Studies with Radioactive Tracers. XII. Further Investigations on the Neutral Extracts from Bread Baked with Sucrose-^{14}C[1]

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ABSTRACT

The neutral fractions from the 70% ethanolic extracts of the crumb and crust of bread made by the straight-dough method with 5% sucrose-^{14}C have been subjected to further investigations. By means of paper chromatography in conjunction with acidic and enzymatic hydrolyses, it was demonstrated that some residual sucrose was present in the crumb extract. Evidence was also obtained indicating that the glucose and fructose derived from the sucrose could undergo degradation as well as condensation or polymerization to give disaccharides and oligosaccharides. The fact that glucose could undergo transformation to these higher saccharides more readily than fructose was suggested as at least in part responsible for the presence of more residual fructose than glucose in the finished bread.

In the previous study on the fate of sucrose-^{14}C during breadmaking (1), it was noted that the neutral fraction of the 70% ethanolic extract of the crumb or crust gave paper chromatograms which indicated the presence of radioactive glucose and fructose. The active peak areas showed greater amounts of residual fructose than glucose in both the crumb and crust, a finding which is in general agreement with the observations of Koch, Smith, and Geddes (2) for bread made from the straight-dough process. However, from the tracer work (1), the activity distributions on the chromatograms of the neutral extracts also showed the presence of additional radioactive peaks, besides those of glucose and fructose, corresponding to unknown neutral compounds derived from the original sucrose-^{14}C. Since the neutral fractions should contain all residual soluble carbohydrates, it was considered worth while to undertake a further study on the unknown components. The present paper reports the results from such investigations.

MATERIALS AND METHODS

The neutral fractions obtained by Lee and Chen (1) from the 70% ethanolic extracts of the crumb and crust of bread made by the straight-dough method with 5% sucrose-^{14}C were utilized in the present work.

Paper-chromatographic studies were carried out with 1-butanol, ethanol, water (BEW) at the ratio of 10:1:2 (v./v.) as solvent (1). The technique employed was analogous to that described by Liau and Lee (3), involving the division of the original chromatogram into various subfractions which were eluted and separately chromatographed again.

Some of the subfractions were subjected to hydrolysis in 1*N* H_2SO_4 before being rechromatographed. To the aqueous eluate (about 0.5 ml.) was added an equal volume of 2*N* H_2SO_4. The resulting solution was placed in a sealed tube and heated in boiling water for 1 or 3 hr. The hydrolysate was then chromatographed in the usual way.

[1]Contribution from the Department of Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. For paper XI, see Lee, C.C., and Lai, T-S., Cereal Chem. 44:620–630 (1967).

The disaccharide fraction from the crumb extract was also subjected to enzymatic hydrolysis, carried out on the paper which was subsequently used for chromatographic study. The aqueous eluate was spotted on Whatman No. 3MM paper, and a solution of about 1 mg. of invertase, emulsin, or maltase was placed on the same spot. After being allowed to incubate at room temperature for 8 hr. in a moist atmosphere over water in a closed chamber, the chromatogram was developed in the usual way.

RESULTS AND DISCUSSION

Paper-chromatographic Studies on the Entire Neutral Extracts

The activity distributions on the chromatograms of the neutral extracts of the crumb and the crust, developed by the BEW solvent system for 14 hr., are shown in Fig. 1. Also indicated are the divisions into six subfractions

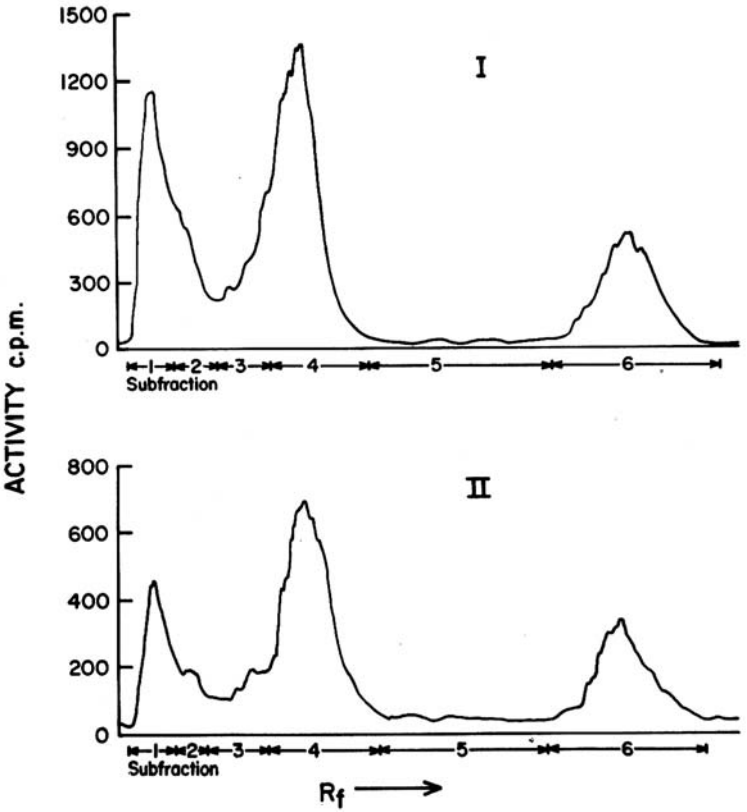

Fig. 1. Activity distributions on paper chromatograms of neutral extracts of crumb (I) and crust (II) developed by the BEW solvent system for 14 hr.

which were subsequently eluted and separately rechromatographed. It may be noted that with the relatively short development time of 14 hr., glucose and fructose were not separated. A new peak, designated subfraction 6, was observed in the present work. This component constituted about 21 and 26%, respectively, of the total activities in the extracts of the crumb and the

crust. Previously, this active peak was not observed because of its high R_f value and the longer development time of 92 hr. used in the earlier work (1).

When the development of the paper chromatograms was extended to 120 hr., the resulting activity distributions were as shown in Fig. 2. As ex-

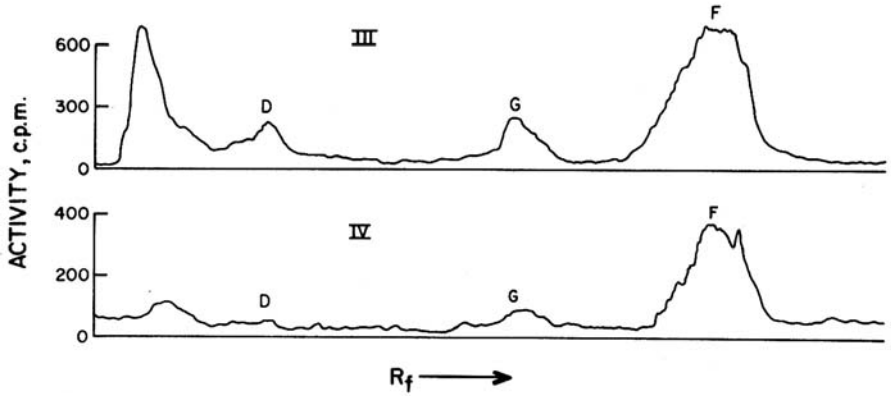

Fig. 2. Activity distributions on paper chromatograms of neutral extracts of crumb (III) and crust (IV) developed by the BEW solvent system for 120 hr. D, disaccharides; G, glucose; F, fructose.

pected, the unknown component corresponding to subfraction 6 has disappeared. Four areas of radioactivity, corresponding to fructose (F), glucose (G), disaccharides (D), and oligosaccharides (made up of three or more monosaccharide units) were noted. The positions of peaks F, G, and D were identified, in this and in subsequent experiments, by comparison with the chromatographic behavior of authentic samples of fructose, glucose, and sucrose. After the loss of subfraction 6 was taken into account, the areas of the peaks shown in Fig. 2 made possible an estimation of the relative composition, in the neutral extracts, of active materials derived from the originally added sucrose–^{14}C. The results are given in Table I.

TABLE I

RELATIVE COMPOSITIONS OF RADIOACTIVE COMPONENTS IN NEUTRAL EXTRACTS FROM THE CRUMB OR CRUST OF BREAD MADE WITH 5% SUCROSE-^{14}C

COMPONENT	R_f fructose	RELATIVE COMPOSITION	
		Crumb	Crust
		%	%
Subfraction 6	3.20	21	26
Fructose	1.00	40	52
Glucose	0.67	11	10
Disaccharides	0.22	8	3
Oligosaccharides		20	9

Studies of Various Subfractions

When the various subfractions (Fig. 1) were eluted and separately rechromatographed for various lengths of time up to 120 hr., the results led to the following conclusions. Subfraction 1 contained all the higher-molecular-weight polymeric materials including oligosaccharides; subfraction 2 con-

tained the disaccharides and possibly some oligosaccharides; all of the glucose and some of the fructose were included in subfraction 3; subfraction 4 was essentially all fructose; and subfraction 6 was an unknown component.

Subfraction 6 was unchanged when subjected to hydrolysis in 1*N* H_2SO_4. Preliminary studies by means of thin-layer chromatography using Kieselguhr G and Silica Gel G layers (4) failed to cause any separation. After elution by water, its UV spectrum indicated that it was not 5-hydroxymethylfurfural. Although the nature of this component is yet unknown, its high mobility under chromatographic conditions suggested that it may be a degradation product derived from radioactive glucose or fructose.

Hydrolytic Studies on the Disaccharides and Oligosaccharides

Figure 3 shows the activity distributions on the chromatograms obtained after 1 or 3 hr. of acid hydrolysis of subfractions 1 and 2 of the crumb extract. Very similar results were observed for subfractions 1 and 2 from the

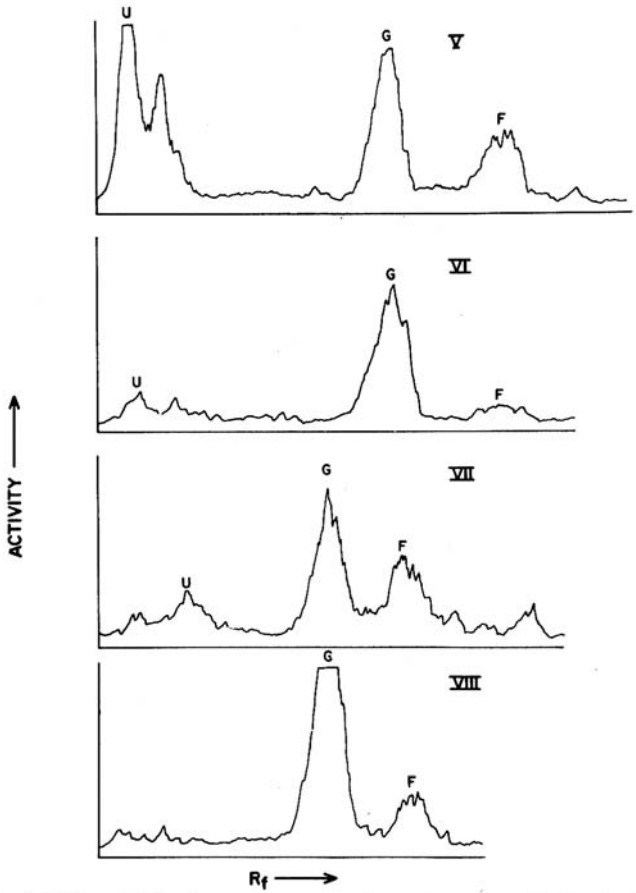

Fig. 3. Activity distributions on paper chromatograms obtained after acid hydrolysis: V and VI from hydrolysis of subfraction 1 of the crumb extract for 1 and 3 hr., respectively; VII and VIII from hydrolysis of subfraction 2 of the crumb extract for 1 and 3 hr., respectively. U, unhydrolyzed material; G, glucose; F, fructose.

extract of the crust. It can be seen that hydrolysis was incomplete after 1 hr., whereas after 3 hr. essentially all of the original subfraction 2, which presumably contained chiefly disaccharides, has been hydrolyzed. Not surprisingly, the hydrolysis products were glucose and fructose. Worthy of note, however, is the fact that acid hydrolysis liberated much more glucose than fructose, especially after a reation time of 3 hr. (Fig. 3). Interestingly, this finding is in contrast to the relative amounts of residual monosaccharides in the original extracts of the crumb and crust, where the amount of residual fructose was several times greater than the amount of residual glucose (compare Fig. 2).

When the disaccharide fraction (D) from the crumb extract (Fig. 2) was treated with maltase, emulsin, or invertase, it was found that no change occurred with maltase or emulsin. On the other hand, treatment with invertase liberated essentially equal amounts of glucose and fructose, as shown in Fig. 4, confirming the presence of some residual sucrose. Part of the disac-

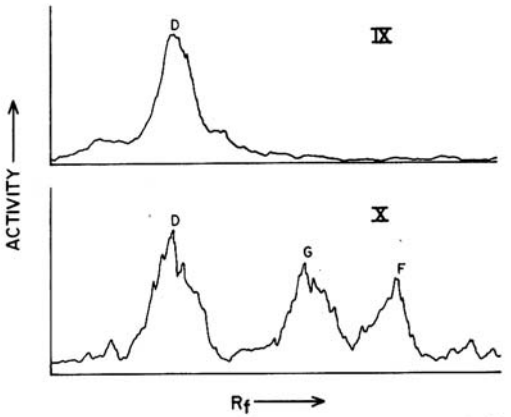

Fig. 4. Activity distributions on paper chromatograms before (IX) and after (X) hydrolysis of the disaccharide fraction with invertase. D, disaccharides; G, glucose; F, fructose.

charides (D), however, was not hydrolyzed by the invertase, even after a second treatment with a fresh solution of the enzyme. Since subfraction 2, which should contain all of the disaccharides, was completely hydrolyzed in 1*N* H_2SO_4 after 3 hr., the behavior with invertase suggested that in the crumb extract, disaccharides other than sucrose were also present. From the peak areas of X in Fig. 4, the relative amounts of residual disaccharides to sucrose were estimated to be about 1.5:1.0.

General Discussion

The present work provided new information on the fate of the sucrose originally added in the baking formula. In the earlier work of Geddes and coworkers (2, 5), it was concluded that the sucrose was rapidly hydrolyzed and none was detectable in the finished bread. With the higher sensitivity of the present tracer technique, some residual sucrose in the neutral crumb extract was demonstrated by its behavior toward invertase. A rough estimation of the amount of residual sucrose could be calculated. The data of Lee

and Chen (1) showed that the aqueous extracts of the crumb and the crust contained 19.7 and 11.1%, respectively, of the original sucrose-^{14}C activity. After treatment with ion-exchange resins, about 70% of the extracted activity was recovered, and of this, 92.1 and 91.3% respectively were in the neutral fractions from the crumb and the crust. Thus, $0.92 \times 0.70 \times 19.7 = 13\%$ and $0.91 \times 0.70 \times 11.1 = 7\%$ of the originally added sucrose-^{14}C activity would be present in the neutral aqueous extracts of the crumb and the crust, respectively. From the present work, it is seen that the disaccharide fraction constituted about 8% of the neutral crumb extract and about 3% of the neutral crust extract (Table I). Also, the ratio of sucrose to total disaccharides was estimated to be about $1.0/(1.0 + 1.5)$. Hence, $13 \times 0.08 \times 1/2.5 = 0.4\%$ and $7 \times 0.03 \times 1/2.5 = 0.08\%$ of the initially added sucrose-^{14}C has survived in the neutral extracts of crumb and crust, respectively. The total residual sucrose in the bread extract would be about 0.5% of the 5.0 g. sucrose per 100 g. flour used in the baking formula. This amount is likely undetectable by conventional means without tracers, especially when the residual sucrose would probably be masked by the presence of other disaccharides.

The presence in the neutral extracts of the more mobile subfraction 6 and the less mobile subfractions 1 and 2 suggest that the glucose and fructose derived from sucrose could undergo degradation as well as condensation or polymerization during the baking of bread. It is known from the literature that the action of heat and acid on glucose or fructose led to the formation of sugar anhydrides and higher saccharides (6,7). The present finding that subfractions 1 and 2, on acid hydrolysis, gave rise to glucose and fructose constituted an unequivocal demonstration that during the making of bread, the monosaccharides derived from sucrose could undergo condensation or polymerization reactions to give higher saccharides. It is of significance to emphasize again that hydrolysis of these higher saccharides gave more glucose than fructose. Earlier workers (2,5) have concluded that glucose is more rapidly fermented than fructose; this results in a greater amount of residual fructose than glucose in the finished bread. The present results further suggest that, under breadmaking conditions, another important factor contributing to the more rapid disappearance of glucose than fructose may be the greater tendency for glucose than fructose to undergo transformation to higher saccharides.

Acknowledgment

The financial support given by the Canada Department of Agriculture is gratefully acknowledged.

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[Received April 17, 1967. Accepted August 25, 1967]