IDENTIFICATION OF CARBONYL COMPOUNDS IN AN ETHANOL EXTRACT OF FRESH WHITE BREAD[1]

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ABSTRACT

A number of carbonyl compounds were isolated from an ethanol extract of fresh white bread by the formation of their 2,4-dinitrophenylhydrazone derivatives. Through these derivatives five carbonyl compounds were identified by conventional analytical procedures: acetaldehyde, ethyl pyruvate, furfural, hexanal, and 2-methylbutanal. Three additional carbonyl compounds were identified by means of paper chromatography in three varied systems and by ultraviolet spectrum data; these compounds are acetone, methyl ethyl ketone, and formaldehyde. Isobutyraldehyde and n-valeraldehyde, which were found in baking-bread vapors, appeared to be absent in the ethanol extract of bread.

The flavor and aroma of freshly baked bread deteriorate rapidly, resulting in a much less appealing product if distribution from baking centers requires more than a few hours. If fresh flavor could be stabilized, a better product could be made available to consumers. A major approach to the problems of improvement and stabilization of bread flavor is to isolate and identify the various compounds in bread which contribute to its flavor. Once the flavor components have been identified, research on the stabilization and enhancement of desirable bread flavor could be conducted more efficiently.

The flavor of bread has been discussed in several recent reviews (4,9,10,11), and intensive research on the problem is under way in a number of laboratories. Recently, Wiseblatt and Kohn (12), Fortmann (2), and Rotsch and Dörner (7) reported new compounds isolated and identified in their research on bread flavor.

The present paper describes the isolation and identification of several volatile carbonyl compounds occurring in fresh white bread. It presents an analytical approach different from those described in the recent literature, and expands the list of carbonyl compounds positively identified in bread so far.

Materials and Methods

Because white bread made by different formulations and/or analyzed by different procedures may give different analytical results, the

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materials and methods used in this work are described in detail.

Bread Extraction and 2,4-Dinitrophenylhydrazone Formation. The bread was made according to the following formulation:

	g
Unbleached baker's patent flour	1,000
Sugar	50
Yeast	30
Nonfat dry milk	30
Shortening	30
Salt	20
Yeast food	2.5
Water, 700 ml.	
Mixing time, 6 minutes	
Dough temperature, 27°C. (80°F.)	
Straight dough, 2 punches	
Fermentation time, 2 hours, 20 minutes, total	
Pan proof, 1 hour at 35°C. (95°F.)	
Baking time, 25 minutes at 218°C. (425°F.)	

Twenty-four 1-lb. loaves of the freshly-baked bread were immersed in 20 liters of carbonyl-free absolute ethanol while the loaves were still hot. After standing overnight at about 40° F., the bread was broken up into smaller pieces with a stirrer, and the alcoholic bread suspension was filtered through a large Büchner funnel.

The filtered alcoholic bread extract was passed through 1 by 40-in. Dowex 50[3] (H^+ form, 20–50 mesh) ion exchange column, then through a 1 by 40-in. Amberlite IR-4B (OH^- form) column. The extract was then distilled in 1½- to 2-liter batches at atmospheric pressure through a 14-in. Vigreux column, until the combined pot residue was about 1½ liters. The distillate was condensed into a 2,4-dinitrophenylhydrazine reagent (50 ml. of a saturated 80:20 ethanol-concentrated hydrochloric acid (v/v) solution for each 2 to 3 liters of distillate). This reaction mixture then was distilled at atmospheric pressure down to 1½ liters. It was further concentrated on a steam bath; most of the ethanol had now boiled away, leaving a precipitate of 2,4-dinitrophenylhydrazones (hereafter abbreviated 2,4-DNPH's) in excess 2,4-dinitrophenylhydrazine solution.

The 1½-liter portion of the bread extract remaining in the pot was further distilled, until the material in the pot would no longer distill over without becoming dark. The distillate was collected in a second portion of 2,4-dinitrophenylhydrazine reagent.

After overnight standing, the precipitated 2,4-DNPH's of each portion were filtered off separately, washed thoroughly, and dried

[3] Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

slowly at room temperature in air. Second crops of precipitated 2,4-DNPH's were filtered off after the filtrates had stood at room temperature for about a week. Before the 2,4-DNPH's were analyzed by chromatographic techniques, each second crop of 2,4-DNPH's was combined with its corresponding main crop.

Column Chromatographic Analyses. By the use of three-column chromatographic systems, five carbonyl compounds were isolated and identified. The three systems are described below.

The 2,4-DNPH samples from bread were chromatographed on a series of 2:1 silicic acid-Celite (w/w) columns according to the method of Pippen *et al.* (5), care being taken not to exceed the capacity of each column. Chloroform was the sample solvent, and diethyl ether-Skellysolve F mixtures, with the ether concentration progressively increasing, were the developing solvent mixtures. A large part of each of the 2,4-DNPH samples was insoluble in the sample solvent, chloroform. This chloroform-insoluble part, believed to be mostly bis-2,4-DNPH's of vicinal-dicarbonyl compounds, was not analyzed in the present work.

The second column chromatographic method used is an adaptation of a method by Ramsey and Patterson (6). Nitromethane (6 to 8 ml.) was ground into activated silicic acid (10 g.) with mortar and pestle. The silicic acid mixture was made into a slurry with Skellysolve B (in place of hexane), and the slurry was packed into a 9-mm.-diameter glass column.

The third method employed a 9-mm.-diameter column containing unactivated silicic acid (Mallinckrodt No. 2847 analytical reagent, 100 mesh), which was packed as a slurry with 3:7 benzene-Skellysolve B (v/v, all solvents redistilled) and developed with this solvent mixture under nitrogen pressure of 8 to 10 p.s.i.

Paper-Chromatographic Analyses. Three paper-chromatographic systems for analyzing 2,4-DNPH's were used. These systems, together with ultraviolet spectrum data, helped identify seven carbonyl compounds, of which some identities are more nearly certain than others. (Two of these were also identified through column chromatography.)

The three systems employed include those of Nonaka *et al.* (3); Sundt and Winter (8), but applied to 2,4-DNPH's on Whatman No. 1 paper, impregnated with 25% dimethylformamide in acetone; and Ellis *et al.* (1). For each of the systems ultraviolet spectrum data were obtained directly on the paper chromatograms after the manner of Nonaka *et al.* (3). For the last two systems the chromatograms were thoroughly air-dried before their ultraviolet spectra were determined.

2,4-Dinitrophenylhydrazones. Generally, 2,4-DNPH's of carbonyl

compounds isolated from bread were recrystallized from 95% ethanol whenever enough 2,4-DNPH was available. Authentic carbonyl 2,4-DNPH's were obtained according to Pippen *et al.* (5), prepared mostly from commercially available carbonyl compounds. Ethyl pyruvate was prepared by conventional esterification methods, in which pyruvic acid was refluxed with an excess of absolute ethanol and a trace of sulfuric acid.

Oven Vapor Condensate. Some preliminary work was done on oven vapors from baking white bread. The vapors were condensed into a trap containing a saturated solution of 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid, and the resulting 2,4-DNPH's were analyzed by column and paper chromatography as described previously.

Results and Discussion

Approximately 0.19 g. of chloroform-soluble 2,4-DNPH's of carbonyl compounds isolated from bread was characterized. Not analyzable by the methods used in this study was approximately 0.74 g. of 2,4-DNPH's which are predominantly sparingly soluble in most common organic solvents. These sparingly soluble 2,4-DNPH's probably include biacetyl bis-2,4-DNPH, methylglyoxal bis-2,4-DNPH, and additional furfural 2,4-DNPH.

Five of the more than a dozen different 2,4-DNPH bands which separated on each chromatographic column were identified as follows after similar bands were combined:

Acetaldehyde was isolated from bread as its 2,4-DNPH (140 mg.), which melted at 158.5°–168°C. (Probably the derivative is a mixture of two crystalline forms of acetaldehyde 2,4-DNPH, or contains a trace of impurities.) The mixed melting point with authentic acetaldehyde 2,4-DNPH (m.p. 167.5°–169°C.) was 159°–168°C. The infrared spectra of the natural and authentic materials appear to be identical.

Ethyl pyruvate was identified by the fact that a mixed melting point of its 2,4-DNPH (40 mg., m.p. 158.5°–159°C.) with authentic ethyl pyruvate 2,4-DNPH (m.p. 157.3°–158°C.) was undepressed (157.3°–158°C.). The infrared spectra of the natural and synthetic materials are identical. It is not known whether the ethyl pyruvate existed in bread as such or as pyruvic acid which was esterified during the extraction.

Furfural was identified through its 2,4-DNPH (3 mg., m.p. 219°C.), which was recrystallized from hot pyridine plus water. When it was mixed with authentic furfural 2,4-DNPH (m.p. 227°C.), the mixed melting point was not depressed (m.p. 220.5°–221.5°C.). Infrared spectra are essentially identical.

Hexanal and 2-methylbutanal were identified as a result of the separation of their 2,4-DNPH's on a nitromethane-silicic acid column. The isolated hexanal 2,4-DNPH (2 mg., m.p. 109°–110°C.), when melted with authentic hexanal 2,4-DNPH (m.p. 109°C.), showed no melting point depression (m.p. 110.5°–111.5°C.). Not enough material was left for infrared analysis, but co-chromatography of the sample with hexanal 2,4-DNPH produced only one zone on a silicic acid column impregnated with nitromethane, and only one spot on a 2-phenoxyethanol-impregnated paper chromatogram.

2-Methylbutanal 2,4-DNPH (0.7 mg.) was identified by melting point (m.p. 122°–126°C.), by R_f and ultraviolet spectra obtained on 2-phenoxyethanol-impregnated paper, and by the observation that only one spot was formed when the sample was mixed with synthetic 2-methylbutanal 2,4-DNPH and chromatographed together on 2-phenoxyethanol-impregnated paper. Unfortunately the sample was depleted before a mixed melting point could be taken with authentic 2-methylbutanal 2,4-DNPH (m.p. 131°–131.2°C.).

The major carbonyl components in freshly baked white bread, not considering chloroform-insoluble derivatives, appear to be principally acetaldehyde (1 part), ethyl pyruvate (0.25 part), furfural (0.02 part, probably a low estimate, since furfural 2,4-DNPH borders on the chloroform-insoluble class of 2,4-DNPH's), and lesser amounts of hexanal and 2-methylbutanal. The quantities are not very meaningful, because the procedure used in the extraction of bread may not afford quantitative results.

Additional small quantities of 2,4-DNPH's, of generally less than 1 mg. each, constituted the remainder of the 2,4-DNPH bands from the chromatographic columns. The bands, when eluted, had indistinct melting points and were often oily solids. Several of the substances present in such small quantities were identified by use of a microanalytical technique involving paper chromatography coupled with ultraviolet spectrum analysis (3). It was felt that adequate proof of the identities of these minute samples was obtained if the R_f values and ultraviolet spectrum data of an unknown, obtained from at least three widely differing paper chromatographic systems, agreed closely with those of an authentic 2,4-DNPH.

Table I compares data of 2,4-DNPH's of carbonyl compounds isolated from bread with those of authentic 2,4-DNPH's. Bread carbonyl compounds identified by use of the microanalytical technique include acetone, formaldehyde, and methyl ethyl ketone, as well as the previously identified acetaldehyde and ethyl pyruvate. Interestingly, isobutyraldehyde and n-valeraldehyde were identified (together

with acetaldehyde and acetone) in the same manner in oven vapors from baking bread, but were not found in the ethanol extract of bread.

TABLE I

COMPARISON OF R_f VALUES AND ULTRAVIOLET SPECTRUM MAXIMA AND MINIMA OF 2,4-DINITROPHENYLHYDRAZONES OF ISOLATED BREAD CARBONYLS AND AUTHENTIC CARBONYLS

Compound Suspected	R_f of 2,4-DNPH		Absorption Maxima of 2,4-DNPH		Absorption Minima of 2,4-DNPH	
	Isolated	Authentic	Isolated	Authentic	Isolated	Authentic
			mμ	mμ	mμ	mμ
	2-Phenoxyethanol:Heptane System					
Formaldehyde	0.16	0.16	362	360–1	301	299–300
Acetaldehyde	0.20	0.20	368	368	300	301
Ethyl pyruvate	0.44	0.44	369	368	304	303
Acetone	0.49	0.49	372	372	305	302
Methyl ethyl ketone	0.73	0.74	374	374	308	308
Isobutyraldehyde[a]	0.63	0.63	369	368	305	301
n-Valeraldehyde[a]	0.70	0.70	368	368	305	301
	N, N-Dimethylformamide:Decalin System					
Formaldehyde	0.09	0.09	361–3	358–9	297	297–9
Acetaldehyde	0.17	0.16	366	365–6	301	300
Ethyl pyruvate	0.29	0.27	365–6	365–6	301	302
Acetone	0.27	0.27	368	368–9	302	301
Methyl ethyl ketone	0.49	0.51	369	368	308	307
Isobutyraldehyde[a]	0.38	0.39	364	364	302–4	299
n-Valeraldehyde[a]	0.43	0.44	365	364	299	299
	Propylene Glycol:Methanol:Skellysolve C System					
Formaldehyde	0.15	0.18	364	364	295	294
Acetaldehyde	0.39	0.39	372–3	372	305	306–7
Ethyl pyruvate	0.66	0.74	368	367–8	302	298
Acetone	0.68	0.65	373	372–3	302	297
Methyl ethyl ketone[b]	0.92	0.91	368	369	319	317
Isobutyraldehyde[a]	0.82	0.82	368–9	368	302–4	302–3
n-Valeraldehyde[a]	0.93	0.93	367–8	367	299	300–2

[a] Found in oven vapors from baking bread, but not in the ethanol extract of bread.
[b] Used 34% ethylene glycol in place of 20% propylene glycol, because of streaking with latter.

In the different paper chromatographic systems used, most of the trace samples of bread carbonyl 2,4-DNPH's resolved into many spots each. Most of these spots gave ultraviolet spectra which are incongruous with those of known 2,4-DNPH's at hand. Probably some are decomposition products, but many are probably still unresolved mixtures of 2,4-DNPH's. Others may yet be unknown 2,4-DNPH's. The seven compounds listed in Table I are the only ones whose spectrum data and R_f values in several systems agree fairly well with those of known 2,4-DNPH's. The known carbonyls whose 2,4-DNPH's

were used for comparisons included those mentioned previously as being found in bread (4,12).

The R_f values in Table I are not absolute; the R_f of a 2,4-DNPH occasionally varied from run to run. Thus it was necessary to run unknowns and suspected knowns side by side on the same chromatogram. However, the R_f value of one 2,4-DNPH relative to another remained in all cases fairly constant.

When used alone, the ultraviolet spectrum maxima and minima are not sufficiently definitive, since similar carbonyl compounds in general give similar maxima and minima. Thus, for any one chromatographic system, all normal aliphatic aldehyde 2,4-DNPH's (except the first member, formaldehyde 2,4-DNPH) have the same maximum and minimum.

Of the ten carbonyl compounds identified in this work, 2-methylbutanal has to our knowledge not yet been reported for bread. Acetaldehyde, ethyl pyruvate, furfural, hexanal, acetone, and formaldehyde were mentioned in recent literature (see 2,7,12); but methyl ethyl ketone, isobutyraldehyde, and n-valeraldehyde have been mentioned only in early literature and with evidence probably insufficient to establish their specific identity.

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