mg, bound 0.1888 mg. The total percentage recoveries were 98.6 and 101.8%. When the residues from 10 g of liver of pigs No. 6 and 7 previously extracted for free gossypol were extracted with hexane without the addition of aniline and the extracts were used as the respective reference solutions for bound gossypol, the total recoveries were 88.4 and 91.2%.

The modification of the original method for total gossypol (4) by substituting hexane for chloroform resulted in bright yellow liver tissue-extracts for bound gossypol from gossypol-consuming pigs. These extracts gave spectral curves characteristic of curves prepared from the conversion of pure gossypol to dianilinogossypol under the conditions of this method. In contrast, chloroform-extracts for bound gossypol at times have a pinkish cast which affects the absorbance values. Some extracts of spleen tissue have a slight pinkish-brown tint even when the extraction is made with hexane. This extraneous material affects the spectral curve slightly and shows a peak at approx 380 mμ.

By these methods, free and bound gossypol have been found and measured in liver, kidney, spleen, heart, lung, pancreas, lymph nodes and diaphragm muscle tissues of swine which have consumed diets containing free gossypol. The methods also have been applied to tissue of rats and goats with positive results.

ACKNOWLEDGMENTS

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Vernonia anthelmintica (L.) Willd. The Effect of Storage on the Epoxy Content of the Seed Oil and Trivernolin[1]

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Abstract

Accessions of *Vernonia anthelmintica* (L.) Willd. seed from India and Pakistan varied somewhat in the amt of oil that they contained, but the oils did not vary significantly in their epoxy content. Storage of the whole seed for periods up to three years did not affect the quality of the oil, but the activity of the seed enzyme system seemed to increase with time. The data also show a rapid development of free fatty acid once the seed is ground. The epoxy content of low FFA *Vernonia* oil and trivernolin changed only slightly when these products were stored at room temp for six months. However, the viscosity of the samples that were exposed to light increased greatly indicating changes in the physical nature of the products. Similar behavior was exhibited by both products when they were stored under nitrogen at 100C and by trivernolin at 4C.

Introduction

THE PRODUCTS OBTAINED from controlled utilization of the enzyme activity of *Vernonia anthelmintica* (L.) Willd. seed and the methods of deactivating and of activating the enzyme system have been investigated and discussed previously (1,2). In these investigations it was found that the enzyme system was very highly active in crushed or ground seed and produced gross changes in the composition of the oil obtained from such seed. However, it was not known if the enzymes were active during prolonged storage of mature whole seed; if so, lipolytic activity in the whole, uncrushed seed would affect adversely the composition of the oil. Another unknown was the effect that storage had on the epoxy content of *Vernonia* oil and its chief component, trivernolin. This knowledge is essential since these natural products are potential stabilizers and plasticizers of poly(vinyl chloride) (3).

The purpose of this paper is to present the results of the following studies on *V. anthelmintica* seed oil: 1) a comparison of the composition of the oil obtained from several seed accessions; 2) the effect of storage of the whole seed on the composition of the oil; and 3) the effect of storage on the epoxy content of low free fatty acid (FFA) *Vernonia* oil and trivernolin. Also, some observations on the effect of storage of the whole seed on the activity of the seed enzymes were made.

Experimental Procedures

Materials and Methods. Seed used in these studies was collected in India and Pakistan. Some of the seed was supplied by Quentin Jones of the Crops Research Division, ARS, USDA; some was obtained through a commercial seed broker. Consequently, the complete history of the seed is not known.

AOCS procedures were used to obtain analytical data. A 40-hour Soxhlet extraction using a previously described technique (4) was used to determine the amt of oil in the seed. Since this extraction technique degrades the oil, composition analyses were done on oil obtained by the rapid extraction technique (4). Meth-

TABLE I

Comparison of Aanalyses of *V. anthelmintica* Seed Accessions

Origin and date rec'd.	Moisture when rec'd.	Oil in seed (mfb)[a]	Analysis of oil[b]		
			FFA[c]	Oxirane oxygen	I.V. (Wijs)
	%	%	%	%	
India					
Feb. 1960	6.21	23.9	2.0	3.90	105.8
March 1961	7.80	25.5	2.0	3.90	106.3
Aug. 1961	9.20	23.3	2.1	3.95	104.4
July 1962	7.70	22.0	1.6	3.92	104.3
Pakistan					
Sept. 1962	8.04	27.2	2.0	3.95	103.4
June 1963	8.00	26.4	1.9	3.77	106.1

[a] Yield obtained by exhaustive extraction of ground seed. mfb—moisture free basis.
[b] Oil for analyses obtained by rapid extraction technique.
[c] Calc. as epoxyoleic acid.

[1] Presented at the AOCS Meeting, Minneapolis, 1963.
[2] A laboratory of E. Utiliz. Res. & Dev. Div., ARS, USDA.

TABLE II
Effect of Storage of Whole *V. anthelmintica* Seed on Oil Composition

Storage time	Moisture	Oil in seed (mfb)	Analysis of oil		
			FFA[c]	Oxirane oxygen	I.V. (Wijs)
months	%	%	%	%	
6[a]	7.80	24.0	2.0	3.91	106.7
11[a]	7.26	23.8	1.9	3.92	104.2
21[a]	8.16	23.6	1.3	3.89	104.3
12[b]	6.21	23.9	1.9	3.90	105.0
31[b]	7.96	23.2	2.0	3.88	104.9
38[b]	7.50	23.8	2.0	3.95	106.8

[a] Stored at 4C.
[b] Stored at 27C.
[c] Calc. as epoxyoleic acid.

ods similar to those described in our extraction studies (5) were used to obtain the large amt of oil and trivernolin needed for the storage tests.

Storage of Whole Seed. Seed was stored at 4C and at 27C (room temp). At 4C, the canvas bags containing seeds were put in plastic bags to protect the seeds from moisture. At 27C, the seeds were stored in the canvas bags without any other protective covering.

Storage of Low FFA Vernonia Oil and Trivernolin. Low FFA *Vernonia* oil and trivernolin were prepared for storage in the following three ways: 1) Samples were sealed under a N_2 atmosphere; 2) samples were put in wide-mouthed loosely-covered containers that allowed the products to be exposed to the surrounding atmosphere; 3) samples to which a stabilizer, 0.3% *tert*-butylhydroquinone, had been added were also put in wide-mouthed loosely-covered containers. Samples from each of the above three groups were stored under the following four conditions: exposed to light at room temp; not exposed to light at room temp, at 100C, and at 4C.

Results and Discussion

The data in Table I show that the only significant differences among seed accessions were in the moisture content and in the amt of oil in the seed. In general, higher yields of oil were obtained from seed grown in Pakistan than from seed grown in India. The analyses of the oils of the six seed accessions show no significant differences with respect to FFA and oxirane oxygen content and iodine values (I.V.).

Storage of the whole seed at 4C for periods up to 21 months and at 27C for periods up to 38 months did not affect the quantity or quality of the oil as shown in Table II.

Whole seeds were stored at 27C and at 4C for various times. After storage the seeds were ground; some of the ground seed was aged (exposed to atmospheric conditions at room temp) before it was extracted and some was extracted immediately (aged 0 days). The data in Table III show that the FFA did not develop in the intact seed during storage, but developed rapidly in the ground seed that was aged. In fact, this

TABLE III
Effect of Storage of Whole Seed on Enzyme Activity

Whole seed stored		Ground seed aged	FFA[a] in oil
Time	Temp		
months	°C	days	%
0	....	4	14.5
11	4	0	1.6
21	4	0	0.5
21	4	21	49.3
21	27	0	1.3
21	27	4	22.8
21	27	21	47.9
38	27	4	27.4

[a] Calc. as epoxyoleic acid.

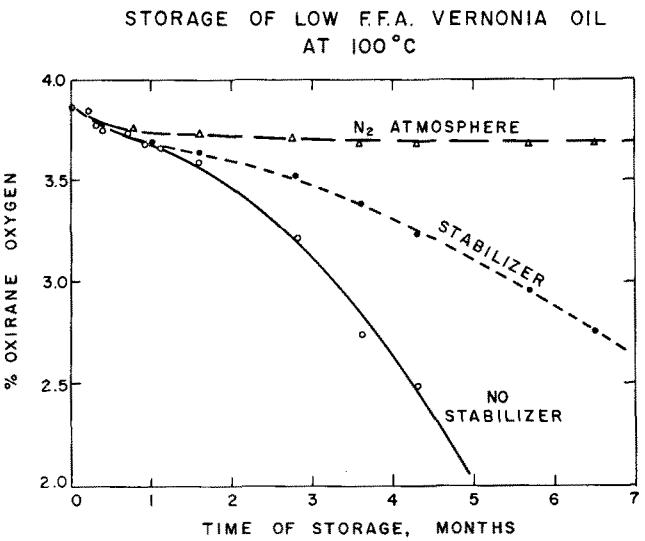

FIG. 1. The effect on the oxirane oxygen content of low FFA *Vernonia* oil stored at 100C in the following ways: 1) sealed in a nitrogen atmosphere; 2) exposed to surrounding atmosphere; 3) stabilizer added, exposed to surrounding atmosphere.

rapid development of FFA and the higher values obtained after the whole seeds were stored for a number of months indicates the possibility that enzyme activity increases with storage time. However, more complete study under carefully controlled conditions of storage, grinding and aging would be necessary to determine if this finding is significant.

The studies to determine the effect of storage on the epoxy content of low FFA *Vernonia* oil and trivernolin showed that the oxirane oxygen content cannot be used as the sole measure of the stability of these products. When low FFA *Vernonia* oil and trivernolin were stored at room temp for six months, the oxirane oxygen content of both of these products decreased a max of 2% from the original 3.82 and 5.04%, respectively. The epoxy content of the samples that were protected from light did not decrease as much as that of the samples that were exposed to light. This would not have been considered relevant because of the very small max loss, but after six months the

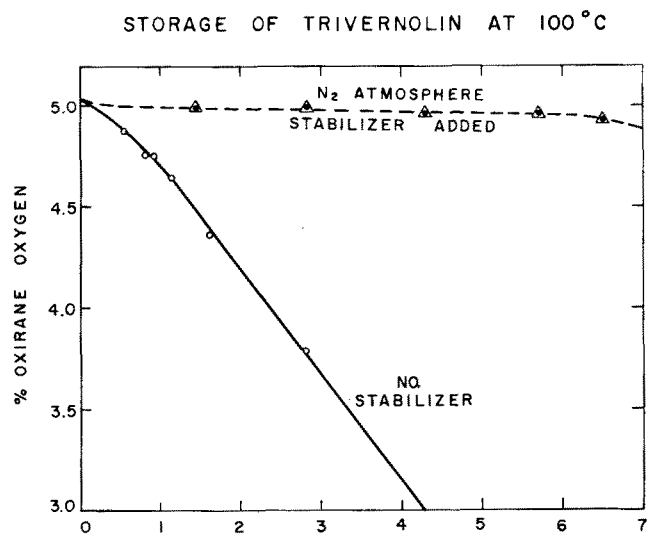

FIG. 2. The effect on the oxirane oxygen content of trivernolin stored at 100C in the following ways: 1) sealed in a nitrogen atmosphere; 2) exposed to surrounding atmosphere; 3) stabilizer added, exposed to surrounding atmosphere.

viscosities of the samples that were exposed to light had more than doubled their original values of 120.7 and 125.0 (at 26C) centistokes, respectively, while those of the oil and trivernolin that were protected from light had increased a max of 27% and 4%, respectively. Also, a nitrogen atmosphere provided the most protection against loss of oxirane oxygen. In fact, the oil samples that were stored under nitrogen retained their original level of epoxy component.

As shown in Figures 1 and 2, storage of low FFA *Vernonia* oil and trivernolin for six months at 100C in a nitrogen atmosphere had little effect on their epoxy contents. Addition of a stabilizer, *tert*-butylhydroquinone (0.3%) also prevented any appreciable loss of epoxy from trivernolin. However, the viscosities of these samples increased greatly in six months to 300.4, 292.1 and 298.0 centistokes, respectively. The oil and the trivernolin that had no stabilizer added were completely polymerized, and the oil that had stabilizer added was too viscous to measure. The oxirane oxygen content of these samples decreased considerably in six months (Figs. 1 and 2).

The epoxy content of low FFA *Vernonia* oil was not affected adversely by storage at 4C for six months and had a max increase in viscosity of 21%. The epoxy content of trivernolin decreased only 2–4%, but the viscosities of the samples stored with and without stabilizer increased to 215.7 and 209.7 centistokes, respectively, while that of the sample stored under nitrogen increased to 168.5.

These results show that under certain conditions of storage *Vernonia* oil and trivernolin undergo changes in their physical nature that are not always indicated by the oxirane oxygen values. On the other hand, a decrease in oxirane oxygen content was accompanied by an increase in viscosity.

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Determination of Refining Loss in Oil of Pistacia Seeds

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Abstract

The laboratory refining loss and the neutral oil content of the crude oil of Pistacia seeds are determined by the Wesson, acetone-insoluble and chromatographic methods and the results are compared.

For a variety of samples with a different free fatty acid content, the refining loss by the chromatographic method is determined and an expression for the relationship between laboratory refining loss and free fatty acid content of Pistacia oil is proposed.

Introduction

THE REFINING EFFICIENCY of a vegetable oil is given by the ratio $\frac{N}{N + N_1}$, where N is the refinery yield of neutral triglycerides calculated as the wt percentage of the crude oil and $N + N_1$ the actual neutral oil, i.e., the actual percentage of neutral triglycerides in the crude oil, determined by analysis. To check refining efficiency, both N and $N + N_1$ are required. From these data, the amt of neutral oil which is lost (N_1) through the saponification and emulcification may be determined.

The refinery yield of neutral oil may be determined by various methods (1–3). On the other hand actual percentage of neutral triglycerides is usually determined by one of three generally recognised methods, Wesson (4–6), acetone-insoluble (7–9) and chromatographic (10–16).

Since the actual results of any of these three methods differ, attempts have been made to establish a correlation between them. Purdum and Werber (17) determined the refining loss of many samples of cotton seed and soybean oil by the Wesson and acetone-insoluble methods and found relationships between them and the cup-test (3); but though the chromatographic method is receiving increasing attention very few attempts have been described to correlate it with other methods.

This work concerns correlation of the refining loss determined by Wesson, acetone-insoluble and chromatographic method. Also the chromatographic refining loss of Pistacia seed oil is compared with its free fatty acid (FFA) content.

Since *Pistacia lentiscus L.* and *Terebinthus L.* are abundant in Greece, the oil from their seeds has multiple uses. There is no technological research reported on this oil in the literature, therefore this oil was chosen for the above determination and correlation.

Procedures

The Refining Loss

To check the refining loss, five different kinds of Greek Pistacia oils were analysed. For each one, various specimens were examined and average values for phosphatides, FFA, moisture and volatile matter, and neutral oil were determined.

1. *Acetone-Insoluble Method.* The sum of moisture and volatile matter, FFA and phosphatides give the laboratory refining loss according to this method.

TABLE I

Neutral Oil by the Acetone-Insoluble Method

Sample No.	% FFA	% Most. and vol. m.	% Phosph.	Total % ref. loss A	% Neutral oil
1	6.31	0.12	0.03	6.46	93.54
2	6.88	0.17	0.05	7.10	92.90
3	19.02	0	0.06	19.08	80.92
4	3.32	0.46	0.05	3.83	96.17
5	5.60	1.77	0.05	7.42	92.58

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