SOME VOLATILE CARBONYL COMPOUNDS ARISING DURING PANARY FERMENTATION[1]

F. E. KOHN[2], L. WISEBLATT, AND L. S. FOSDICK[3]

ABSTRACT

Volatile carbonyl compounds produced during dough fermentation were isolated and identified. Straight doughs from normal ingredients and from ingredients specially treated to attain minimal bacterial populations were compared as to the types and amounts of carbonyl compounds recoverable. The compounds identified in both normal and low-bacteria doughs are acetone, acetaldehyde, pyruvaldehyde, n-hexanal, n-butyraldehyde, isovaleraldehyde, and 2-hexanone. Benzaldehyde, which was identified in normal doughs, was not detected in low-bacteria doughs. Quantitative estimates of these substances showed no notable order-of-magnitude differences between the doughs. These compounds are not detectable in the ingredients, and are clearly fermentation by-products.

The importance of panary fermentation in determining the flavor and aroma of white pan bread has been well established by the investigations of Baker and associates (1,2). These workers have shown that unfermented doughs are incapable of producing products having a flavor resembling bread derived from fermented doughs.

Baker has attempted to account for this in terms of yeast action upon amino acids and sugars, resulting in the formation of fusel oils and "browning" precursors respectively. Other investigators (5) have implicated volatile products formed by the action of the bacterial flora known to be present in compressed yeast, flour, and other ingredients of doughs.

In the light of present knowledge of the volatile carbonyl constituents recoverable from fresh bread (6) it was considered of potential value to determine whether any of these compounds actually originated during fermentation. If possible, any compounds identified would also be related either to the action of yeast or normal bacterial flora, or to both.

Materials and Methods

Dough Formulation. The following formula was used in the preparation of all doughs.

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[2] Present address: Industrial Bio-Test Laboratories, Northbrook, Illinois. This paper is taken from a portion of a thesis submitted by F. E. Kohn to the Graduate School of Northwestern University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] Professor of Chemistry, Northwestern University School of Dentistry.

	g
Flour (nonbromated)	1,000
Water	700
Yeast, compressed	30
Salt	20
Glucose	60
Nonfat dry milk	40
Lard	30

Fermentation Conditions. Doughs were no-punch straight doughs, fermented for 4 hours at a temperature of 24°C. and relative humidity of 85%.

Pure Yeast. The pure yeast culture had a total bacteria count of 20 per g.

Sterile Ingredients. Nonfat dry milk and flour were sterilized with ethylene oxide. Lard was autoclaved 15 minutes at 121°C. Sugar and salt solutions and water were subjected to Seitz filtration. After these treatments the ingredients were found to be sterile.

Recovery of Volatiles from Doughs. During fermentation, all evolved gases were aspirated under mild vacuum through a train of three gas scrubbing columns, each containing 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid, to trap any volatile carbonyl compounds which might otherwise be lost. By allowing fresh air to pass across the entire dough surface on its way to the gas scrubbers, such carbonyls were trapped very effectively. The recovered hydrazones were combined with those obtained from the vacuum strippings.

At the completion of the desired fermentation period, 2,800 ml. of carbonyl-free isopropanol were added to the dough (final alcohol concentration approximately 80% by volume) to precipitate the proteins. This resulted in a granular precipitate which was easily separated from the medium by centrifugation and subsequent decantation of this supernatant liquid. The solids were then washed with three 50-ml. portions of carbonyl-free isopropanol and finally with two 50-ml. portions of distilled water. All washings were combined with the original supernatant liquid.

The system for recovering the volatiles consisted of a rotary flash evaporator connected to a vacuum pump through a series of four cold traps. Three of these traps were chilled by dry ice and alcohol mixtures; the final trap was chilled with liquid nitrogen. The pump was fitted with a needle valve which acted as a bleed mechanism to allow precise pressure control during the distillation. The pressure was maintained in the region of 50 to 200 mm. mercury throughout most of the distillation. After complete removal of the aqueous alcohol phase, the operating pressure was reduced to 50 μ mercury and maintained at this level for 30 minutes. After this treatment

the residue was devoid of any discernible odor.

Derivatization and Separation of Carbonyl Compounds. The condensate collected in the cold traps was treated with an excess of 2,4-dinitrophenylhydrazine in hydrochloric acid sufficient to bring the final acid concentration to approximately 2*N*. The dinitrophenylhydrazones (DNPH) were allowed to form during 24 hours at room temperature.

The isopropyl alcohol was then removed by flash evaporation at 40°C. and the acid was neutralized with sodium bicarbonate. Water was next removed by flash evaporation and the DNPH's were completely extracted with warm benzene. This extract was concentrated, then treated with four volumes of petroleum ether to precipitate the dicarbonyl compounds and excess reagent, then filtered. The filtrate was separated into its components by a combination of chromatography on a silicic acid column and paper chromatography, using decalin and dimethylformamide. This procedure has been described in detail (6).

Identification of Carbonyl Compounds. The DNPH's were next recrystallized from aqueous ethanol to yield products suitable for melting-point determinations.

Quantitative Determinations. For quantitative determinations, known aliquots of the components separated by column chromatography were stripped of solvent, dissolved in benzene, and purified further, if necessary, by paper chromatography. The resolved fractions were eluted quantitatively from the paper with warm benzene, cooled, and diluted to volume. Quantitative determinations were made by ultraviolet spectrophotometry at 360–370 mμ, using the findings of Jones, Holmes, and Seligman (3) that all saturated carbonyl hydrazones have essentially the same molar absorbancy and that the absorption of hydrazone solutions follows the Lambert-Beer law.

Testing of Ingredients. Samples of the ingredients were examined for the presence of volatile carbonyl compounds by distilling alcoholic extracts and testing the distillates with 2,4-dinitrophenylhydrazine solution followed by alcoholic alkali, according to Lappin and Clark (4). In all cases, the results were essentially negative, so that those carbonyl compounds reported here can be considered as fermentation by-products.

Results and Discussion

The volatile compounds isolated from the dough made with commercial yeast and nonsterile ingredients, together with their melting

points and melting points of mixtures with equal quantities of pure reference derivatives, are listed in Table I. The amount of pyruvaldehyde was very small. In addition, a small amount of a component was found whose DNPH melted at 120°–122°C. This did not correspond to any available reference DNPH and the compound could not be identified.

TABLE I

VOLATILE CARBONYL COMPOUNDS FORMED IN THE FERMENTATION OF DOUGH

DESCRIPTION	M.P.	CORRESPONDING REFERENCE HYDRAZONE	M.P.	MIXED M.P.
	°C		°C	°C
Yellow-orange rods	106–107	2-hexanone	107	106–107
Yellow needles	102–103	n-hexanal	107	103–105
Orange rods	115–116	isovaleraldehyde	120	117–118
Orange-yellow plates	115	n-butyraldehyde	122	116–117
Yellow needles	125	acetone	127	126
Deep orange needles	238	benzaldehyde	243	241
Yellow needles	155–157	acetaldehyde	163	157–159
Deep orange needles	292	pyruvaldehyde	308	300–306

Acetaldehyde, acetone, 2-hexanone, and pyruvaldehyde have also been reported in bread (6). The remaining compounds in Table I may have reacted during baking, or volatilized in the oven, to be absent in bread.

The effects of the bacteria normally present in the yeast and other ingredients on the formation of carbonyl compounds during fermentation were studied both qualitatively and quantitatively. For this purpose the ingredients used in the dough formula, except yeast, were sterilized. Yeast with a very low bacteria count was used. The bacteria count in the yeasts and doughs is given below.

	Bacteria per g. Control dough	*Low-bacteria dough*
Yeast	1.3×10^6	20
Dough after fermentation	52×10^6	1,200

To ensure that doughs made with sterile ingredients and yeast of low bacteria count were not abnormal, they were baked into bread. The bread was comparable in quality characteristics with that made from the control dough.

The same volatile compounds were obtained from the fermentation of the low-bacteria dough as were obtained from the control dough (Table I). Pyruvaldehyde and benzaldehyde were not determined quantitatively because the amounts present were too small for accurate analysis. Because the DNPH's of 2-hexanone and n-hexanal

could not be separated quantitatively in pure form they were determined together. Quantitative determinations were made on duplicate doughs and the results shown in Table II are the averages.

TABLE II

AMOUNTS OF CARBONYL COMPOUNDS FORMED IN FERMENTATION OF NORMAL DOUGH AND ONE OF LOW BACTERIAL CONTENT

COMPOUND	NORMAL DOUGH	LOW-BACTERIA DOUGH
	ppm	*ppm*
2-Hexanone n-Hexanal	246	263
Isovaleraldehyde	24	22
n-Butyraldehyde	22	25
Acetone	70	49
Acetaldehyde	33	15

There was a considerable difference in results between duplicates, so that the data of Table II should be interpreted as giving only the order of magnitude. Since Table II shows no differences in the orders of magnitudes of the individual compounds, the normal bacterial flora of the fermenting dough apparently have little effect on the relative amounts of the carbonyl compounds formed. These compounds appear to be essentially the results of yeast fermentation.

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Literature Cited

1. BAKER, J. C., and MIZE, M. D. Some observations regarding the flavor of bread. Cereal Chem. **16:** 295–297 (1939).
2. BAKER, J. C., PARKER, H. K., and FORTMANN, K. L. Flavor of bread. Cereal Chem. **30:** 22–30 (1953).
3. JONES, L. A., HOLMES, J. C., and SELIGMAN, R. B. Spectrophotometric studies of some 2,4-dinitrophenylhydrazones. Anal. Chem. **28:** 191–198 (1956).
4. LAPPIN, G. R., and CLARK, L. C. Colorimetric method for determination of traces of carbonyl compounds. Anal. Chem. **23:** 541–542 (1951).
5. ROBINSON, R. J., LORD, T. H., JOHNSON, J. A., and MILLER, B. S. The aerobic microbiological population of pre-ferments and the use of selected bacteria for flavor production. Cereal Chem. **35:** 295–305 (1958).
6. WISEBLATT, L., and KOHN, F. E. Some volatile aromatic compounds in fresh bread. Cereal Chem. **37:** 55–66 (1960).