

Studies with Radioactive Tracers. XIII. The Fate of Starch-¹⁴C during Breadmaking¹

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ABSTRACT

When bread was made by the straight-dough method with some uniformly labeled starch-¹⁴C included in the baking formula, only about 1.2% of the activity was recovered as volatile materials. Allowance for the possible losses of volatiles gave an estimate of a maximum of about 3% of the starch being fermented to volatile compounds. Extraction and fractionation studies showed the conversion of some of the starch to soluble dextrin as well as to basic, acidic, and neutral materials soluble in 80% alcohol. Paper-chromatographic examination of the neutral fraction of the alcoholic extracts indicated the presence of substantial amounts of active maltose with only a very minute quantity of active glucose. It was postulated that the chief source of residual glucose in the fermented dough was not starch but was the sucrose originally present in the baking formula. Chromatographic data also suggested that the oligosaccharides in the fermented dough might undergo further hydrolytic degradations during the process of baking.

It is generally expected that enzymatic action on the starch during bread-making results in hydrolytic degradations which will lead to the formation of some reducing sugars that will undergo fermentation to maintain or supplement gas production. In the words of Pazur and Sandstedt (1), "Amylolytic activity in bread-dough results in the formation of reducing sugars of low molecular weight and in the modification of the physical and chemical properties of the residual starch. The reducing sugars are essential for production of carbon dioxide during fermentation, while the modified starch enhances the texture and quality of the baked product." Studies with radioactive tracers should be capable of verifying these general concepts and should also be able to provide more detailed information. As an extension of the work already reported from this laboratory on the fate of sucrose-¹⁴C and of glycine-1-¹⁴C during the making of bread (2-4), analogous studies on the fate of uniformly labeled starch-¹⁴C have been carried out and the results are reported in this paper.

MATERIALS AND METHODS

Uniformly labeled starch-¹⁴C was obtained from Atomic Energy of Canada, Limited. According to the supplier, this starch-¹⁴C was isolated from tobacco leaves which had been allowed to photosynthesize in the presence of ¹⁴CO₂ for 10 to 12 hr.

Bread was made by the straight-dough method, with 100 g. flour, 5.00 g. sucrose, 3.00 g. yeast, 3.00 g. shortening, 1.75 g. NaCl, 4.00 g. milk powder, 0.30 g. nondiastatic malt, 0.10 g. NH₄H₂PO₄, 63.5 ml. water, and 8.84 or 5.94 mg. of starch-¹⁴C, respectively, for loaf I or II. The starch-¹⁴C was mixed with the flour by vigorous shaking in a closed container prior to the making of the dough.

The volatile fractions were collected during fermentation and baking as previously reported (2). The method of assay of the ¹⁴C-activity, in millicuries (m μ C), has also been described (3).

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Loaf I was divided into crust and crumb and extracted, first with 80% alcohol and then with water (5). The aqueous extract would contain the soluble dextrin (5). The 80% alcoholic extract was further fractionated, by means of ion-exchange resins, into basic, acidic, and neutral fractions (2). The neutral fraction, which should contain all the lower-molecular-weight carbohydrates, was subjected to paper-chromatographic studies. For comparison, a sample of the fermented dough from loaf I was taken just before baking, extracted with 80% alcohol, and fractionated to give a neutral fraction for paper-chromatographic examination.

Paper-chromatographic work was carried out with two sets of solvents. One was the 1-butanol, ethanol, water (BEW) system previously used in this laboratory (2,4), and the other was 1-butanol, acetic acid, water, ethyl acetate (BAWE) at the ratio of 6:3:4:6 (v./v.) recently employed by Huber and co-workers (6). Identifications of the maltose and glucose spots were made by comparison with the chromatographic behaviors of authentic samples.

RESULTS AND DISCUSSION

Activity Distributions

The distributions of activity in the volatile fractions collected during fermentation and baking of bread originally containing some starch-¹⁴C are shown in Table I. It should be pointed out that no accurate estimate of the

TABLE I
ACTIVITIES IN VOLATILE FRACTIONS DURING BREADMAKING WITH STARCH-¹⁴C

FRACTION	ACTIVITY		PERCENT	
	Loaf I	Loaf II	Loaf I	Loaf II
Starch- ¹⁴ C added	<i>mμc</i> 134,000	<i>mμc</i> 90,800	% 100	% 100
Fermentation condensate	21	15	0.02	0.02
Fermentation CO ₂	356	193	0.27	0.21
Oven-vapor condensate	834	590	0.62	0.65
Oven-vapor CO ₂	389	254	0.29	0.28
	Total volatiles		1.20	1.16

residual activity in the resulting bread could be made, because different samples of the crust or crumb gave widely varying results. Apparently, owing to the insolubility of starch, the originally added activity was not uniformly distributed in the finished bread. The data in Table I, however, represented the total activities in the various fractions and should, therefore, reflect the behavior of all the starch during the making of bread.

It is of interest to note that only a little over 1% of the originally added starch was recovered as volatile materials during the entire process of bread-making. Because of some experimental difficulties discussed earlier (2), not all of the volatile materials are expected to be recovered. From the work with sucrose-¹⁴C (2), about 42% of the activity remained in the finished bread and the total volatiles recovered were 24–26%. Thus the loss in activity was 32–34% when bread was made from a formula originally containing sucrose-¹⁴C. If the losses were entirely due to uncollected volatile materials, these would amount to about 1.5 times the volatiles that were actually recovered. If such a correction were applied to the present data, a rough estimate of the

maximum amount of starch that could have been fermented to give volatile materials, under the present breadmaking conditions, would be no more than about $1.2 \times 2.5 = 3\%$.

Because of the nonuniform distribution of the activity in the finished bread, the entire sample of the crust or crumb from loaf I was extracted first by refluxing for 2.5 hr. with 400 ml. of 80% alcohol and then for 2 hr. with 500 ml. of water at 30°C. The 80% alcoholic extract was subsequently separated into basic, acidic, and neutral fractions and the observed activity data are summarized in Table II. Similar results were found for both the

TABLE II
DATA FROM EXTRACTION AND FRACTIONATION OF CRUST AND CRUMB OF LOAF I

	SOLUBLE DEXTRIN	80% ALCOHOLIC EXTRACT	FRACTION FROM 80% ALCOHOLIC EXTRACT		
			Basic	Acidic	Neutral
Crust					
Activity (μc)	8,831	3,664	100	441	1,949
Relative distribution ^a	2.41	1.00	0.03	0.12	0.53
Percent based on original starch- ¹⁴ C	6.59	2.73	0.07	0.33	1.45
Crumb					
Activity (μc)	8,326	3,551	51	360	2,034
Relative distribution ^a	2.34	1.00	0.01	0.10	0.57
Percent based on original starch- ¹⁴ C	6.21	2.65	0.04	0.27	1.52

^aRelative to the activity of the 80% alcoholic extract as 1.00.

crust and crumb. Thus the soluble dextrin derived from the starch-¹⁴C amounted to 2.3–2.4 times the materials extracted by 80% alcohol. As expected, after fractionation, the neutral fraction contained the largest proportion of the recovered activity. The fact that some basic and acidic compounds were obtained is of interest in that they indicated that the hydrolysis products of starch have undergone more complex transformations, which likely included processes leading to browning reactions (3,7).

Paper-Chromatographic Studies

Chromatographic work was carried out with the neutral fraction from the 80% alcoholic extract of the dough, crumb, or crust of loaf I. As expected, the major component in this fraction was maltose. Initial studies with the BEW solvent system indicated the possible absence, or the presence of only very small amounts, of radioactive glucose. Figure 1, I, shows the activity distribution in the chromatogram of the neutral fraction from the fermented dough developed in the BEW system for 5 days. Although a color spot corresponding to glucose was found, this spot showed an essentially negligible amount of radioactivity. However, if a much larger sample was chromatographed, the presence of active glucose, though small in comparison to maltose, could be confirmed. This is illustrated in Fig. 1, II.

The finding of a substantial amount of maltose derived from starch-¹⁴C, whereas only a very minute amount of radioactive glucose was present, is in general agreement with the observations of Geddes and co-workers (8,9) and of Griffith and Johnson (10) that glucose would be fermented much more rapidly than maltose, especially in straight doughs. Of interest also is the finding, reported in the preceding paper (4), that the rapid decrease in glu-

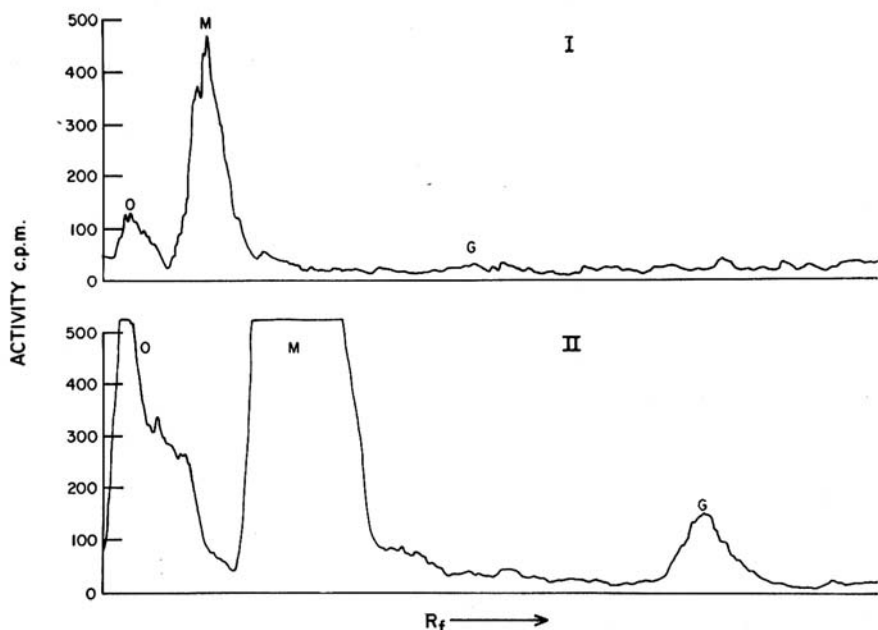


Fig. 1. Activity distributions on paper chromatograms of the neutral fraction of the dough extract. I, development in the BEW solvent system for 5 days; II, development in the BAWE solvent system for 3 days; O, oligosaccharides; M, maltose; G, glucose.

cose might also involve condensation and polymerization reactions. Pazar and Sandstedt (1) have reported that alpha-amylase action on starch gave a series of saccharides including glucose, maltose, amylotriose, amyloetraose, and amylopentaose, whereas the action of beta-amylase resulted in the formation of maltose as the only low-molecular-weight saccharide. Flour is known to contain a large amount of beta-amylase (5,11,12), and it is thus not surprising that in the present work, the chief low-molecular-weight carbohydrate derived from starch- ^{14}C was found to be maltose. From Fig. 1, I, while the radioactivity of the glucose in comparison to that of maltose was extremely small, the color intensity of the glucose spot was not negligible. It might, therefore, be further suggested that the chief source of residual glucose in the fermented dough was not starch, but was the sucrose originally present in the baking formula (2,4).

In an attempt to separate the oligosaccharides in the neutral extracts, the paper chromatograms were developed over an extended period of time. Figure 2 shows the activity distributions of the chromatograms developed for 24 days in the BEW solvent system. While no definite separation of the oligosaccharides was achieved, the relative areas of the peaks did indicate that in the neutral fraction of the 80% alcoholic extract of the crumb or crust, the ratio of active maltose to active oligosaccharides derived from starch- ^{14}C was approximately 3.5:1.0.

Huber and co-workers (6) have recently reported paper-chromatographic

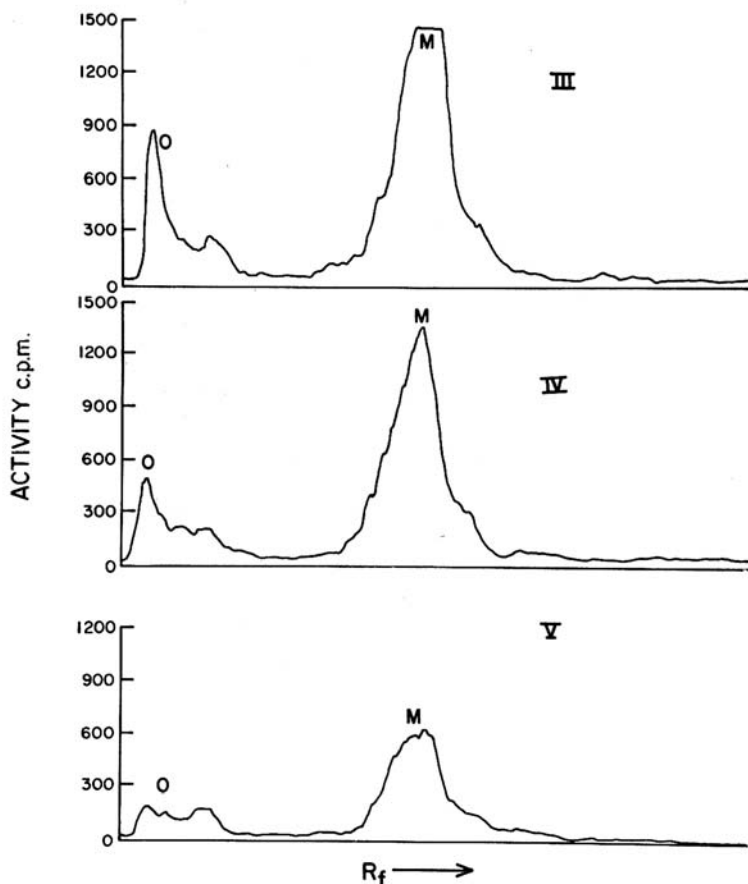


Fig. 2. Activity distributions on paper chromatograms developed for 24 days in the BEW solvent system. III, IV, and V from neutral fractions of extracts of dough, crumb, and crust, respectively; O, oligosaccharides; M, maltose.

separation of the oligosaccharides of corn syrup with the use of the BAWE solvent system. When the various neutral extracts from the present work were chromatographed in this solvent system for 10 days, the resulting activity distributions were as shown in Fig. 3. The most definitive chromatogram was obtained with the neutral extract of the fermented dough, which showed the presence of various peaks of lesser mobility than maltose. These presumably were attributable to tri- to nonasaccharides (6), while the activity remaining near the origin indicated the possible presence of deca- or higher saccharides. The analogous chromatograms for the neutral extracts of the crumb or crust differed considerably from that of the dough, the most pronounced difference being the absence of any appreciable active peaks near the original spot. These observations suggest that further hydrolytic degradations of the oligosaccharides in the fermented dough likely have taken place during the process of baking.

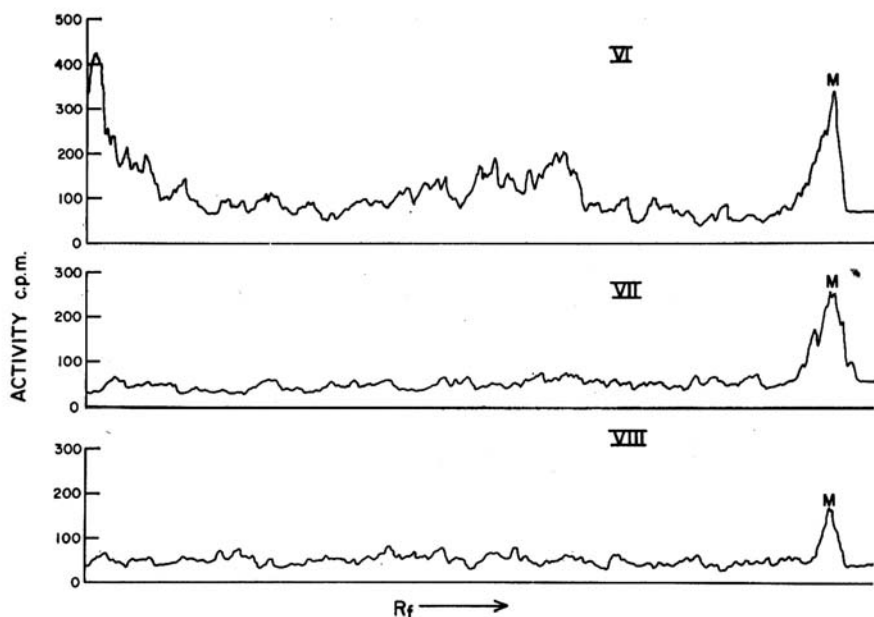


Fig. 3. Activity distributions on paper chromatograms developed for 10 days in the BAWE solvent system. VI, VII, and VIII from neutral fractions of extracts of dough, crumb, and crust, respectively; M, maltose.

General

The above discussions are based on the assumption that the starch- ^{14}C used in the present experiments behaved during breadmaking in the same way as the starch of the flour. Since the starch- ^{14}C was derived from tobacco leaves and not from wheat, it may be of interest to undertake similar studies with labeled wheat starch. It is, however, reasonable to expect that differences in behavior, if any, between starches derived from different sources would not be extremely great. The present results should at least be regarded in a general way as valid indications of the fate of starch during breadmaking.

Acknowledgment

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Literature Cited

- PAZUR, J. H., and SANDSTEDT, R. M. Identification of the reducing sugars in amylolysates of starch and starch-oligosaccharides. *Cereal Chem.* 31: 416-422 (1954).
- LEE, C. C., and CHEN, C-H. Studies with radioactive tracers. IX. The fate of sucrose- ^{14}C during breadmaking. *Cereal Chem.* 43: 695-705 (1966).
- LIAU, Y. H., and LEE, C. C. Studies with radioactive tracers. X. The fate of glycine- ^{14}C during breadmaking. *Cereal Chem.* 43: 706-715 (1966).
- LEE, C. C., and LIAU, Y. H. Studies with radioactive tracers. XII. Further investigations on the neutral extracts from bread baked with sucrose- ^{14}C . *Cereal Chem.* 45: 66-72 (1968).
- BECK, H., JOHNSON, J. A., and MILLER, B. S. Studies on the soluble dextrin fraction and sugar content of bread baked with alpha-amylase from different sources. *Cereal Chem.* 34: 211-217 (1957).
- HUBER, C. N., SCOBELL, H., and TAI, H. Determination of saccharide distribution

- of corn syrup by direct densitometry of thin-layer chromatograms. *Cereal Chem.* **43**: 342-346 (1966).
7. HODGE, J. E. Chemistry of browning reactions in model systems. *J. Agr. Food Chem.* **1**: 928-943 (1953).
 8. KOCH, R. B., SMITH, F., and GEDDES, W. F. The fate of sugars in bread doughs and synthetic nutrient solutions undergoing fermentation with baker's yeast. *Cereal Chem.* **31**: 55-72 (1954).
 9. LEE, J. W., CUENDET, L. S., and GEDDES, W. F. The fate of various sugars in fermenting sponges and doughs. *Cereal Chem.* **36**: 522-533 (1959).
 10. GRIFFITH, T., and JOHNSON, J. A. Chromatographic analysis of sugars in bread. *Cereal Chem.* **31**: 130-134 (1954).
 11. ERLICH, V. I., and BURKERT, G. M. Beta-amylase activities of barley, wheat, and rye. *Cereal Chem.* **27**: 423-437 (1950).
 12. LEE, J. W., and GEDDES, W. F. The role of amylase activity in the rapid increase of the maltose content of wheat flour doughs during mixing. *Cereal Chem.* **36**: 554-558 (1959).

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