Storage of Cottonseed and Peanuts Under Conditions Which Minimize Spectrophotometric Changes in the Extracted Oil

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IN a previous paper (1) it was shown that cottonseed and peanuts could be stored without change in the free fatty acid content of the oil by adjusting the moisture content to 8% or less and placing the seed in a closed container at 1° or —18°C. In the past the development of free fatty acids and loss of germination have been almost the only indexes used for following the deterioration of seeds in storage. The work to be reported has been directed toward the establishment of other indexes of storage deterioration and toward the further verification of previous recommendations (1) for the storage of experimental samples of cottonseed and peanuts.

Since it seemed likely that the development of the red gossypol of Podolskaja (2) would serve as a useful index of storage changes in cottonseed, this was investigated. Podolskaja has shown that this red pigment increases during the storage of cottonseed and the present work demonstrates its usefulness in cottonseed storage investigations. According to Boatner (6), the red gossypol preparations of Podolskaja were mixtures of gossypol and a purple pigment which Boatner purified and named gossypurpurin. The term red gossypol is used in this paper as a convenient name for a substance or substances which exhibit absorption maxima in chloroform at 528 and 565 mμ, with no intention of implying a relationship to gossypol.

It was also found that an increase in the absorption of the extracted oil in the region of diene conjugation, 236 mμ, is another index of storage change. Since it was found that gossypol has its strongest absorption at 236 mμ, it was necessary to determine the spectrophotometric characteristics of this cottonseed pigment so that the nongossypol absorption at 236 mμ could be calculated. This increased absorption at about 236 mμ is not limited to cottonseed since the extracted oil from peanuts stored at room temperature for over four years also showed a much higher absorption in this region than that from peanuts stored for the same period at 1° or —18°C. The increase in this nongossypol absorption at 236 mμ is, most likely, a result of the development of diene conjugated systems within the unsaturated fatty acid molecules of the oil, by isomerization of the linoleic acid or by peroxidation and probably accompanying dehydration of the oleic acid.

Description of Cottonseed and Peanuts

The varieties of cottonseed used and the moisture contents at the time of storage are given in Table I. Lots 1 to 10, from the U. S. Cotton Field Station at Greenville, Texas, were from cotton picked the day the boll opened. The seed cotton was dried indoors before ginning. Lots 1 to 5 were from early opening bolls and lots 6 to 10 from late opening bolls collected 10 days later. Lots 11 to 16, cottonseed grown at Stoneville, Mississippi, were from the 1945 crop and were shipped to this laboratory as soon as possible after picking.

Lots 17 and 18 were peanuts in the shell from the Georgia Agricultural Experiment Station. The varieties were Tennessee Red and Spanish, respectively. Some storage experiments on these lots of peanuts have been reported previously (1).

TABLE I

Variety of Cottonseed and Moisture Content of Seed at Time of Storage

Lot No.	Variety	Moisture
		%
1	Deltapine 14-060	9.4
2	Station C-42	9.5
3	Acala W-29-1	8.9
4	AOL 16-5-8	9.0
5	Mebane Buckellew	9.1
6	Deltapine 14-060	4.6
7	Station C-42	4.3
8	Acala W-29-1	5.1
9	AOL 16-5-8	4.8
10	Mebane Buckellew	5.0
11	Bobshaw 1	7.0
12	Coker 100-9	6.6
13	Delfos 531 C	6.4
14	Deltapine 14-060	7.1
15	Stoneville 2B	6.8
16	Wilds 17	5.5

Methods

In order to investigate the development of red gossypol during storage a chloroform extraction was used since red gossypol is relatively stable in this solvent. However, chloroform extracts could not be used for measurements in the far ultraviolet where chloroform absorbs, and optically pure cyclohexane extracts were used for measurements in this region. In some preliminary work Soxhlet extractions were made and the extracted oil dissolved in cyclohexane prior to spectrophotometric measurements.

Chloroform extraction. The cottonseed was cracked in a Bauer mill without drying or preheating and was screened to separate the meats. The meats were ground in a small Wiley mill using a 20-mesh screen. Immediately after grinding, a 2-g. or 5-g. sample of the ground meats was placed in a Waring Blendor with 30 ml. of chloroform and blended for 15 minutes. The material adhering to the sides was washed down three times with more chloroform during the extraction. The slurry was washed into a 100-ml. wide-mouth volumetric flask, adjusted to room temperature in a beaker of water, made to volume with chloroform, and filtered through a coarse filter paper, rejecting the first portion of the filtrate. The cloudy filtrate was refiltered. A portion of the filtrate, 20 ml., was pipetted into a tared flask, the chloroform evaporated on a steam bath, the weight of the oil determined and calculated to oil concentration in grams per liter. Spectrophotometric determinations of the optical densities at various wave lengths were made on the extracts with a Beckman spectrophotometer using a 1-cm. cell. The data were calculated to

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extinction coefficients based on the oil concentration of the extracts in grams per liter and a 1-cm. cell length. The extractions and spectrophotometric measurements were made on a strict time schedule.

Cyclohexane extraction. This procedure was identical with the above chloroform extraction except that optically pure cyclohexane replaced the chloroform.

Soxhlet extraction with ethyl ether or with low-boiling petroleum ether. The cottonseed meats or peanut kernels were ground in a food cutter provided with a 19-tooth blade. Immediately after grinding, a 10-g. sample was weighed into a paper thimble and extracted in a medium-sized Soxhlet extractor for four hours with peroxide-free ethyl ether or with low-boiling petroleum ether (A.O.C.S. Specification H-2-41). The material was removed from the thimble and ground to a fine powder in a mortar. It was then returned to the original thimble and the extraction continued for an additional 16 hours. Most of the solvent was evaporated on a steam bath and the last traces were removed in a vacuum desiccator. A 1-g. sample of the extracted oil was weighed into a 25-ml. beaker, dissolved in optically pure cyclohexane, filtered into a 25-ml. volumetric flask, the paper was washed with cyclohexane, and the filtrate made to volume. This solution, which had a concentration of 40 g. of oil per liter, was used for spectrophotometric measurements in the near ultraviolet and in the visible regions. A 0.1-g. sample of the oil was treated in the same way to give a solution having a concentration of 4 g. per liter. Portions of this were diluted quantitatively to give solutions having concentrations of 0.4 g. per liter and 0.04 g. per liter. These were used for spectrophotometric measurements in the far ultraviolet.

Preparation of Pure Gossypol

In order to calculate the nongossypol absorption of the cyclohexane extracts of cottonseed, it was necessary to know the spectrophotometric characteristics of pure gossypol in cyclohexane. Pure gossypol was prepared by the following procedure from cottonseed pigment glands separated by the flotation process (3, 4).

Cottonseed pigment glands, 250 g., were extracted with 1 liter of acetone for three hours on a mechanical shaker. After filtration through a hardened paper, the volume of the filtrate was reduced to 600 ml. under reduced pressure. The filtrate was mixed with 600 ml. of glacial acetic acid and the mixture allowed to stand overnight. The precipitate was separated by filtration, washed by suspension in low-boiling petroleum ether, and again separated by filtration. Its weight was 101 g. This crude gossypol acetic acid was dissolved in 800 ml. of acetone, filtered, and 400 ml. of glacial acetic acid was added with stirring. After 2 hours the precipitate was separated by filtration, washed by suspension in low-boiling petroleum ether, and refiltered. The precipitate was again dissolved in acetone and again precipitated with acetic acid in the same way. The weight of the precipitate was 91 g.

The gossypol acetic acid was converted to free gossypol by suspending it in 600 ml. of peroxide-free ethyl ether and pouring it into a large evaporating dish containing 500 ml. of water and a small amount of sodium hydrosulfite (5). The ether was removed on the steam bath, while stirring with a thermometer, care being taken that the temperature of the water did not exceed 50°C. After the ether was removed, the lumps of free gossypol were crushed with a pestle, transferred to a Büchner funnel, and washed with water. The weight of material was 87 g.

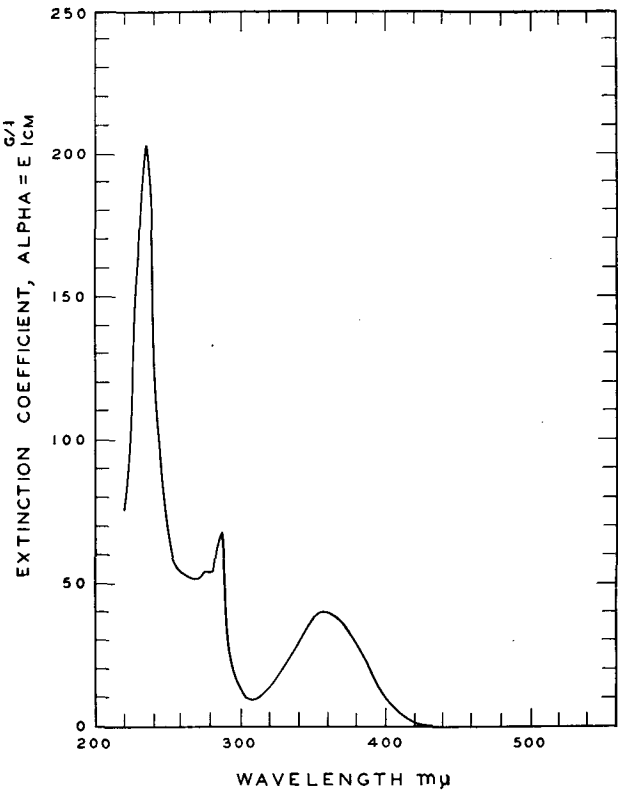

FIG. 1. Ultraviolet absorption spectrum of gossypol in cyclohexane.

The crude gossypol was dissolved in 800 ml. of peroxide-free ether, an equal volume of low-boiling petroleum ether was added, and the solution concentrated under reduced pressure until crystallization began. One-half volume of low-boiling petroleum ether was then added, and after one hour the precipitate was separated by filtration. A second crop of material was obtained by evaporation to 500 ml. under reduced pressure. This second crop was combined with the first, dissolved in ether, and precipitation by the addition of low-boiling petroleum ether was carried out two more times. The weight of material was now 58 g.

To effect further purification, the material was dissolved in ethyl ether, converted to gossypol acetic acid, brought back to free gossypol, and recrystallized three times from a mixture of ethyl ether and petroleum ether in a manner similar to that given above. It was then dissolved in acetone and precipitated as gossypol acetic acid. The gossypol acetic acid was dissolved in ethyl ether and converted to free gossypol in the manner described. The pure gossypol was dried in a vacuum oven at 50°C. for four hours and placed overnight in a vacuum desiccator over phosphorus pentoxide. The yield was 33 g.

The analysis (micro) of this material gave the following values:

Carbon 69.0, hydrogen 5.9. Calculated for $C_{30}H_{30}O_8$: carbon 69.5, hydrogen 5.8. The nitrogen content by Kjeldahl digestion and Nesslerization was 0.05%.

A spectrophotometric curve made on this pure gossypol in cyclohexane is shown in Fig. 1. Spectrophotometric data to establish the extinction coefficients [2] at the three maxima are given in Table II. For comparison, data on a preparation made by the method of F. H. Smith (5), on a preparation supplied by F. H. Smith, and on two preparations supplied by Roger Adams, are given. The close agreement of the spectrophotometric data for all these preparations is good evidence for the purity of all of them. The agreement in absorption characteristics of freshly prepared and old samples is evidence that gossypol is reasonably stable when protected from light under ordinary laboratory conditions.

Calculation of Nongossypol Absorption at 236 mμ

If we assume that practically all of the absorption of the extracts of cottonseed in cyclohexane at 358 mμ or at 286 mμ is due to gossypol, it is possible to calculate the absorption due to gossypol at 236 mμ by use of the appropriate factors and obtain the nongossypol absorption at 236 mμ by difference. Although it is realized that the values so obtained are somewhat empirical, their relative values are valid and serve as a good index of change in storage. The data reported in Table II for the absorption of gossypol in cyclohexane show that the ratio of extinction at 236 mμ to that at 286 mμ is 2.96. However, 2.9 has been used in the calculations in order to avoid negative values for nongossypol absorption. It is likely that a very small part of the absorption at 286 mμ is due to substances other than gossypol; so the calculated gossypol absorption at 236 mμ based on the value at 286 mμ is likely to be higher than the true absorption. The ratio of extinction at 236 mμ to that at 358 mμ averages 5.19; so for purposes of calculation the value 5.2 has been used to obtain the gossypol absorption at 236 mμ from the absorption at 358 mμ. Thus two formulas have been used to calculate the nongossypol absorption at 236 mμ.

1. Nongossypol absorption at 236 $m\mu = a_{236} - 2.9\ a_{286}$

2. Nongossypol absorption at 236 $m\mu = a_{236} - 5.2\ a_{358}$

a=extinction coefficient (concentration in g. per liter, solution depth 1 cm.).

[2] All quantitative values of absorption referred to in this paper are given as extinction coefficient (alpha) defined as $\text{Log } \frac{I_0}{I}/\text{cl.}$ numerically equal to the expression $E^{\text{g./l.}}_{\text{1 cm.}}$ where I_0 and I are the intensities of the incident and transmitted light respectively, c is the concentration in grams per liter, and l is the cell length in centimeters.

Results with Cottonseed

Considerable preliminary work, not to be reported in detail, was done by Soxhlet extraction with peroxide-free ethyl ether or low-boiling petroleum ether. The nongossypol absorption values at 236 mμ obtained by these methods indicated that storage at low temperatures retarded or prevented the increase in nongossypol absorption. However, the values were quite variable and the data contained a number of inconsistencies. Therefore, only values for nongossypol absorption at 236 mμ obtained by the cyclohexane method are given since this method gives more reproducible and consistent results. However, some of the ethyl ether extraction values are of interest in relation to the gossypol content of the seed since this method has been used for the quantitative extraction of gossypol. Table III shows the absorption values

TABLE III

Spectrophotometric Characteristics of Ethyl Ether Extracts of Cottonseed Stored at Two Different Temperatures

Lot No.	Alpha 560 mμ			Alpha 358 mμ		
	Orig.	14 Months		Orig.	14 Months	
		27°C.	1°C.		27°C.	1°C.
1	0.002	0.021	0.001	0.91	0.77	0.88
2	0.001	0.017	0.001	0.91	0.85	0.96
3	0.001	0.017	0.001	0.83	0.70	0.86
4	0.001	0.024	0.001	0.98	0.97	0.96
5	0.001	0.015	0.001	1.10	0.90	1.11
Average	0.001	0.019	0.001	0.95	0.84	0.95
6	0.003	0.002	0.001	0.91	0.97	1.02
7	0.002	0.002	0.001	0.88	0.88	0.87
8	0.001	0.002	0.001	0.92	0.86	0.91
9	0.002	0.002	0.001	1.03	0.99	0.96
10	0.002	0.003	0.001	1.05	0.97	1.00
Average	0.002	0.002	0.001	0.96	0.93	0.95

at 358 mμ, one of the maxima of gossypol. These indicate that little or no change in gossypol content of the cottonseed took place during storage for 14 months at 27° or at 1°C. Table III also shows that an increase in absorption of the ethyl ether extracted oil at 560 mμ occurred in lots 1 to 5 stored at 27°C. These lots, from the early opening bolls, had a moisture content of about 9% when stored. This increase in absorption at 560 mμ is probably related to the increase in red gossypol, which is better demonstrated by chloroform extraction. This increase in absorption at 560 mμ did not occur in seeds stored at 1°C., nor did it occur in lots 6 to 10, the late opening bolls of the series, which had a moisture content of about 5% when stored.

The spectrophotometric characteristics of the chloroform extracts for lots 1 to 10 are shown in Table

TABLE II

Spectrophotometric Characteristics of Various Preparations of Pure Gossypol in Cyclohexane

Description of Preparation	Alpha			Ratios		
	236 mμ	286 mμ	358 mμ	236 mμ / 358 mμ	236 mμ / 286 mμ	286 mμ / 358 mμ
As described in this paper	211.3	72.8	40.2	5.26	2.90	1.81
As described in this paper	211.1	72.6	39.6	5.33	2.91	1.83
As described in this paper	207.5	70.1	39.8	5.21	2.96	1.76
As described in this paper	216.3	72.4	39.8	5.43	2.99	1.82
As described in this paper	217.5	72.9	40.4	5.38	2.99	1.80
As described in this paper	203.6	68.2	40.0	5.09	2.99	1.71
As described in this paper	193.8	65.5	39.7	4.88	2.96	1.65
As described in this paper	193.8	65.2	39.5	4.91	2.97	1.65
As described in this paper	210.3	70.5	40.1	5.24	2.98	1.76
Average	207.2	70.0	39.9	5.19	2.96	1.75
As described by F. H. Smith	207.3	70.6	39.8	5.21	2.94	1.77
From F. H. Smith	204.6	69.2	39.2	5.22	2.96	1.77
From Roger Adams; m.p. 199°	203.0	67.9	39.1	5.19	2.99	1.74
From Roger Adams; m.p. 214°	209.1	69.8	39.7	5.27	3.00	1.76

IV. A definite increase in red gossypol, as indicated by absorption at 565 mμ or at 528 mμ, took place at 27°C. This increase occurred in all 10 lots of seed at 27°C. but was much larger in the seed from the early opening bolls, which had a rather high moisture content. No significant change in the red gossypol absorption took place in the seed stored at 1°C. The absorption values at 365 mμ do not indicate any very large change in the gossypol content of the seed under either of the storage conditions.

Extensive data were obtained on lots 11 to 16 which were stored at 27°, 1°, and —18°C. and analyzed at intervals of 5, 7, and 18 months. Complete spectrophotometric curves were made on the chloroform extracts of lot 11. Some of these are shown in Fig. 2 to illustrate the large increase in red gossypol, absorption maxima at 565 mμ and 528 mμ, which took place during storage for 18 months at 27°C. Fig. 2 also shows that very little change in red gossypol took place in the samples stored at 1° and at —18°C. This is borne out by the data in Table V. A significant increase in absorption at 565 mμ and at 528 mμ is evident after five months at 27°C. and is quite large after 18 months at this temperature. It is worthy of note that lot 16, which showed the smallest increase in red gossypol at 27°C., had the lowest moisture content at time of storage (Table I). No significant change can be demonstrated at 1° or at —18°C. The data for the absorption of the chloroform extracts at 365 mμ are indicative of little or no change in the amount of gossypol.

Table VI shows Evelyn colorimeter measurements in the region of red gossypol absorption of independent chloroform extracts of lots 11 to 16. They are entirely in agreement with the spectrophotometric measurements and show that a photoelectric colorimeter can be used to follow the development of red gossypol during storage of cottonseed.

Fig. 3 shows spectrophotometric curves on the cold cyclohexane extract of lot 11 at the time of storage and after 18 months at 1° and at —18°C. An increase in absorption at 236 mμ is evident after storage at 27°C. That this change is real and significant is brought out by Tables VII and VIII. Table VII gives the spectrophotometric absorption characteristics of the cyclohexane extracts of lots 11 to 16. Although an increase in absorption at 236 mμ is evident in storage at 27°C., such a large part of the 236 mμ absorption is due to gossypol, variable amounts of which may be extracted by the cyclohexane, it is best

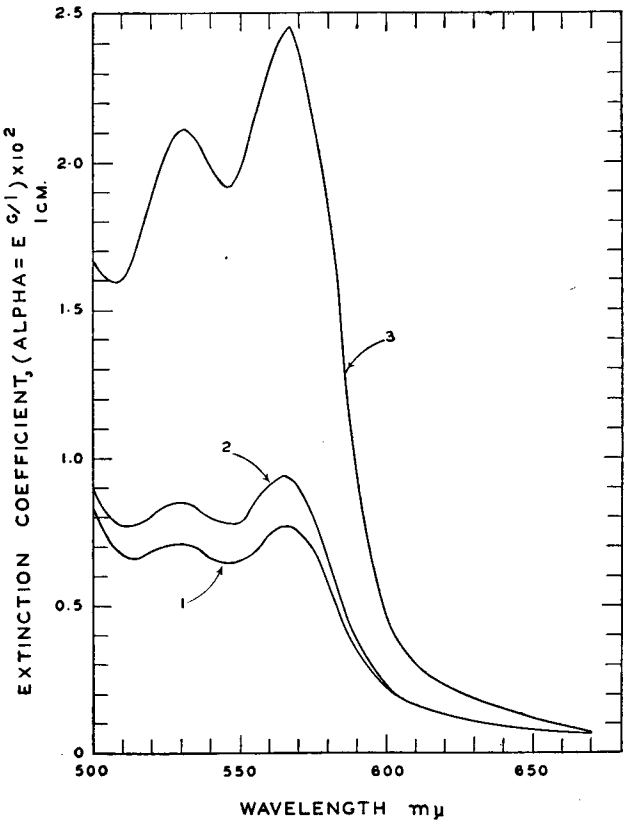

FIG. 2. Absorption spectra of chloroform extracts of lot 11 cottonseed.
Curve 1. Original seed.
Curve 2. After storage for 18 months at 1° and —18°C.
Curve 3. After storage for 18 months at 27°C.

to calculate the nongossypol absorption by the formulas given earlier in this paper and base the conclusions on these calculations. Table VIII shows the nongossypol absorption values at 236 mμ. A significant increase in the nongossypol absorption at 236 mμ, the region of diene conjugation, is evident after five months at 27°C. This increase is much greater after 18 months. No significant increase in the nongossypol absorption at 236 mμ took place at either 1° or —18°C. However, the average value at 1°C. tends to be a little higher after 18 months than the original value. It would probably be best, therefore, to store cottonseed at —18°C. if the storage period was long and one wished to be sure that no change took place in the nongossypol absorption of the extracted oil at 236 mμ.

TABLE IV

Spectrophotometric Characteristics of Chloroform Extracts of Cottonseed Stored at Two Different Temperatures

Lot No.	Alpha 565 mμ			Alpha 528 mμ			Alpha 365 mμ		
	Orig.	14 Months		Orig.	14 Months		Orig.	14 Months	
		27°C.	1°C.		27°C.	1°C.		27°C.	1°C.
1	0.006	0.014	0.005	0.006	0.013	0.006	0.82	0.96	0.94
2	.....*	0.018	0.006	.....*	0.016	0.006	.....*	1.01	0.98
3	0.006	0.023	0.007	0.006	0.019	0.006	0.82	0.84	0.86
4	0.005	0.017	0.005	0.005	0.016	0.005	0.93	0.99	0.97
5	0.005	0.018	0.004	0.006	0.015	0.005	1.01	1.12	1.15
Average	0.006	0.018	0.005	0.006	0.016	0.006	0.90	0.98	0.98
6	0.003	0.006	0.005	0.004	0.006	0.006	1.04	0.64	0.70
7	0.005	0.006	0.005	0.005	0.006	0.005	0.94	0.53	0.63
8	0.004	0.008	0.006	0.004	0.008	0.006	0.99	0.81	0.79
9	0.006	0.009	0.006	0.007	0.009	0.005	1.01	0.83	0.78
10	0.005	0.011	0.008	0.005	0.010	0.008	1.10	0.96	0.81
Average	0.005	0.008	0.006	0.005	0.008	0.006	1.02	0.75	0.74

* Oil concentration not obtained due to accident.

TABLE V

Spectrophotometric Characteristics of Chloroform Extracts of Cottonseed Stored for Various Times at Different Temperatures.

Lot No.	Orig. Sample	5 Months		7 Months			18 Months		
		27°C.	−18°C.	27°C.	1°C.	−18°C.	27°C.	1°C.	−18°C.
Alpha 565 mμ									
11	0.008	0.012	0.009	0.013	0.009	0.010	0.025	0.009	0.009
12	0.011	0.013	0.012	0.013	0.010	0.010	0.018	0.011	0.010
13	0.007	0.011	0.010	0.012	0.009	0.008	0.018	0.009	0.008
14	0.009	0.012	0.010	0.010	0.008	0.008	0.021	0.009	0.009
15	0.009	0.014	0.011	0.015	0.010	0.010	0.025	0.011	0.010
16	0.005	0.008	0.005	0.005	0.005	0.004	0.008	0.005	0.005
Average	0.008	0.012	0.010	0.011	0.009	0.008	0.019	0.009	0.009
Alpha 528 mμ									
11	0.007	0.011	0.008	0.012	0.008	0.009	0.021	0.009	0.009
12	0.010	0.012	0.010	0.012	0.009	0.009	0.016	0.010	0.009
13	0.007	0.010	0.009	0.010	0.009	0.007	0.015	0.008	0.007
14	0.009	0.010	0.009	0.009	0.008	0.008	0.018	0.008	0.008
15	0.008	0.012	0.009	0.012	0.009	0.009	0.021	0.010	0.009
16	0.005	0.008	0.005	0.005	0.005	0.005	0.008	0.005	0.005
Average	0.008	0.011	0.008	0.010	0.008	0.008	0.017	0.008	0.008
Alpha 365 mμ									
11	1.05	1.16	1.22	1.13	1.13	1.08	1.21	1.20	1.25
12	1.01	1.08	1.18	1.06	1.12	1.11	1.16	1.21	1.16
13	0.71	0.78	0.88	0.88	0.79	0.87	0.84	0.90	0.88
14	1.11	1.08	1.02	1.04	1.06	1.02	1.11	1.09	1.10
15	0.90	1.06	1.04	1.04	0.99	0.97	1.04	1.03	0.98
16	0.59	1.04	0.67	0.65	0.70	0.61	0.79	0.78	0.85
Average	0.90	1.03	1.00	0.97	0.97	0.94	1.03	1.04	1.04

Results with Peanuts

In order to see whether the increase in absorption at about 236 mμ took place in some oil seed other than cottonseed, two lots of peanuts that had been in storage over four years were investigated. It was found that the maximum for extinction in this region was 227 to 234 mμ in the peanut extracts. The interpretation of the data was much simpler than with cottonseed since gossypol was absent. The data in Table IX show that the absorption in the region of diene conjugation is much higher in the oil extracted from the peanuts stored at 27°C. than from those stored at 1° or −18°C. Unfortunately, no original value was available for these samples.

Preliminary experiments on the stability of the oil from these peanuts indicated that the oil from peanuts stored at 27°C. for over 4 years was much less stable than that from peanuts stored at the lower temperatures. The oils all had low peroxide values at the start of the stability experiments.

TABLE VI

Evelyn Colorimeter Measurements of Chloroform Extracts of Cottonseed Stored at Different Temperatures

Lot No.	"E" Values with 565 Filter						
	Orig.	7 Months			18 Months		
		27°C.	1°C.	−18°C.	27°C.	1°C.	−18°C.
11	0.010	0.014	0.010	0.011	0.030	0.014	0.011
12	0.013	0.015	0.012	0.012	0.023	0.014	0.014
13	0.009	0.013	0.010	0.009	0.019	0.011	0.010
14	0.010	0.014	0.011	0.009	0.025	0.011	0.010
15	0.011	0.016	0.012	0.012	0.026	0.012	0.012
16	0.005	0.007	0.005	0.006	0.009	0.006	0.006
Av.	0.010	0.013	0.010	0.010	0.022	0.011	0.011

The peanuts stored at 27°C. for over four years were not viable while those stored at 1° and −18°C. germinated 100%. The testa of the kernels of the

TABLE VII

Spectrophotometric Characteristics of Cyclohexane Extracts of Cottonseed Stored for Various Times at Different Temperatures.

Lot No.	Orig. Sample	5 Months		7 Months			18 Months		
		27°C.	−18°C.	27°C.	1°C.	−18°C.	27°C.	1°C.	−18°C.
Alpha 236 mμ									
11	2.04	1.82	1.81	1.91	2.12*	1.80	2.75	2.23	2.26
12	1.94	2.00	1.93	1.90	1.94	2.03	2.73	2.48	2.37
13	1.16	1.44	1.09	1.30	1.29	1.17	1.75	1.49	1.43
14	1.19	2.12	1.41	1.93	1.44	1.48	2.41	1.81	1.86
15	1.19	1.77	1.62	1.75	1.73	1.56	2.48	1.92	1.84
16	1.41	0.83	1.21	1.43	1.16	1.32	1.84	1.63	1.58
Average	1.48	1.66	1.51	1.70	1.61	1.56	2.33	1.93	1.89
Alpha 286 mμ									
11	0.68	0.55	0.61	0.61	0.66*	0.58	0.73	0.74	0.73
12	0.67	0.63	0.64	0.64	0.65	0.65	0.80	0.79	0.76
13	0.41	0.44	0.34	0.42	0.45	0.43	0.48	0.47	0.49
14	0.38	0.66	0.47	0.56	0.46	0.49	0.63	0.61	0.62
15	0.44	0.52	0.53	0.49	0.58	0.53	0.66	0.62	0.64
16	0.46	0.23	0.40	0.44	0.39	0.43	0.53	0.54	0.54
Average	0.51	0.51	0.50	0.53	0.53	0.52	0.64	0.63	0.63
Alpha 358 mμ									
11	0.36	0.28	0.32	0.32	0.36*	0.30	0.37	0.39	0.40
12	0.36	0.33	0.34	0.33	0.34	0.34	0.42	0.43	0.42
13	0.22	0.23	0.18	0.21	0.23	0.22	0.24	0.24	0.26
14	0.19	0.34	0.24	0.29	0.24	0.26	0.32	0.32	0.33
15	0.22	0.27	0.27	0.26	0.30	0.28	0.33	0.33	0.33
16	0.25	0.10	0.21	0.23	0.21	0.22	0.27	0.29	0.29
Average	0.27	0.26	0.26	0.27	0.28	0.27	0.33	0.33	0.34

* These values obtained after eight months, since the values at seven months were in error.

TABLE VIII

Nongossypol Absorption of Cyclohexane Extracts of Cottonseed Stored for Various Times at Different Temperatures

Lot No.	Orig. Sample	5 Months		7 Months			18 Months		
		27°C.	−18°C.	27°C.	1°C.	−18°C.	27°C.	1°C.	−18°C.
Nongossypol Absorption at 236 mμ Calculated from Formula 1									
11	0.07	0.22	0.04	0.14	0.21*	0.12	0.63	0.08	0.14
12	0.00	0.17	0.07	0.04	0.05	0.14	0.41	0.19	0.17
13	−0.03	0.16	0.10	0.08	−0.02	−0.08	0.36	0.13	0.01
14	0.09	0.21	0.05	0.31	0.11	0.06	0.58	0.04	0.06
15	−0.09	0.26	0.08	0.33	0.05	0.02	0.57	0.12	−0.02
16	0.08	0.16	0.05	0.15	0.03	0.07	0.30	0.06	0.01
Average	0.02	0.20	0.07	0.18	0.06	0.06	0.48	0.10	0.06
Nongossypol Absorption at 236 mμ Calculated from Formula 2									
11	0.17	0.36	0.15	0.25	0.25*	0.24	0.83	0.20	0.18
12	0.07	0.28	0.16	0.18	0.17	0.26	0.55	0.24	0.19
13	0.02	0.24	0.15	0.21	0.09	0.03	0.50	0.24	0.08
14	0.20	0.35	0.16	0.42	0.19	0.13	0.75	0.15	0.14
15	0.05	0.37	0.22	0.40	0.17	0.10	0.76	0.20	0.12
16	0.11	0.31	0.12	0.23	0.07	0.18	0.44	0.12	0.07
Average	0.10	0.32	0.16	0.28	0.16	0.16	0.64	0.19	0.13

* These values obtained after eight months, since the values at seven months were in error.

peanuts stored at 27°C. were much darker than the testa of those stored at the lower temperatures.

TABLE IX

Summary of Spectrophotometric Absorption of Oil Extracted from Peanuts Stored at Different Temperatures

Lot No.	Method of Extraction	Alpha 227 mμ - 234 mμ Storage Time 4⅓ Years		
		27°C.	1°C.	−18°C.
17	Ethyl Ether—Soxhlet	1.01	0.48	0.34
	Petroleum Ether—Soxhlet	0.92	0.41	0.32
	Cyclohexane—Cold	0.88	0.52	0.33
18	Ethyl Ether—Soxhlet	1.25	0.43	0.33
	Petroleum Ether—Soxhlet	1.21	0.47	0.31
	Cyclohexane—Cold	1.27	0.40	0.33
Average		1.09	0.45	0.33

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Summary

1. Cottonseed was stored for over a year without appreciable change in red gossypol content by adjusting the moisture content to 8% or less and placing in closed containers at 1° or at −18°C. Cottonseed stored at room temperature, 27°C., showed a large increase in red gossypol content.

2. Cottonseed stored in the above manner at 1° or at −18°C. showed little or no increase in the nongossypol absorption of the extracted oil at 236 mμ, the region of diene conjugation. This property of the extracted oil showed a large increase when cottonseed was stored at room temperature, 27°C.

3. Gossypol dissolved in cyclohexane has spectrophotometric extinction coefficients (concentration in g. per liter, solution depth 1 cm.) of 207, 70, and 40 at the maxima at 236 mμ, 286 mμ, and 358 mμ, respectively.

4. The extracted oil from peanuts stored for over four years at room temperature, 27°C., showed a much greater absorption in the region of 227 mμ to 234 mμ than did peanuts stored for the same time at 1° or at −18°C.

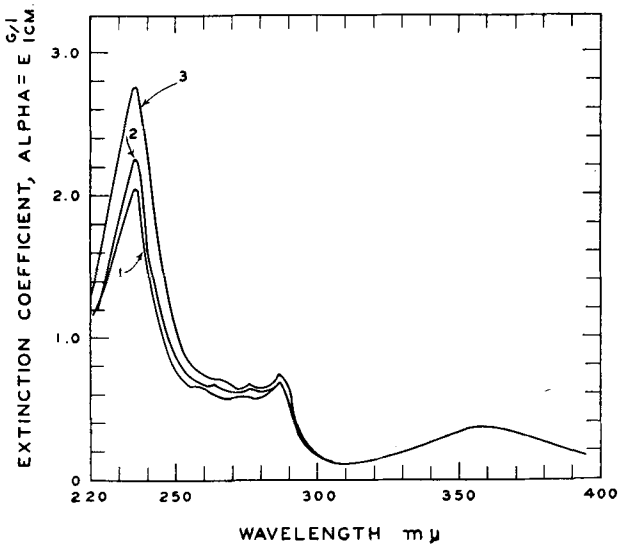

FIG. 3. Absorption spectra of cyclohexane extracts of lot 11 cottonseed.

Curve 1. Original seed.
Curve 2. After storage for 18 months at 1° and −18°C.
Curve 3. After storage for 18 months at 27°C.

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