not been determined whether the difference in migration rate is due to variation in the carbohydrate or aglycone moiety.

Ionophoresis of the sapogenin portion is successful only if an organic solvent is added to the buffer to increase the solubility of the triterpene organic acids. The sapogenin from the hydrolysis of saponin in fraction A and pure oleanolic acid migrate at the same rate in a buffer composed of 60 volumes of ethyl alcohol and 40 volumes of pH 10 buffer. However, as a mixture of ursolic and oleanolic acids, which are isomeric triterpene acids, was not resolved, further work along this line was abandoned.

The use of these techniques in this investigation has been extremely helpful, because valuable information can be gained from only a few milligrams of material in a relatively short time. The application of some of these methods to the rapid quantitative determination of floc in refined sugar has been considered, but has not yet been investigated thoroughly.

Discussion

The work on floc at this laboratory has been concerned with the material actually formed when a refined sugar is dissolved in water and acidified. The authors have, therefore, chosen to consider that all materials isolated by filtration (other than obvious gross foreign particles) are components of the floc. It is probably true that the acid-insoluble saponin material is responsible for the formation of the floc on acidification. Because of its colloidal nature, however, it tends to act as a scavenging agent as it slowly forms aggregates, picking up impurities present in the original sugar or in the water used to prepare the sirup. For example, one sample of isolated floc contained colloidal decolorizing carbon particles passed by the filters in the refining process. The silica content and the brownish coloring matter of the floc are probably picked up in the same manner. The fat component of the floc may come from the oils added to the processing liquors to prevent foaming during the boiling operations. The saponin, acting as a soap in neutral solution, may carry along some of the oil which ordinarily remains in the crystallizing liquors.

Investigation of fraction A will be continued to try to complete the knowledge of its constitution and properties. The results obtained thus far are similar to those of previous workers (*5*) on sugar beet saponin, who also failed to account quantitatively for all the hydrolysis products. It is hoped that more adequate information about the nature of fraction A may suggest improvements in the methods for its elimination from processing juices.

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Literature Cited

(1) Achaya, K. T., and Saletore, S. A., *Analyst*, **77,** 375 (1952).
(2) Assoc. Offic. Agr. Chemists, "Official Methods of Analysis," 7th ed., Secs. 29.61–29.62, 1950.
(3) Consden, R., and Stanier, W. M., *Nature*, **169,** 783 (1952).
(4) Eis, F. G., Clark, L. W., McGinnis, R. A., and Alston, P. W., *Ind. Eng. Chem.*, **44,** 2844 (1952).
(5) Haar, A. W. van der, *Rec. trav. chim.*, **46,** 775, 793 (1927).
(6) Hodgman, C. D., ed., "Handbook of Chemistry and Physics," 27th ed., pp. 1180–2, Cleveland, Ohio, Chemical Rubber Publishing Co., 1943.
(7) Kunkel, H. G., and Tiselius, A., *J. Gen. Physiol.*, **35,** 89 (1951).
(8) McComb, E. A., and McCready, R. M., *Anal. Chem.*, **24,** 1630 (1952).
(9) Schlenk, W., *Ann.*, **565,** 204 (1949).
(10) Steinle, J. V., and Kahlenberg, L., *J. Biol. Chem.*, **67,** 425 (1926).
(11) Van Etten, C. H., *Anal. Chem.*, **24,** 1697 (1951).
(12) Williams, K. T., and Bevenue, A., *J. Assoc. Offic. Agr. Chemists*, **34,** 817 (1951).

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BROMIDE RESIDUES

Determination in Fresh Fruits after Fumigation with Ethylene Dibromide

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A method is described for the determination of ethylene dibromide and bromide residues in fresh fruits and vegetables which were fumigated with ethylene dibromide. Papaya and pineapple flesh showed no increase in ethylene dibromide or bromide content 6 days after fumigation. Avocado flesh retained 7.3 p.p.m. of ethylene dibromide and 5.5 p.p.m. of bromide. Banana flesh retained no ethylene dibromide, but an excess of 28 p.p.m. of bromide.

ETHYLENE DIBROMIDE (1,2-dibromoethane) is an excellent fumigant for destroying infestations of the oriental fruit fly (*Dacus dorsalis* Hendel) and the melon fly (*Dacus cucurbitae* Coq.) in fresh fruits and vegetables. The potential usefulness of this material was first demonstrated in laboratory screening tests made by Balock and Lindgren (*3*), and its adoption as an approved treatment was the result of subsequent developmental research conducted by Balock (*2*). The use of ethylene dibromide permits the treatment of most fruits without injuring them, as was often the case with earlier methods, and it has already increased the movement of Hawaiian crops to mainland markets.

Before this fumigant could be used commercially it was necessary to demonstrate that no significant bromide residues remained in the fruits after treatment. The results of this investigation are reported in this paper.

Experimental Work

Prior to the development of ethylene dibromide as a fumigant, methyl bromide was used in fumigation experiments. Bromide residues in the treated fruits were determined satisfactorily by a method described by Shrader *et al.* (*8*). This method consists of the destructive hydrolysis of the fruit sample with alcoholic potassium hydroxide, followed by sodium hydroxide fusion and ashing. The ash is analyzed for bromide by oxidation to bromate and iodometric determination of bromate as originally described by van der Meulen (*6*, *7*) and recently reintroduced by Alicino *et al.* (*1*).

When this same procedure was applied to fruit samples to which known amounts of ethylene dibromide had been added, bromide recoveries ranged from 28 to 61% and averaged about 40%.

This result, surprising at first, can be explained readily when one considers the ways by which alcoholic potassium hydroxide can react with ethylene dibromide. Whereas methyl bromide can yield only methanol and potassium bromide, ethylene dibromide may give rise to various proportions of ethylene bromohydrin, ethylene glycol, vinyl bromide, or acetylene. Bernoulli and Kambli (*4*) found no ethylene bromohydrin or acetylene among the reaction products. When they carried out the reaction in 50% aqueous ethyl alcohol, the chief product was vinyl bromide. This compound became the sole product when ethylene dibromide was treated with sodium hydroxide in absolute ethyl alcohol.

It is a well-known experimental fact that vinyl bromide, which should be the major product under the conditions of Shrader's method (*8*), is resistant to further hydrolysis, as are other vinyl halides. The explanation for this behavior may be found in the partial double bond character of the carbon-to-halogen bond in vinyl halides. The length of this bond has been measured by electron diffraction (*9*).

After a number of unsuccessful attempts to modify the Shrader method, attention was directed toward work by Hanson (*5*), who in 1950 described the analysis of soil samples after fumigation with ethylene dibromide. In this procedure water is added to the samples and the mixture is distilled into 95% ethyl alcohol. The distillate is then refluxed with a large excess of potassium iodide in an acidic medium and the resulting triiodide is titrated against sodium thiosulfate.

To determine the feasibility of this method for fumigated fruit samples, measured amounts of ethylene dibromide were added to the fruit. However, recoveries were erratic, ranging from 33 to 90%. A number of possible explanations may be advanced for this failure: Ethylene dibromide may suffer partial hydrolysis at the temperature prevailing during the distillation; fruits contain substances (absent in soil) which promote this hydrolysis; and reducing materials present in fruits may codistill, reduce free iodine, and thus lead to low recoveries. This last explanation was kindly suggested by Paul F. Sharp, director, Agricultural Experiment Station, College of Agriculture, University of California at Berkeley.

In order to lower the distillation temperature and to minimize hydrolytic action during distillation, benzene instead of water was added to the fruit samples. This change resulted in recoveries of 73.3 ± 2.8% when 1 mg. of ethylene dibromide was added to 10-gram samples of untreated fruit. Although 100% recovery could not be achieved, the results were sufficiently consistent to allow the use of this method. The details of this test are reported in Table I.

By this procedure unchanged ethylene dibromide may be removed from the fruit samples and determined. The samples remaining after the distillation can then be analyzed for bromide (present as fruit constituents or derived from ethylene dibromide during or after fumigation) by the method of Shrader (*8*).

Procedure

The fumigation was carried out in accordance with Balock's (*2*) description. Ten to twenty fruits of each species were fumigated separately with 0.5 pound of ethylene dibromide per 1000 cubic feet of space in a 240-cubic-foot gas-tight chamber. The ethylene dibromide was vaporized by heating over an electric hot plate and the vapor was circulated in the chamber by an electric fan for 2 hours. During the fumigation the temperature was kept at 70° ± 2° F. After fumigation some fruits were selected at random for immediate analysis and the others were placed under light refrigeration (50° F.) (except for bananas, which were kept at room temperature) to be analyzed after 1 and 6 (or 7) days. The fruits tested were papaya, pineapple, avocado, and banana. Untreated fruits of each species were analyzed as controls.

Each fruit to be analyzed was separated into skin and flesh parts and both portions were diced into pieces measuring 3 to 5 mm. along the edge and thoroughly mixed. Duplicate 10-gram samples were taken from each fruit; each sample was placed in a 100-ml. flask to which 15 ml. of C.P. benzene was added. The flask having a 24/40 neck was connected by a gooseneck adapter to a Friedrich condenser which dipped into a 125-ml. beaker charged with 25 ml. of ethyl alcohol. The flasks were heated in an oil bath kept at 90° to 100° C. until the fruit residues were dry (1.5 to 2 hours). The distillate was then refluxed with 5 grams of potassium iodide and 0.4 ml. of acetic acid (to render the solution acidic) and the triiodide was determined exactly as described by Hanson (*5*). The fruit residues were transferred from the flasks to nickel crucibles, digested with alcoholic potassium hydroxide, fused, and ashed, and the bromide content of the ash was determined in exactly the manner reported by Shrader *et al.* (*8*).

Table II summarizes the results of these analyses, which are expressed in parts per million of ethylene dibromide or bromide residue based on fresh fruit weight.

Table I. Recovery of Ethylene Dibromide

Fruit	No. of Determinations	% Recovery
Papaya		
Skin	2	71.6 ± 3.6
Flesh	4	74.4 ± 1.6
Pineapple		
Skin	3	78.8 ± 5.0
Flesh	3	73.2 ± 8.1
Avocado		
Skin	1	71.5
Flesh	1	71.5
Banana		
Skin	2	69.8 ± 1.9
Flesh	2	76.0 ± 1.0
Average for all fruits	18	73.3 ± 2.8

Discussion and Results

In considering the results it must be borne in mind that an extremely light fruit load was used during the fumigation (less than 1% of the total fumigating space). As ethylene dibromide is very readily adsorbed by fruits and vegetables, the results of the residue tests would tend to be higher than would be expected under commercial fumigating conditions.

Furthermore, a fruit is not a homogeneous substance. If a blender is used to macerate the fruits, the fleshy parts are adequately homogenized but the skins are not. Dicing and mixing were therefore chosen for all the samples. In addition, each set of two samples came from a different fruit with an individual history prior to fumigation. Although this is not conducive to consistent results, it is a more realistic procedure than to analyze fruit which was stored in a diced condition for as long as 7 days after fumigation.

Papaya. As much as 8.3 ± 2.3 p.p.m.

Table II. Ethylene Dibromide and Bromide Residues in Fumigated Fruits

Days after Fumigation	Skin				Flesh			
	No. of detns.	Ethylene dibromide, p.p.m.	No. of detns.	Bromide, p.p.m.	No. of detns.	Ethylene dibromide, p.p.m.	No. of detns.	Bromide, p.p.m.
				Papaya				
0	2	8.3 ± 2.3	2	9.4 ± 0.8	1	2.3	2	7.4 ± 0.6
1	3	3.4 ± 1.1	3	15.8 ± 1.4	3	5.7 ± 2.0	3	9.4 ± 0.9
6	5	2.7 ± 2.1	5	13.5 ± 3.7	5	2.7 ± 2.1	4	10.7 ± 1.1
Untreated	5	1.0 ± 1.2	5	7.0 ± 0.6	5	1.6 ± 0.8	3	14.2 ± 2.9
				Pineapple				
0	2	18.4 ± 0.0	3	29.7 ± 1.9	2	5.5 ± 0.5	2	14.6 ± 1.0
1	3	2.6 ± 1.3	3	37.7 ± 1.3	2	4.8 ± 2.6	3	9.9 ± 1.4
6	3	0.0 ± 0.0	3	25.0 ± 2.3	3	0.3 ± 0.4	3	9.7 ± 0.5
Untreated	10	2.0 ± 1.7	9	22.8 ± 8.1	9	1.6 ± 1.1	8	15.4 ± 5.7
				Avocado				
0	4	78.4 ± 4.4	2	4.7 ± 0.1	4	6.3 ± 0.8	4	3.1 ± 0.6
1	6	13.8 ± 5.8	6	11.8 ± 4.0	7	12.0 ± 3.6	7	9.8 ± 5.5
7	3	1.1 ± 0.7	3	7.9 ± 0.7	3	9.3 ± 4.5	3	7.7 ± 0.6
Untreated	3	2.0 ± 0.4	3	3.0 ± 0.6	3	3.8 ± 1.3	3	2.2 ± 0.2
				Banana				
0	2	11.6 ± 0.2	1	30.3	2	8.4 ± 1.0	1	26.3
1	3	2.2 ± 1.5	3	33.5 ± 1.4	3	3.2 ± 2.1	3	27.9 ± 0.6
7	3	0.0 ± 0.0	3	32.2 ± 1.4	3	0.0 ± 0.0	3	36.9 ± 0.4
Untreated	4	0.0 ± 0.0	4	33.3 ± 0.8	4	0.0 ± 0.0	4	8.8 ± 0.2

of unchanged ethylene dibromide was found in papaya skins immediately after fumigation. In 6 days most of the ethylene dibromide had disappeared. The bromide content in excess of that of untreated fruit was about 2.5 p.p.m. immediately after fumigation; it rose to about 9 p.p.m. and remained close to that level. The flesh of fumigated papaya contained 2.3 ± 0 p.p.m. of ethylene dibromide immediately after treatment. After 1 day 5.7 ± 2.0 p.p.m. was found, and after 6 days 2.7 p.p.m. Fumigation did not increase the bromide content in the edible (flesh) part of papaya.

Pineapple. Qualitatively the results obtained with pineapple skin were the same as with papaya skin. The ethylene dibromide content fell off sharply during the first day after fumigation and none could be detected after 6 days. Bromide variation among the fruit samples and the escape of volatile bromine compounds from the skin are possible explanations for the temporary rise in bromide content.

The ethylene dibromide determination in pineapple flesh was not very satisfactory. Every now and then fruits, treated and untreated, of unusually high apparent ethylene dibromide content were found. A reasonable explanation of this phenomenon might be that some pineapples are rich in one or more volatile oxidizing agents, and that these substances are distilled along with ethylene dibromide and oxidize the iodide to give a high titration value. If these erratic results are disregarded, pineapple flesh appears to be free from ethylene dibromide after 6 days, and from bromide in excess of that found in untreated pineapple flesh, although again the bromide content of untreated pineapple flesh was high and variable (8 to 20 p.p.m.).

Avocado. The skins of avocado adsorbed much more ethylene dibromide during fumigation than did any of the other fruits tested, but none was left after 7 days. As would be expected, most of it had evaporated, but apparently some was changed to bromides of a different nature and caused a bromide excess of 5 p.p.m. Avocado flesh retained an excess of 5.5 p.p.m. of ethylene dibromide and of bromide after 7 days. On the basis of 75% recovery in the ethylene dibromide determination, the corrected ethylene dibromide value would be 7.3 p.p.m.

Banana. No unchanged ethylene dibromide was found in either banana skin or flesh 7 days after treatment. The bromide content of the skin samples was in the range of the untreated samples throughout the experimental period. Banana flesh, however, was left with a relatively large amount of excess bromide, which rose to about 28 p.p.m. after 7 days.

Experiments with these four fruits showed clearly that ethylene dibromide was concentrated largely on the fruit skins, from which it evaporated. Small amounts of ethylene dibromide were apparently transformed into other bromine compounds and retained in the skin. The edible (flesh) parts of the fruits did not retain significant amounts of ethylene dibromide except in the case of avocado; with the exception of banana there was no evidence of any accumulation of bromide. This different behavior among fruits indicates a necessity for new experiments if other fruits are to be fumigated with ethylene dibromide.

Considerable average deviations from the mean are to be expected in this type of analysis, as fruits pick up different amounts of ethylene dibromide in a chamber, depending on ripeness, injury, genetics, and nutritional and climatic conditions during growth.

Literature Cited

(1) Alicino, J. F., Crickenberger, A., and Reynolds, B., *Anal. Chem.*, **21,** 755 (1949).

(2) Balock, J. W., *Science*, **114,** 122 (1951).

(3) Balock, J. W., and Lindgren, D. L., *J. Econ. Entomol.*, **44,** 657 (1951).

(4) Bernoulli, A. L., and Kambli, W., *Helv. Chim. Acta*, **16,** 1187 (1933).

(5) Hanson, W. J., *Advances in Chem. Ser.*, No. **1,** 202 (1950).

(6) Meulen, J. H. van der, *Chem. Weekblad.*, **28,** 82 (1931).

(7) *Ibid.*, **31,** 333 (1934).

(8) Shrader, S. A., Beshgetoor, A. W., and Stenger, V. A., *Ind. Eng. Chem., Anal. Ed.*, **14,** 1 (1942).

(9) Wheland, G. W., "The Theory of Resonance and Its Application to Organic Chemistry," p. 288, New York, John Wiley & Sons, 1944.

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