[CONTRIBUTION FROM DIVISION OF FOOD TECHNOLOGY, UNIVERSITY OF CALIFORNIA]

Deterioration of Dried Fruits. II. Identification of Furfurals

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In this paper confirmatory evidence is presented for the occurrence of furfurals in darkened apricot extracts. The proof for hydroxymethylfurfural rests on conventional evidence, including characterization of derivatives. Furfural was present in minute amounts insufficient for the necessary recrystallizations of the derivatives isolated. Its presence was, therefore, demonstrated by its absorption spectrum, chromatographic behavior of its 2,4-dinitrophenylhydrazone, partition between ether and water, volatility in steam and specific qualitative tests. Evidence for methylfurfural was inconclusive and must be considered tentative.

Pure Furfurals.—Pure furfurals and their derivatives were prepared for comparison with compounds isolated from the darkened apricot extracts. Furfural (Quaker Oats Co.) was purified by distillation under reduced pressure. Methylfurfural was prepared by distillation from acidified rhamnose solution[1] and hydroxymethylfurfural was obtained by the method of Haworth and Jones.[2]

2,4-Dinitrophenylhydrazones.—These derivatives were selected because they are very insoluble in water and can be isolated from dilute solutions. They are colored compounds of relatively high molecular weight and can be easily separated by chromatographic techniques. However, confusion exists as to their melting points. The various values reported for the furfural derivative are: 202°,[3,4] 222°,[5,6] 230° (cor.),[7] 229°[8]; for that of hydroxymethylfurfural 184°,[9] 198–200° and 197–198°.[10] The melting point of the methylfurfural derivative is still more uncertain.[6]

Bredereck[7] and Bryant[11] attributed these discrepancies to stable and metastable forms, while Campbell[8] did not observe such forms in the case of the acetaldehyde derivative and ascribed the phenomenon to impurities formed during preparation of the derivative. Brady[12] explained such discrepancies in terms of formation of thin films of the solvents, around the crystals, from which these derivatives were crystallized.

The hydrazones were prepared by precipitation with a 2 *N* hydrochloric acid solution of 2,4-dinitrophenylhydrazine.[12] The precipitates were filtered, washed with 2 *N* hydrochloric acid followed by water, and then dried.

The furfural derivative on recrystallization twice from pyridine decomposed at 229° (cor.). When Bredereck's method for the red form[7] was followed, the melting point could not be raised above 222° (cor.) even after six crystallizations. The present method is simpler and gives a reproducible melting or decomposition point. During this work, the melting point of 184°, which is supposed to be the melting point of the mixture of the two forms postulated by Bredereck, has never been obtained. The methylfurfural derivative recrystallized from 95% ethanol showed slight shrinkage at 135°, a color change at 170° and an unsharp melting point at 180°, 183.3° (cor.). Recrystallized from glacial acetic acid, it decomposed at 212°, 217° (cor.), *cf.* ref. 6. The hydroxymethyl furfural derivative was twice crystallized from 95% ethanol. Great shrinkage was observed at 184°. When the temperature was raised 1° per minute there was further shrinkage and change in color. At 193–194°, if held at this temperature for three minutes, it melted at 197–198° (cor.). On further heating the substance decomposed at 197°, 200.3° (cor.). These observations indicate that the hydroxymethylfurfural derivative undergoes a partial decomposition at 184° without melting and finally decomposes at 197–198° (cor.), and the decomposed products melt at 200–203° (cor.). This accounts for the reported melting points of 184°, 197–198° and 200°. These decomposition points are highly reproducible and serve to characterize the compound.

Partition Experiments.—The pure furfurals were each partitioned between water and ether, the two solvents being saturated with respect to each other. Concentrations used in the case of furfural and hydroxymethylfurfural were of the same order of magnitude as those found in darkened apricot extracts, calculated to be about 100 mg. per liter. After partition, 1 ml. of the aqueous phase was diluted to 25 ml. for spectroscopic determination. The blank was prepared by diluting 1 ml. of ether-saturated water to 25 ml. with water. Optical densities of the original and the subsequent aqueous phases were measured at several wave lengths between 320 mμ and 210 mμ. Concentrations were calculated at 277, 292 and 282 mμ for furfural, methylfurfural and hydroxymethylfurfural, respectively. Percentage extractions by ether (with an ether-water ratio of 1:1) were 77, 82 and 24 in the three cases.

Chromatography of 2,4-Dinitrophenylhydrazones.—The mixture of the three hydrazones

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(1) Runde, Scott and Johnson, THIS JOURNAL, **52**, 1288 (1930).

(2) Haworth and Jones, *J. Chem. Soc.*, 667 (1944).

(3) Pergutti, *Gazz. chim. ital.*, **24**, 564 (1894).

(4) "Dictionary of Organic Compounds," Vol. II, Eyre and Spottiswoode, London, 1936.

(5) Simon, *Ber.*, **66**, 320 (1933).

(6) Mainzer, *Biochem. Z.*, **247**, 171 (1932).

(7) Bredereck, *Ber.*, **65**, 1833 (1932).

(8) Campbell, *Analyst*, **61**, 391 (1936).

(9) Blanksma and Wackers, *Rec. trav. chim.*, **55**, 658 (1936).

(10) Scallet and Gardener, THIS JOURNAL, **67**, 1934 (1945).

(11) (a) Bryant, THIS JOURNAL, **55**, 320 (1933); (b) Bryant, *ibid.*, **60**, 2815 (1938).

(12) Brady and Elsmie, *Analyst*, **51**, 77 (1926).

was dissolved in carbon disulfide and passed over a column of anhydrous magnesium sulfate (General Chemical Co., New York). Two zones were formed. After eluting the first zone with carbon disulfide, the column was washed with petroleum ether to remove carbon disulfide. On further development with dry benzene the second zone separated into two bands. The lower one was eluted readily with this solvent, while the remaining band was eluted easily by a mixture of 30% ethyl acetate and benzene. The whole separation took twenty-five minutes, with a column 10 cm. long and 2 cm. in diameter under 60 cm. pressure. The three substances thus separated were characterized by rechromatographing with known derivatives as the 2,4-dinitrophenylhydrazones of furfural, methylfurfural and hydroxymethylfurfural, respectively. The type of the adsorbent and the solvents had a marked influence on the separation and elution of the three zones.[13]

Distillation Experiments.—When it was realized that it would be necessary to identify furfural in very dilute solution, enrichment procedures were sought. Partitioning was inadequate, so a distillation technique was applied. Furfural solutions (154 ml.) in concentrations of 21 and 42 ml. per liter were rapidly distilled without refluxing and the first two fractions (44 ml., two-sevenths of the total) contained two-thirds of the furfural present in both cases.

Isolation of Furfural from Black Apricots

Dried apricots containing 5000 p. p. m. of sulfur dioxide were stored at 55° for three weeks until black. They were then ground and extracted with 50% ethanol in the ratio of 100 g. of fruit to 500 ml. of the solvent by steeping for twenty-four hours. The extract was filtered and the black filtrate was passed over cation and anion exchange resins (Duolites C-3 and A-3, respectively),[14] to remove nitrogenous compounds, salts and acids. The neutral fraction thus obtained was distilled under 20–30 mm. pressure at about 35° until two-thirds was distilled. The distillate thus obtained was called solution A. As will be shown, it contained most of the furfural, and traces of the relatively non-volatile hydroxymethylfurfural.

The non-distillable residue was then extracted continuously for fourteen hours with ether. The non-extractable residue was discarded. The ether extract should contain most of the hydroxymethylfurfural originally present together with slight amounts of furfural not removed by the distillation. To separate most of the residual furfural from the hydroxymethylfurfural, the ether extract was partitioned with water. According to the previous partition experiments, the aqueous phase (called solution B) should contain 75% of hydroxymethylfurfural, whereas the ether phase, solution C, should contain 75% of the furfural.

To establish the effectiveness of the above separation, solutions A, B and C, as well as solution D, prepared from known freshly distilled furfural, were adjusted to the same optical density at 280 mμ. To each solution 25 ml. of freshly distilled xylidine and 12.5 ml. of glacial acetic acid were added.[15] In the cases of A and D a pink coloration developed which faded slowly. With C an orange color with a reddish tinge developed, and in B it was light orange. This showed that solution A contained mostly furfural. Solution B contained only traces of furfural while solution C contained somewhat more furfural than solution B. These observations were in agreement with expectation.

Proof for Hydroxymethylfurfural (Solution B).—Solution B gave a weak xylidine test, as anticipated, due to traces of furfural, and the solute was non-volatile in steam. Spectroscopic examination showed a high absorption maximum at 282.5 mμ, *cf.* ref. 16.

Partitioned with ether, the percentage extracted was *ca.* 25. Results are shown in Table I, and demonstrate the efficiency of the extraction procedure employed.

TABLE I

SOLUTION A PARTITIONED FOUR SUCCESSIVE TIMES WITH ETHER (DATA IN TERMS OF THE OPTICAL DENSITIES)

Wave length	Before extraction	Extraction number			
		1	2	3	4
290	0.839	0.201	0.152	0.125	0.093
285	1.066	.255	.176	.134	.101
280	1.050	.285	.177	.134	.101
275	1.170	.282	.162	...	.095
270	1.040	.252	.135	.112	.085
240	0.224	.060	.027	...	.041
230	.305	.081	.031	...	.052
Percentage extracted (calculated at 280 mμ)		72.9	37.9	24.2	24.2
SOLUTION B SIMILARLY PARTITIONED WITH ETHER					
290	1.020	0.768	0.570	0.422	0.312
285	1.110	.828	.615	.458	.340
280	1.060	.797	.596	.439	.325
240	0.138	.100	...	...	...
230	0.190	.141	...	...	...
Percentage extracted (Calculated at 285 mμ)		25.4	25.7	25.5	25.7[a]

[a] With fifth and sixth extractions the percentages fell to 24.7 and 21.7%, respectively.

The 2,4-Dinitrophenylhydrazone.—The same details were followed as for the known, already described. The same transition and decomposition points were observed as with the known, and a mixed melting point showed no difference. The absorption curves of the known and the unknown derivatives completely coincided with one another. Finally, no separation was observed when

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(13) Paper III of this series, THIS JOURNAL, **70**, 3583 (1948).

(14) Unpublished data.

(15) Suminokura and Nakahara, *Tottori Soc. Agr. Sci.*, 1–3 (1927–1931).

(16) Paper No. IV of this series, THIS JOURNAL, **70**, 3586 (1948).

the known and the unknown derivatives were chromatographed.

The Phenylhydrazone.—This derivative was prepared from the residue obtained by distilling the ether from the ether extract of the non-distillable residue after adding a few ml. of water to avoid darkening.[17] (A concentrated solution B might equally well have been used.) When crystallized from 33% ethanol, it melted at 136° (uncor.). On recrystallizing twice from toluene it melted at 140–141° (*cf.* ref. 18), (cor.) 142.8°.

Benzoylation.—The benzoyl derivative was prepared from the residue obtained as in the case of phenylhydrazone. Benzoylation was performed in the presence of 10% alkali by vigorous shaking and cooling.[19] The derivative melted at 57°[20] after four crystallizations from 95% ethanol.

The equivalent weight of the benzoyl derivative was determined by hydrolyzing 19 mg. of the derivative with a known volume of standard alkali solution and titrating the excess of alkali against standard hydrochloric acid. The volume of 0.01 *N* alkali used was 8.87 ml. (microburet) giving an equivalent weight of 217 1 (calculated, 218).

The above evidence constitutes an unequivocal proof of the presence of the hydroxymethylfurfural.

Proof for Furfural in Solution A

The positive xylidine test and the steam distillability substantiate the presence of furfural. Solution A showed a pronounced absorption maximum *ca.* 277 mμ, *cf.* ref. 16.

Partition with Ether.—Solution A, partitioned with ether, and examined as in the case of solution B, was clearly a mixture. The first extraction eliminated 72.9% of the absorption at 280 mμ, and a shift in the position of the maximum is definitely discerned, the residue presumably containing small quantities of hydroxymethylfurfural, since the percentage falls to that yielded by the residue, solution B, in which this component has been identified (Table I).

To avoid interference by other substances, the partitioning technique for solution A was then modified. Solution A was made alkaline with caustic soda and extracted with ether. The ether was partitioned with water to remove hydroxymethylfurfural. To the ether extract, 50 ml. of water was added to avoid darkening of furfural and the ether was distilled. The aqueous solution was then rapidly distilled and the first 14 ml. collected, which should contain two-thirds of the furfural. This was diluted to the desired concentration (*ca.* 100 mg./l.), and 20 ml. of it was partitioned with water-saturated ether. Instead of successive partitioning of the aqueous portion, the partitioning was reversed and carried on with the ether portion. This was then partitioned with ether-saturated water. Percentage extractions were determined spectroscopically at 277 mμ. Results are shown in Table II.

TABLE II

PARTITION OF ETHER EXTRACT OF SOLUTION A WITH WATER

	Optical densities		
Water extraction	Water extract	Calculated equivalence in ether phase	Per cent. remaining in ether
Original	1.28	...	..
1st	0.350	0.930	72.6
2nd	.249	.681	73.2
3rd	.179	.502	73.9
4th	.140	.362	71.9
5th	.112	.250	69.0
6th	.090	.160	64.0

The 2,4-Dinitrophenylhydrazone.—Approximately 3 liters of solution A were redistilled and the first 400 ml. collected. This solution was purified by partitioning with ether. To the ether phase a few ml. of water were added and the ether distilled. From the residue the 2,4-dinitrophenylhydrazones were prepared as already described. These were crystallized from pyridine and chromatographed in dry benzene over a talc column.[21] A number of substances separated on the column, but the second one which was eluted with dry benzene gave an absorption curve which coincided with that of the known. This substance when mixed with the pure furfural derivative and chromatographed formed a single band, thus confirming the presence of furfural.

Methylfurfural.—The enriched distillate from solution A gave a very faint greenish coloration when the Maquenne test[22] was applied, indicating that methylfurfural can only be present in trace amounts.

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Summary

The formation of hydroxymethylfurfural and furfural has been established during the darkening of dried apricots. The dinitrophenylhydrazone, phenylhydrazone and benzoyl derivatives of the former have been prepared. The evidence for furfural itself is based on its steam volatility specific qualitative test, spectroscopic analysis, partition between ether and water and the behavior of its 2,4-dinitrophenylhydrazone on a Tswett column.

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[17] Wolff, *Ann.*, **394**, 101 (1912).

[18] Reichstein, *Helv. Chim. Acta*, **9**, 1066 (1926).

[19] Middendorf, *Rec. trav. chim.*, **138**, 1 (1919).

[20] Beilstein, Heterocyclische Reihe, **18**, p. 15, 4th edition.

[21] Talc was used prior to development of magnesium sulfate as an adsorbent (paper III of this series). It was later abandoned owing to variability of different lots of talc.

[22] Maquenne, *Compt. rend.*, **109**, 573 (1889).