

(2) The nature of the coupling reactions between the phenols and diazotised *p*-nitraniline is discussed, and a modified test is described of considerably greater sensitivity and reliability; the test involves the removal of soaps from the saponified mixture by salting out, distillation of the acidified filtrate, and coupling the distillate with stable *p*-nitrobenzene diazotate in alkaline buffer soln.

(3) The test is a general one for the lower phenols and their esters; the azo-colour formed can be separated and identified by pptn. as lakes on magnesium hydroxide.

I am obliged to the Director, Medical and Health Department, Mauritius, for permission to publish this paper.

REFERENCE

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The Absorption of Diphenyl and *o*-Phenyl-phenol by Oranges from Treated Wraps

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ROTTING of citrus fruits due to green mould can be reduced by wrapping the fruit in paper treated with the volatile fungicides diphenyl^{1,2,3} and *o*-phenyl-phenol.^{4,5,6}

Methods of estimating the small amounts of these fungicides absorbed by oranges from treated wraps have now been devised and some of the factors affecting the extent of absorption are discussed below.

Oranges were wrapped in papers impregnated with diphenyl, or *o*-phenyl-phenol and hexamine, and then stored in partly-open tins at constant temperature. The amount of diphenyl (or *o*-phenyl-phenol) taken up was estimated at intervals. In most of the expts. each treated wrap contained about 100 mg of diphenyl or *o*-phenyl-phenol, which is more than is required to reduce wastage. Consequently the quantities of the fungicides absorbed by the fruit were probably greater in our experiments than would be expected under commercial conditions.

ESTIMATION OF DIPHENYL IN ORANGES—A sample (4 or 5) of the oranges which had been stored in diphenyl-treated wraps was weighed and the peel was separated from the pulp. The tissue (peel or pulp) finely minced, was then steam-distilled in the usual manner until about 2 litres of distillate had been collected (2 hr.). The distillate was extracted three times with chloroform (90 ml), which removed both the diphenyl and any orange oil present. Of the chloroform soln. 45 ml, containing about 2 ml of oil, were shaken in a separating funnel with sulphuric acid (sp.gr. 1.84, 45 ml). Moderately vigorous shaking of the mixture was necessary for the efficient destruction and removal of the orange oil, but the formation of a too persistent emulsion should be avoided. After standing for 20 min. the mixture separated into two layers, the upper light yellow and the lower dark red. The latter (sulphuric acid) was removed, and the upper layer (chloroform) again shaken with an equal vol. of sulphuric acid (sp.gr. 1.84). This procedure was repeated four times, chloroform being added to the upper layer when necessary to maintain its vol. at 45 ml. After the last treatment the chloroform soln. was shaken with water (120 ml in two portions), dried over anhydrous calcium chloride and filtered. The colour of the resulting soln. was a very pale yellow, indicating the almost complete destruction of the orange oil. The diphenyl in this solution was then estimated according to Mulliken 1904,⁷ "Drop a hard lump of sublimed aluminium chloride weighing 0.2–0.3 g into a clean 6–8 in. test-tube that has just been taken from a hot drying-oven. Stopper the tube loosely. Hold it in a nearly horizontal position, and by means of a small flame placed under one end slowly sublime the chloride until it forms a thin light yellow coating covering a considerable portion of the glass surface. Allow to cool. Drop in 0.5 ml of a soln. containing 0.05 g of the hydrocarbon dissolved in 2.5 ml of chloroform. Stopper the tube tightly. Lay it on to its side upon a sheet of white paper that rests upon and partly covers the colour standard. Then roll it back and forth so that the solution shall flow over and wet all parts of the sublimate. Observe the colour after a few seconds, and again after 15–20 minutes." The colour given by diphenyl was blue. (Orange oil, if present, completely masked the colour given by the diphenyl.) The chloroform soln. of the diphenyl obtained from the oranges was diluted until 0.5 ml gave a similar colour intensity under standard conditions to that produced by 0.5 ml of chloroform containing 0.05 mg of diphenyl. Distilled dry chloroform was used, and every precaution taken to exclude traces of moisture.

It was found convenient to take a large fragment of aluminium chloride and break it into smaller pieces under chloroform to ensure fresh substance for the test.

The method must be considered only roughly quantitative, the results being subject to an error of about 20%, as shown in Table I.

TABLE I

Procedure	Amount of diphenyl added mg	Amount of diphenyl recovered mg
1. Added to CHCl_3 , treated with H_2SO_4 , etc.	5 10 20	4.5 10 16
2. Added to 2 ml* of oil, the oil dissolved in CHCl_3 and then treated with H_2SO_4 , etc.	5 10 20	4.5 8.4 14
3. Added to H_2O , steam-distilled, and distillate extracted with CHCl_3 , etc.	10	8.9
4. Added to orange oil* (4 ml) steam-distilled and distillate extracted with CHCl_3 , etc.	10	9.5
5. Added to minced peel from 5 oranges, steam-distilled, and distillate extracted with CHCl_3 , etc.	5 10 20	4.8 8.9 18.2
6. Orange* oil (2 ml) steam-distilled, etc.	nil	nil

* The orange oil had been previously prepared from orange peel by steam-distillation.

WRAPS—The treated wraps (2 or 3, each approx. 10 in. $\times$ 10 in.) were extracted with chloroform (3 $\times$ 30 ml) and the amount of diphenyl present was determined as described above. As no orange oil was present, the treatment with conc. sulphuric acid was omitted.

ESTIMATION OF o-PHENYL-PHENOL IN ORANGES—A sample (4 or 5) of the oranges which had been stored in o-phenyl-phenol treated wraps was weighed and the peel was separated from the pulp. The finely-minced tissue (pulp or peel) was then steam-distilled in the usual manner until the volume of the distillate collected was about two litres (2 hr.). The distillate was acidified (5 ml of 2 N hydrochloric acid) and then shaken vigorously with light petroleum (10 ml, B.P. 40–60° C.) in a separating funnel. After standing a short time the mixture separated into two layers; the light petroleum layer was removed and the aqueous layer was again shaken with light petroleum (10 ml). It was found that at least ten extractions with light petroleum were necessary to remove all the o-phenyl-phenol from the aqueous phase. The combined light petroleum extracts were then shaken vigorously with sodium hydroxide soln. (N/10, 33 ml) in a separating funnel. After the mixture had stood for a short time for it to separate, the sodium hydroxide was removed, and the light petroleum soln. was again shaken with fresh sodium hydroxide (N/10, 33 ml). This procedure was repeated once more. The o-phenyl-phenol now in solution in the sodium hydroxide soln., was estimated colorimetrically by coupling with diazotised sulphanilic acid as described by Hanke and Kressler⁸ for phenols and related compounds. The colour was compared with that produced by a standard solution of o-phenyl-phenol (0.01 mg/ml) under similar conditions.

Table II shows the degree of accuracy which may be expected by this method:

TABLE II

Procedure	Amount of o-phenyl-phenol added mg	Amount of o-phenyl-phenol found mg
1. Added to light petroleum (100 ml.) and extracted with sodium hydroxide soln.	5 10 20	5 10 20
2. Added to water (2 litres), and extracted with light petroleum. The light petroleum extracted with sodium hydroxide soln.	10	8.6
3. Added to water, steam distilled, and the distillate extracted with light petroleum, etc.	5 10 20	4.0 8.3 16.8
4. Added to orange oil (4 ml) dissolved in light petroleum and extracted with sodium hydroxide soln.	5 10 20	5 10 20
5. Added to the peel (minced) from 5 oranges (allowed to stand overnight) and then steam-distilled, etc., as described in the full procedure.	5 10 20	3.75 9.1 16.9
6. Untreated orange peel (5 oranges).	nil	nil

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WRAPS—The treated wraps (2 or 3, approx. 10 in. × 10 in.) were extracted with sodium hydroxide soln. (N/10, 100 ml in two portions). The aqueous soln., which contained the sodium salt of *o*-phenyl-phenol, was acidified and extracted with light petroleum as described above.

AMOUNTS OF DIPHENYL OR *o*-PHENYL-PHENOL RECOVERED FROM ORANGES WRAPPED IN PAPER IMPREGNATED WITH DIPHENYL OR *o*-PHENYL-PHENOL AND HEXAMINE—The effects of temperature of storage and of the amount of fungicide in the wrap are shown in Tables III, IV, VI and VII. Practically no diphenyl or *o*-phenyl-phenol (less than 0.1 mg) was recovered from the pulp, the amounts recorded below having been recovered from the peel.

These and other expts. showed that both fungicides are absorbed by oranges from treated wraps in quantities which were related to the concentration in the wraps, and duration of storage. In most expts. absorption increased with temperature. The amounts absorbed also varied with oranges from different countries and was about 4 to 20 mg of diphenyl or 20 to 30 mg of *o*-phenyl-phenol per 100 g. Aeration of the fruit after removal from treated wraps resulted in a very slow loss of the absorbed fungicides (Tables V and VIII).

TABLE III
UPTAKE (mg per orange) OF DIPHENYL BY JAFFA ORANGES
(Each wrap containing 100 mg of diphenyl)

Temperature of storage °C.	Days					
	14	27	34	48	62	71
18	3.2	10.5	4.8	6.4	7.2	8.0
10	4.0		6.4	8.0	8.9	9.4
5	2.4		3.9	4.5	6.5	7.3

TABLE IV
UPTAKE OF DIPHENYL (mg per orange) AT 10° C. FROM WRAPS CONTAINING
DIFFERENT AMOUNTS OF DIPHENYL

Amount of diphenyl in wraps	Days				
	14	36	48	62	71
100 mg per wrap ..	4.0	6.4	8.0	8.9	9.4
50 " " " " ..	2.4	4.0	5.2	5.6	6.7
20 " " " " ..	1.9	2.8	2.8	2.9	2.8

TABLE V
LOSS OF DIPHENYL FROM FRUIT PREVIOUSLY WRAPPED IN DIPHENYL TREATED WRAPS
(The fruit was stored in a desiccator through which a slow stream of air was passing)

Diphenyl, mg per orange	Days in air				
	0	11	17	23	36
	7.6	7.2	4.6	4.6	4.8

The odour of diphenyl soon disappeared from these oranges.

TABLE VI
UPTAKE (mg per orange) OF *o*-PHENYL-PHENOL BY ORANGES AT DIFFERENT TEMPERATURES
Each wrap contained 95 mg *o*-phenyl-phenol plus 25 mg hexamine

Temperature of storage °C.	Days				
	17	42	57	68	77
18	4.8	9.1	11.4	10.0	10.0
10	2.4	4.6	5.0	8.9	8.0
5	3.0	4.0	4.0	4.4	5.0

TABLE VII
UPTAKE OF *o*-PHENYL-PHENOL BY ORANGES FROM WRAPS CONTAINING DIFFERENT AMOUNTS
OF *o*-PHENYL-PHENOL AND HEXAMINE. TEMPERATURE 10° C.

Amount of <i>o</i> -phenyl-phenol and hexamine per wrap	Days				
	17	41	57	68	76
96 mg of <i>o</i> -phenyl-phenol + 25 mg of hexamine	2.4	4.6	5.0	8.9	8.0
48 " " " + 125 " "	3.0	5.7	3.3	4.2	4.4
24 " " " + 6.2 " "	1.6	2.9	2.5	3.3	3.4
85 " " " + 30 " "	4.3	8.1	8.3	—	8.5

TABLE VIII

LOSS OF o-PHENYL-PHENOL FROM FRUIT PREVIOUSLY STORED IN o-PHENYL-PHENOL TREATED WRAPS (the fruit was stored in a desiccator through which a slow stream of air was passing)

Amount of o-phenyl-phenol, mg per orange	Days in air				
	0	12	16	22	37
	6.7	7.7	6.7	10.0	5.7

TABLE IX

EXAMPLES OF AMOUNTS OF DIPHENYL OR o-PHENYL-PHENOL FOUND IN THE PEEL AND PULP (mg per orange)

Diphenyl		o-Phenyl-phenol	
Peel	Pulp	Peel	Pulp
6.1	nil	17.4	nil
4.8	0.1	12.8	0.04
4.7	nil	10.0	nil
4.6	0.11	10.0	..
4.2	0.1	6.65	0.03
3.4	0.17	5.7	nil
1.8	nil		

COMMERCIAL STORAGE WITH TREATED WRAPS—Three cases of Jaffa oranges (each orange wrapped in a paper containing diphenyl) were sent from Palestine to England. The amount of diphenyl originally included in the papers is not known. No diphenyl was found in the papers on arrival in this country, but the amounts recovered from the fruit taken from each of the three cases was 3.9, 4.2 and 3.8 mg per orange respectively. The average weight of the oranges was 230 g and the average weight of the peel 109 g. The pulp was found to contain no diphenyl. Thus the concentration for the whole orange was 17 p.p.m., while the peel contained about 37 p.p.m.

MARMALADE FROM ORANGES PREVIOUSLY STORED IN TREATED WRAPS—Sour oranges were stored for three weeks in treated wraps, and then made into marmalade (8 sour oranges, 2 lemons, 7½ lb. of sugar and 8 pints of water made 12 lb. of marmalade). The amount of diphenyl originally present in the 8 sour oranges (1265 g) was estimated to be 48 mg; of this, less than 0.6 mg remained in the marmalade (1 part per 10,000,000).

In a similar expt. with o-phenyl-phenol, 25 mg remained of 84 mg originally present in the 8 sour oranges, which weighed 1253 g (4.5 parts per 1,000,000).

SUMMARY—Methods have been devised for the estimation of diphenyl and o-phenyl-phenol absorbed by oranges from treated wraps. The amounts of diphenyl and o-phenyl-phenol found to be absorbed from wraps containing 100 mg of these substances were 4–20 mg of diphenyl or 20 to 30 mg of o-phenyl-phenol per 100 g of peel (= approx. 1 orange).

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