The Acetic Acid Content of Sour French Bread and Dough as Determined by Gas Chromatography[1]

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ABSTRACT

The total acidity of sour French bread, dough, and starter was determined by extraction of these materials with acetone followed by titration of the extracts with standard alkali. The results were compared with extracts of conventional bread and dough. There was ten times more acid in the sour bread than in the conventional bread. Percentage of acetic acid present in the acid fractions was determined by gas chromatography. Approximately half of the total acidity of sour French bread and three-fourths of the total acidity of conventional bread was acetic acid.

Sour-dough French bread, made only from flour, water, salt, and the all-important starter sponge, appears to be unique in being produced only in northern California, although its popularity is widespread. The starter sponge provides both the leavening and the souring action and is maintained commercially by rebuilding approximately every 6 to 8 hr. Although this process has been perpetuated in this manner for over 100 years, nothing has been reported, to our knowledge, either on the identity of the microorganisms involved or on the nature

[1]Contribution from Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Albany, Calif. 94710.

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of the acids and other fermentation products produced by them. This study is part of a general study on the nature of this process and deals specifically with the acetic acid content of sour doughs and breads. The acetic acid content of conventional breads and doughs is also reported for comparative purposes.

MATERIALS AND METHODS

Sour French Bread Preparation

The "starter" or mother sponge used was one which had been carried through many transfers from the original sponge obtained from a local sour-dough bakery. Transfers were made about four times a week with the following formulation:

Previous sponge	100 parts
Hi-gluten flour (14% protein)	100 parts
Water	53 parts

The freshly prepared mother sponge was incubated 7 to 8 hr. at 80°F., during which time the pH value dropped from 4.5 to 3.9. Sponges were held at 50°to 55°F. between transfers.

The bread was prepared with the following formulation:

Bread patent flour (12% protein)	100 parts
Water	60 parts
Salt	2 parts
Mother sponge	18 parts

The bread dough was rounded and allowed approximately 1 hr. floor time before scaling and moulding. It was allowed to proof either directly on canvas or in perforated metal pans. Proofing was for 7 to 8 hr. at 86°F., during which time the pH value of the bread dough dropped from 5.3 to between 3.9 and 4.0. The dough was baked in a rotary hearth oven for 45 min. at 375°F. with low-pressure steam bled in for the first half of the baking time.

Conventional Bread Preparation

Conventional white bread was prepared by the straight-dough procedure as described by Kline and Sugihara (1).

Extraction of Organic Acids and Preparation for Analysis

From doughs and starters: Fifty grams of frozen dough and 100 ml. of acetone were placed in a 1-pint Osterizer jar whose blade assembly was modified with an "O" ring and Teflon seal to prevent leakage. Samples were ground for approximately 5 min. at low speeds to prevent the temperature of the mixture from rising. Jar and contents were then placed in a refrigerator for approximately 30 min. The cold mixture was then filtered on a Buechner funnel through Celite. The precipitate was washed several times with acetone, and washings and filtrate were combined and made up to a volume of 200 ml. with acetone. A 50-ml. aliquot of the acetone solution was placed in an Erlenmeyer flask and 50 ml. of water was added. The mixture was then titrated to pH 8 with 0.1N sodium hydroxide, and total acidity calculated as meq. of acetic acid. The remainder of the stock solution was neutralized with 0.1N sodium hydroxide, and then evaporated to approximately 30 ml. at 30° to 50° under vacuum. The alkaline residue was

extracted twice with equal volumes of dichloromethane to remove any nonacidic, nonvolatile components that might interfere with subsequent chromatography; the dichloromethane extract was in turn extracted once with water, and the aqueous extract thus obtained was added back to the original extracted alkaline solution. The latter was then evaporated to a volume of 1 to 2 ml. and the residue transferred to a 12-ml. centrifuge tube with about 4 ml. of water, to which was added 1 ml. of dilute HCl (1:3 acid:water); the mixture was shaken well, centrifuged, and then made up to a volume of 7 ml. with water, reshaken and recentrifuged; a portion of the supernatant was poured into a 5-ml. volumetric flask which was then capped with a serum bottle rubber septum.

From baked bread: The procedure for extracting organic acids from baked bread was similar to that used for extracting acids from dough except for the following modifications. The baked loaf, usually weighing a little under 500 g., was cut into four quarters. A quarter was then cut into 1/4-in. pieces which were placed in an Osterizer jar with 150 ml. of acetone, ground to a slurry, cooled in the refrigerator, and then filtered on a Buechner funnel through Celite. The precipitate of crumbs was washed with acetone; the washings were combined with the filtrate and made up to a volume of 350 to 400 ml. with acetone. An aliquot of the total solution was placed in an Erlenmeyer flask, and an equal volume of water was added. The titer was determined as for the doughs in the previous section and then the total acidity and acidity per g. were calculated. The stock solution was made alkaline, reduced in volume, extracted, further reduced in volume, made acid, centrifuged, and transferred to a 5-ml. volumetric flask that was capped with a rubber serum bottle closure.

Determination of the Acetic Acid Content of Extracts of Bread and Dough by Gas Chromatography

The gas chromatography apparatus used in this study consisted of a heated Barber-Colman flame ionization detector, a Beckman Thermotrac oven, a Jarrel-Ash electronic amplifier, and a 5-mv. recording potentiometer. Separations were made on a stainless-steel column (10 ft. × 1/8 in. o.d.) packed with 10% LAC-1-R (296) on Gas-Chrom Q (80 to 100 mesh). Column temperature was 100°C. Nitrogen for the column (50 ml. per min. at 38 psig. monitored by a gage at the head of the column) was passed through a toggle valve connected to a self-storing nylon hose, which in turn was connected to the head of the column by a quick-connect tube fitting with double end shut-off.

Samples for analysis were taken from the septum-covered 5-ml. volumetric flasks containing the free organic acids. As the needle was withdrawn from the flask its tip was wiped clean by the rubber septum. Prior to injection of the sample, the nitrogen supply to the column was cut off with the toggle valve, the quick connects leading to the column were separated, and the pressure at the head of the column slowly dropped to zero. The other end of the quick-connect tube was emptied of nitrogen and the quick connects were reconnected. The sample was injected on the column through an on-column injection port heated to 150°C. The toggle valve was flipped on and the inlet checked for leaks by means of the device described by Hunter and Walden[2]. Retention time for the acetic acid peak under the conditions

[2]Manuscript in preparation.

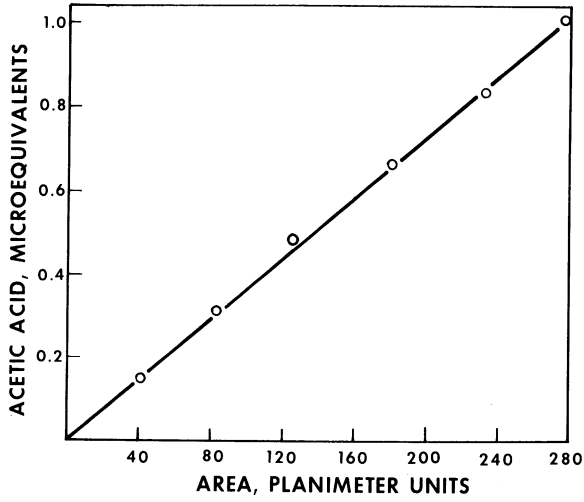

Fig. 1. Calibration curve of acetic acid in microequivalents vs. area in planimeter units.

described was 5.5 min. Peak areas were determined by planimeter. A linear response (see Fig. 1) for acetic acid was obtained with the gas chromatograph when 2 to 5 μliters of a standard solution in water, containing 10.45 γ of acetic acid per μliter, was injected. Initial injections of the acid mixtures gave a slightly smaller response than did subsequent injections. After two or three injections the response remained constant. Only those data obtained after the response stabilized were used.

Recovery values for acetic acid by gas-chromatographic analysis of the

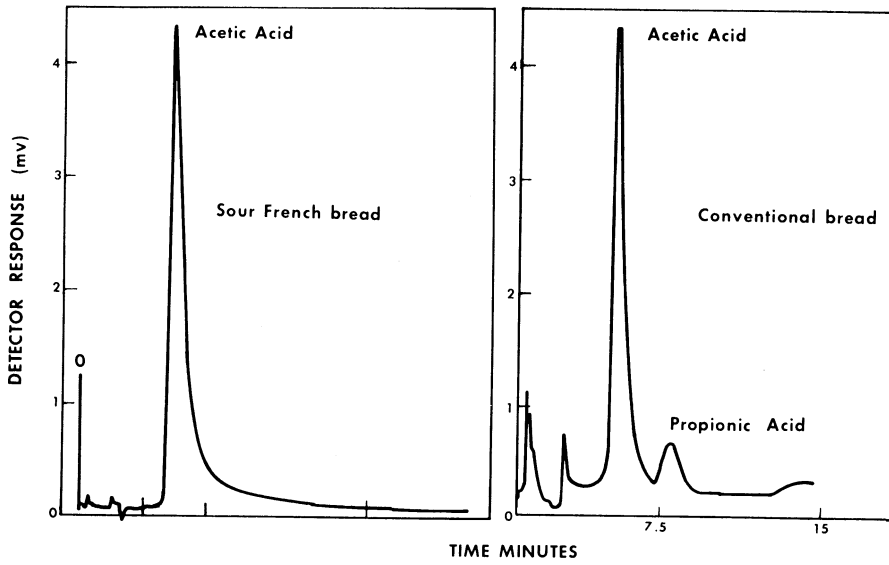

Fig. 2. Typical chromatograms of extracts of sour French bread and conventional bread.

concentrated extracts of bread and dough were determined by adding to a dough or bread sample known amounts of acetic acid. After grinding and extracting, the final concentrate was assayed for acetic acid. Average recovery ranged between 98 and 102%.

Chromatograms prepared from extracts of sour bread and dough showed only acetic acid peaks, whereas chromatograms of extracts of conventional breads and doughs indicated the presence of substantial amounts of propionic acid as well as acetic acid. Typical chromatograms are shown in Fig. 2.

Percent acetic acid present in the total acids extracted was determined by the following formula:

$$\text{Percent acetic} = \frac{\text{microequiv. acetic acid}}{\text{microequiv. total acidity}} \times 100$$

RESULTS AND DISCUSSION

The results of the assays for total acidity and acetic acid content of sour and conventional breads and doughs are shown in Table I. Acetic acid represented from

TABLE I. ACETIC ACID CONTENT OF BREAD AND DOUGH[a]

Type	Total Acidity		Acetic Acid	
	A[b]	B[b]	A	B
	meq./g.		% of total acidity	
Sour mother sponge	0.0472	0.0496	48.9	50.1
Sour French bread dough	0.0384	0.0384	53.5	51.4
Baked sour French bread	0.0476	0.0468	49.4	51.1
Conventional bread dough	0.00982	0.00780	57.0	56.1
Baked conventional bread	0.00527	0.00504	75.8	75.8

[a]All values are an average of two or more determinations.

[b]Sample designation.

56 to 76% of the total acidity in conventional breads and doughs and about 50% of the acidity in the sour breads and doughs. However, the total acidity of the sour bread samples was, not surprisingly, 5 to 10 times that in the conventional breads. The predominance of acetic acid suggests that acetic acid may contribute in an important way to the flavor of sour-dough French bread.

Acknowledgment

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Literature Cited

1. KLINE, L., and SUGIHARA, T. F. Factors affecting the stability of frozen bread doughs. I. Prepared by the straight dough method. Bakers Dig. 42(5): 44 (1968).

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