THE VOLATILE ORGANIC ACIDS FOUND IN DOUGH, OVEN GASES, AND BREAD[1]

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ABSTRACT

The volatile aliphatic acids found in fermented dough, bread, and oven vapors, with their concentrations as estimated by vapor-phase chromatography, are (milligrams per thousand grams of flour basis): acetic, 198, 150, 15; n-butyric, 23.1, 10.6, 0; isovaleric, 13.1, 6.1, 0; n-caproic, 6.2, 0, 0. The acids comprise only those which are steam-distillable from fermented dough and bread extracts, and that found in oven vapors. The amounts in the bread and oven gases do not add up to the corresponding amounts in dough, leading to speculation regarding the fates of the dough acids during baking.

The nature of the contribution of volatile aliphatic acids to bread aroma is not well understood, and in fact the identities, amounts, and sources of these acids have received inadequate study heretofore. The present study was undertaken as part of a broad investigation into various aspects of bread flavor, some results of which have been presented elsewhere (2,3).

Materials and Methods

The doughs used in this work were made up from this formulation:

Flour (NW Patent)	1000 g.
Yeast	30 g.
Sucrose	60 g.
Salt	20 g.
Lard	20 g.
Water	630 ml.

The same formulation was used whether the fermented dough or the bread was to be analyzed. After mixing in a Hobart A-120 mixer fitted with a McDuffee bowl and fork, fermentation was carried out at 30°C. (86°F.). Two punches were given, at 75 and 105 minutes, and the doughs were taken at 135 minutes (from the start of the mixing). If the dough was to be studied as such, it was scaled, molded, and proofed normally in pans, and then the proofed dough was treated as described below. Otherwise, the doughs were baked in a Blue M Model VO 18S electric laboratory oven. This oven has a low heat-recovery rate, so it was loaded at 255°C. (491°F.); the

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oven temperature quickly fell to about 203°C. (397°F.) and recovered slowly to about 230°C. (446°F.) in 30 minutes. The loaves baked out satisfactorily in the 30 minutes. One set of loaves provided material both for the oven-gas analysis and analysis of the bread itself, when the gases were drawn into an efficient Friedrichs condenser followed by an ice-salt cold trap.

Recovery of Acids from Dough. The fermented and proofed dough from 1,000 g. of flour was stirred with 2 liters of reagent-grade acetone until completely disintegrated, and filtered with mild vacuum on a large Büchner funnel. The cake was ground up with 1 liter of acetone and filtered again; this sequence was repeated three times. The combined filtrates were distilled through an 18-in. Vigreaux column to remove most of the acetone, leaving an aqueous residue with a layer of liquid fat on top. The fat layer was free of volatile acids.

The residue was diluted with water, the fat layer drawn off as completely as possible, and the aqueous phase was distilled rapidly, with addition of water to maintain the volume in the still at over 1 liter. Two liters of distillate were collected, and were titrated with saturated barium hydroxide solution to the phenol red end-point (pH 8.4). The solution containing the barium salts of the acids was vacuum-evaporated to dryness, using a rotary evaporator. The dry residue of barium salts was taken up in sufficient 2*M* sulfuric acid (calculated from the titer) to ensure a final pH below 2.5, and the mixture, after saturation with solid sodium sulfate, was extracted repeatedly with reagent-grade diethyl ether to remove the free, un-ionized acids. The ether extract was evaporated carefully under an air condenser, and was then diluted up to 5.00 ml. with ether for analysis.

Recovery of Acids from Bread. The same scheme as detailed above was followed, the fresh bread having been previously disintegrated by the blades of a Waring Blendor. (CAUTION: not more than about 2 oz. at a time!) The quantities given are suitable for three 1-lb. loaves of bread. Even with the previous disintegration, the filter cake from bread tended to be loose, and the use of a rubber dam over the filter funnel is recommended to improve the expression of occluded acetone. The suction flask was also chilled in an ice bath to improve the vacuum, and hence the pressure applied to the filter cake.

Recovery of Acids from Oven Gases. The oven-gas condensate was made alkaline as above with barium hydroxide solution, and subsequent steps followed the scheme described.

Calibration of Gas Chromatograph. The instrument used was the Aerograph Model A-100, with a 4-filament thermal conductivity de-

tector cell. Filament power (200 ma.) was supplied by a Beckman model 2695 hydrogen-lamp power source, whose output was dropped through a heavy voltage-divider resistor and restabilized at about 13 volts by a 10-watt zener diode (Texas Instruments Co., Type 1N 1816) shunted across the input to the filament circuit. Used in this way, the zener diode dissipated about 1.3 watts to the instrument case, on which it was mounted by its anode stud using mica insulating washers. The column used throughout was a stainless steel column, 10 ft. by ¼ in., packed with a silicone-stearic acid mixture on an inert support, as described by James and Martin (1). It was operated at 120°C., rather than the 137°C. used by these workers, and showed much improved stability at the lower temperature. Good resolution of the peaks was obtained for the normal alphatic acids in the C_2 to C_6 range, although tailing prevented measurement of total peak areas. It was also possible to distinguish between the retention times of the normal and iso C_4 and C_5 acids, although in mixtures of the normal and iso acids single, broadened peaks were obtained.

To calibrate the column, 10% (w/v) solutions of acetic, propionic, isobutyric, and isovaleric acids in benzene were prepared. Using the standard conditions of helium flow rate, 120°C. and 25 ml. per minute, samples of each solution were injected from a 50 μl. Ham-

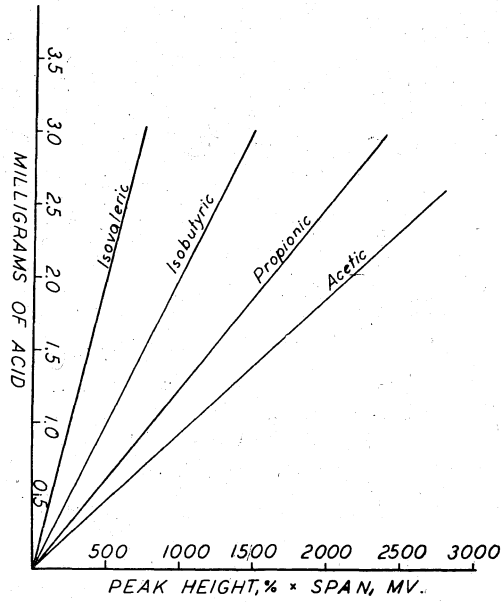

Fig. 1. Calibration plots for estimating volatile acids by vapor-phase chromatography.

ilton syringe. Because of tailing, the peak heights only were recorded. Figure 1 shows the plots of mg. acid against (peak height × span) for the various acids, using samples of 5, 10, 15, etc. microliters of the solutions. The "span" referred to is the number of millivolts required to drive the recorder pen to 100% of scale at the particular sensitivity attenuation used. Thus all the samples are compared on a common basis, independent of the individual sensitivity settings used.

Although complete runs were not done with n-butyric, n-valeric, and n-caproic acids, it was noted that the calibration line for n-butyric would fall very nearly midway between those for propionic and isobutryric, while that for n-valeric almost coincided with isobutyric and that for n-caproic with isovaleric. Interpolation, if necessary, would be sufficiently accurate for the reproducibility of this method of analysis.

Several mixtures of known C_2 to C_6 acids, made up accurately by weight, were applied to the gas chromatograph, and the amounts of the component acids estimated from the calibration plots. The accuracy for acetic and propionic acids was excellent, normally better than 5%, and decreased as expected for the higher acids, whose peaks are lower and broader. The results cited below show, by the agreement between gas chromatographic and titration data, that the procedure used is of practical utility, although the theoretical basis for using peak heights alone is weak. Identities of the acids were based on retention times.

Analysis of Acids from Dough, Oven Gases, and Bread. The ether solutions of the volatile organic acids were applied to the gas chromatograph column, determining by trial the size of sample and the sensitivity required to record the detectable peaks adequately. It was usually necessary to reduce the sensitivity by a considerable factor to keep the acetic acid peak on scale, and to increase it again to get adequate peak heights for the other acids. The peak heights and spans were noted, and the equivalent milligrams of each were read or interpolated from Fig. 1.

Results

Volatile Acids in Dough. By titration with barium hydroxide, the acidity of the distillate from a fermented and proofed dough (1,000 g. of flour) was 3.73 meq. From the peak heights of the acids, the sample volumes, and their retention times, the following data for the total acids were obtained:

	mg.		meq.
Acetic	198.0	or	3.3
n-Butyric	23.1	or	0.26
i-Valeric	13.1	or	0.13
n-Caproic	6.2	or	0.05
	Total		3.74

The agreement with the titration value is excellent.

Volatile Acids in Oven Gases. As reported elsewhere (2), only acetic acid was found. On the basis of 1,000 g. of flour, the amount of acetic acid was 15 mg.

Volatile Acids in Bread. The acidity of the distillate from bread representing 1,000 g. of flour, by barium hydroxide titration, was 2.79 meq. By gas chromatography, the following data were obtained:

	mg.		meq.
Acetic	150.0	or	2.5
Butyric	10.6	or	0.12
i-Valeric	6.1	or	0.06
	Total		2.68

The agreement was not as good as found for dough. Further, no caproic acid was detected in the bread.

Discussion

If we add the acetic acid found in bread to that found in the oven gases from the same batch, we obtain a total of $150 + 15 = 165$ mg. The equivalent dough contained 198 mg. Comparing the n-butyric and isovaleric acid contents for the dough and bread, we see a reduction of rather more than half of each acid. It would be pure speculation to assume that the differences represent esterification of these acids during baking; the conditions are probably more conducive to hydrolysis. The general procedure used in these studies was evolved after trial and rejection of other extraction methods, including water extraction of the dough and bread at pH 2 or lower. Separation and estimation of the acids by partition column chromatography showed no notable advantages in selectivity or accuracy over the vapor-phase chromatographic method, while being far slower and more costly in material and solvents.

What these analyses may imply as regards the flavor of bread will depend to some extent on how the amounts of the various acids change during the storage of bread, and how these changes correlate with flavor alterations. The volatile acids composition of a fermented dough can be shown to vary greatly, depending not only on the physical conditions but also on the ingredients. Not the least

important of these ingredients is the yeast; unpublished preliminary experiments in this laboratory, using simple liquid brews fermented by different commercial baker's yeasts, have shown striking differences in the volatile acids compositions, and also in the aromas of the brews.

Literature Cited

1. JAMES, A. T., and MARTIN, A. J. P. Gas-liquid partition chromatography: the separation and microestimation of volatile fatty acids from formic acid to dodecanoic acid. Biochem. J. **50:** 679–690 (1952).
2. WISEBLATT, L. Some aromatic compounds present in oven gases. Cereal Chem. **37:** 728–733 (1960).
3. WISEBLATT, L., and KOHN, F. E. Some volatile aromatic compounds in fresh bread. Cereal Chem. **37:** 55–66 (1960).