third one have been proposed to account for the course of the oxidation of hydrocarbons in the cycloparaffin series.

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[CONTRIBUTION FROM THE SUBTROPICAL HORTICULTURE LABORATORY OF THE UNIVERSITY OF CALIFORNIA, LOS ANGELES]

Identification of Acetaldehyde in the Steam Distillate of the Peel of Citrus Fruits

BY J. B. BIALE AND F. T. WEISS[1]

A preliminary study on the relation of acetaldehyde to physiological disorders of citrus fruits under cold storage conditions suggested the advisability of determining whether this substance is produced in the rind of the fruit. Vapors of acetaldehyde have been observed by Nelson[2] to produce brown spot and storage spot on oranges and grapefruit. Hall and Wilson[3] have detected traces of this aldehyde in the aqueous residue from ether extraction of Valencia orange juice. Positive identification of acetaldehyde in the apple has been established by Power and Chesnut[4] and by Thomas,[5] and in the pear by Harley and Fisher.[6] The latter have found an accumulation of larger quantities of aldehyde in pear tissue affected with scald and breakdown than in normal fruit. These facts, coupled with the demonstration of toxicity symptoms produced by acetaldehyde vapors, suggested the possibility that acetaldehyde may play an important role as an intermediate product in the oxidation of the respiratory substrates of citrus fruits.

Experimental

Samples of finely ground peel from 10 oranges, 15 lemons, and 6 grapefruit were steam distilled from a 5-liter flask in an all-glass apparatus. By using two receiving flasks in series containing a solution of 1.2% sodium bisulfite it was found that all the aldehyde was absorbed in the first receiver. Since total aldehyde was determined by Tomoda's[7] modification of Ripper's[8] procedure, the loss of bisulfite through the escape of sulfur dioxide or oxidation by air did not interfere.

TABLE I

ALDEHYDE CONTENT OF CITRUS PEEL IN MILLIEQUIVALENTS

	Oranges W. navel	Oranges Valencia	Eureka lemon	Marsh grapefruit
Grams peel	730	635	695	615
Fraction no. 1	0.302	0.352	0.529	0.391
2	.042	.077	.063	.053
3	.019	.072	.038	.030
4	.015	.042	.025	.030
5	.009			
Total	.387	.543	.655	.504
Per 100 g. of peel	.053	.086	.094	.082

Exact comparisons cannot be made between the several varieties because of different stages of maturity of the samples. In the W. navel 800 ml. of distillate was obtained for all fractions, while in the other varieties 500 ml. was caught for each fraction. The rapid drop from the first fraction to the next suggests that most of the aldehyde is produced in the rind during the process of respiration and is not formed by the breakdown of substances during the course of distillation. In one case the rind residue after distillation was kept under sterile conditions for one week and then subjected to a second distillation. Each of the four fractions taken gave about the same amount of aldehyde as the last fraction of the first distillation. Further substantiating evidence on the formation of the aldehyde in the process of fruit metabolism has been obtained by collecting the vapors from air passed through frozen ground peel or over whole fruit and into bisulfite. In one case nearly 0.05 milliequivalent of aldehyde was recovered from each 100 g. of frozen ground lemon rind for a period of two weeks. The recovery of aldehyde from air passed over whole fruit was much less. Apparently the aldehyde is present in the fruit in a form which is more readily released by distillation.

For a colorimetric test of the aldehydes present in the steam distillate the Rimini reaction[4] was employed. Indigo blue changing to yellow or brown indicates the presence of acetaldehyde,

(1) The assistance of the junior author was made available by a research grant from the California Fruit Growers' Exchange, Los Angeles.
(2) Nelson, *J. Agr. Research*, **46**, 695 (1933).
(3) Hall and Wilson, THIS JOURNAL, **47**, 2575 (1925).
(4) Power and Chesnut, *ibid.*, **42**, 1511 (1920).
(5) Thomas, *Biochem. J.*, **19**, 927 (1925).
(6) Harley and Fisher, *J. Agr. Research*, **35**, 983 (1927).
(7) Tomoda, *J. Soc. Chem. Ind.*, **48**, 76 (1929).
(8) Ripper, *Monatsh.*, **21**, 1079 (1900).

TABLE II

MELTING POINTS (IN °C.) OF THE CRYSTALLINE DERIVATIVES

(All Temperatures Corrected)

Fraction	CH_3CHO mg./cc.	2,4-Dinitrophenylhydrazone Unmixed	2,4-Dinitrophenylhydrazone Mixed with CH_3CHO deriv.	Methone derivative Unmixed	Methone derivative Mixed with CH_3CHO deriv.
		W. Navel Orange Peel			
1	2.36	160–161	161–161.5	142–143	142–143
2	1.18	161–162	161–163	141–143	142.5–143.5
3	0.53			140.5–141.5	140–143
4	.17			139–141	140.5–142.5
		Eureka Lemon Peel			
1	.76	161–162.5	161–164	141–142	141–142.5
2	.50	122–133		138–140.5	139–141
3	.08	Failed to precipitate			
4	.05	Failed to precipitate			

citral gives a red color, citronellal yellow. This test is sensitive to 1 in 10,000 and can be used for the bisulfite complex as well as for the free aldehyde. When applied to the first and second fractions of the concentrated distillate (of the samples reported immediately below) this test gave a positive result for acetaldehyde, but it was negative for the third and fourth fractions.

In order to obtain crystalline derivatives for melting point determination the peel of 400 oranges and 300 lemons was steam distilled, and the distillate was concentrated by repeated distillations from a saturated sodium bicarbonate solution into sodium bisulfite. We found that upon concentration 80% of the aldehyde came over in the first 5% of the distillate. The oil was removed by means of decolorizing carbon. The concentrated bisulfite aldehyde complex was saturated with sodium bicarbonate and distilled into an acid solution of 2,4-dinitrophenylhydrazine or into an alcoholic solution of dimethylcyclohexanedione (commonly known as dimethyldihydroresorcinol, and as methone). All melting points were checked by mixing the unknown derivative with that of pure acetaldehyde.

The m. p. of the derivative prepared from pure acetaldehyde with 2,4-dinitrophenylhydrazine was found to be 159–161°. Similarly, the methone derivative with acetaldehyde gave a m. p. 141.5–142°. In the first two fractions of the oranges and in the first one of the lemons no recrystallization was required in either one of the two derivatives. This fact, coupled with the narrow range of the m. p., furnishes evidence that the major portion of the aldehyde in these fractions is acetaldehyde. The m. p. of the derivatives of some essential oil aldehydes that might interfere, such as citral, are different[9] from that of acetaldehyde. The m. p. of the formaldehyde derivative of dinitrophenylhydrazine is in the neighborhood of that of acetaldehyde, but the methone derivative of formaldehyde is nearly 50° higher than that of acetaldehyde. Though the essential oil aldehydes are present in relatively large quantities in the rind, their boiling points are much higher than that of acetaldehyde, and consequently remain mostly in the residue after repeated distillations. Of the predominating aldehydes, Poore[10] reports a higher content of citral in lemon oil than of decyl aldehyde in orange oil, which difference may account partially for the poorer results we have obtained with the lemon samples. The third and fourth fractions of the lemon distillate failed to form a precipitate with 2,4-dinitrophenylhydrazine and with methone even when they were combined. However, from a physiological standpoint the first fraction is of chief concern to us, because it appears to contain most of the aldehyde produced in the process of fruit respiration.

Summary

1. The aldehyde content of the peel of oranges, lemons, and grapefruit has been found by steam distillation to be of the order of 0.05 to 0.1 milliequivalent per 100 g. of fresh rind.

2. By means of preparation of derivatives of the aldehyde obtained in the distillate with 2,4-dinitrophenylhydrazine and with dimethylcyclohexanedione it has been established that most of the aldehyde found in the first two fractions of orange peel and in the first fraction of lemon peel is acetaldehyde.

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(9) Weinberger, *Ind. Eng. Chem., Anal. Ed.*, **3**, 365 (1931).

(10) Poore, U. S. D. A. Tech. Bull. 241 (1932).