LACTIC AND VOLATILE (C_2–C_5) ORGANIC ACIDS OF SAN FRANCISCO SOURDOUGH FRENCH BREAD[1]

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ABSTRACT

Changes were observed in the pH, total titratable acidity (TTA), and lactic and volatile (C_2–C_5) organic acid contents of commercially prepared sour starter sponges, bread doughs, and fully baked bread. The results showed that lactic and acetic acids composed most of the total TTA. Gas-liquid chromatography, however, showed that six other minor acids—propionic, isobutyric, butyric, α-methyl *n*-butyric, isovaleric, and valeric acids—contributed to the TTA of the fully fermented starter sponge, the fully proofed bread dough, and the baked bread—1.19, 0.64, and 0.59%, respectively. Baking increased the pH negligibly but decreased the TTA by 9%, mainly due to the loss of acetic acid.

Cereal Chem. 55(4): 461-468

Previous investigations concerning the acidity of San Francisco sourdough French bread revealed that lactic and acetic acids were the only acids present (1–3). The unique sour taste and aroma of this bread was suspected to be due to these two acids, which are produced by the heterofermentative microorganisms that Kline and Sugihara (4) identified. The partition and gas-liquid chromatography (GLC) methods may have prevented previous investigators from identifying other organic acids because of small initial extracted samples, low volumes of applied or injected samples, low detector sensitivity in the case of GLC, highly dilute extracts, or both of the last two. Excessively dilute extracts containing a large proportion of acetic acid tend to mask minute amounts of other organic acids.

Therefore, we studied commercially prepared sourdough samples to

[1]Presented at the 62nd Annual Meeting, San Francisco, Oct. 1977.
Journal Article No. 78-15-j, Kansas Agricultural Experiment Station, Manhattan, KS 66506.

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0009-0352/78/000052$03.00/0

determine if other organic acids contributed to the flavor of this particular specialty bread. This investigation was designed to study the organic acids that microorganisms release into the surrounding media during the various processing steps in commercial production of San Francisco sourdough French bread.

MATERIALS AND METHODS

Three sponge samples at 0, 4, and 8 hr of fermentation; three dough samples after 30 min of floor time (rest) and at 2 and 4 hr of proofing; and fully baked bread were obtained from Larraburu Baking Company (San Francisco, CA 94107). Samples were kept frozen until analyzed.

The Larraburu Company produces San Francisco sourdough French bread by the sponge and dough process. Each day a piece of straight dough or starter sponge known as the "Mother" is saved and refrigerated to be used as a starter sponge the following day. This starter sponge is used to make more starter sponges as well as sponges for bread production. The starter sponge consists of 100 parts of clear flour (14% protein), approximately 50 parts of water, and 50 parts of the starter sponge. The ingredients are mixed and fermented for 9–10 hr at 80° F. The bread dough is made by mixing 100 parts of flour (12% protein), 60 parts of water, 15 parts of sponge, and 1.5–2% salt. The dough rests 1 hr and then is divided, molded, and deposited on canvas dusted with corn meal or rice flour. The dough is proofed for 4 hr at 105° F (41° C) and 96% relative humidity and baked at 420° F (216° C) for 40–50 min in a Perkins oven with direct injection of low pressure steam (5 psi). Oven shelves were covered with Carborundum.

Determining Moisture Content, pH, and Total Titratable Acidity

Moisture content was determined according to the modified AACC two-stage air oven method (5). The pH was determined according to the official method (5) except that 10 drops of formaldehyde were added to stop fermentation. Total titratable acidity (TTA) was measured by neutralizing dough suspensions to pH 6.6 using *N* NaOH (6).

Lactic acid Determination

Lactic acid was extracted from the sourdough samples in a liquid extractor with diethyl ether according to the AACC method (5). Colorimetric determinations were made by converting the lactic acid to acetaldehyde and then reacting the acetaldehyde with *p*-diphenyl phenol. The developed color was measured at 630 nm by a Varian Tectron spectrophotometer (7). Results were expressed in micrograms and microequivalents lactic acid per gram of sample.

Analysis of Short-Chain (C_2–C_5) Volatile Organic Acids

The method of Faridi and Johnson (8) was used to extract the organic acids from the sponge, dough, and bread samples. The pH of the samples, however, was adjusted to 8 by adding 1.0*N* NaOH to avoid any violatilization of the free organic acids during blending and handling of the extracts. The combined acetone extracts were concentrated to approximately 500 ml in a rotary evaporator at room temperature. Extracts were distilled after adjusting the pH to ~2 using 1*N* HCl. The condensates were evaporated to 40–50 ml under vacuum

at room temperature in a rotary evaporator. Organic acids and their salts were finally purified by applying the concentrated condensates on a bed of Dowex 1 × 2 resin (chloride form).

GLC analysis was done with a Barber Coleman Gas Chromatograph. The column (U-shaped, 120 cm long, 3.5 mm ID) was packed with 60–80-mesh Carbopack A modified with 0.3% sp-1,000 and 0.3% H_3PO_4. The injected volumes were adjusted for an optimum response of acetic acid (1–2 μl) and a maximum response of the other minor acids (up to 10 μl). GLC conditions were as follows: oven temperature, initially 105° C for 5 min, then raised 5° C/min to a final temperature of 140° C; injector temperature, 180° C; detector temperature, 350° C; flow rate of nitrogen, 20 ml/min; sensitivity, 1×10^{-8}, 3×10^{-9} amp. A standard containing 0.1% each of acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids was used as a reference. One milliliter of the standard plus 1.05 μl of α-methyl *n*-butyric acid (0.941 g/ml) was used for both qualitative and quantitative analysis of this acid.

RESULTS AND DISCUSSION

Moisture Content, pH, and TTA

The moisture content, pH, and TTA of the starter sponge, the sourdough, and the bread samples are shown in Table I. The starter sponge contained less moisture than did the bread dough, because the flour/water ratio was greater in the sponge than in the dough. Moisture content differed negligibly between starter sponge samples after 0, 4, and 8 hr of fermentation, or between bread dough samples after 0.5, 2, and 4 hr of proofing. Nevertheless, the moisture contents of the whole bread differed appreciably from the crust-free bread crumb. These differences are atrributed to the thick, crispy (almost dry) crust that constitutes a high proportion of the whole bread.

The decreases in the pH of the starter sponge with fermentation time were not so rapid as they were in the bread dough. The pH of the starter sponge dropped from 4.58 at 0 hr of fermentation to 4.15 and 3.8 after 4 and 8 hr of fermentation,

TABLE I
Moisture Content, pH, and Total Titratable Acidity of Sour Sponge, Dough, and Fully Baked Bread Crumb

Aspect Measured	Sponge Fermentation Time (hr)			Dough Fermentation Time (hr)			Bread
	0	4	8	0.5	3	5	
Moisture content (%)	41.47	41.55	41.32	45.13	44.96	45.02	Crumb 41.72 Whole 36.44
pH	4.58	4.15	3.80	5.03	3.98	3.85	3.9
Total titratable[a] acidity (μeq/g)	71.76	114.62	158.80	46.16	112.64	144.93	131.55

[a]Dry basis.

respectively. The pH of the dough dropped drastically from 5.03 to 3.98 in the first 3 hr of proofing, and then changed little during the last 2 hr. The higher temperature, pH, and moisture content of the sourdough compared with the starter sponge may have increased the microbial activity. Moreover, the environmental conditions in the dough (pH, temperature, moisture content) may have accelerated the flour amylases and increased the fermentable sugars, especially maltose. This would concomitantly increase acid production and further decrease the pH.

The pH of the final bread was 3.9; baking, therefore, did not appreciably affect the pH. This result agrees with earlier investigations (2,9).

Baking decreased the TTA 9%. This does not agree with the observation that pH changed little during baking. This is probably due to the loss of acetic acid, which has a pK_a of 4.74: at pH 3.9, most of it is undissociated, which contributes to the TTA rather than altering the pH.

Isolating Organic Acids

The initial trial to isolate the short-chain volatile acids involved extracting 50- to 75-g samples according to the TTA values and proceeding as mentioned in the

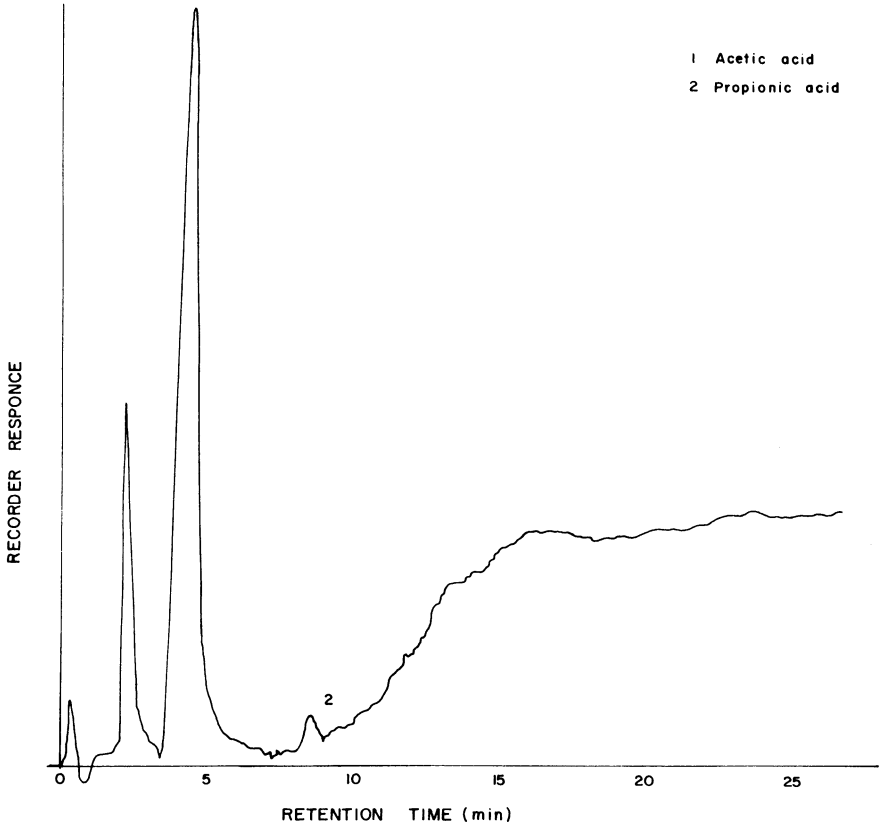

Fig. 1. Chromatogram of bread dough after 5 hr of proofing.

methods. The extracted sample was concentrated to a volume of 5–6 ml. Injected volumes of up to 5 μl of acids extracted from starter sponges, bread doughs, and bread showed that acetic acid was the only short-chain volatile acid that could be positively identified (Fig. 1). This result agreed with previous findings reported on the short-chain volatile organic acids of San Francisco sourdough French bread (1).

An additional small peak corresponding to the propionic acid retention time also was observed in Fig. 1; this suggested the presence of other short-chain acids. No positive peak identifications could be made, however, beyond the 8-min retention time; this was thought to be due to the small sample size, the detection limits of the instrument, and the conditions under which the samples were run, i.e., temperature programming sharply elevated the baseline when switching to a higher sensitivity after the acetic acid peak had been resolved. Therefore, the sample size in the extraction step was increased to 500 g, and the final volume was concentrated to ~10 ml to detect other acids that may be present. These modifications resulted in the detection of acetic acid as well as six additional short-chain volatile organic acids (Fig. 2). The acids were tentatively identified as propionic, isobutyric, butyric, α-methyl *n*-butyric, isovaleric, and valeric acid. The presence of α-methyl *n*-butyric acid was confirmed by spiking the sample with this acid and rechromatographing. This increased the α-methyl *n*-butyric acid peak of the sample.

Identification of six additional organic acids in the sourdough samples is an

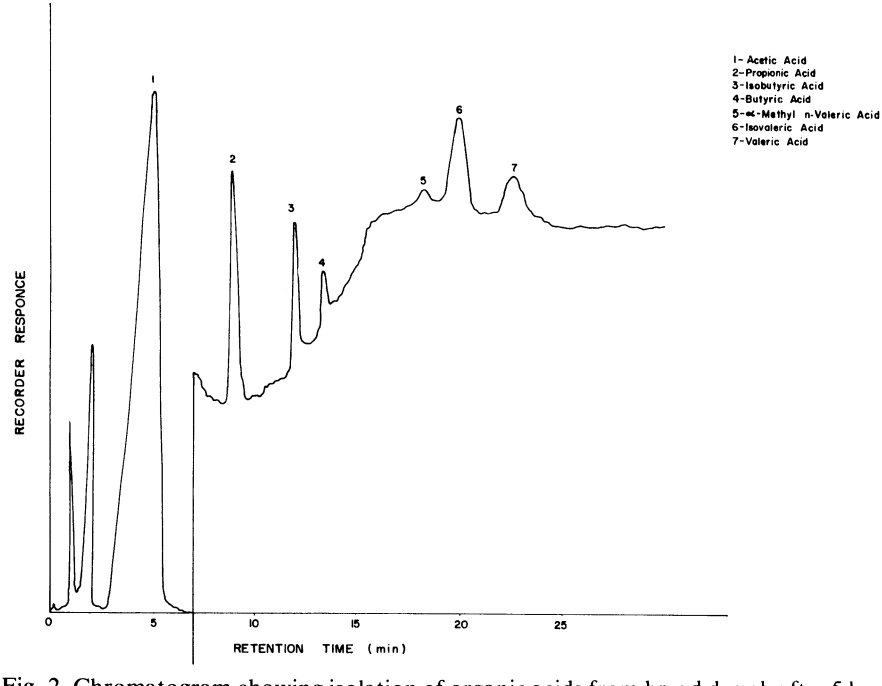

Fig. 2. Chromatogram showing isolation of organic acids from bread dough after 5 hr of proofing using modified method.

important finding, because previous investigators were unable to isolate any volatile organic acids from San Francisco sourdough samples except acetic acid. The specific amounts of the organic acids found in the samples are shown in Table II. Lactic and acetic acids were the predominant organic acids in all of the samples analyzed, and the other six acids were found only in minute amounts. Among the identified minor acids, isovaleric acid appeared in the greatest amount in the starter sponge. Once the starter sponge was used to make the dough and the dough was allowed to proof for 4 hr, the level of propionic acid exceeded the level of isovaleric acid.

The shift in the amounts of minor acids present in the starter sponges and doughs may be due to a number of factors. For example, if the Mother used as the inoculum for the starter sponge was stored for a long period, the action of microbial enzymes may have contributed to the high proportions of isovaleric acid found in the starter sponge. Other factors such as differences between the fermentation and proofing times for the starter sponge and the dough and differences in holding temperature also may have contributed to variations in enzyme activity and subsequent differences in the quantities of the acids found in the samples.

Microbial enzymes probably are responsible for producing the organic acids in the sourdough system The exact origins of these free fatty acids, however, have not been identified. Two possible biochemical mechanisms in the formation of these acids may be postulated: lipid degradation, or deamination or transamination of amino acids, or both.

Production of substantial amounts of free fatty acids caused by lipolysis can be disregarded in the sourdough system, because the optimum pH for lipases is between 7.0 and 8.0 (10), depending on the substrate; the pH of the sourdough system, however, varies from 3.8 to 5.0. Lipase activity would be minimal in this pH range.

The main biochemical mechanisms producing free fatty acids most likely

TABLE II
Lactic and Volatile (C_2–C_5) Organic Acids of Sour Sponge, Dough, and Fully Baked Bread Crumb (μeq/g, dry basis)

Acid	Stage of Processing						
	Sponge, Fermentation Time (hr)			Dough, Proof Time (hr)			Bread
	0	4	8	0.5	3	5	
Lactic	48.98	79.27	112.11	30.37	77.11	98.84	97.22
Acetic	19.58	29.25	38.93	13.40	30.23	35.48	31.31
Propionic	0.466	0.504	0.681	0.222	0.277	0.450	0.369
Isobutyric	0.128	0.142	0.215	0.061	0.111	0.119	0.101
n-Butyric	0.042	0.080	0.100	0.026	0.036	0.046	0.038
α-Methyl *n*-Butyric	0.031	0.037	0.039	0.014	0.017	0.023	0.019
Isovaleric	0.700	0.627	0.590	0.157	0.141	0.174	0.169
n-Valeric	0.129	0.245	0.268	0.055	0.081	0.101	0.085
Total	70.056	110.155	152.933	44.305	108.003	135.233	129.311

involve the α-keto acids supplied by the glycolytic pathway or the α-keto acids produced by the transamination of amino acids or both. The α-keto acids produced by both routes are decarboxylated, and the resulting aldehydes are oxidized to form the free fatty acids. In addition, free fatty acids may be formed through the deamination of amino acids.

The content of free amino acids and sugars present in the dough system affect organic acid formation. One source of these amino acids in the starter sponge and dough may be through the action of proteases on wheat gluten.

Proteases derived from malted wheat and fungal sources have a pH optimum between 3.0 and 4.0 for the hydrolysis of gluten. In addition, the autodigestion of flour proteins has been shown to decrease rapidly when the pH of the system is higher than the optimum of 4.0 (11). This may explain why proteases have little effect on the gluten proteins at the normal pH of conventional bread dough. Proteases in the sourdough system, however, are undoubtedly acting on gluten when the Mother is stored for 16.5 hr at a low pH of 3.825, particularly without salt. The literature indicates that increments of salt up to 3% strongly inhibit flour proteases. Beyond that level, the proteases are completely inhibited (12).

Proteolytic degradation of wheat gluten produces amino acids that may be degraded further by transamination and decarboxylation reactions. For example, isovaleryl COA, a precursor of isovaleric acid, could be produced from leucine in the sourdough starter. Similar mechanisms can be suggested for producing other acids, such as α-methyl *n*-butyric acid from isoleucine, isobutyric acid from valine, and propionic acid from threonine and methionine.

The percentages of TTA that the various organic acids contributed are shown in Table III. The percentage of TTA that lactic acid contributed increased with starter-sponge fermentation time and dough proofing time. The percentage of TTA that acetic acid contributed, however, decreased in all stages of processing, even though the amounts of both lactic and acetic acids increased.

Differences in the proportions of lactic and acetic acid in sourdoughs may be due to differences in the degree of aeration. Ng (3) has shown that the degree of aeration is important in producing lactic and acetic acids by sourdough bacteria in broth cultures. Applying this to a dough system, one could hypothesize that

TABLE III
Percentage of Total Titratable Acidity Contributed By Various Acids Present in Starter Sponge, Dough, and Bread

Stage of Processing (hr)		Lactic Acid Percentage of TTA	Acetic Acid Percentage of TTA	Acetic/ Lactic	Lactic/ Acetic	Other Volatile Acids Percentage of TTA	Total Determined Acids Percentage of TTA
Starter sponge fermentation time	0	68.24	27.30	0.40	2.50	2.09	97.64
	4	69.15	25.52	0.37	2.70	1.43	96.10
	8	70.60	24.52	0.35	2.88	1.19	96.31
Dough proofing time (min)	0.5	65.77	29.02	0.44	2.27	1.16	95.95
	3	68.45	26.82	0.39	2.55	0.51	95.86
	5	69.06	24.79	0.36	2.79	0.64	94.49
Bread		73.91	23.80	0.32	3.10	0.59	98.30

incorporating oxygen into the starter sponge or dough during mixing would enhance the aerobic production of acetic acid. As fermentation or proofing times are increased, however, the oxygen potential in the starter sponge or dough decreases and the level of CO_2 increases. These essentially anaerobic conditions favor the production of lactic acid at the expense of acetic acid.

The data presented in Table III also indicate that the percentage of TTA contributed by the minor volatile organic acids was about two times greater at 0 starter-sponge fermentation time than it was after the dough was allowed 30 min of floor time. In addition, the minor volatile acids of the starter-sponge samples were higher than those of the dough samples at any stage of the process. This could be due to the long storage time of the Mother, which resulted in the continued production of organic acids by microbial enzymes. The consumption of metabolizable sugars by the microorganisms during the long fermentation time compared with the shorter proofing time may result in the catabolism of amino acids by the microorganisms. In addition, the initial high pH of the dough as well as the high temperature of proofing favors amylase activity and diminishes protease activity.

The combined amounts of propionic, isobutyric, *n*-butyric, α-methyl *n*-butyric, isovaleric, and *n*-valeric acid constitute less than 1% of TTA of the sourdough bread. Although this is only a small fraction of TTA, these acids may contribute significantly to the flavor of the San Francisco sourdough French bread, because these minor acids are 1.5 times more prevalent in sourdough than in conventional white pan bread (13). Organoleptic studies, however, would have to be conducted to determine the specific contribution of these minor acids to the total flavor.

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[Received August 31, 1977. Accepted December 7, 1977]