Table IX. Effect of Cysteine on Irradiation of Methyl Linoleate

(Concentration of methyl linoleate. 2.55 × 10^{-2}M. Dosage. 1000 r. at 25 r./min.)

Concn. of Cysteine, M × 10^2	Concn. of Conjugated Diene, M × 10^5	Ionic Yield
0	20.2	101
0.11	3.23	16.2
1.01	0	0

concentrations which inhibit the autoxidation in vitro are considerably greater than those which exist in plasma.

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CHEMICAL RESIDUES

Determination of Perchloroethylene in Strawberries

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The vapors of perchloroethylene have been found effective in controlling rot-producing mold on strawberries and other fresh fruit. A method is described which involves the extraction of the perchloroethylene from the berries with diethyl ether, followed by an evaporation in the presence of ethylbenzene, and the determination of the chloride by a nephelometric method. Experimental data on known mixtures and on fumigated berries are given.

THE VAPORS OF PERCHLOROETHYLENE have been found effective under certain conditions in controlling rot-producing mold on strawberries and other fresh fruits (*2, 3*). One factor which must be considered before any foodstuff is treated chemically is the hazard that may be associated with residual fungicide. In order to evaluate this problem, it is necessary to establish the quantity of perchloroethylene remaining after treatment. The analytical method described herein is applicable to the determination of small amounts of perchloroethylene in treated strawberries which do not contain other chlorinated organic compounds.

As no sensitive and selective chemical test for perchloroethylene was available, it was found necessary to concentrate the compound by extraction and careful evaporation and to base the analysis on a chlorine determination. The perchloroethylene is extracted from the strawberries with diethyl ether. Ethylbenzene is added to the extract, the ether is removed by evaporation, and the ethylbenzene containing the perchloroethylene is burned in an oxygen bomb. The resulting chloride is determined by a nephelometric procedure.

Apparatus. A Parr oxygen bomb (*1*, No. 1102), with electrical ignition unit, is required for burning the ethyl benzene. The chloride is measured with a photoelectric colorimeter (Lumetron Model 402EM) using 20 × 40 mm. nephelometric cells.

Test tubes, 15 × 150 mm., marked to contain 3 ml., are used for the final evaporation.

Reagents. Oxygen gas (from cylinder); diethyl ether, anhydrous; ethylbenzene, redistilled; alcohol, Formula 30, chloride-free.

Silver nitrate solution. Dissolve 1.7 grams of silver nitrate (reagent grade) in water. Add 12.5 ml. of nitric acid (concentrated C.P.) and dilute to 1 liter with water.

Standard chloride solution. Dissolve 1.6485 grams of dry sodium chloride in water and dilute to 1 liter. Dilute 10 ml. of this solution to 1 liter with water. One milliliter of the final solution contains 10γ of chloride.

Reference solution. Add 2 ml. of standard chloride solution (20γ of chloride) to 23 ml. of water in a 50-ml. volumetric flask, and proceed as directed in the procedure for final test.

Sodium carbonate, 0.5% aqueous solution.

Preparation of Standard Curve

Clean six 50-ml. volumetric flasks with dilute nitric acid and rinse with distilled water. Measure a 0-, 1-, 2-, 3-, 4-, and 5-ml. portion of standard chloride solution into each flask from a microburet. These correspond to 0,

10, 20, 30, 40, and 50γ of chloride. Add water to make a total of 25 ml. and proceed as in the final test procedure. Use the 20-γ standard to adjust the Lumetron instrument to a 30-unit deflection. Plot the number of micrograms in each aliquot against the galvanometer deflection on ordinary graph paper.

Procedure

Preparation Of Sample Place a 100-gram sample of strawberries in a 16-ounce wide-mouthed bottle, add 100 ml. of diethyl ether, and seal with a tinfoil-lined cap. Shake and allow to stand at least overnight. Take a 25-ml. portion of the ether and place in a 50-ml. beaker containing 3 ml. of ethylbenzene. Evaporate the ether slowly on a steam bath until the volume is about 10 ml.

Table I. Recovery of Added Perchloroethylene

Perchloroethylene Added, Mg.	Perchloroethylene Recovered, Mg.	Recovery, %
0.0235	0.0208	88.5
0.047	0.0413	87.9
0.0705	0.0724	102.7
0.094	0.0834	88.7
0.117	0.01070	91.1
0.0234	0.0196	83.8
0.0234	0.0209	89.3
0.0585	0.0563	96.2
0.0585	0.0535	91.5
0.117	0.116	99.1
0.117	0.110	94.0

Take a previously marked test tube containing two or three 10-mesh Carborundum boiling chips and transfer the remaining solution to it with diethyl ether. Place the test tubes in a water bath at 50° C. and allow the evaporation to proceed until approximately 3 ml. remains. Cool, weigh, and record the weight of ethylbenzene as A. Cap the test tube with a tinfoil-covered rubber stopper and place in a deep freeze. From this sample, weigh 1 ml. into an oxygen bomb cup that has been cooled with dry ice. Record the weight as B and immediately place the cup in the bomb, which should contain 5 ml. of the 0.5% sodium carbonate solution. Add 35 atmospheres or 500 pounds pressure of oxygen and ignite.

Cool, carefully open the bomb, and with small portions of water wash the contents into a clean 50-ml. volumetric flask. Dilute to volume, take a 25-ml. aliquot, or another suitable portion such that the galvanometer deflection will be within the limits of the standard curve, and place the aliquot in a 50-ml. volumetric flask. If less than 25 ml. is taken, the total volume in the flask should be diluted to 25 ml.

Final Test To each solution add 20 ml. of chloride-free Formula 30 alcohol and 5 ml. of the silver nitrate solution. Mix the contents and place the flask in a water bath at 40° C. for 15 minutes. Remove, mix again, and cool to room temperature. Standardize the Lumetron instrument to read 30 units' deflection with the prepared reference solution in a nephelometric cell. Transfer the sample to a similar cell and read the deflection. Determine the number of micrograms of chloride present from the standard curve. A new reference solution should be made for each set of samples. If the chloride content is too high to be run conveniently by turbidity it may be determined by a micro-Volhard titration.

Calculation

Micrograms of perchloroethylene (C_2Cl_4) = micrograms of chloride × 1.17 p.p.m. of C_2Cl_4 =

$$\frac{\text{micrograms of } C_2Cl_4 \text{ in final aliquot} \times 100 \times 50 \times A}{\text{Final aliquot, ml.} \times 25 \times B \times \text{grams of original sample}}$$

Unless perfectly matched cells are used, the same cell should be employed each time for the reference solution and the cell used for the sample should be the same as that used in making the standard curve.

Results of Known Mixtures

Table I shows the recovery of perchloroethylene added to strawberries and analyzed by this method. The mixtures were prepared by weighing the perchloroethylene in a microampoule and breaking the ampoule in a 100-ml. volumetric flask containing ether. The volume was diluted to 100 ml. and a 5- to 10-ml. aliquot of the mixture was added to 100 grams of strawberries. Sufficient ether was added to give a total volume of 100 ml. of solvent, and the mixture was allowed to stand overnight in a closed container and then analyzed by the above procedure.

In the data presented untreated control samples were carried through the procedure to obtain the correct per cent recovery.

Results of Experimental Fumigation

Fresh strawberries in pint, wooden boxes were fumigated in an air-tight 22-liter fumigation vault. Approximately one third of the space was occupied by the berries. After closure a slight vacuum was drawn on the vault. The desired amount of perchloroethylene (U.S.P. grade) was weighed out in a small flask having an air inlet and outlet. The outlet of the flask was connected to the inlet of the vault and the valve opened. The vapors of the perchloroethylene were swept into the vault along with the air necessary to satisfy the vacuum in bringing the vault to atmospheric pressure. The flask was gently warmed to aid in the complete vaporization of the liquid fumigant.

Table II. Recovery of Perchloroethylene After Experimental Fumigation

Treatment	Hours Aerated	Perchloroethylene, P. P. M.
1/10,000	0	133
	4	27
	8	22
	12	22
	24	13
1/20,000	0	122
	4	19
	8	12
	12	2
	24	1.5
Control	...	0.7

The fumigation was carried out at 80° F. with an exposure period of 16 hours. At the completion of the fumigation the vault was opened, and one portion of the berries taken for immediate analysis. The remainder of the boxes of berries were allowed to air for the time indicated under normal room conditions at 80° F. Results are shown in Table II.

The dosage given as 1 to 10,000 means 1 ml. of liquid perchloroethylene per 10,000 ml. of vault volume.

A few experiments were made to test the applicability of the method to compounds other than perchloroethylene. The recovery of ethylene bromide was 80 to 90% while the recovery of ethylene chloride was less than 70%. These results would probably be improved by carrying out the evaporation under conditions different from those given for this method. The low recovery of ethylene chloride is due to loss by evaporation.

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