SOME CHEMICAL CHANGES WHICH ACCOMPANY THE BROWNING OF CANNED BREAD DURING STORAGE[1]

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ABSTRACT

The rate of browning of canned bread, as determined at 600 mμ with a Beckman Model DU spectrophotometer equipped with a reflectance attachment, increased with the temperature of storage. At 25°C. there was only very slight browning after 120 days, but at 35°C. appreciable browning had occurred at 100 days' storage. At 50° and 75°C. browning was progressively much more rapid. No appreciable biochemical changes were noted in bread stored at −15° and 25°C. At 35°C., however, the pH decreased, whereas the total soluble nitrogen, titratable acidity, and amino nitrogen increased in the samples stored for 100 days. These biochemical changes were much more marked at 50° and 75°C. Lysine nitrogen decreased with an increase in storage time at 50° and 75°C. Total reducing substances remained essentially constant. Bacteriological tests indicated that these chemical changes were not likely to be due to bacterial action. The brown pigment could not be extracted with water or with a number of organic solvents. Acetylation gave a brown reaction mixture with both white and brown crumb; water then extracted the brown pigments from both samples, yielding solutions of similar absorbance and amino nitrogen content.

The development of a satisfactory method of canning white bread by the Quartermaster Corps in 1946 provided a means of supplying bread in field rations[3]. Although canned bread remained palatable for several months, it eventually developed a yellowish-brown color and acquired a slightly unpleasant odor and taste. These phenomena are likely caused by nonenzymic browning reactions which commonly occur upon the storage of heat-processed foods.

Several theories have been proposed for the mechanism of the chemical reactions which lead to the development of nonenzymatic browning in heat-processed foods. As early as 1912, Maillard (16) observed that upon heating, a mixture of amino acids and reducing sugars became dark in color. Hodge (6) has recently reviewed various lines of evidence concerning the details of the reaction. Lea and

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[3] Holton, Paul; from a lecture before Midwest Section, AACC, and given in AACC News Letter No. 67, May 1946, p. 81.

Hannan (11,12,13), employing casein and glucose as model reactants, observed a decrease in the ϵ-amino groups of lysine and the formation of bound glucose; later, the complex darkened in color, and lysine could not be recovered upon acid hydrolysis of the reaction product (10).

Protein-sugar reactions of the Maillard type are frequently involved in the browning of processed foods. Among other factors, it is generally accepted that increases in active acidity retard the rate of discoloration of some food products (2,9,19). The researches of Haas and Stadtman (5) and of Lewis *et al.* (14) led to the postulation that organic acids may be involved in the browning of dehydrated fruits.

To serve as a basis for a later investigation of the effect of formula variations on the browning of canned bread, the preliminary exploratory studies reported in this paper were undertaken. The study is divided into two sections: 1) gross changes in the chemical composition of canned white bread as browning occurred during storage at different temperatures, and 2) attempts to extract the brown pigments from the bread after storage.

Materials and Methods

Preparation and Storage of Canned Bread. The canned bread was prepared according to the following formula[4], supplied by the Quartermaster Food & Container Institute.

Ingredients[a]	*Parts by Weight*
Flour	100.00
Salt	1.75
Sucrose	4.00
Shortening	4.00
Nonfat dry milk	2.00
Monocalcium phosphate	0.25
Calcium propionate	0.125
Yeast, compressed	2.00
Water	60.00

[a] The flour, a low-protein short patent, milled from hard red winter wheat, and hydrogenated vegetable shortening (100-hour stability) were supplied by the Institute.

The bread was made by a 100% sponge procedure; 2,835 g. of flour and the proportional quantities of salt, monocalcium phosphate, yeast, and water were placed in an 8-qt. round-bottomed bowl and mixed for 2.5 minutes at slow speed in a Model C-10 Hobart mixer

[4] Subsequent to this work, the Institute developed a new formula for canned bread (Chicago Quartermaster Depot, Military Specification; Bread, Canned, MIL-B-1070B; 1953) which was designed to yield a product having a lower pH and moisture content than that produced by the formula used in this study.

equipped with two hooks. The sponge was fermented 135 minutes at 30°C.; the remaining ingredients were added and mixed for 1 minute at low speed and 3 minutes at second speed. The remixed dough was immediately scaled into 15 pieces each weighing 326 g., which were then rolled in hydrogenated shortening and placed in No. 2½ lacquered cans. The cans were clinched and proofed (about 44 minutes) at 30°C. until the dough was 1.5 in. from the top of the cans. The dough was baked at 232°C. for 30 minutes. The bread was allowed to cool for 5 minutes after which the cans were hermetically sealed and immersed in running tapwater for 45 minutes.

Storage and Preparation of Bread Crumb. The canned bread was stored at temperatures of −15°, 25°, 35°, 50°, and 75°C. After various periods of storage the cans were opened, the crust was removed from the bread, and the crumb cut into thin slices and dried at room temperature to less than 10% moisture. The crumb was then ground in a semimicro Wiley mill to pass a No. 40 sieve.

Changes in the Chemical Composition of Canned Bread with Browning

The extent of browning of the dried crumb was measured and analyses were made for total soluble nitrogen, free amino nitrogen, titratable acidity, ϵ-amino groups of lysine, reducing substances, and pH.

Methods. A measure of the browning of the bread crumb was obtained by means of a Beckman Model DU spectrophotometer with a reflectance attachment. A block of magnesium carbonate with a freshly scraped surface was used as a standard (reflectance = 1.000). The samples were prepared by packing the finely ground crumb into dishes 1.5 in. square and 0.5 in. deep and carefully smoothing the surface with a spatula. Reflectance was measured at 600 mμ[5]. Good reproducibility of surfaces was obtained, the deviation in the readings taken from the same lot of bread being less than 1%.

[5] Reflectance measurements were first made on a control sample of dried bread crumb and on a heavily browned sample (stored at 75°C. for 35 days) at a series of wave lengths between 350 and 700 mμ. Representative values were:

	Reflectance	
Wave length	*Control crumb*	*Browned crumb*
mμ		
350	0.303	0.035
500	0.684	0.094
600	0.766	0.237
700	0.814	0.446

Since the accuracy of any photoelectric measurement falls off rapidly when the transmission of light is less than 13%, a wave length of 600 mμ was selected. After the completion of this work Larsen *et al.* (8) have utilized the Hunter Color and Color-Difference Meter for following the browning of bread crumb, a method which probably gave more accurate results.

Total soluble nitrogen was determined by extracting 4.0 g. of dried crumb in a 125-ml. Erlenmeyer flask with 20 ml. of water for 60 minutes at room temperature with intermittent shaking. After centrifugation, the supernatant was filtered and analyzed for nitrogen by a micro-Kjeldahl procedure. Digestion was carried out with selenium oxychloride in sulfuric acid (perchloric acid was omitted) according to the method of Pepkowitz and Shive (18). Distillation of the digest and titration were conducted by the procedure of Ma and Zuazaga (15).

The titratable acidity and free amino nitrogen of the dried crumb were determined on a 20-g. sample by the modified Sorenson formol titration method given in *Cereal Laboratory Methods* (1).

Basic lysine nitrogen of the crumb was determined by a microbiological assay procedure[6] described by Zittle and Eldred (22).

Reducing substances were determined in a 2.5-g. sample by the alkaline ferricyanide procedure for reducing substances in flour (1). Since numerous reducing substances, in addition to maltose, were probably present in the bread crumb, the results are reported in terms of the quantity of ferricyanide reduced.

The pH of the crumb was measured by the method for bread (1), employing a Coleman Model 3 pH electrometer.

To ascertain the possible role of bacteria as a cause of the chemical changes occurring in canned bread, plate counts were made on bread crumb from two cans of bread which had been stored 2 years at 25°C. using dextrose-tryptone agar[7] which is recommended by the National Canners' Association for the determination of flat-sour organisms in flour, starch, and sugar. Inoculations were made with successive dilutions of the bread crumb (as removed from the cans) in sterile water. A fluid thioglycollate medium which is capable of supporting many types of aerobic and anaerobic organisms was also inoculated with successive dilutions of the bread-crumb dispersion.[8]

Results

The results of the reflectance and biochemical measurements on the bread crumb are recorded in Table I. The reflectance data were in accord with the browning of the crumb, as judged visually. At 25°C. there was only very slight browning after 120 days of storage, but at 35°C. appreciable browning had occurred at 100 days of storage.

[6] The authors are indebted to the General Mills Research Laboratories, Minneapolis, Minn., for carrying out these analyses.

[7] Difco Laboratories, Inc.: "Manual of Dehydrated Culture Media and Reagents" (7th ed.). Detroit, Mich. (1944).

[8] Dr. J. J. Jezeski, Department of Dairy Husbandry, University of Minnesota, kindly performed these studies.

With storage temperatures of 50° and 75°C., browning was progressively much more rapid; at the latter temperature, browning was so extensive that the measurements were discontinued after 35 days of storage.

No appreciable biochemical changes were noted in the bread crumb stored at −15° and 25°C. At 35°C., however, the pH decreased, whereas the total soluble nitrogen, titratable acidity, and amino nitrogen increased in the samples stored for 100 days, a time which corresponded with the development of a brown color. At storage temperatures of 50° and 75°C. these biochemical changes were much more marked, particularly at 75°C. The rate of increase in total soluble nitrogen was the same as the rate of increase in amino nitrogen at the three highest storage temperatures, revealing a break-

TABLE I

EFFECT OF TIME AND TEMPERATURE OF STORAGE OF CANNED WHITE BREAD ON THE BROWNING AND THE BIOCHEMICAL PROPERTIES OF THE CRUMB OF THE BREAD[a]

TEMPERATURE	DAYS	pH OF CRUMB EXTRACT	TOTAL SOLUBLE NITROGEN	TITRATABLE ACIDITY[b]	AMINO NITROGEN	REDUCING SUBSTANCES[c]	REFLECTANCE[d]	LYSINE NITROGEN
°C			mg		mg			mg
..	0	5.7	9.1	0.50	0.13	28	....	...
−15	14	5.7	8.9	0.50	.16	27	....	...
−15	48	5.7	9.6	0.51	.15	27	....	...
−15	100	5.6	9.1	0.51	.17	28	....	...
−15	200	5.6	8.6	0.57	.18	28	....	...
..	0	5.7	9.4	0.50	.16	29	0.760	2.43
25	11	5.7	8.5	0.51	.15	29	.765	...
25	31	5.7	8.4	0.50	.16	29	.767	...
25	60	5.7	8.3	0.49	.17	30	.768	...
25	120	5.5	9.2	0.55	.17	30	.755	2.51
25	200	5.5	9.4	0.58	.18	30	.750	...
..	0	5.8	9.3	0.46	.17	29	.763	2.45
35	10	5.7	9.1	0.50	.20	29	.752	...
35	27	5.6	8.8	0.53	.15	29	.774	...
35	50	5.7	8.6	0.48	.16	28	.771	2.64
35	100	5.4	13.2	0.66	.24	28	.738	...
35	150	5.2	14.1	0.81	.26	28	.720	2.84
..	0	5.7	8.7	0.35	.13	27	.762	2.73
50	0	5.7	8.8	0.38	.14	27	.744	...
50	11	5.6	8.9	0.39	.15	28	.706	...
50	20	5.5	9.8	0.43	.21	27	.666	2.45
50	40	5.4	11.0	0.47	.21	26	.665	...
50	60	5.4	13.0	0.51	.18	27	.635	2.21
..	0	5.8	8.9	0.35	.17	28	.766	2.81
75	5	5.4	14.5	0.49	.24	27	.601	...
75	10	5.2	21.0	0.67	.34	27	.526	...
75	17	5.0	28.6	0.80	.44	26	.417	1.61
75	25	4.9	37.6	0.93	.67	26	.328	...
75	35	4.8	45.5	1.12	0.85	24	0.237	1.03

[a] All data are expressed on the basis of 1 g. dry crumb.
[b] Expressed as ml. 0.14*N* base neutralized.
[c] Expressed as ml. 0.1*N* ferricyanide reduced.
[d] Measured at 600 mμ.

down of the protein. Lysine nitrogen decreased with an increase in the storage time at 50° and 75°C., particularly at the latter temperature.

Total reducing substances remained essentially constant except, perhaps, for a very slight decrease after 35 days of storage at 75°C.

Limited bacteriological tests indicated that the chemical changes which occurred during storage could not be ascribed to bacterial action. A can of bread stored 2 years at room temperature which had a distinctly yellow crumb and a cheeselike odor gave negative tests with both the dextrose-tryptone agar and the fluid thioglycollate medium. Another can stored under similar conditions had a bacterial count of only about 16,000 per g.

Attempted Extraction of the Brown Pigments of Dried Bread Crumb

Investigators have succeeded in extracting the brown pigments from such food products as nonfat dry milk, dried whey, dehydrated whole egg, dried apricots, and darkened orange juice (3,4,7,21). Attempts were therefore made to remove the pigments from browned bread crumb. Two methods were tried: 1) direct extraction of the pigment by means of various solvents; and 2) extraction after the crumb was first acetylated.

Methods. Direct extraction of the crumb (2.0 g.) by intermittent shaking with 50 ml. of various redistilled solvents—water, pyridine, methanol, ethanol, isobutanol (2-methyl-1-propanol), benzaldehyde, ethyl acetate, chloroform, toluene, and dioxane (1,4-dioxane)—at room temperature for 24 hours was tried. For the acetylation procedure, the crumb was first extracted with a benzene-ethanol mixture (60 + 40) to remove most of the lipids, after which it was dispersed and dried by the method used by Pacsu and Mullen in preparing starch for acetylation (17). Four grams of the extracted crumb were dispersed in a mixture of 60% pyridine and 40% water and this was allowed to stand for 20 hours. The water was then removed by distillation as an azeotropic mixture with pyridine in the ratio of 60% pyridine and 40% water. An excess of pyridine was added to ensure the removal of all the water while the crumb was allowed to remain in a dispersed state in the pyridine. To this dry pyridine suspension of crumb, 30 ml. of acetic anhydride were added and the mixture was allowed to react for a week at room temperature. To decompose the unreacted acetic anhydride, 95% ethanol was added to the reaction mixture and allowed to stand overnight. The solvents and excess reactants were removed by distillation at reduced pressure.

The acetylated crumb was then extracted with water and other solvents to ascertain whether the brown pigment could be dissolved.

To obtain an estimate of the amount of nitrogen present in the extracts, their hydrochloric acid content was adjusted to 20% and they were refluxed for 24 hours. The amino nitrogen of the hydrolyzed solution was determined with the Van Slyke apparatus and expressed as mg. nitrogen per g. dry matter.

Results. Direct extraction with any of the solvents tried was quite ineffective in removing the pigment from browned bread crumb. Such poor extraction was obtained that no quantitative measurements were undertaken. From visual examination of the extracts and the residual crumb, the efficiency of extraction with the various solvents was classified into the following groups:

1. Very little extraction (solvent not colored) — benzaldehyde, chloroform, dioxane, ethyl acetate, isobutanol, toluene, mixture of benzene and ethanol (1 + 1).
2. Slight extraction — various aqueous methanol solutions containing from 20 to 100% of methanol.
3. Some extraction — water,[9] pyridine.

Acetylation gave a brown reaction mixture with both browned and white bread crumb. Extraction of the acetylated crumb with water, after removal of the excess reactants and solvents, left a nearly-white residue. Analysis of the water extract after hydrolysis gave the following values for amino nitrogen:

Sample	*Nitrogen per g. dry matter*[a]		
	mg Expt. 1	*mg* Expt. 2	*mg* Mean
Nonbrowned crumb	6.7	9.4	8.0
Browned crumb (stored 17 days at 75°C.)	8.0	9.4	8.7

[a] Amino nitrogen values for each extract were determined in duplicate.

Despite marked differences in the extent of browning of the two samples, no significant differences in the nitrogen content of the water-soluble fractions of the acetylated crumb were found. The absorbance values for aqueous and chloroform extracts of acetylated "fresh" and browned crumb at wave lengths of 240 to 600 mμ were not materially different.

Discussion

These exploratory studies on canned bread reveal that rather drastic storage conditions in regard to temperature and time are necessary to produce significant browning. Moreover, crumb discoloration was quite extensive before any marked chemical changes

[9] A 4% solution of Penetrant 1, an alkyl aryl sulfonate (Arnold Hoffman and Co., Providence, R. I.) also failed to extract any appreciable quantity of the pigment, as judged by the color of the crumb.

were detected, and no clear-cut evidence was obtained of the nature of the reactions which were involved. The amino nitrogen content of the crumb increased as browning developed; these values paralleled the increases in soluble nitrogen, and when they were corrected for the changes in solubility, there was no significant change in free amino nitrogen. Moreover, the total reducing substances remained rather constant. The oxidizing reagent, potassium ferricyanide, employed in these experiments is not specific for reducing sugars but is also reduced by carbonyl compounds which result from the degradation of carbohydrates. The only specific evidence that the Maillard reaction may be involved in the discoloration of bread crumb was the decrease in lysine nitrogen, but this did not occur until browning was quite extensive.

Although the first step in the Maillard reaction is sugar-amine condensation, this may not be reflected by a loss in free amino groups as determined by formol titration. The N-glycoside is in equilibrium with the original sugar, amine, and water. Because of the reversible nature of the reaction, N-glycosides of amino acids do not show a loss of amino nitrogen. Taufel and Iwainsky (20) found that they had to use a copper-complexing method to determine the extent of amino acid combination with sugars.

The marked increases which occurred in titratable acidity and the decreases in pH during storage favor the postulation that organic acids may play a role in the browning of canned bread, as has been proposed as a result of studies with other foods. Although the composition of the brown pigment would provide some clue regarding the nature of the reactants which produce it, the attempts which are made to extract it were entirely unsuccessful.

These preliminary experiments indicate that investigations of the effect of formula variations on the browning of bread may be carried out at 75°C. In view of the very limited changes in the quantity of reducing substances during browning, it would seem advisable to study the nature of the carbonyl substances before and after browning occurred.

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