

The Sugars of Flour and their Involvement in the San Francisco Sour Dough French Bread Process¹

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ABSTRACT

No sugars are added in the making of San Francisco sour dough French bread. Therefore, it is of interest to determine the sources and types of carbohydrates utilized by the two microorganisms recently reported to be involved in this process. The principal carbohydrate available, approximately 5.5% maltose based on dry weight of flour, is produced after the flour-water mixture is formulated, presumably by amylase action on free starch. The flour itself contributes approximately 1.7% of additional carbohydrates other than maltose. The sour dough bacterium, a previously undescribed *Lactobacillus*, utilizes only about 56% of the maltose, conveniently leaving the remainder as a necessary ingredient in crust browning. The sour dough yeast, *Saccharomyces exiguus*, which does not utilize maltose, consumes virtually all of the flour carbohydrates including glucose, fructose, and two families of glucofructans: glucose₁-fructose_n (n 1 thru 4). The contribution of the yeast to the acidity produced (lactic and acetic) is shown to be negligible.

San Francisco sour dough French bread has been made continuously in the San Francisco region for over 100 years. Only recently, workers in our laboratory have unraveled the microbiological nature of this unique process (1-4). These reports have identified the active yeast moiety of the system as *Saccharomyces exiguus* (3), and a previously undescribed sour dough bacterium as *Lactobacillus sanfrancisco* (suggested name) (4). Interesting aspects of this system are that the yeast and bacteria coexist in a pH range of 3.8 to 4.5, and that the bacteria preferentially utilize maltose as a source of carbon whereas the yeast does not utilize maltose at all. Thus they are not competitive for the same carbohydrate in the dough.

By analysis of the flours and uninoculated flour-water mixtures, as well as the separate and combined action of the two microorganisms in flour-water cultures, this paper describes the sources and types of carbohydrates utilized in the process as well as the contribution of the two microorganisms to the lactic and acetic acids produced.

MATERIALS AND METHODS

The two flours used in the process and analyzed were, typically, a strong, high-gluten flour used in preparing the starter sponge (Montana Spring, approximately 14% protein, as-is basis), referred to by its trade name "Titan" (Terminal Mills, Portland, Oreg.), and a topping flour from the same source known as "White Spray" (approximately 11.5% protein, as-is basis). The formulation and method for using these flours in sour dough French bread are described by Kline et al. (1). Carbohydrate analyses on the two flours were virtually identical, so only the analysis of Titan is reported.

¹Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

The sugars were extracted from flour as follows: 20 g. of flour was heated under reflux in 100 ml. of 70% ethanol for 1 hr. The mixture was cooled and centrifuged, and the supernatant retained. The solids were washed once with 100 ml. of 70% ethanol and centrifuged. The combined supernatants were concentrated under vacuum to about 20 ml. and extracted with 40 ml. of chloroform. The aqueous phase was retained. One-half of this extract was chromatographed on a Dowex 50W X2 (K^+) column (120 × 4.5 cm.) as previously described (5). One-fourth-milliliter aliquots of fractions collected from the columns were analyzed for carbohydrate by the phenol-sulfuric acid method (6) and elution patterns were determined. Paper chromatography, and invertase and mild acid hydrolyses were carried out as described for the sugars of wheat bran (5).

Flour-water mixtures were prepared as follows: 100 g. of flour was blended with 250 ml. of water and 2.2 g. of sodium chloride. Dilute hydrochloric acid was added, to pH 5.0 to 5.3, to simulate the natural starting pH of sour bread dough, and the mixture was inoculated with 0.5 to 1.0 g. of sour dough yeast (B-98) cake, 2.5 to 3.0 g. of bacteria (B-1) cake, or both, prepared as described below. The mixtures were incubated at 75° to 80°F. for 6 and 11 to 12 hr. Studies have shown that microbial activities and acid production in the flour-water system are comparable to those observed in the sour doughs (unpublished results, Kline and Sugihara). After this incubation period the samples were immediately frozen. Samples for sugar analysis were lyophilized, and extracted as described above for flour. Frozen samples were also thawed, centrifuged, and the supernatant fluid analyzed for organic acids on a Celite column as described previously (4). An uninoculated control was also prepared and incubated in the same manner; adjustment of its pH to 5.0 with dilute hydrochloric acid served to retard growth of flour contaminants. In all cases sugar investigations were carried out only on those samples subjected to 6 hr. incubation. All carbohydrate contents are reported either as percent of raw flour or as percent of the incubated flour-water mixtures after freeze-drying.

The yeast cake was obtained by growing yeast 18 to 24 hr. on a medium containing 2% glucose, 1% trypticase, and 0.5% yeast extract; centrifuging, washing with sterile 1% NaCl solution, and using the packed cells (cake) immediately. The medium for growing the bacteria and the methods for counting yeast and bacteria also have been described previously (3,4).

RESULTS AND DISCUSSION

Figure 1 illustrates the sugars present in wheat flour (Titan) before mixing with water and incubating. Based on distribution coefficient in this column-chromatographic system (7), and on paper chromatography before and after mild acid or enzyme (invertase) hydrolysis, the peaks were assigned as follows. Peak A², glucofructans, glucose, fructose_n, where n is 4 or greater; Peak B, tetrasaccharide glucose-fructose₃; Peak C, trisaccharide glucose-fructose₂ and a trace of raffinose; Peak D, sucrose; Peak E, maltose; Peak F, glucose; Peak G, fructose. When peaks A, B, and C were carefully examined by paper chromatography, and the distances traveled were plotted logarithmically against

²The alphabetical designations refer to the same sugars in all figures.

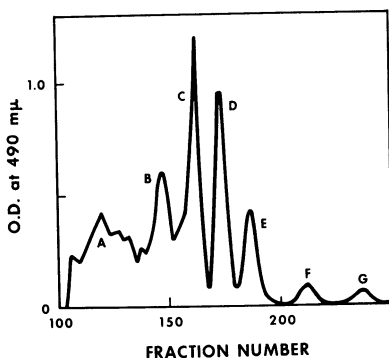


Fig. 1. Chromatography on Dowex 50W (K^+) of sugars extracted from wheat flour.

chain size, two distinct families of glucofructans were present. This phenomenon has been found previously in sugar extracts of flour by White and Secor (8) and more recently by Medcalf and Cheung (9). No attempt was made to investigate further the structural configuration of these two oligosaccharide families, since this is clearly beyond the scope of the present paper. Knowledge of the components and peak areas permitted calculation of the sugar distribution in wheat flour; the results are listed in Table I.

The total concentration of sugars reported here is higher than has been recorded previously for wheat flour, although the relative amounts are somewhat similar (10). The arbitrarily defined "levosine" in the earlier work (10) corresponds to our Peaks A plus B.

Figure 2 illustrates the elution pattern of flour after incubation with water but not inoculated. Because of the large amount of maltose now present, the sucrose peak is obscured. An increase in glucose and a decrease in trisaccharide can be seen. Presumably the maltose has been produced from damaged starch by amylase activity in the flour; glucose could arise from amylolysis or breakdown of glucofructans. In a quantitative assay, the concentration of extracted sugars increased from 1.82 to 7.19%, all of which (5.37%) can be accounted for as maltose produced by amylolysis.

TABLE I. PEAK COMPONENTS IN FIG. 1: THE SUGARS EXTRACTED WITH 70% ETHANOL FROM WHEAT FLOUR, AND THEIR DISTRIBUTION

Peak	Sugars	% Distribution of Sugars	% Sugars in Flour (g./100 g. flour-dry)
A	Glucofructans	37.7	0.72
B	Glu_1-Fru_3	14.5	0.26
C	Glu_1-Fru_2	21.8	0.40
	Raffinose trace		
D	Sucrose	14.2	0.26
E	Maltose	6.4	0.12
F	Glucose	1.2	0.02
G	Fructose	2.2	0.04
	Total sugars		1.82

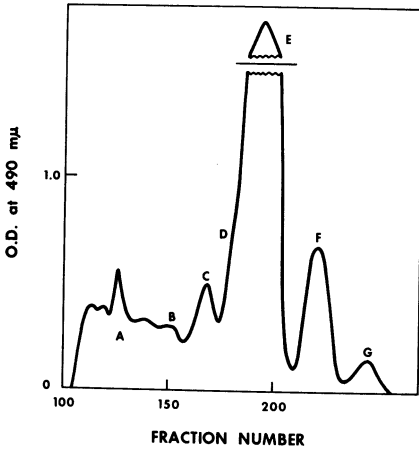


Fig. 2 (left). Chromatography on Dowex 50W (K⁺) of sugars extracted from a flour-water-salt mixture incubated at 75° to 80°F. for 6 hr.

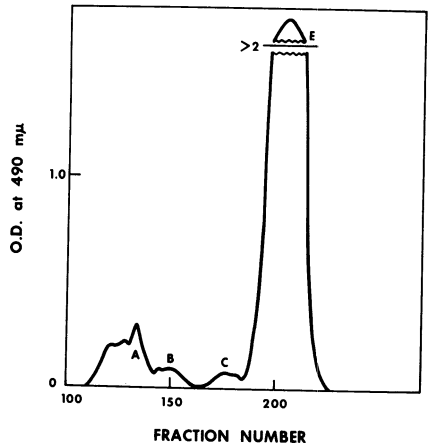


Fig. 3 (right). Chromatography on Dowex 50W (K⁺) of sugars extracted from a flour-water-salt mixture inoculated with *Saccharomyces exiguus* and incubated at 75° to 80°F. for 6 hr.

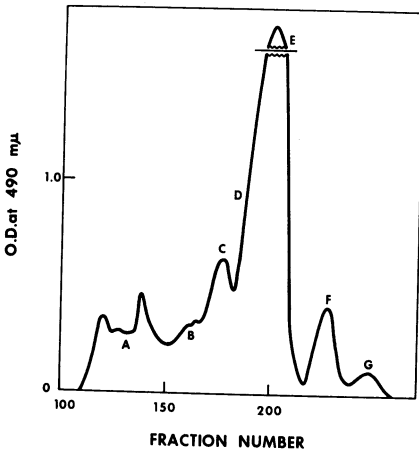


Fig. 4 (left). Chromatography on Dowex 50W (K⁺) of sugars extracted from a flour-water-salt mixture inoculated with sour dough bacteria and incubated at 75° to 80°F. for 6 hr.

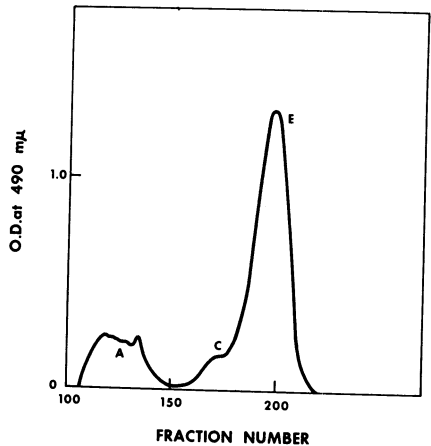


Fig. 5 (right). Chromatography on Dowex 50W (K⁺) of sugars extracted from a flour-water-salt mixture inoculated with *S. exiguus* and sour dough bacteria and incubated at 75° to 80°F. for 6 hr.

Figure 3 illustrates the sugar-elution pattern of the flour-water mixture inoculated only with yeast and incubated. All the fructose-containing oligosaccharides have decreased substantially; and glucose and fructose have completely disappeared, whereas maltose concentration has remained constant and high. These results substantiate the report (3) that the yeast, *S. exiguus*, does not

TABLE II. CHANGES TAKING PLACE ON INCUBATION OF FLOUR-WATER MIXTURE INOCULATED WITH YEAST OR SOUR DOUGH BACTERIUM ALONE OR TOGETHER

Experiment	pH			Yeast Count			Bacteria Count			μeq. Organic Acids/ml. After 11-12 hr.		Total Sugar Concentration, % after 6 hr.
	0	6 hr.	11-12 hr.	0	6 hr.	11-12 hr.	0	6 hr.	11-12 hr.	Total (acetic + lactic)	% of Total as acetic	
Flour, control	5.0	5.0	5.1	...	$<1 \times 10^4$	$<1 \times 10^4$...	$<1 \times 10^4$	3×10^6	3.1	33.3	7.19
Flour + yeast	5.0	4.8	4.8	140×10^5	400×10^5	700×10^5	...	$<1 \times 10^4$	$<1 \times 10^4$	5.7	38.6	5.26
Flour + bacteria	5.3	...	3.5	210×10^7	...	370×10^7	80.9	18.1	4.59
Flour + yeast and bacteria	5.3	...	3.5	100×10^5	355×10^7	69.6	18.5	2.84

utilize maltose, and indicate that the substrates are glucose, fructose, and glucofructans.

Figure 4 illustrates the sugars remaining after incubation of the flour-water mixture inoculated only with sour dough bacteria. Little change has occurred in the amounts of glucose, fructose, and glucofructans. Quantitatively, maltose concentration is estimated to be about 45% of that in the similarly incubated but uninoculated control. This corroborates the earlier finding (4) that this bacterium shows a marked preference for maltose as a source of carbon.

When the flour was incubated with the yeast and bacteria combined, the result was essentially a composite of yeast and bacteria separate incubations. Thus, Fig. 5 shows the marked decrease in glucofructans, a decrease in maltose, and the complete disappearance of monosaccharides.

Table II shows the total sugar content (i.e., 70% ethanol extractable), pH, yeast and bacteria counts, and organic acids produced in the incubated flour-water mixtures which had been inoculated with either yeast, bacteria, or both, as well as the uninoculated control. It is apparent that an appreciable drop in pH and production of organic acids occurred only in the presence of the bacteria. The yeast alone produces almost no acids, and when combined with the bacteria neither increased the amounts of acids nor altered the ratio of acetic acid to lactic acid produced by the bacterium alone. This table also shows that when the yeast is grown in combination with the bacteria it is slightly inhibited when compared to the yeast growing by itself, probably because of the lower pH as a result of the acid produced by the bacteria.

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[Received July 21, 1971. Accepted September 27, 1971]