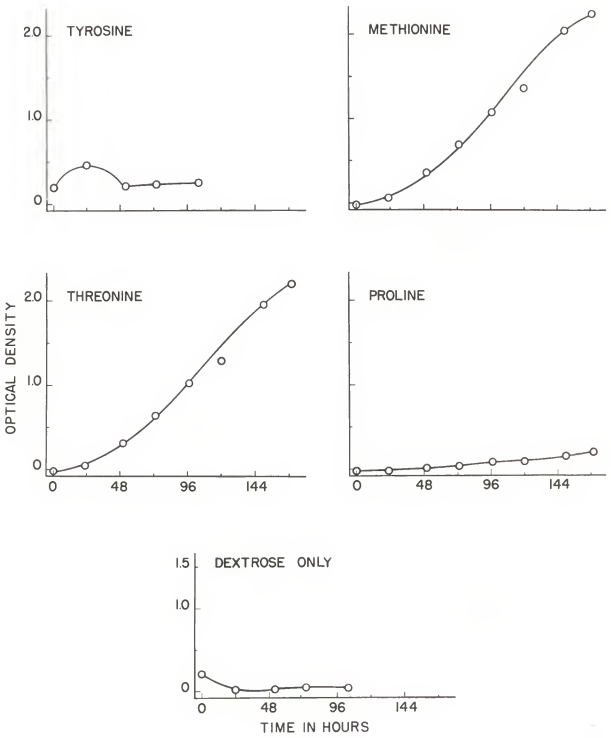


Fig. 2. The interaction of the amino acid and dextrose at 70° C., measured by light absorption in the ultraviolet spectrum.

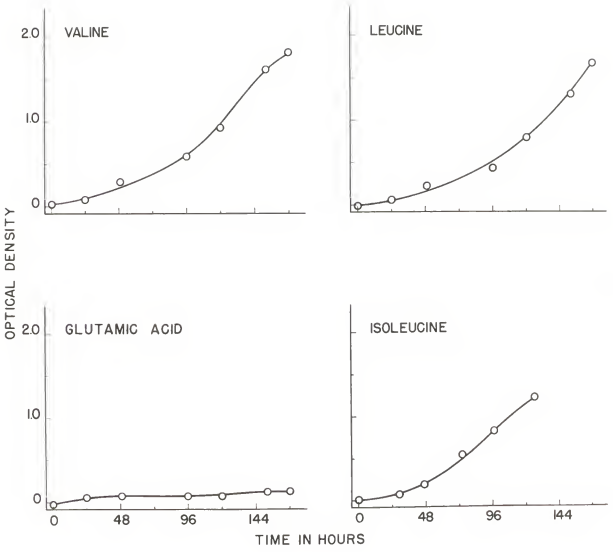


Fig. 3. The interaction of the amino acid and dextrose at 70° C., measured by light absorption in the ultraviolet spectrum.

Table 2. The final pH and wave length of maximum light absorption of reaction mixtures of amino acids and dextrose.

Amino acid	Wave length of maximum absorption, m	Necessary dilution of reaction mixture	Final pH
Glutamic acid	282	1-100	2.7
Valine	285	1-100	2.8
Leucine	285	1-100	2.9
Isoleucine	285	1-100	3.0
Histidine	285	1-100	3.1
Arginine	285	1-100	3.0
Lysine	282	1-100	2.7
Glycine	285	1-100	2.8
Phenylalanine	282	1-100	2.8
Tryptophan	278	1-500	2.9
Tyrosine	275	1-400	0.9
Threonine	285	1-100	3.0
Methionine	285	1-100	3.1
Proline	281	1-100	3.1
None (Control)	280	1-100	2.8

methylated derivatives and glycine. Dextrose alone developed more color than did the methylated derivatives and glycine. A Benedict's qualitative test on the alpha methylglucoside and tetramethylmethylglucoside solutions indicated that no free

reducing sugar was present after 151 hours of incubation at the pH of 3.0.

Table 3 shows the wave length at which the curves of Fig. 4 were made, and the final pH of the reaction mixtures. The wave length at which the maximum absorption occurred was slightly lower for the glucosides, both with glycine and without glycine, than for glucose and the other methylated derivatives.

Table 3. The final pH and wave length of maximum light absorption of systems containing methylated glucose derivatives.

Reactants	Wave length: m	Final pH
Tetramethylglucose and glycine	283	3.4
Tetramethylglucose only	281	3.4
Tetramethylmethylglucoside and glycine	280	3.3
Tetramethylmethylglucoside only	278	3.3
Alpah-methyl D-glucoside and glycine	275	3.3
Alpha-methyl D-glucoside only	275	3.4
D-glucose and glycine	285	3.2
D-glucose only	283	3.2

The results of the reaction of acetaldehyde and acetone with glycine demonstrated that the aldehyde group was much more reactive than the ketone group. The acetaldehyde-glycine reaction mixture produced very dark products, darker even than

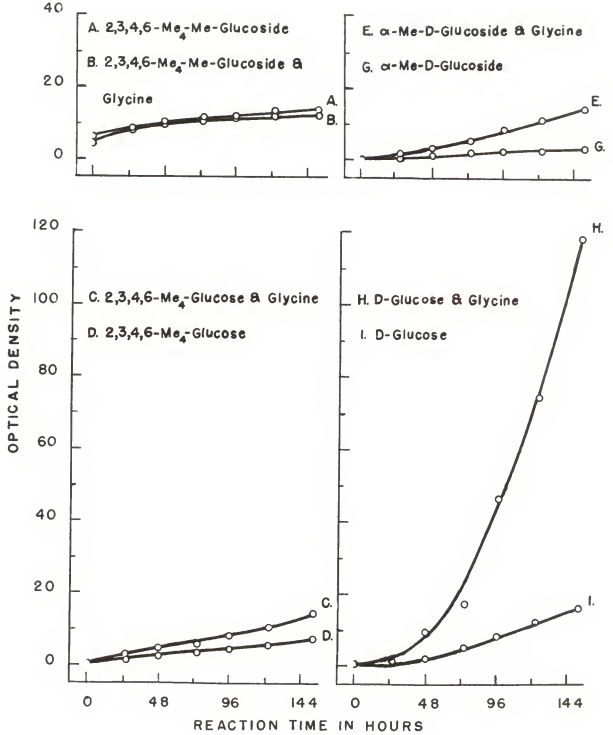


Fig. 4. The interaction of dextrose and methylated dextrose derivatives with glycine at 70° C., measured by light absorption in the ultraviolet spectrum.

those produced by glucose and glycine under identical conditions. The absorption curve in the ultraviolet from 220 to 350 mμ was very similar to those of the dextrose-amino acid reaction mixtures, producing a peak at 276 mμ for three successive days. The acetone-glycine reaction mixture under identical conditions showed a minor light absorption peak at 270 mμ. This peak increased little with reaction time, and visible coloration of the mixture was not observed.

In order to determine the character of the chromophoric group responsible for the light absorption peaks between 275 and 285 mμ of the reaction mixtures studied, a study of simpler compounds, for which the structures are known, was made. Two oximes, acetaldoxime and butyraldoxime, known to contain a cyano group, were prepared and light absorption curves run on their water solutions. The absorption curve between 220 and 350 mμ was not similar to that of the dextrose-amino acid mixtures, and no peak was found between 275 and 285 mμ. Instead, the absorption peak was near 250 mμ.

Attempts to separate the reactants from the reaction products of the forementioned synthetic systems by dialysis were unsuccessful. The colored material passed the membrane as well as the reactants. The only substance retained was the insoluble colored material which could be separated by filteration.

Production and Treatment of Bakery Products

In order to determine the extent of the browning (Maillard) reaction in bread, it was necessary to extract the brown pigment from the crust. Extraction with non-polar solvents was not successful. Toluene and benzene extracted little or no pigment; carbon tetrachloride, petroleum ether, diethyl ether, and acetone revealed slight extraction; and water-saturated N-butyl-alcohol showed somewhat greater extraction. Salting out with magnesium chloride and sodium bicarbonate in acetone and diethyl ether-ethyl alcohol suspensions resulted in no extraction or precipitation of the colored components. The best solvent found for the brown pigment of bread was water, even though extraction with water was not complete.

Kjeldahl determinations on the crust extract revealed the presence of a small amount of nitrogen, as indicated in Table 4. The extract was allowed to sit at room temperature for several days, whereupon a precipitate formed. A portion of the colored material was bound in this precipitate. Kjeldahl determinations were made on the original extract, the precipitate, and the filtrate. These determinations were made on the extract of bread crust that contained milk and on that of crust containing no milk.

Table 4. Nitrogen content of bread crust extracts.

	Nitrogen content in milligrams/10 cc solution		
	Original extract	Precipitate	Filtrate
Milk bread	.9900	.0071	.9700
Bread with no milk	.9600	.0067	.7600

These extracts did not give a positive protein test. The milk bread contained slightly more water-extractable nitrogen in the crust than did the non-milk bread.

The light absorption curve of the water and absolute ethanol extracts of bread crust were very similar to those of the synthetic systems containing amino acids and glucose. The light absorption maxima in all cases fell between 275 and 285 mμ. In water the maximum light absorption was at 285 mμ, compared to 280 mμ in 95 percent ethanol. When the water extracts of bread containing different concentrations of dextrose were studied, it was found that those containing the higher concentration of dextrose tended to have the peak located at a slightly higher wave length. Where 1.0 percent dextrose was used, the peak was located at 275 mμ; if 3.0 percent dextrose was used, the peak was at 282 mμ.

The light absorption peak of absolute ethanol extract of pre-dried bread crust was at 280 mμ.

Attempts to correlate the visual color of bread to optical

density measurements at 340 mμ were unsuccessful.

The use of amino acids as a color stabilizer in bread, with dextrose used as the sugar agent, was attempted. The milk was omitted from the bread formula, and the amino acids substituted in approximately the same quantity in which they are found in casein. Table 5 illustrates the results of the substitution. The optical densities of the bread extract are not too reliable a criterion of the color, since the color varied somewhat on different portions of the crust.

Tryptophan and tyrosine produced bread with a dark crust, as was expected from the study of the synthetic systems. Certain other amino acids, and combinations of amino acids, also produced dark color. Many of the combinations produce a color equal to that of milk bread. In some cases the amino acids caused undesirable flavor and aroma in the baked bread. These are recorded in Table 5.

Factors Affecting Coloration of Sugar Cookies

Statistical analysis of the color differences measured by the reflectometer of samples containing 0, 0.5, 3.0, 5.0, and 8.0 percent dextrose showed a significant effect of dextrose concentration on the color of the cookies, except between 5.0 and 8.0 percent dextrose. These samples containing 10.0, 15.0, 25.0, and 50.0 percent dextrose did not differ in color or pH. Plate I illustrates the effects observed by varying dextrose concentration. Table 6 shows the difference

Table 5. The color variation of bread samples containing amino acids.

Substitutions in sample	Wt. of amino acid, g	Visual coloration	Optical density	Remarks
Sucrose	None	2.5	.306	
Cerelose	None	2.0	.252	
Alanine Glycine	0.7 0.7	4.0	.409	Slight musty odor
Serine Threonine Tyrosine	1.5 1.5 1.5	5.0	.419	Slight odor
Valine Leucine Isoleucine	1.5 1.5 1.5	5.0	.432	Strong odor
Alanine Tyrosine Tryptophan	1.1 1.1 1.1	5.5	.379	Strong cheese odor
Methionine Cystine	0.4 0.4	2.0	.282	Strong hydrogen-sulfide odor
Lysine Arginine	1.3 1.3	4.0	.513	Strong heartsease honey odor
Aspartic acid Glutamid acid	2.0 2.0	3.0	.518	Slight odor
Tryptophan Glutamic acid Methionine	1.5 1.5 1.5	5.0	.452	Mercaptan odor
Tryptophan Glutamic acid Methionine Histidine	1.5 1.5 1.5 1.5	5.0	.498	Strong mercaptan odor
Tryptophan	2.0	5.0	.418	
Glutamic acid	2.0	4.0	.453	
Aspartic acid	2.0	3.0	.364	
Control (containing milk)	-	5.0	.336	

in color found by the reflectometer expressed as the International Commission on Illumination (ICI) values, and those found in pH and spread factor. The pH decreased as the concentration of dextrose increased to a concentration of 5 percent dextrose. The spread factor was not affected materially by dextrose concentration.

Table 6. The effect of dextrose concentration on sugar cookies.

Percent dextrose	ICI values			pH	Spread factor diameter thickness
	X	Y	Z		
0.0	.36	.37	.63	9.1	8.16
0.5	.40	.39	.47	8.6	9.02
1.0	.40	.39	.43	8.5	8.39
3.0	.42	.39	.35	8.4	7.78
5.0	.43	.39	.33	8.0	7.86
8.0	.43	.39	.33	7.7	7.74

Storage studies for a 90-day period were made on sugar cookies with two variables, sugar agent and leavening agent. Color, pH, and fat acidity determinations were made immediately after preparation, after 30 days, after 60 days, and after 90 days. Upon storage the color of the cookies faded slightly, but was inconsistent within the storage period studied. Conclusions based on the statistical analysis indicate that this change in color is so small that a larger sample or a longer storage period would be necessary to prove conclusively that the color

EXPLANATION OF PLATE I

The effect of dextrose concentration on the color of sugar cookies.

Number	Concentration (percent)
11	0.0
12	0.5
13	1.0
14	3.0
15	5.0
16	8.0

PLATE I

of the cookies becomes lighter as a result of chemical action during storage. The results obtained are given in Tables 7, 8, 9, and 10.

Sugar cookies for which dextrose, alpha methylglucoside, tetramethylmethylglucoside, and tetramethylglucose were substituted for 5.0 percent of the sugar, sucrose being used for the remaining 55.0 percent, are illustrated in Plate II. The blocking of the active aldehyde group of glucose, as in the case with alpha methylglucoside and tetramethylmethylglucoside, greatly reduced the color produced in cookies as compared to dextrose. Tetramethylglucose, in which the active group was not blocked, also produced a cooky of much lighter color than did the dextrose. The pH of the cookies prepared from the three methylated glucose derivatives and of those prepared from dextrose differed very little. The cookies prepared from dextrose had a slightly lower pH.

The top grain and spread factor of these cookies baked with different sugar agents differed only slightly, presumably due to granulation and state of the sugar agent. These data are given in Table 11.

Table 7. The effect of leavening agent and storage on the color, pH, and fat acidity of sugar cookies when sucrose is the only sugar agent used.

Leavening agent	Storage days	ICI value* X	Y	Z	pH	Acid number
1.0% Sodium bicarbonate and 0.75% Ammonium bicarbonate	0	.57	.59	.44	9.3	.29
	30	.59	.61	.48	9.4	.89
	60	.63	.62	.48	9.6	.83
	90	.60	.62	.50	9.2	1.29
1.75% Sodium bicarbonate	0	.55	.58	.40	9.9	.31
	30	.57	.59	.42	10.1	.54
	60	.57	.59	.43	10.1	.67
	90	.62	.60	.45	10.0	.56
1.75% Ammonium bicarbonate	0	.63	.66	.59	7.1	1.49
	30	.64	.67	.61	7.5	1.05
	60	.65	.66	.60	7.0	1.10
	90	.65	.67	.63	6.4	1.32

* Approximate International Commission on Illumination values.

Table 8. The effect of leavening agent and storage on the color, pH, and fat acidity of sugar cookies when 0.5 percent of the sucrose has been substituted for by dextrose.

Leavening agent	Storage days	ICI value* X	Y	Z	pH	Acid number
1.0% Sodium bicarbonate and 0.75% Ammonium bicarbonate	0	.51	.51	.32	9.4	.45
	30	.52	.52	.34	9.2	.56
	60	.52	.52	.35	9.5	.49
	90	.55	.55	.40	9.2	.75
1.75% Sodium bicarbonate	0	.51	.51	.31	9.8	.32
	30	.54	.55	.38	9.7	.60
	60	.54	.53	.35	9.9	.69
	90	.52	.54	.34	9.7	.64
1.75% Ammonium bicarbonate	0	.55	.56	.42	7.1	1.40
	30	.59	.60	.48	7.3	.87
	60	.61	.63	.51	7.3	.78
	90	.61	.61	.51	6.4	.80

* Approximate International Commission on Illumination values.

Table 9. The effect of leavening agent and storage on the color, pH, and fat acidity of sugar cookies when 5.0 percent of the sucrose has been substituted for by dextrose.

Leavening agent	Storage days	ICI value* X	Y	Z	pH	Acid number
1.0% Sodium bicarbonate and 0.75% Ammonium bicarbonate	0	.33	.31	.15	8.3	.56
	30	.38	.36	.18	8.7	.73
	60	.38	.36	.18	8.8	.87
	90	.40	.38	.20	8.4	.59
1.75% Sodium bicarbonate	0	.30	.27	.12	9.3	.49
	30	.34	.31	.15	9.3	.67
	60	.36	.34	.16	9.4	.40
	90	.36	.34	.16	9.3	.36
1.75% Ammonium bicarbonate	0	.49	.48	.30	7.2	.61
	30	.50	.49	.31	6.9	1.01
	60	.52	.52	.34	6.9	.80
	90	.51	.51	.32	7.5	.66

* Approximate International Commission on Illumination values.

Table 10. The effect of leavening agent and storage on the color, pH, and fat acidity of sugar cookies when 5.0 percent of the sucrose has been substituted for by dextrose.

Leavening agent	Storage days	ICI value* X	Y	Z	pH	Acid number
1.0% Sodium bicarbonate and 0.75% Ammonium bicarbonate	0	.34	.32	.16	8.2	.43
	30	.37	.34	.17	8.4	.74
	60	.35	.33	.16	8.6	.62
	90	.36	.34	.17	8.1	.61
1.75% Sodium bicarbonate	0	.31	.28	.13	9.2	.42
	30	.30	.28	.12	8.8	.54
	60	.33	.30	.14	9.2	.49
	90	.33	.30	.14	9.0	.68
1.75% Ammonium bicarbonate	0	.49	.49	.30	6.8	1.05
	30	.48	.48	.30	7.0	.89
	60	.49	.49	.29	7.1	.84
	90	.50	.48	.31	6.6	.87

* Approximate International Commission on Illumination values.

EXPLANATION OF PLATE II

The effect of 5.0 percent dextrose or methylated dextrose derivative on the color of sugar cookies

Number	Sugar agent
1	Dextrose
2	Methylglucoside
3	Tetramethylmethylglucoside
4	Tetramethylglucose
5	Sucrose

PLATE II

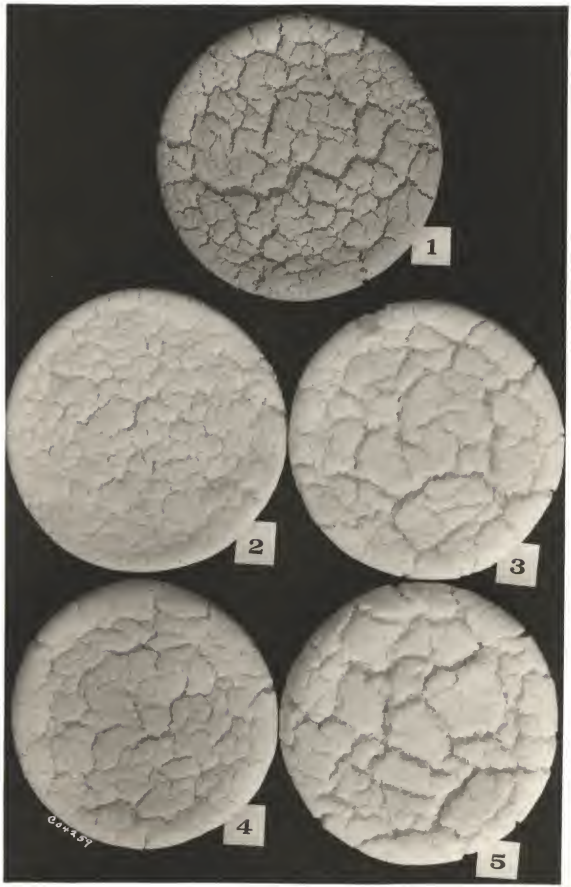


Table 11. The effect of dextrose and glucose derivatives on the color, pH, and spread factor of sugar cookies.

Sugar source	Number corresponding to Fig. 6	pH	Spread factor: D/T	ICI value* X	Y	Z
Dextrose	1	8.3	10.61	.38	.38	.22
Methylglucoside	2	9.1	11.79	.52	.60	.46
Me_4-Me-glucoside	3	9.1	9.50	.52	.61	.48
Me_4-glucose	4	9.1	9.51	.51	.61	.48
Sucrose	5	9.1	10.15	.53	.63	.53

* Approximate International Commission on Illumination values.

DISCUSSION

In the study of synthetic systems, the results showed that tryptophan and tyrosine are the greatest color-producing amino acids present in flour gluten. The tryptophan reaction mixture demonstrated fluorescence values which were many times greater than those of the amino acid causing the next greatest value. It has been suggested that a method for the determination of tryptophan content of gluten hydrolysate could be devised from this principle (4). All the amino acid-dextrose mixtures increased in fluorescence as the reaction time increased, but the differences in gain were extremely variable, depending upon the amino acid used. The fluorescence values for some of the mixtures (numbers 1, 8, 11, and 12 of Table 1)

even decreased after varying lengths of reaction time. This probably was due to the intense color which had developed and was interfering with the fluorescence measurements. In the water extracts of bread, the fluorescence was affected more by sugar concentration in the bread than it was by the length of time that the bread was baked.

Light absorption peaks of systems containing the 15 amino acids of flour gluten and glucose, at low concentrations, are found only in the ultraviolet spectrum. In some cases more than one light absorption peak was found when unbuffered systems were employed. The use of a slightly more concentrated reaction mixture which was buffered caused the minor peaks to disappear. The light absorption peak was observed to shift slightly to an increased wave length as the reaction proceeded. This phenomenon probably was due to concentration change of the products. Barham (4) also observed this shift while studying the reaction of gluten hydrolysate and glucose between 350 and 600 mμ. Proline, which possesses an imino group, was shown to react very little, if any, with glucose.

It was observed that the glucose-amino acid reaction mixtures and the bread extracts in water and ethyl alcohol produced light absorption peaks between 275 and 285 mμ. This could be caused by a common chromophoric group with different radicals attached. Brode (9) suggested that the chromophoric group responsible for absorption within this range is the carbonyl group. He indicated, however, that changes in the radical

attached influence the point of maximum absorption considerably. The present theories of the mechanism of the browning reaction postulate that the initial reaction involves the production of a cyano group. Other organic compounds, such as oximes, contain this group. The study of the absorption spectra of acetaldoxime and butyraldoxime revealed, however, that this group could not be responsible for the light absorption peaks observed between 275 and 285 mμ.

The use of amino acids in bread causes the crust to become increasingly darker during baking. This suggests that the amino acids are involved in the browning reaction. Likewise, the addition of reducing sugars in the presence of available amino groups intensifies the browning reaction. The lack of either available amino groups or reducing sugars will limit the extent of the browning reaction. Although tryptophan and tyrosine were most reactive in the synthetic systems, when used in bread did not appear more reactive than some other mixtures of amino acids. When lactose was substituted for dry milk solids, which contain both soluble amino acids and reducing sugar, little effect on crust color was observed. This provides further evidence that both reducing sugars and available amino groups, either as protein or amino acids, must be present to produce brown colored products.

The use of dextrose in sugar cookies has demonstrated that when 5 percent of the sucrose has been substituted for by dextrose, the maximum color is produced. Additional amounts of

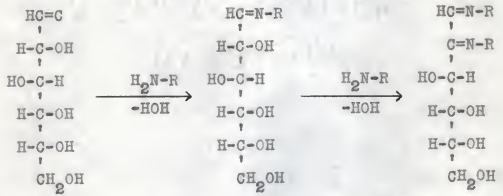
dextrose had no added effect. This suggests that there are only sufficient available amino groups to react with 5 percent of dextrose. Additional amounts of dextrose, therefore, would not enter into the color formation. As little as 0.5 percent dextrose produced a significant effect on the color of the cookies. Intensity of this color was increased as the dextrose concentration was increased up to 5 percent (Table 6). These observations suggest that a reducing sugar is involved in the brown coloration. If the browning in cookies was due to caramelization, it would not be expected that substitution of dextrose for sucrose would have any effect on coloration. These observations lend strong support to the view that the browning reaction in baked products involves the reducing group of the sugar and available amino groups. The pH of the cookies (Table 6) decreased as the concentration of the dextrose increased up to 5 percent. The pH did not change beyond that concentration where color differences were not evident. This is further evidence that the browning reaction in cookies is similar to the classical Maillard reaction, since in synthetic systems involving amino acids and reducing sugars there is concomitant lowering of the pH.

The study of cookies stored for 60 days reveals that the brown color is essentially stable. The pH and acid number tend to go up as would be expected. The color tends to become lighter, but the change is so small that either a larger sample or longer storage period would be required to demonstrate a statistically-

significant difference. Dried milk solids and eggs were not used in these cookies. However, their presence might be effective in promoting or retarding the physical and chemical properties of cookies.

The synthetic systems containing glycine and the methylated glucose derivatives, tetramethylmethylglucoside, tetramethylglucose, and alpha methylglucoside, show that color production is retarded almost completely. Sugar cookies produced from these derivatives also demonstrated their ability to prevent color production. Color production is retarded by blocking either or both the active carbonyl group and the hydroxyl positions of the glucose molecule; this would indicate that at least two groups are involved in the reaction. A system involving both the active group and the hydroxyl positions could be responsible for color production, and any interruption would disturb this system in such a way that color production would be inhibited. If this is the case, the blocking of any of the groups on the glucose molecule would prevent discoloration in the presence of an amino acid. Another possible mechanism would be that the active carbonyl group is essential for color production. A condensation would occur between the amino group of the amino acid and the carbonyl group of the glucose. Further interaction would occur and the carbon atom adjacent to the carbonyl group be involved, producing a secondary condensation between another molecule of amino acid and the second carbon atom of the glucose. Decarboxylation would

occur on the amino acid. Color would not be produced until this occurs. Reaction of the second carbon atom could not take place prior to condensation on the carbonyl group. Polymerization of these units would bring about the large insoluble browning products. The following configuration illustrates the proposed mechanism:



Decarboxylation and polymerization would occur following this initial phase of the reaction. The formation of osazones lends support to this postulation. The initial reaction is similar. This theory explains why a reducing sugar is necessary for this reaction. According to the results with methylated derivatives, the reducing group would not be involved, since color was retarded when tetramethylglucose and glycine were reacted. The inhibition of color, when alpha methylglucoside and glycine were reacted, demonstrated that the reducing group plays an important role in color production.

SUMMARY

An investigation was conducted on the browning reaction in baked goods. Fundamental approaches have been made in an attempt to determine the mechanism of this reaction.

1. Of the 15 amino acids present in flour gluten, tryptophan and tyrosine were the most reactive in the production of brown pigments, as was indicated by fluorometric and spectrophotometric studies. Proline, an imino acid, displayed very little if any activity.

2. Aqueous buffered reaction mixtures of each of the 15 amino acids and dextrose produced only one light absorption maximum. This maximum was located between 275 and 285 mμ in all cases. This provides a means of following the browning reaction.

3. Browning products in bread crust are partially soluble in water, ethanol, and acetone. The solubility is much greater in water than in the other two; however, the extraction was not complete in any case. This suggested that the products of the browning reaction may form high polymers.

4. Water and absolute ethanol extracts of bread crust show light absorption maxima in the ultraviolet region between 275 and 285 mμ.

5. The color of sugar cookies was influenced considerably by the addition of dextrose between the concentration of 0.1 and 5.0 percent. Greater concentrations of dextrose

had no added effect on the color of the cookies. This provided evidence in favor of the Maillard reaction's being responsible for browning in baked goods.

6. Storage studies on sugar cookies revealed that no changes in color were produced during 60 days' storage.

7. The use of amino acids, as a substitute for milk in bread, resulted in a dark crust color. This supported the view that available amino groups are involved in the browning reaction.

8. The use of methylated derivatives of dextrose in systems containing only glycine and in sugar cookies greatly retarded color production as compared to the use of dextrose. These data indicated that the reducing group and second carbon atom of dextrose are involved in the formation of brown products in baked goods.

9. It is postulated that the primary browning reaction involves the alpha amino group and the numbers 1 and 2 carbon atoms of the reducing sugar. This may be followed by condensation to form high polymers.

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APPENDIX

Suggestions for Future Study

Approach to the problem of browning reaction through fundamental research is essential. Many practical browning problems would be greatly simplified if the mechanism of this reaction could be proven.

In order to identify the nature of the browning products, chromatographic systems should aid in the discovery and provide further evidence for their mode of formation. Chromatographic techniques could be used to separate and identify the browning reaction products. In this connection, a method of control of the browning could be developed. This phase has not been extensively investigated previously.

Further study of the blocking of reactive centers by means of methylation or other blocking agent on the glucose molecule should provide evidence for the mechanism of the browning reaction. Blocking of reactive centers on the amino acid or protein molecule would also be of great importance in this study.

The use of infra-red absorption would aid in the identification of linkages in simple systems containing amino acids and dextrose. Much study would be necessary to develop the use of this instrument, due to the insolubility of the products in solvents other than water. Perhaps a combination of two methods, such as chromatography and infra-red spectroscopy, would be necessary.

Further study of ultraviolet absorption could result in a simple method for measuring the degree of browning. Identification of the grouping responsible for absorption between 275 and 285 mμ would be extremely useful in determining the mechanism of the reaction. Combinations of this method and chromatography might be necessary.

BROWNING REACTION IN BAKED PRODUCTS

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The course of the browning reaction at 70° C. between 15 amino acids found in wheat gluten and dextrose was followed fluorometrically and spectrophotometrically. Fluorescence was many times greater in the tryptophan and tyrosine reaction mixtures than in the other reactions. Light was absorbed strongly in the ultraviolet region only. Absorption peaks were located between 275 and 285 mμ. Tryptophan and tyrosine demonstrated the most activity.

Browning was greatly retarded when the methylated glucose derivatives, alpha methylglucoside, tetramethylglucose, and tetramethylmethylglucoside, were substituted for the dextrose in reaction with glycine and in sugar cookies. This suggests that color is produced through a system involving both the aldehyde group and one or more of the hydroxy groups. A primary mechanism involving first the aldehyde group, then the hydroxy position on the carbon atom adjacent to the aldehyde group, has been suggested.

Browning products of baked goods demonstrated considerable solubility in water, but very little solubility in nonpolar solvents.

The brown coloration increased and the pH decreased as the concentration of dextrose was increased in sugar cookies between 0 and 5.0 percent dextrose. Increasing the concentration of dextrose beyond 5.0 percent had no added effect on the color or pH of the cookies. The availability of amino groups limits the amount of dextrose which can be utilized in color

formation. If caramelization were responsible for this color formation, the color would increase proportionally to the concentration of dextrose beyond 5.0 percent. The control cookies containing sucrose as the only sugar source would also form caramel products.

The addition of amino acids to bread increased crust color, indicating that color is controlled in some cases by the availability of the amino groups. The flavor and aroma of the bread also were controlled by the amino acids.