STUDIES ON ACCELERATED BROWNING IN STARCH PASTES CONTAINING VARIOUS BREAD INGREDIENTS[1]

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ABSTRACT

Wheat starch pastes (10%) with and without single and multiple additions of wheat gluten, glucose, lactose, shortening, and nonfat dry milk, prepared at pH values of 4.4, 5.4, and 6.4, were stored at 75°C. for 25 days. The sugars were the principal ingredients which caused browning, as measured by reflectance of the dried pastes at 600 mμ. Browning was accompanied by increases in reducing substances, even in pastes made without sugars. Aqueous ethanol extracts of the stored pastes exhibited absorption maxima at approximately 275 mμ, a region where carbonyl compounds show strong absorption. Chromatographic separation on magnesium sulfate of the 2,4-dinitrophenylhydrazones prepared from ethanol extracts of a browned paste made with wheat starch, gluten, and sucrose at pH 6.4 yielded 13 "nonacid" and 17 "acid" carbonyl derivatives. The absorbance of these derivatives did not correspond to that of the corresponding hydrazones of 5-hydroxymethylfurfural or levulinic acid.

Exploratory studies conducted by the authors with canned bread revealed that rather drastic conditions of time and temperature were necessary to produce any appreciable browning (16). The development of a brown color was paralleled by increases in total soluble nitrogen, amino nitrogen, and titratable acidity, whereas reducing substances remained essentially constant. Lysine N, however, decreased with an increase in storage time at the highest storage temperatures employed (50° to 75°C.), but browning was already quite extensive before any decrease was noted.

In the light of these preliminary studies, it was decided to investigate the effects of different bread ingredients on the browning phenomena by using model systems containing various combinations of ingredients stored at 75°C. Since 5-hydroxymethylfurfural is a recognized intermediate in browning (6,7) and has been reported to increase in bread in proportion to the amount of sugar used in the formula (9), several of the systems were examined for the presence of

[1] Manuscript received January 13, 1960. Contribution from the Department of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota. Paper No. 4210 of the Scientific Journal series, Minnesota Agricultural Experiment Station. The data in this paper represent part of a thesis presented to the Graduate School of the University of Minnesota by Russell J. Stenberg in partial fulfillment of the requirements for the Ph.D. degree, May 1950.

This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, and has been assigned number 1079 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense.

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carbonyl compounds. The experiments were carried out with wheat starch pastes containing one or more of the other ingredients used in making bread.

Materials and Methods

The following materials were used:

Wheat starch. A specially prepared product (General Mills Research Laboratories, Minneapolis, Minn.) washed from a hard winter wheat flour dough, without the use of sulfur dioxide. This starch was processed to contain not more than 0.4% protein (N × 5.7, dry basis).

Wheat gluten. Prepared by washing starch from a spring wheat flour dough. The gluten in thin sheets was dried at room temperature (about 23°C.) in moving air and then ground in a semi-micro Wiley mill to pass a 40-mesh sieve.

Sucrose, reagent grade (General Chemical Co.)

D-glucose—C.P. (Mallinckrodt)

Lactose (U.S.P.)

Nonfat dry milk, specially prepared for breadmaking by the spray process.

Shortening: leaf lard, kettle-rendered.

Preparation and Storage of Starch Paste Mixtures. Pastes were prepared by stirring 10 g. of starch and one or more of the other ingredients with 90 ml. of distilled water or buffer solution for 25 minutes at 75°C. Biphthalate buffers (0.05*M*) were used for pH values of 4.4 and 5.4 and phosphate buffer (0.05*M*) for pH 6.4.

With the exception of nonfat dry milk, where 0.25 g. was used, 0.5 g. of each additional ingredient was employed in preparing the pastes. They were stored in 250-ml. Erlenmeyer flasks for 25 days in a circulating-air oven maintained at 75°C.

Methods of Analysis. At the beginning and end of the storage period, a portion of each paste was frozen, crushed, and lyophilized. The dried material containing 4 to 5% moisture was ground in a semi-micro Wiley mill fitted with a 20-mesh screen. The color of the ground product was measured at 600 mμ by means of a Beckman Model DU spectrophotometer with a reflectance attachment, using a freshly scraped block of magnesium carbonate as a reference standard. All other analytical determinations were made on the pastes without drying them.

Reducing and nonreducing substances were determined by a modification of the procedures for reducing and nonreducing sugars described in *Cereal Laboratory Methods* (1). The modification consisted of stirring 25 g. of the paste with 10 ml. of 95% ethanol and conduct-

ing the sugar analysis on a 10-ml. aliquot. Results were expressed as the ml. of 0.1*N* ferricyanide reduced.

Spectrophotometric measurements in the ultraviolet region were made on solutions prepared from some of the pastes. The well-mixed paste (25 g.) in a 125-ml. Erlenmeyer flask was extracted with 35 ml. of 95% ethanol which had been purified by distillation over potassium hydroxide and zinc dust. The extract was clarified by filtration and its absorbance determined in a Beckman Model DU spectrophotometer at wave lengths from 220 to 320 mμ. For comparative purposes, the absorbances of levulinic acid and 5-hydroxymethylfurfural in the ultraviolet region were also determined. Levulinic acid was purified by redistilling twice under reduced pressure in an atmosphere of nitrogen. The resulting colorless product crystallized readily at 7°C., melted at 31°C. with refractive index $n_D^{27} = 1.4388$. The method described by Haworth and Jones (5) was used to prepare 5-hydroxymethylfurfural. The product was distilled twice under high vacuum to yield a light yellow liquid which crystallized readily at 0°C. (refractive index $n_D^{25} = 1.5602$).

Separation of Carbonyl Compounds. For the isolation and attempted characterization of carbonyl compounds, a browned paste made with wheat starch, wheat gluten, and sucrose buffered at pH 6.4 and stored for 25 days at 75°C. was employed. The paste (400 g.) was extracted with 400 ml. of purified 95% ethanol and the extract removed by filtration. A second filtrate was obtained by extracting the residue with 100 ml. of 95% ethanol for 1 hour. The filtrates were combined and concentrated to about 200 ml. by heating at 35°C. under reduced pressure, after which 10 g. of sodium bicarbonate were added to neutralize any organic acids which were present. Continuous extraction with redistilled diethyl ether in a modified Soxhlet apparatus for 6 hours removed aldehydes and ketones which did not contain acidic groups ("nonacid" carbonyl compounds). The residual solution was then acidified with hydrochloric acid equivalent to the sodium bicarbonate previously added, and continuously extracted with redistilled diethyl ether to remove the "acid" carbonyl compounds. Each of the ether solutions was concentrated at room temperature under reduced pressure to about 25 ml. Water (10 ml.) was then added and the solutions were reduced to a final volume of 15 to 20 ml.

To prepare the 2,4-dinitrophenylhydrazones of the carbonyl compounds, 2,4-dinitrophenylhydrazine hydrochloride was dissolved in 2*N* hydrochloric acid according to the directions of Brady and Elsmie (2). Each solution of carbonyl compounds was added to 200 ml. of the freshly prepared and filtered reagent and after 3.0 hours the reaction

mixture was extracted three times with redistilled diethyl ether. The extracts were combined and the ether removed under reduced pressure to yield a mixture of any 2,4-dinitrophenylhydrazones which were present and any unreacted reagent which may have been extracted.

The chromatographic technique used in separating those hydrazones was patterned after the method employed by Stadtman (15) in the separation of similar derivatives obtained in studies on dried apricots. After development of the chromatograms, the hydrazones were extracted from each band with purified diethyl ether.

The eluate from each band was evaporated to dryness under reduced pressure. To remove occluded benzene, the residue was dissolved in purified 95% ethanol and once more evaporated to dryness; this process was repeated three times.

The 2,4-dinitrophenylhydrazones of 5-hydroxymethylfurfural melted at 200°C., as compared with 200.3°C. reported by Wahhab (18); the levulinic acid derivative melted at 206°C., the same as reported in the literature (8).

The absorbance of solutions of the 2,4-dinitrophenylhydrazones isolated from the browned pastes and the corresponding derivatives of the two reference compounds was determined with the Beckman DU spectrophotometer at wave lengths from 225 to 450 mμ.

Bacteriological examinations were made on ten samples of the pastes which had been stored by plating on a dextrose-tryptone agar. A fluid thioglycollate medium which is capable of supporting many types of aerobic and anaerobic organisms was also inoculated with successive dilutions of the pastes[3].

Results

Bacteriological Tests. The plate counts and dilution tests carried out on several of the stored samples gave no evidence of bacterial contamination. Bacteria were, therefore, not involved in the chemical changes which occurred in the pastes during storage.

Effect of Ingredients and pH on Color Formation in Pastes during Storage. The reflectance data recorded in Table I do not show any consistent relation between pH and browning over the range investigated. The pH values of the buffered pastes did not change during storage, whereas the acidity of an unbuffered starch paste fell from pH 6.1 immediately after preparation to pH 3.4 after being held for 25 days at 75°C. Studies on the browning of several food products have revealed that the rate of color development decreases with a decrease

[3] The bacteriological tests were carried out by Dr. J. J. Jezeski, Department of Dairy Husbandry, University of Minnesota.

in pH (4,11,17). However, the effect of pH over the range employed with these pastes was slight and inconsistent.

Comparisons of the reflectance changes at corresponding pH values in the different pastes clearly show that the sugars were the major contributors to browning. When added to starch or to mixtures containing starch, gluten, shortening, and nonfat dry milk, sucrose caused more discoloration than glucose. Unfortunately, no pastes were prepared to determine the effect of D-fructose formed along with glucose by the inversion of sucrose. The contribution of lactose to browning appeared to be less than that of the other two sugars.

The effects of the other ingredients on the formation of brown pigments varied, depending on the nature of the other substances

TABLE I

EFFECT OF INGREDIENTS AND pH OF PASTES ON COLOR FORMATION DURING STORAGE FOR 25 DAYS AT 75°C.

COMPOSITION OF PASTE	REFLECTANCE		
	pH 4.4	pH 5.4	pH 6.4
Starch only (not buffered, not stored), 0.91[a]	...	...	...
Starch only (not buffered, stored), 0.92[b]	...	...	...
Starch only	0.71	0.72	0.87
Starch + gluten (control, not buffered, not stored), 0.90[c]	...	...	...
Starch + gluten	.68	.85	.81
Flour	.72	.72	.68
Starch + sucrose	.70	.69	.63
Starch + glucose	.76	.71	.68
Starch + lactose	.69	.74	.72
Starch + NFDM[d] (control, not stored)	.90	..	.88
Starch + NFDM	.71	.74	.82
Starch + shortening (control, not stored)	.86	..	.84
Starch + shortening	.73	.76	.84
Starch + gluten + sucrose	.64	.67	.73
Starch + gluten + glucose	.66	.65	.67
Starch + gluten + lactose	.80	.75	.72
Starch + gluten + NFDM	.80	.80	.77
Starch + gluten + shortening	.75	.77	.75
Starch + shortening + sucrose	.61	.64	.74
Starch + shortening + glucose	.77	.80	.82
Starch + glucose + NFDM	.70	.63	.72
Starch + gluten + shortening + sucrose	.65	.76	.74
Starch + gluten + shortening + NFDM	.65	.77	.84
Starch + shortening + sucrose + NFDM	.73	.77	.83
Starch + gluten + sucrose + NFDM	.62	.70	.78
Starch + gluten + shortening + NFDM + sucrose	.62	.67	.73
Starch + gluten + shortening + NFDM + glucose	.63	.75	.79
Starch + gluten + shortening + NFDM + lactose	0.79	0.81	0.78

[a] This unbuffered paste immediately after preparation had a pH of 6.0.
[b] This unbuffered paste had a pH of 3.4 after 25 days' storage.
[c] This unbuffered paste had a pH of 6.1 immediately after preparation.
[d] Nonfat dry milk.

which were present. Gluten caused an increase in coloration when added to mixtures of starch and sucrose or of starch and glucose. In other pastes, gluten appeared to play a less important role. Nonfat dry milk apparently retarded browning when present in a paste containing starch, shortening, and sucrose. Shortening also retarded coloration in mixtures of starch and glucose and of starch, gluten, and sucrose. In other pastes, shortening and nonfat dry milk had no effect on the development of color during storage.

These data on the marked influence of the sugars on the browning of the pastes have been confirmed by the subsequent work of Larsen *et al.* on the browning of canned bread prepared by formulas varying in sugar content (10).

Effect of Paste Ingredients on Changes in Reducing and Nonreducing Substances during Storage. Reducing and nonreducing substances were determined in pastes stored at pH 5.4, the active acidity which was the closest to that found in canned bread prepared by the original formula.

The results in Table II show that increases in reducing substances occurred even in those pastes made with starch alone, although the

TABLE II

EFFECT OF COMPOSITION OF PASTE ON AMOUNT OF MATERIALS REDUCED BY ALKALINE FERRICYANIDE DURING STORAGE AT pH 5.4 FOR 25 DAYS AT 75°C.

COMPOSITION OF PASTE	REDUCING SUBSTANCES[a]		
	Stored	Control	Gain
Starch only	0.6	0.1	0.5
Starch + gluten	1.4	0.8	0.6
Flour	4.3	2.1	2.2
Starch + sucrose	3.3	0.1	3.2
Starch + glucose	7.4	6.2	1.2
Starch + lactose	5.6	5.0	0.6
Starch + NFDM[b]	2.2	1.4	0.8
Starch + shortening	1.0	0.1	0.9
Starch + gluten + sucrose	3.9	0.5	3.4
Starch + gluten + glucose	7.6	5.6	2.0
Starch + gluten + lactose	6.4	3.1	3.3
Starch + gluten + NFDM	2.1	1.6	0.5
Starch + gluten + shortening	1.0	0.3	0.7
Starch + shortening + sucrose	4.4	0.1	4.3
Starch + shortening + glucose	6.6	6.1	0.5
Starch + NFDM + glucose	8.1	7.6	0.5
Starch + gluten + shortening + sucrose	3.1	0.2	2.9
Starch + gluten + shortening + NFDM	2.6	1.7	0.9
Starch + shortening + NFDM + sucrose	4.8	1.3	3.5
Starch + gluten + NFDM + sucrose	4.3	1.5	2.8
Starch + gluten + shortening + NFDM + sucrose	4.7	1.5	3.2
Starch + gluten + shortening + NFDM + glucose	8.3	7.5	0.8
Starch + gluten + shortening + NFDM + lactose	6.0	6.0	0.0

[a] Reducing substances are expressed as ml. 0.1*N* ferricyanide reduced per 25 g. paste.
[b] Nonfat dry milk.

reducing power was further increased by the addition of other ingredients, particularly the sugars.

The reducing and nonreducing substances in pastes to which sucrose was added are recorded in Table III. These determinations were carried out on a different series of pastes from those for which analyses are given in Table II, and the differences in the gain in reducing sugars for comparable samples in Tables II and III give a measure of the reproducibility of the experiments. The gain in reducing substances during storage was substantially greater than the loss in nonreducing substances, so that reducing substances have arisen from sources other than sucrose. When sucrose solution was stored at pH 5.4, however, the increase in reducing substances equaled the decrease in nonreducing materials.

TABLE III

CHANGES IN REDUCING AND NONREDUCING SUBSTANCES FOUND IN WHEAT STARCH PASTES CONTAINING SUCROSE AND OTHER BREAD INGREDIENTS AFTER STORAGE AT pH 5.4 FOR 25 DAYS AT 75°C.

COMPOSITION OF PASTE	REDUCING SUBSTANCES[a]		GAIN IN REDUCING SUBSTANCE[a]	NONREDUCING SUGARS[a]		LOSS IN NON-REDUCING SUGARS[a]
	Stored	Control		Stored	Control	
Starch + sucrose	2.4	0.2	2.2	3.4	4.7	1.3
Starch + sucrose + gluten	3.3	0.4	2.9	3.0	4.8	1.8
Starch + sucrose + shortening	2.6	0.2	2.4	3.0	3.6	0.6
Starch + sucrose + shortening + gluten	2.9	0.3	2.6	2.8	4.6	1.8
Starch + sucrose + shortening + NFDM[b]	4.1	1.4	2.7	3.1	4.9	1.8
Starch + sucrose + gluten + NFDM	2.6	1.7	0.9	4.0	4.7	0.7
Starch + sucrose + gluten + shortening + NFDM	3.8	1.7	2.1	3.3	4.4	1.1
Sucrose solution (not a paste)	2.9	0.0	2.9	3.1	6.4	3.3

[a] Expressed as ml. of 0.1*N* ferricyanide per 25 g. paste.
[b] Nonfat dry milk.

Spectrophotometric Analyses of Aqueous Ethanol Extracts of Wheat Starch Pastes. Absorbance values determined on 50% ethanol extracts of representative pastes which were made with phosphate buffer (pH 6.4) are recorded in Figs. 1 and 2[4]. These data show that

[4] The biphthalate ion showed strong absorption in the same spectral region as the carbonyl compounds, and hence curves were not made for pastes containing this buffering agent. Absorption of the alcoholic extracts was confined essentially to the ultraviolet region, and the curves have therefore been plotted only for the region from 215 to 320 mμ.

The effect of ethanol concentration on the absorption characteristics of an extract prepared from a wheat starch paste containing gluten and sucrose stored at pH 6.4 for 25 days was investigated. One aliquot was diluted with 50% and another with 95% ethanol. The increase in ethanol concentration had no influence on the character of the absorption curve.

In another experiment the effect of diethyl ether extraction was studied. Twenty milliliters of the undiluted extract from a stored paste which contained starch, gluten, and glucose was further extracted with diethyl ether for 6 hours. The ether was removed by distillation and the residue made up to 200 ml. with 50% ethanol. Extraction with diethyl ether shifted the maximum absorption from 273 to 277.5 mμ and a decrease in the quantity of absorbing material also resulted.

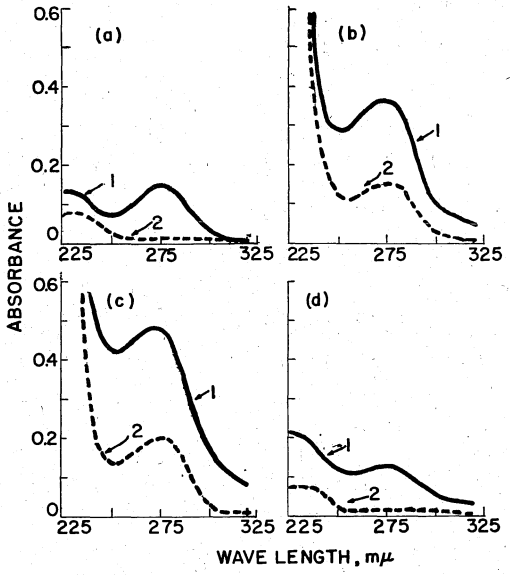

Fig. 1. Ultraviolet absorption curves for 50% ethanol extracts of starch pastes of various composition before and after storage for 25 days at 75°C. Curve 1, after storage; curve 2, before storage; (a) wheat starch only; (b) wheat starch + wheat gluten; (c) wheat flour; (d) wheat starch + sucrose.

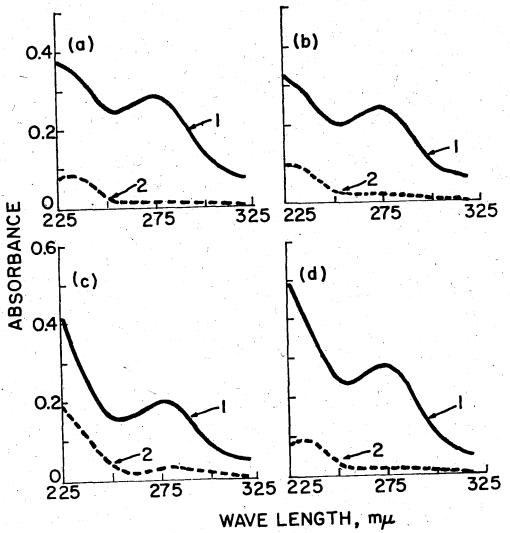

Fig. 2. Ultraviolet absorption curves for 50% ethanol extracts of starch pastes of various composition before and after storage for 25 days at 75°C. Curve 1, after storage; curve 2, before storage; (a) wheat starch + glucose; (b) wheat starch + lactose; (c) wheat starch + nonfat dry milk; (d) wheat starch + shortening.

a substance or group of substances which exhibited an absorption maximum at approximately 275 mμ was produced in each of the pastes during storage.

The increases in absorbance at 275 mμ during storage of the various pastes for 25 days at 75°C. are recorded in Table IV. A paste made with wheat flour showed a great increase in absorbance on storage. The presence of sugars in the pastes is associated with a large increase in absorbance; glucose caused the greatest increase, followed by lactose and sucrose in the order named. Surprisingly, nonfat dry milk which contains about 50% lactose, when present in a paste, depressed the change in absorbance during storage.

Comparison of Absorption Curves for Extracts of Starch Pastes with 5-hydroxymethylfurfural and Levulinic Acid. The absorption curves and constants for 5-hydroxymethylfurfural dissolved in water and in absolute ethanol are given in Fig. 3. Changing the solvent from water to absolute ethanol shifted the maximum absorption from 283 to 280 mμ. When the solvent was 50% ethanol the spectral curve followed that of the water solution very closely.

The absorption curves for pure levulinic acid in water, and in 50%

TABLE IV

EFFECT OF COMPOSITION ON THE INCREASE IN ABSORBANCE OF EXTRACTS OF WHEAT STARCH PASTES AT pH 6.4 DURING STORAGE AT 75°C. FOR 25 DAYS

COMPOSITION OF PASTE	INCREASE IN ABSORBANCE[a] AT 275mμ
Starch only	0.14
Starch + gluten	.22
Flour	.28
Starch + sucrose	.12
Starch + glucose	.27
Starch + lactose	.22
Starch + NFDM[b]	.17
Starch + shortening	.27
Starch + gluten + sucrose	.31
Starch + gluten + glucose	.38
Starch + gluten + lactose	.31
Starch + gluten + NFDM	.16
Starch + gluten + shortening	.17
Starch + shortening + sucrose	.21
Starch + shortening + glucose	.21
Starch + glucose + NFDM	.16
Starch + gluten + shortening + sucrose	.23
Starch + gluten + shortening + NFDM	.17
Starch + sucrose + shortening + NFDM	.16
Starch + gluten + sucrose + NFDM	.17
Starch + gluten + shortening + NFDM + sucrose	.18
Starch + gluten + shortening + NFDM + glucose	.22
Starch + gluten + shortening + NFDM + lactose	0.24

[a] The increases are the difference in absorbance of extracts of pastes before and after storage.
[b] Nonfat dry milk.

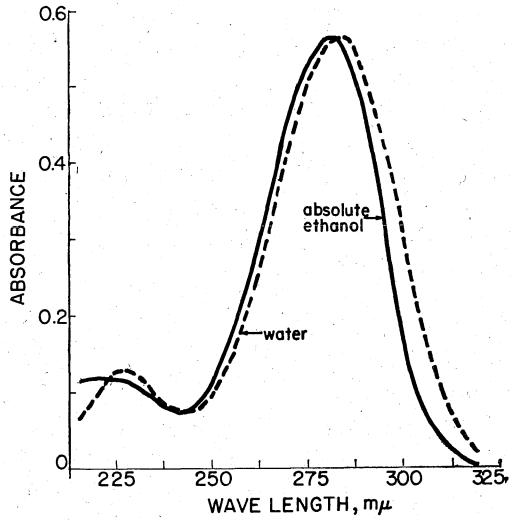

Fig. 3. Ultraviolet absorption curves for 5-hydroxymethylfurfural in aqueous and absolute ethanol solutions.

Absorption constants in water:

Max., 283 mμ
E = 15670

Min. 242.5 mμ
E = 2175

Max. 227 mμ
E = 3600

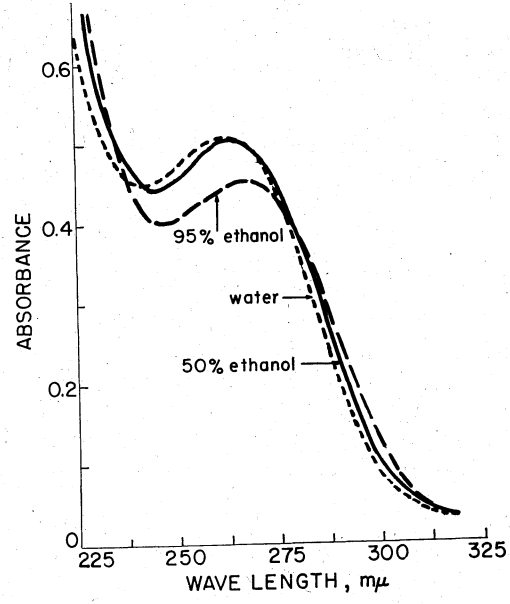

Fig. 4. Ultraviolet absorption curves for levulinic acid in water, 50% and 95% aqueous ethanol.

Absorption constants in water:

Min. 241 mμ
E = 24.7

C = 0.0244 moles/liter

Max. 262.5 mμ
E = 27.8

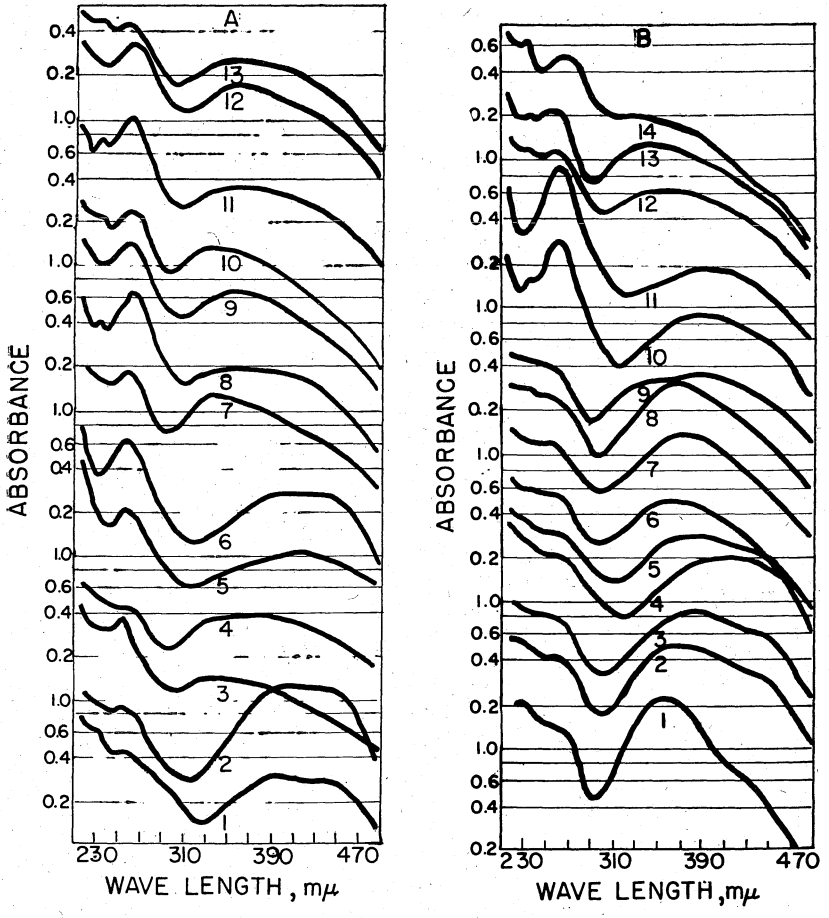

Fig. 5. Absorption spectra of 2,4-dinitrophenylhydrazones of (A) "nonacid" and (B) "acid" carbonyl compounds extracted from a paste prepared from wheat starch, wheat gluten, and sucrose after storage for 25 days at 75°C. Curves are numbered in order of increasing affinity of the derivatives on magesium sulfate.

and 95% ethanol are given in Fig. 4. When absorption data were calculated for a mixture of $2.812 \times 10^{-5}M$ hydroxymethylfurfural and $1.219 \times 10^{-2}M$ levulinic acid, a spectral curve was obtained which was very similar to that for an extract of a paste prepared from starch, gluten, and sucrose. This indicates that both of these carbonyl compounds may be present in the pastes after storage.

Separation and Attempted Characterization of Carbonyl Compounds in Stored Pastes. The method employed for separating the "acid" carbonyl compounds from aldehydes and ketones which do not possess acidic groups was first applied to a known mixture of

levulinic acid and furfural in aqueous solution[5]. The respective maxima of the two fractions were separated by at least 10 mμ. Assuming that all of the furfural was extracted by diethyl ether, only about 40% of this compound was recovered, whereas 90% of the levulinic acid was found in the water fraction. These results indicated that the carbonyl compounds which are extracted from wheat pastes could be separated into acid and nonacid fractions, although losses may occur in carrying out the procedure.

Chromatographic separation of the 2,4-dinitrophenylhydrazones of the "nonacid" carbonyl compounds yielded 13 bands, whereas 17 bands were obtained from the "acid" carbonyl compounds. The absorbances for these two groups of hydrazones from 220 to 490 mμ are shown in Fig. 5, A and B respectively.

None of the spectral curves corresponded to that for the 2,4-dinitrophenylhydrazone of 5-hydroxymethylfurfural. Although curve No. 1 of the acid carbonyl derivatives resembled that of the levulinic acid compound, the absorption affinity of the latter on magnesium sulfate was much greater and it cannot, therefore, be the same compound.

Discussion

Measurements of color development in the pastes show that sugars were the major, but not the only, contributors to browning. Several reactions can occur. They may decompose to furfurals or similar compounds involving the sugars containing an active carbonyl group (5,18). These may either condense with nitrogen compounds or polymerize to form brown, resinous materials. Another possibility is the decomposition of the sugars to organic acids which may react with sugars, nitrogenous materials, or with themselves to produce pigments. Because the pastes, with one exception, were well buffered, any increases in the quantity of acids could not be detected through pH changes; however, the fact that the pH of an unbuffered starch paste fell from pH 6.0 to 3.4 showed that organic acids were produced.

The increase in substances which reduce ferricyanide was associated with the development of browning in the pastes. This increase was not due solely to the hydrolysis of sucrose, since pastes which did not contain this sugar browned and showed increases in reducing substances. Hydrolysis of starch yields maltose and glucose which could decompose to produce such reducing substances as dihydroxyacetone

[5] Sodium bicarbonate was added to form the sodium salt of levulinic acid which is insoluble in diethyl ether. The solution was then extracted four times with diethyl ether, to remove the furfural. After evaporation of the ether at reduced pressure, the extracted material was made up to volume in water and the absorbance of both fractions determined. Sodium bicarbonate had no effect upon absorbance between 260 and 320 mμ.

and glyceraldehyde, which have been postulated as intermediates in the browning reaction by Speck and Ball (14). Reducing substances which may arise from the breakdown of sugars include glyoxal, methyl glyoxal, diacetyl, acetol, acetoin, and the furfurals, all of which may be intermediates in color formation. The furfurals, especially, have received attention as possible precursors of the dark pigments in dried fruit (12,13), and 5-hydroxymethylfurfural has been reported in bread (9).

The marked absorbance of extracts of the pastes at about 275 mμ is characteristic of the carbonyl group, and the chromatographic separation of the 2,4-dinitrophenylhydrazones indicated the presence of 13 carbonyl compounds in the "nonacid" group and 17 in the "acid" group, despite the probable loss of the more volatile carbonyl compounds such as acetaldehyde due to the method of separation employed. It seems unlikely, however, that 30 different hydrazones were isolated. There is a possibility that the separation into the two groups was incomplete, so that some of the same derivatives may be present in both; also, it has been shown that 2,4-dinitrophenylhydrazones which exist as cis, trans isomers may be partially separated by chromatographic methods (20). In the present experiments, the 2,4-dinitrophenylhydrazone of 5-hydroxymethylfurfural yielded two bands of nearly equal color intensity and the same absorption when chromatographed on a magnesium sulfate column. Tautomerization of the 2,4-dinitrophenylhydrazones may also be a source of some of the many compounds observed.

The distribution of the absorption maxima in the range between 330 and 410 mμ (phenylhydrazine absorption) indicates that more than one class of derivative is present. It has been shown that the position of the absorption band attributed to the phenylhydrazine part of the molecule is dependent upon the extent of conjugated unsaturation and, to a lesser degree, upon the amount of alkyl substitution present in the parent carbonyl compound (21,22). General classifications of the 2,4-dinitrophenylhydrazones have been made on this basis. Other characteristics, besides the degree of unsaturation in the parent carbonyl compound, however, may alter the nature of the "phenylhydrazine" absorption; thus λ max. increases considerably for the bis-2,4-dinitrophenylhydrazones of the alpha-diketones where conjugation occurs between the two hydrazone systems (22). From the phenylhydrazine absorption of the derivatives in these experiments it was not possible to arrive at any classification of the parent carbonyl compounds, but it may be concluded that they varied in nature.

The absorption maximum at 260 mμ exhibited by all the deriva-

tives is also shown by 2,4-dinitrophenylhydrazine itself. The fact that derivatives which were most strongly adsorbed by magnesium sulfate had higher ^{E}Max. values in the range of 220 to 300 mμ than the ^{E}Max. value of the phenylhydrazine portion of the molecule cannot be explained. None of the 50 compounds studied by Braude and Jones (3) corresponded to those found in the present studies. The fact that none of the hydrazones corresponded to those of 5-hydroxymethylfurfural or levulinic acid does not mean that they were not formed. These, and other very reactive carbonyl compounds, could have reacted further to form the brown pigments, leaving the less reactive carbonyls free to be isolated as hydrazones.

In a personal communication Hodge[6] has discussed these findings in the light of his investigations of the chemistry of browning reactions (6,7). The first step in the Maillard reaction is sugar-amine condensation. With secondary amines, the amine is then eliminated from carbon 1 of the sugar after 1,2- and 2,3-enolization of the sugar moiety. This generates a terminal methyl group at carbon 1 and releases the amine (amino acid) for further reaction. After this sequence of reactions a methyl-carbonyl-carbonyl arrangement exists on carbons 1, 2, and 3 of the erstwhile sugar, and this d-dicarbonyl compound is considered to be a critical intermediate for browning. It undergoes fission and dehydration without fission to give polymerizing unsaturated carbonyl compounds. Presumably some of these compounds have been isolated as 2,4-dinitrophenylhydrazones in the present study.

The derivatives of the unknown carbonyl compounds which were separated in these experiments cannot be identified until the absorption spectra of the 2,4-dinitrophenylhydrazones of a series of reference carbonyl compounds, which include all those which are present in the pastes, have been studied and classified[7].

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[6] J. E. Hodge, Northern Utilization Research and Development Division, Peoria, Illinois.

[7] Recently, Wiseblatt (19) has succeeded in identifying several of the carbonyl compounds distilled from bread under high vacuum by the formation, chromatographic separation, and characterization of their 2,4-dinitrophenylhydrazones by comparison with authentic hydrazones of known carbonyl compounds which have been prepared since the studies in this paper were undertaken.

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