SOME VOLATILE AROMATIC COMPOUNDS
IN FRESH BREAD

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ABSTRACT

Volatile compounds in freshly baked bread are recovered with minimal
decomposition by vacuum pumping from a steam-jacketed vessel containing
the coarsely shredded bread. Efficient traps are necessary to condense the
more volatile materials. The total condensate is treated to remove organic
acids, and then the carbonyl compounds are converted to 2,4-dinitrophenyl-
hydrazones (DNPH's). Separate aliquots are used to investigate alcohols and
esters. No qualitative evidence has been found for the presence of phenolic
compounds or amines.

The DNPH's have been separated by repeated adsorption chromatog-
raphy on activated silica gel columns and, to a lesser degree, by paper
chromatography. The aldehydes identified in this way are acetaldehyde,
crotonaldehyde, 2-ethylhexanal, and furfural; the ketones are acetone, hexa-
none-2, heptanone-3; dicarbonyl compounds are diacetyl and methylvigloxal;
pyruvic and levulinic acids are also present, possibly in the form of their
ethyl esters. Several other DNPH's have not been identified positively.

Quantitative estimations have been made of some of the volatile com-
ounds, and the data used in attempts to impart a typical breadlike flavor
to a very bland, chemically leavened product. These attempts were unsuccess-
ful, whether the treatment was applied to the dough ingredients or (by
aeration) to slices of the freshly baked product.

The experiments of Baker and associates (1,2) have lent weight to
the view that the flavor of fresh bread is due to a combination of the
enzymatic and chemical reactions during fermentation of the dough
and the thermal changes occurring in the oven, particularly associated
with the formation of a brown crust. These workers believe that the
more volatile compounds originally in the crust are drawn into the
crumb during cooling of bread, where they are transformed by oxida-
tive or other changes to substances imparting a "stale" flavor.

Most of the published information on the chemistry of bread flavor
has been concerned with products other than the white pan bread most
popular in America, notably rye breads. In view of the gross differences
in flavor between these two classes of bread, any inferences drawn from
the studies of European workers (12,15,19) on rye breads must be of
limited application to white pan bread. Apart from the previously

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assigned number 995 in the series of papers approved for publication. The views or conclusions contained
in this report are those of the author(s). They are not to be construed as necessarily reflecting the views
or endorsement of the Department of Defense. Contribution from the laboratories of the American Institute
of Baking.
2 Graduate student, Northwestern University.
cited work of Baker (1,2), the meager published material on flavor substances in white bread includes the report of Visser’t Hooft and deLeeuw (20), one of the first attempts to correlate a specific product of fermentation with the palatability of the bread.

In the work to be described, analytical methods which have yielded excellent results in other areas of food flavor research have been applied to those substances which exert significant vapor pressures in fresh bread. No attempt has been made to decompose nonvolatile complexes which might release volatile substances by chemical treatment (as, for example, splitting out organic acids from their salts or protein complexes). The objective has been to determine what compounds contribute directly to the aroma component of fresh flavor and in what concentration these compounds occur. A further objective, contingent upon the validity of the analytical results achieved, was to attempt the synthesis of a flavoring composition which would impart breadlike flavor to a very bland, chemically leavened product (14).

Materials and Methods

Bread Formula. In order to simplify the investigation as far as possible, only those ingredients absolutely essential to proper baking performance and crust formation were included. Such ingredients as milk solids and malt preparations were deliberately omitted, since they can contribute pronounced flavors of their own. The formula used was as follows (percentages are based on flour weight):

- Flour (bakers patent from S.W. spring wheat) 100.0%
- Water — as needed, usually about 64.0%
- Yeast 2.5%
- Sucrose 5.0%
- Salt 2.0%
- Lard 1.2%

Doughs were no-punch straight doughs, fermented about 3 hours at 24°C. They were scaled at 18.5 oz. and molded; proofed to height at 35°C.; and the loaves were baked at 292°C. (550°F.) for 25 minutes.

Recovery of Volatiles. Initial attempts to isolate volatile aromatic substances were based on steam distillation either of aqueous slurries of fresh bread or of filtrates from such slurries. In either procedure, there was evidence of severe breakdown during steam distillation with the production of foul-smelling artifacts, notably indole. The breakdown products were not as prominent when filtrates were steam-distilled, but some of the volatile aromatics are very poorly watersoluble and were not present in filtrates.

Attempts to omit the distillation step were unsuccessful. Whether
water or any organic solvent was used as the extractant, the extract contained relatively great amounts of sugars, lipids, etc., which reacted with any reagent used to derivatize the volatiles and made any subsequent separations extremely ineffective. Even when ether, benzene, or heptane extracts were dried over sodium sulfate and then distilled under vacuum, decomposition became excessive long before the bulk of the aromatics could be separated.

The most successful technique was to shred the freshly baked bread coarsely and to distill the aromatics (together with moisture) under high vacuum, supplying only sufficient heat to the crumb to maintain room temperature or slightly above. The apparatus comprised an aluminum pressure canner, modified so that the sealing gasket would seal under vacuum rather than pressure and with the pressure gauge and safety valve removed. An aluminum gooseneck vapor-takeoff was fitted to the lid with neoprene sealing gaskets. The canner was set into a steam jacket made from a 30-gallon steel drum to permit heating the charge during distillation. The vapors were led through a train of traps beginning with an ice bath and followed by at least two dry ice-alcohol traps. A mechanical vacuum pump was used as the vacuum source, and, when necessary, it was supplemented by an oil diffusion pump operating with a silicone oil (DC 703). The combination was capable of maintaining as low as 1 micron absolute pressure in the system, but normally such high vacuum was not necessary.

In operating this system, a relatively moderate vacuum (15–30 mm. Hg) was applied until nearly all the moisture had been removed from the bread. Usually, the bread was then pulverized to improve the transfer of heat from the container walls, and pumping was continued until the absolute pressure dropped below 50 microns measured with a McLeod gauge. After about 1 hour in this range, the residual bread was always free of any discernible odor, even if a sample were stirred with boiling water. If allowed to stand exposed to air, the dry residue rapidly developed a rancid odor, however.

_Treatment of Recovered Volatiles._ Of primary interest in these studies were the volatile carbonyl compounds. Regardless of whether these were separated from bread extracts or by direct vacuum distillation, as just described, it was considered important to convert carbonyl compounds into relatively stable derivatives at the earliest moment to minimize their decomposition. The 2,4-dinitrophenylhydrazones offer many advantages; they are formed readily and in almost quantitative yields; they can be separated from one another by chromatographic methods; and the identities of the parent carbonyl compounds can be
learned from melting-point and crystallographic data on the purified derivatives.

Generally, the 2,4-dinitrophenylhydrazones (DNPH’s) were formed by adding an excess of 2,4-dinitrophenylhydrazine reagent in aqueous hydrochloric acid to the mixture containing the carbonyl compounds, so that the final mixture would be about 2N in acid. If the carbonyls were in an organic solvent, this was removed by evaporation after forming of the DNPH’s. The latter were allowed to form overnight at room temperature and were filtered off on a coarse fritted-glass filter. After the solids were washed with warm 2N hydrochloric acid and water, they were dried thoroughly over sodium hydroxide pellets in a desiccator.

In the investigation of volatile acids, the vacuum distillate from fresh bread was titrated with sodium hydroxide until just alkaline, and then it was evaporated to dryness under reduced pressure using a rotary flash evaporator. The sodium salts were dissolved in a little water, acidified with a few drops of sulfuric acid, the solution was saturated with solid sodium sulfate, and the free acids were extracted with benzene. The benzene solution of the organic acids was used to spot chromatograms on Whatman No. 1 paper while the paper was exposed to ethylamine vapor. The chromatograms were also spotted with known organic acids (acetic, propionic, butyric, isobutyric, isovaleric, and caproic) and were developed with n-butanol:water:ethylamine (85:15:1), as described by Hiscox and Berridge (3).

No attempt was made to characterize volatile esters, since qualitative tests with hydroxylamine and ferric ion were negative. This suggested that any study of volatile esters would require amounts of bread too great to be handled successfully with the equipment at hand.

To study the alcohols in the bread volatiles, the vacuum distillate was first titrated to convert free acids to salts; it was then distilled at atmospheric pressure until the undistilled portion was odorless. This new distillate was next treated to form the DNPH’s of the carbonyl compounds; the latter were filtered off and the filtrate was made approximately neutral and distilled at atmospheric pressure. This final distillate was treated according to the procedure of Holley and Holley (4); a portion was reserved for examination by gas chromatography.

Chromatography of DNPH’s. Column adsorption chromatography was considered preferable to paper chromatography for the identification of individual carbonyl compounds via their DNPH’s, since it is possible to isolate amounts sufficient to obtain mixed melting point data. The simplicity and sensitivity of paper chromatography made it a useful adjunct, however. Numerous adsorbents and solvent systems
were tried for column separations, among which may be mentioned alumina (17); magnesium sulfate (16); talc (18); and silicic acid (13). Silicic acid proved to be the most satisfactory adsorbent, when the columns were prepared as follows:

Silicic acid, reagent grade, 100 mesh ("suitable for chromatography") is dried for at least 8 hours at 180°C. and is stored in completely filled, screw-capped jars. To pack a column, the prepared adsorbent is cautiously slurried with anhydrous diethyl ether at the rate of 75 cc. ether per 20 g. of silicic acid (caution — heat is evolved). The slurry is poured into the tube, fitted with a porous retaining plate, and is compacted with 1 p.s.i. of air pressure. When the solvent level has almost reached the top of the packing, another 25 cc. of anhydrous diethyl ether are added, and the solvent is again forced down to the level of the packing. Then the diethyl ether is displaced by petroleum ether (boiling range 30°C–60°C.), using a total of 150 cc. to ensure complete displacement of the diethyl ether. The column is now ready for application of the sample.

It is customary to apply pressure when operating these columns to increase the solvent flow rate. Experience in this laboratory indicated that solvent flow rates much lower than those usually specified (e.g., about 0.5 cc. per minute for a column 19 by 250 mm.) gave much cleaner separations of complex mixtures with improved homogeneity of the bands and freedom from streaking and channeling. Such flow rates could be attained by gravity flow alone, while the time to complete a run could be lessened safely by increasing the solvent system polarity more rapidly than was tolerable when air pressure was used.

Details of the adsorption chromatography have been omitted here; in no instance was it possible to isolate pure DNPH's from a mixture by passage through a single column. Large columns, about 42 mm. inside diameter, were used to achieve rough separations into groups, which in turn were applied to smaller columns and resolved further. As many as four separate columns might be used to purify some of the DNPH's.

Paper chromatography was helpful in examining the eluates from columns for homogeneity and usually indicated when a DNPH was sufficiently pure to be recrystallized for melting-point determinations. The solvent systems employed were mainly the n-heptane:methanol system of Huelin (6) and the decahydronaphthalene:dimethylformamide system of Horner and Kirmse (5). The DNPH's of the keto acids, and a few others which moved very slightly in the above systems, were handled preferably with the system n-butanol:ethanol:water (4:1:5). Examination of the developed chromatograms was facilitated by the
use of a viewing box containing "black light" fluorescent tubes, covered by two sheets of ground glass with a dark blue gelatin optical filter between. As viewed by the ultraviolet light transmitted from beneath the paper sheets, the spots appeared almost black against a pale blue background. In some cases, the sheets were sprayed with dilute alcoholic sodium hydroxide, which rendered the DNPH spots red, brown, or blue (the blue color indicating a bis-DNPH of a dicarbonyl compound).

Reference Derivatives of Known Carbonyl Compounds. In order to identify the DNPH's isolated from the volatile carbonyl compounds of bread, mixed melting points and chromatographic comparisons were made with authentic DNPH's of known carbonyl compounds. In general, the latter were prepared and purified by standard methods from commercially procured carbonyl compounds; the starting compounds were redistilled when there seemed any doubt of their purity (e.g., furfural). Melting points of the reference DNPH's were checked against those reported in Huntress (7), Johnson (10), Strain (18), and Jones et al. (11). Ultraviolet absorption spectra, taken with a Beckman DU Spectrophotometer, were also compared with those reported in the literature where available.

Quantitative Analytical Procedures. Volatile acids were estimated by conventional titration in aqueous medium. Ethanol was estimated by dichromate oxidation, neglecting any errors introduced by other oxidizable compounds. (The concentration of ethanol in the volatiles is greatly in excess of all other compounds detected.) Furfural was estimated by its ultraviolet absorption at 277 m\(\mu\) after ascertaining with aqueous standard solutions that its molar absorbancy of 14,000 at this wave length is about 1000 times greater than the absorbancy of any other carbonyl compound identified. Crotonaldehyde, which exhibits a similarly strong absorption at 223 m\(\mu\), did not interfere at 277 m\(\mu\).

Total alpha-beta dicarbonyls were estimated, under the assumption that no acetoin or dihydroxy compounds were present in the volatiles. Acetoin, if present in the bread, has a low volatility, while glycols would be even less volatile. Periodic acid oxidation was therefore assumed to account only for diacetyl plus methylglyoxal, the two dicarbonyl compounds identified in the volatiles. The method followed is described by Jackson (8).

Diacetyl was estimated colorimetrically by its reaction with creatine and alkaline alpha-naphthol (21). It was determined first that, under the specified reaction conditions, methylglyoxal in equivalent amounts produced no measurable color. Methylglyoxal was then estimated by
the difference between total dicarboxyls and diacetyl.

It was not possible to estimate total carbonyls with any confidence. The usual volumetric methods based on oximation could not be applied successfully on the micro scale dictated by the small amounts of volatiles which were obtainable. Numerous methods based on measurement of the color developed by DNPH’s in alcoholic alkali have been reported, but a careful survey of such methods in this laboratory\(^8\) showed that they are uniformly incapable of yielding accurate results with known mixtures of carbonyl compounds and are useful only in comparative studies.

**Supplementation of Chemically Leavened Bread.** Before it could be assumed that any mixture of substances simulating the chemical composition of bread flavor might be useful as an additive to chemically leavened doughs, it was felt advisable to try an actual distillate from conventional white bread of good flavor. Sixteen loaves, baked from the formula already given with the addition of 4% nonfat dry milk, were stripped under vacuum, and the condensed volatiles were redistilled at atmospheric pressure. The original strippings, as was usual by the method employed, had an odor best described as “rubbery”; following the atmospheric distillation, the condensate had a more breadlike aroma. A sevenfold concentration of the flavorful material was thus obtained in the first 13.5% of distillate; the undistilled portion was essentially odorless. The concentrated distillate (360 ml.) was used as part of the dough water to make up a chemically leavened dough (14) based on 750 g. of flour. Two loaves were baked from this dough, along with two loaves from an unsupplemented dough.

A second approach was to expose slices of freshly baked, chemically leavened bread, in a vacuum desiccator, to the vapor from a bread distillate or from a synthetic “flavor” mixture. Such treatment would, it was felt, obviate losses and alteration of the volatiles through baking. The general procedure consisted of evacuating the desiccator with its contained slices to about 20 mm. absolute pressure and connecting the desiccator to a small vacuum oven containing the aromatic substances in a dish, previously evacuated to about ½ atmosphere. As the oven-venting cock was opened, the system slowly came to equilibrium at about 200 mm. absolute; the oven was being heated during this time up to a maximum of perhaps 90°C. Then nitrogen from a cylinder was allowed to bleed slowly into the oven and thence into the desiccator, sweeping volatiles into the latter. When the system reached atmospheric pressure, the desiccator was opened, and the slices were

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\(^8\) Kohn, F. E. Unpublished data. American Institute of Baking, 1957.
sampled. They had acquired a pronounced aroma in every instance.

**Results and Discussion**

*Identification of Carbonyl Compounds.* When converting the carbonyl compounds of fresh bread volatiles to the DNPH's, an amount of 2,4-dinitrophenylhydrazine reagent (in 2N hydrochloric acid) sufficient to ensure a considerable unreacted excess was always used. As keto-acid hydrazones are somewhat soluble in aqueous media, the filtrate from a derivative mixture was normally extracted with ethyl acetate to recover any keto-acid DNPH's, and these were re-extracted into dilute sodium bicarbonate solution, returning the ethyl acetate-soluble neutral DNPH's, if any, to the main bulk of precipitated derivatives.

Paper chromatography of the keto-acid DNPH's revealed two spots, whose Rf values corresponded to those of pyruvic acid DNPH and levulinic acid DNPH. By streaking the mixture on large sheets of Whatman 3MM paper, it was possible to resolve and recover sufficient of the two hydrazones to obtain mixed melting points with authentic samples of the supposed compounds and thereby to confirm their identities.

*Pyruvic Acid DNPH.* Pale yellow needles from hot water, m.p. 218°C. Mixed m.p. with authentic sample, 219°C.; m.p. of authentic sample, 220°C.

*Levulinic Acid DNPH.* Orange crystals from hot water, m.p. 206°C. Mixed m.p. with authentic sample, 206°C.; m.p. of authentic sample, 206°C.

The latter keto acid was found in the form of its ethyl ester by chromatographic isolation of the DNPH.

*Ethyl Levulinate DNPH.* Orange-yellow crystals from ethanol, m.p. 102°C. Mixed m.p. with authentic sample, 102°C.; m.p. of authentic sample, 103°C.

Among the 2,4-dinitrophenylhydrazones of the neutral carbonyl compounds, the most easily purified were those of the saturated aliphatic aldehydes and ketones, which are eluted from silicic acid adsorption columns with small concentrations of diethyl ether in petroleum ether.

*2-Ethylhexanal DNPH.* Orange-yellow needles from cold methanol and water, m.p. 120°C. Mixed m.p. with authentic sample, 122°C.; m.p. of authentic sample, 124°C.

*Acetone DNPH.* Orange-yellow needles from methanol, m.p. 127°C. Mixed m.p. with authentic sample, 126°C.; m.p. of authentic sample, 127°C.

*Hexanone-2 DNPH.* Orange plates from cold methanol and water, m.p. 100°–101°C. Mixed m.p. with authentic sample, 103°C.; m.p. of authentic sample, 104°C.

*Heptanone-3 DNPH.* Yellow needles from petroleum ether (−29°C.), m.p. 79°C. Mixed m.p. with authentic sample, 80°–82°C.; m.p. of authentic sample, 81°C.
The DNPH of acetaldehyde was always detectable on paper chromatograms, but this compound could not be resolved on adsorption columns. Orange needles which crystallized readily and melted at 134°–137°C. were obtained regularly and could not be identified until a paper chromatogram of these needles revealed two spots corresponding to the DNPH’s of acetaldehyde and crotonaldehyde. Rapid heating of the orange needles to 170°C. caused vigorous ebullition, and fine yellow needles were obtained as a sublimate. These proved to be acetaldehyde DNPH. The melt resolidified after driving off this compound, and the brilliant red residue was shown to be crotonaldehyde DNPH. These two derivatives appear to form a complex of rather definite structure during desorption from silicic acid, while a certain amount of crotonaldehyde DNPH could be eluted in a pure state.

_Acetaldehyde DNPH._ Sublimate of fine yellow needles, m.p. 157°C. Mixed m.p. with resublimed authentic sample, 157°C. M.p. of sublimed (metastable form) authentic sample, 157°C.


The most difficult carbonyl compounds to isolate in the form of their 2,4-dinitrophenylhydrazones were furfural, diacetyl, and methylglyoxal (pyruvic aldehyde). Furfural DNPH occurs in two isomeric forms, of which the higher-melting form is poorly soluble in many solvents and difficult to elute from an adsorption column or to move on a paper chromatogram. Both diacetyl and methylglyoxal form bis-DNPH’s, which are very difficultly soluble and which are held tenaciously on adsorption columns. These three derivatives were resolved successfully by adsorption on neutral alumina from a chloroform solution (necessarily dilute); the high-melting form of furfural DNPH was then displaced with 5% methanol in chloroform and the two bis-DNPH’s with 10% pyridine in chloroform. Methylglyoxal bis-DNPH was the last to emerge, and only the 2,4-dinitrophenylhydrazine reagent itself is more strongly held by the adsorbent.

_Furfural DNPH (high-melting isomer)._ Dark red crystals from pyridine, m.p. 225°C. Mixed m.p. with authentic sample, 227°C. M.p. of authentic sample, 233°C.

_Diacetyl bis-DNPH._ Orange powder from chloroform and petroleum ether, m.p. 317°C. Mixed m.p. with authentic sample, 316°C. M.p. of authentic sample, 318°C.

_Methylglyoxal bis-DNPH._ Very small orange needles from pyridine, m.p. 305°–309°C. Mixed m.p. with authentic sample, 308°C. M.p. of authentic sample, 308°C.

_Identification of Organic Acids._ Paper chromatograms of the ethylamine salts of volatile organic acids revealed the presence of acetic
acid with only traces of propionic acid. Since there was no evidence of free pyruvic or levulinic acids being present, it has been assumed that these occurred in the volatiles as esters. Not readily explainable is the fact that no other esters could be detected in a portion of the filtrate after removal of the carbonyl compounds as DNPH's.

Identification of Alcohols. The distilled filtrate from the preparation of the DNPH's contained a considerable amount of ethanol, easily detected by its odor. Treatment with 3,5-dinitrobenzoyl chloride and chromatography by the procedure of Holley and Holley (4) indicated that ethanol was by far the chief alcoholic constituent in the volatiles; no other alcohol could be detected with confidence. A portion of the filtrate was saturated with magnesium sulfate, extracted with dry ether, the extract dried over anhydrous sodium sulfate and applied to a 5-foot column in a gas chromatograph (Aerograph Model A-100). The column packing was Carbowax 400 on C-22 firebrick, and operation was at 110°C. with helium as the carrier gas, at a flow rate of 24 ml. per minute. One distinct peak was obtained having a retention time identical with ethanol under the same conditions; at maximum sensitivity there was a barely perceptible "shoulder" on the peak, whose estimated retention time corresponded to that of isopropanol.

Quantitative Results. Expressed on the basis of one loaf of bread, the following concentrations were estimated: Titratable volatile acids (as acetic acid), 41 mg.; ethanol, 2.33 g.; furfural, 1.7 mg.; total dicarbonyls, 0.30 millimole; diacetyl, 1.44 mg. (0.017 millimole); methylglyoxal (0.30–0.017) = 0.28 millimole or 20 mg.

"Flavor Supplementation" Experiments. In the case where volatiles from conventional white bread were added as part of the ingredients for the chemically leavened bread, there was no significant effect on the flavor of the latter when compared with an unsupplemented control. The same was true when slices of the chemically leavened bread were treated with the vapors from conventional white bread.

Although the scanty quantitative data above are insufficient to permit formulation of a "synthetic flavor" composition, several such mixtures were made up arbitrarily, and the slice-aeration technique was used to assess their effects, if any, on flavor. The first mixtures tried comprised the compounds whose concentrations are listed above, together with various proportions of acetaldehyde, crotonaldehyde, 2-ethylhexanal, and hexanone-2. None of the mixtures was in the least breadlike in aroma. Substitution of pyruvic acid for acetic acid seemed to improve the odor, and the following mixture was used to aerate 12 slices of "instant" bread:
Ethanol (95%) & 100.0  
Furfural & 0.37  
Pyruvic acid & 2.0  
Diacetyl & 0.27  
Methylglyoxal (30% aq.) & 0.72  
2-Ethylhexanal & 0.1  
Crotonaldehyde & 0.1

There was general objection to the overly prominent diacetyl odor of these slices. In view of the high volatility of diacetyl, this compound probably distilled into the slices in a disproportionate concentration. Two more mixtures were made up and tried, with this basic formulation:

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<td>Furfural</td>
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<td>Pyruvic acid</td>
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<td>Methylglyoxal (30% aq.)</td>
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<td>Acetaldehyde</td>
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<td>2-Ethylhexanal</td>
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<td>Crotonaldehyde</td>
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This mixture was divided into two equal portions. To one was added 0.015 ml. diacetyl; to the second, 0.025 ml. Slices of “instant” bread were aerated with the two mixtures, and the flavors were compared with untreated slices by experienced personnel of the American Institute of Baking. No preferences were shown for any of the samples, although the aerated slices could be distinguished easily from the controls.

From the results of these experiments, it must be concluded that flavor fortification of chemically leavened bread by the means used is not practicable, at least with our present knowledge of bread flavor. It may be that some of the subtle characteristics of bread flavor are due to minute amounts of unidentified volatile substances. Furthermore, the natural states of flavorants in conventional bread may have much to do with the observed aroma and taste; it is extremely unlikely that compounds crudely sorbed onto bread slices will have the same relative vapor pressures as they would when produced naturally during fermentation and baking.

**Summary**

Several volatile compounds have been recovered and identified from fresh white bread, and some of these have been estimated. Neither the actual distillate containing these compounds nor several synthetic blends of them have proved of any value in enhancing the
palatability of a bland, chemically leavened bread. One conclusion
seems inescapable; that certain products of fermentation, not neces-
sarily the same as those identified here, are essential at the time a bread
dough goes into the oven and are altered by oven heat (both in nature
and amounts) to give rise to the actual constituents of flavor.

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