VARIATIONS IN THE GOSSYPOL AND OIL CONTENT OF COTTONSEED

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GOSSYPOL is a normal fat-soluble constituent of the cotton seed. Its importance in the technology of cottonseed oil has been discussed in a recent paper by Royce and Lindsay (5). These investigators have shown that the alkali refining loss of certain grades of crude cottonseed oil may be reduced 1 to 3 per cent by the addition of fractional percentage amounts of gossypol. This property of gossypol which produces a good break in the refining process is attributed to the reaction of gossypol with naturally occurring protein degradation products which act as emulsifying agents. From this viewpoint gossypol may be considered a valuable constituent of crude cottonseed oil.

It was in connection with an investigation of the nutritive value of the press cake as a stock feed that a study was made of factors which affect the gossypol content of cottonseed meal. The results of a study dealing with heat and moisture as essential factors in the rapid destruction of gossypol in the seeds and meal have previously been published (3). Obviously the total gossypol content of crude cottonseed oil and meal will depend primarily upon the amount contained in the original seeds whereas a distribution of the gossypol between these two products is influenced largely by the methods employed in recovering the oil. Heating the seeds previous to the expression of the oil promotes destruction of gossypol and decreases its solubility in oil.

In studies of the quantitative variation of gossypol and oil in cotton seeds, Schwartze and Alsberg (6) found that cotton seeds varied in their gossypol content by as much as 300 per cent. An annual variation of 200 per cent was found in seeds of a single variety which indicated that in the development of gossypol factors other than those of a varietal character were influential. It was also observed that the seeds which showed a high gossypol content were usually high in oil. Since seed of high oil content usually follows seasons of heavy rainfall (1), it seems reasonable to believe that weather conditions during growth may likewise influence the development of gossypol.

The present investigation was undertaken to determine the extent to which the quantity of gossypol in cotton seeds may be influenced by factors pertaining to the nutrition of the plant. In view of the economic importance of high oil bearing seeds it appeared desirable to determine the oil content as well as the gossypol content of the seeds and to determine whether or not changes in one constituent were accompanied by changes in the other. The results are believed to be of particular interest to the cottonseed oil industry in that they make possible certain predictions with respect to the value of the seeds for oil from a knowledge of their source and the season in which they were produced.

Previous studies have shown that during the development of the cotton seed the formation of gossypol is concurrent with that of oil. The formation of oil takes place during the first 30 days of growth of the seed and is complete some time before other parts of the cotton boll have matured. The formation of gossypol proceeds more rapidly than that of oil and continues at a slow rate even after the seed has reached its full size and oil development has ceased (2). It is evident, therefore, that immature seeds between 30 and 40 days old may contain less gossypol but approximately the same amount of oil as older seeds which have reached a more mature stage.[2]

Experimental Material and Methods

Cotton seeds of Oklahoma Triumph 44 variety were used throughout this investigation. During a two-year study of the effect of fertilizers on gossypol and oil content, the cotton seeds were secured through the courtesy of the Agronomy Department from commercial fertilizer test plots located in four sections of Oklahoma. During a third year it was possible to obtain similar samples of seed from only two of these localities.[3] Four different fertilizer treatments and two checks were represented in the seeds obtained from each locality. The test plots were located in Greer, Payne, Bryan and McIntosh Counties. The commercial fertlizers used were sodium nitrate, superphosphate, and potassium chloride (kainit). They were applied to the various plots at the time of planting in the following amounts per acre: Plot 2 received sodium nitrate 100 lbs., plot 5 received superphosphate 200 lbs., plot 7 received sodium nitrate 100 lbs. and superphosphate 200 lbs., and plot 10 received superphosphte 160 lbs., sodium nitrate 30 lbs., and potassium chloride 10 lbs. At the time the cotton was picked representative samples of seed cotton were obtained from each plot for Agronomic studies and the seeds from these samples were reserved for gossypol and oil determinations.

Since the cotton plant blooms over an extended period of time samples of mature seeds from an entire plant will include some seeds which developed early and others which developed late in the season. Cotton seeds as they are received at the oil mill will include these early and late matured seeds and a certain amount of seed which, due to drought or other adverse growing conditions, is undeveloped and immature. To determine whether or not the gossypol and oil content varied with time of development, collections of seed were made from early developed bolls by selecting fully matured bolls which were close to the main stem and near the base of the plant; those which formed at the end of the branches and near the top of the plant were taken as representing late development. To obtain equally representative samples the same number of bolls, usually 4 or 5, were taken from the top of the plant as were taken from the bottom.

The effect of under nutrition on the development of gossypol and oil in the seed was determined by selecting bolls from poorly developed plants growing in parts of the field subject to considerable erosion and where vegetative growth had been markedly retarded. These were obtained the first two years from test plots on and adjacent to the experiment station farm and in the last year from similar plots located a few miles distant. The seeds from approximately 100 bolls formed a composite sample.

The various lots of seeds were delinted with sulphuric acid, air-dried and ground previous to analysis. The amount of ether-soluble material which was taken as representing oil was determined by extracting 2-gram samples of the dry ground seeds with anhydrous di-ethyl ether for 24 hours. Gossypol was determined by Carruth's method as modified by Schwartze and Alsberg (6). The results of these determinations are given in the tables which follow.

Results and Discusson

The results presented in Table 1 show that the gossypol content of the cotton seeds under investigation was determined largely by the locality in which they were produced and that it was influenced to a small extent by the additional supply of plant nutrients effected through the application of commercial fertilizers. During two consecutive years the seeds produced in McIntosh County contained larger quantities of gossypol than seeds from similarly fertilized plots in the other localities considered. Furthermore, the seeds produced on the unfertilized check plots in McIntosh County contained more gossypol than seeds from fertilized plots in the other localities with but one exception, that being Greer County, second year, complete fertilizer plot. This evidence of an apparent relationship between the gossypol content and the locality of growth of the seed even in so restricted an area as is represented here is in agreement with the findings of Schwartz and Alsberg (6), who conducted studies with different varieties of cotton seeds grown in variour localities of the United States. That the relationship is not absolute, however, even under carefully controlled conditions, is apparent from a consideration of the average gossypol content of seeds produced on the check plots in the different counties during the first two years. During the first year seeds from the check plots in Bryan County contained next to the highest gossypol content and in the second year the lowest. The average percentage of gossypol in seeds produced on check plots in Greer, Payne, and Bryan Counties during the first year was 0.561, 0.549 and 0.626 respectively, and during the second year, 0.671, 0.574 and 0.438, respectively.

The influence of locality on the composition of the seed is also brought out in the results of the oil determinations. With the exception of the seeds produced on one of the check plants in Bryan County during the first year, none showed as high an oil content as those produced on the six different plots in McIntosh County during the two years considered. The average percentage oil content of the seeds from the check plots in Greer, Payne, and Bryan Counties for the first year was 25.46, 25.00 and 28.12 respectively, and for the second year, 25.93, 24.03 and 23.67, respectively. From this it may be readily discerned that if the seeds are arranged by counties in order of increasing oil content they take the same relative position as when listed in order of increasing gossypol content for the two years considered. This fact emphasizes the relationship between oil and gossypol content.

A consideration of the effect of fertilizers on the composition of the seeds shows that during three years, the application of a complete fertilizer tended to raise the gossypol content of the seeds in all localities. It was particularly effective in the case of seeds produced in Greer County in the first two years and resulted in an appreciable lowering of the gossypol content in only one instance (Payne County, 2nd year). The oil content of the seeds was likewise increased by this treatment in 7 of the 10 cases.

The addition of either nitrogen or phosphorus generally resulted in a decrease of gossypol and in only 3 of the 16 cases produced an appreciable increase. Likewise, the oil content was decreased more often than it was increased by the addition of either of these fertilizer constituents. Nitrogen when used alone decreased the oil content of the seeds in 6 of the 7 cases cited. A combination of nitrogen and phosphorus decreased the gossypol content in all but one instance (Bryan County, 2nd year) and in that case the resulting increase was so small as to be negligible. The effect of this combination of fertilizers on the oil content was variable.

In view of the results presented by Garner, et al (4) and those obtained in this study, it is evident that applications of nitrogen tend to decrease the gossypol and oil content of the seeds. The increased gossypol and oil content of seeds produced on the complete fertilizer plots suggests that potassium is an influential factor; or possibly that certain fertilizer ratios are more favorable to gossypol and oil development than are others.

An explanation of the consistently high gossypol and oil content of seeds from all plots in McIntosh County was sought in climatological data recorded for this and the other localities. Data on rainfall and temperature for these localities are given in Table II.

Reference to Table II shows that the amount of rainfall during the growing season was considerably above normal in Bryan County during the first year and in McIntosh County during the second year. There was but little departure from the normal in the other localities during these years. The mean temperature for the various counties show no conspicuous differences during either year.

Confirmatory evidence of a positive correlation between the amount of rainfall during the growing season and the oil content of the seeds is found in the case of seeds produced in Bryan County during the first year. When rainfall was above normal the percentage oil content of the seeds was high and closely approached that of seeds from McIntosh County. In the following year when rainfall was approximately normal, the oil content was conspicuously lower. A similar correlation is not found in the case of seeds produced in McIntosh County. The oil content of seeds from that locality was high both years although rainfall varied, being normal the first year and extremely high the second year. Evidently the development of oil in these seeds was influenced by factors other than rainfall, and since they contained over 28 per cent oil it seems reasonable to believe that this is close to the maximum amount attainable.

An examination of the figures presented in Table III reveals no consistent differences between the gossypol content of seeds from bolls which developed early and those from bolls which developed late in the season. There are indications of a decreased oil content in seeds from late developing bolls, but this difference is small and may be of no real significance.

The seeds from poorly developed plants were obtained from those which had been dwarfed as a result of unfavorable soil conditions. As previously stated, the plants were growing in eroded sections of the field where they had developed considerably less foliage and fewer bolls than had adjacent healthy plants from which the early and late developing bolls were secured. Similarly eroded areas are not uncommon in certain sections of the cotton belt and are clearly distinguished from the rest of the field by scanty plant growth. As shown in Table III, the seeds from these plants contained approximately the same percentage of oil as those from normally developed plants; in fact, the oil percentages approach those which might be expected of composite samples of seeds taken from the entire field. The actual amount of oil produced by these plants was of course less than that produced by the healthy plants since there were fewer bolls. Unlike the oil content, the percentage gossypol content was decreased slightly in the seeds from the poorly developed plants. The result might be anticipated from the fact that the formation of oil takes place early in the development of the seed and reaches completion previous to that of gossypol.

Oil: Gossypol Ratio

As previously pointed out, gossypol generally follows what has been termed the "rule of the oil" (6). Seeds of inferior quality as indicated by their oil content are correspondingly low in gossypol. What appears to be of most importance in this relationship is brought out by a comparison of the ratio of oil to gossypol as found in seeds of low oil content with that of seeds of high oil content. In the present study the average rate of oil to gossypol shown by the seeds of low oil content was approximately 55:1. The seeds of high oil content had a ratio of approximately 35:1. When a similar calculation is applied to the results of Schwartze and Alsberg (6) even wider differences in these ratios are revealed. During the years 1918, 1917 and 1919, the percentage of oil in the meats of cotton seeds of Trice variety produced in Bells, Tennessee, was 28.37, 32.51, and 35.85, respectively. The oil:gossypol ratio of the meats which had the lowest oil content was approximately 70:1 as compared to 31:1 for the meats of the highest oil content.

Summary

The gossypol and oil content of cotton seeds is known to vary in different sections of the United States. In the present study, similar variations were observed in cotton seeds produced in different years in various sections of a small cotton growing area. These variations were apparent in seeds of a single variety and appeared to be related to environmental factors particular to the region in which the seeds were produced. The seeds from one locality were consistently high in gossypol. In most instances small increases in gossypol were produced by increasing the fertility of the soil with a complete fertilizer. The results suggest that an increased supply of potassium was beneficial to the development of gossypol. Gossypol was also increased in the seeds of one locality following a season of heavy rainfall. Seeds which developed early in the season had about the same gossypol content as those which developed late. Seeds from poorly developed plants which had produced few bolls showed only a slight decrease in gossypol content.

Factors which altered the gossypol content of the seeds produced a corresponding although less proportionate change in their oil content. The oil:gossypol ratio was much wider in seeds of low oil content than in seeds of high oil content. The practical significance of this ratio relates to the decreased refining loss shown by certain grades of crude cottonseed oil on increasing their gossypol content. Similar studies carried out over a period of years in other localities should make it possible to predict the relative value of different lots of cotton seed for oil from a knowledge of their source.

TABLE I.

Relation of Fertilizer to the Percentage Gossypol and Oil Content of Cotton Seeds Based on the Dry Weight of the Delinted Seed.

Locality of Growth of the Seed	Fertilizer Addition: Nitrogen	Phosphorus	Nitrogen and phosphorus	Nitrogen, phosphorus and potassium	Check	Check
	Percentage Gossypol					
1st year—						
Greer Co.	0.552	0.515	0.571	0.638	0.592	0.531
Payne Co.	....	0.524	....	0.578	0.560	0.538
Bryan Co.	0.503	0.538	0.577	0.626	0.642	0.611
McIntosh Co.	0.756	0.728	0.744	0.738	0.762	0.735
2nd year—						
Greer Co.	....	0.690	....	0.714	0.658	0.685
Payne Co.	0.512	....	0.530	0.533	0.583	0.564
Bryan Co.	0.465	0.441	0.442	0.478	0.438	0.437
McIntosh Co.	0.687	0.714	0.638	0.777	0.708	0.707
3rd year—						
Greer Co.	0.350	0.366	0.364	0.391	0.355	0.408
Payne Co.	....	0.430	....	0.414	0.314	....
	Percentage Oil					
1st year—						
Greer Co.	24.30	23.86	25.00	25.02	25.73	25.20
Payne Co.	....	24.79	....	25.32	25.10	24.91
Bryan Co.	26.98	26.77	27.45	27.16	28.45	27.78
McIntosh Co.	27.96	28.79	27.74	28.23	28.65	28.14
2nd year—						
Greer Co.	....	25.76	....	27.05	25.96	25.90
Payne Co.	24.10	....	24.40	24.60	24.24	23.83
Bryan Co.	23.17	23.13	24.92	24.37	23.82	23.52
McIntosh Co.	27.03	28.23	27.00	28.70	27.10	27.75
3rd year—						
Greer Co.	23.19	23.55	24.30	24.39	22.86	24.49
Payne Co.	....	24.06	....	24.32	23.69	....

TABLE II.

Rainfall and Mean Temperature at Stations Located in Greer, Payne, Bryan, and McIntosh Counties for Four Months, May to August.

Month	Rainfall at Stations in: Greer Co. In.	Payne Co. In.	Bryan Co. In.	McIntosh Co. In.	Mean Temperatures at Stations in: Greer Co. °F.	Payne Co. °F.	Bryan Co. °F.	McIntosh Co. °F.
1st year—								
May	3.05	2.63	3.62	4.39	71.0	69.7	70.4	69.6
June	1.62	4.74	3.26	3.43	80.1	75.9	77.8	76.4
July	3.74	3.04	9.85	4.02	81.8	78.9	79.2	79.0
August	3.70	1.44	8.50	2.67	81.6	80.6	81.0	82.4
Total	12.11	11.85	25.23	14.51				
Departure from normal	—1.47	—3.46	+8.72	—0.60				
2nd year—								
May	3.25	2.26	1.74	4.56	74.9	71.0	75.2	72.1
June	2.40	7.46	5.20	4.85	78.3	75.6	78.0	75.3
July	3.84	2.33	7.89	7.61	82.0	79.5	81.4	78.4
August	1.50	4.41	1.04	9.84	80.0	76.1	81.3	76.3
Total	10.99	16.46	15.87	26.86				
Departure from normal	—2.59	+1.15	—0.64	+11.75				

TABLE III.
Percentage of Gossypol and Oil (on Dry Delinted Basis) of Cotton Seeds Collected During Three Years from Early and Late Developing Bolls and from Bolls of Poorly Developed Plants.

	First Year		Second Year		Third Year	
Source of Seeds	Gossypol	Oil	Gossypol	Oil	Gossypol	Oil
Early developed bolls	0.632	24.30	0.518	23.86	0.443	23.89
Late developed bolls	0.542	23.69	0.508	23.88	0.498	22.69
Bolls of poorly developed plants	0.535	24.27	0.458	23.60	0.407	23.12

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(6) Schwartze and Alsberg, *J. Agr. Research*, 25, 285 (1923).

FOOTNOTES

[1]Published with the permission of the Director of the Oklahoma Agricultural Experiment Station, Stillwater.

[2]The age of the seeds was calculated from the date of flowering. Usually less than 50 per cent of the cotton bolls were open 40 days after flowering and less than 1 per cent at 34 days.

[3]The seeds obtained the third year, although produced in the same general vicinity as those of previous years, were from different experimental plots and for that reason their analysis should be considered apart from the others.

TEA SEED OIL— A TEST FOR ITS DETECTION IN OLIVE OIL

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THE close similarity between tea seed oil and olive oil in both physical and chemical constants (1, 2, 3, 4), makes it impossible to distinguish readily between the two oils. For sixteen years it has been known that tea seed oil is used as an adulterant of olive oil. In 1920 Ernest Millian (5) noted: "Adulteration of olive oil is practiced on a large scale in England." Since both oils have practically the same physical and chemical constants some additional test is necessary to detect the presence of tea seed oil.

Furthermore, as this adulterant has become more highly refined, *none* of the tests previously proposed for its detection remains sufficient. Cofman-Nicoresti suggested a color test (6) which H. A. Caulkin (7) showed was of no value with highly refined tea seed oil.

Another color test was proposed by several investigators, Biebers Test (3), H. Blins' Test (8), Dybowsky & Millia (4), the only apparent difference between them being the volume of reagents used. This color test, quoted from Allen's Organic Analysis, Vol. II (4), is: "4 cc. of oil are shaken with a mixture of 5 cc. H_2SO_4, 3 cc. HNO_3 and 3 cc. water for 30 seconds: immerse the whole mixture at 5°C for 5 minutes and note color after 15 minutes. The test as applied should result in a deep black and turbid layer for pure tea seed oil and light straw and clear for olive oil." The authors of this article have applied this test to a sample of pure tea seed oil and substantiated the conclusion of E. Richard Bolton (3), and Allen (4), that "the test is of little value in detecting tea seed oil in olive oil."

The above two color tests are not the only methods which have been published and subsequently proven practically useless in view of the present degree of refinement of tea seed oil. Other methods have been proposed since.

One of these, the Bolton and Williams (9) method of grouping fatty oils with reference to the determination of the iodine number of the Unsaponifiable Matter, divides the oils into four groups. The fact that olive oil is the only oil in one of these groups is supposed to distinguish it from all other oils. A report of the Olive Oil Committee of the American Oil Chemists Society (9), however, finds the method to be inaccurate.

Both E. Richard Bolton (3) and George S. Jamieson (2) state that 25% or more of tea seed oil would reduce notably the Titer below that given by olive oil alone. Olive oil, quoting Jamieson, has a Titer range of 18°C-25°C, tea seed oil has a range of 13°C-15°C. The authors have found 17.0°C to be the Titer on the tea seed oil they used. The U. S. P. XI gives the following Titer range for olive oil, "Not less than 17°C nor more than 26° C." With this wide range for olive oil and its slight variation from tea seed oil, it does not seem probable that even 50% tea seed oil in olive oil could be detected by the Titer test.

Constants of the samples of oils used in the experiments reported herewith were as follows:

Tea Seed Oil—	
Iodine Value	84.3
Saponification Value	191.5
Acid Value	0.2
Titer Test	17.0° C.

This sample of refined tea seed oil conforms to the requirements of the U. S. P. XI FOR A PURE OLIVE OIL.

Italian Olive Oil—	
Iodine Value	83.8
Saponification Value	191.7
Acid Value	1.80
Titer Test	22.6° C.
Spanish Olive Oil—	
Iodine Value	84.8
Saponification Value	191.0
Acid Value	1.15
Titer Test	19.6° C.
French Olive Oil—	
Iodine Value	83.7
Saponification Value	191.0
Acid Value	0.88
Titer Test	21.6° C.
California Olive Oil—	
Iodine Value	84.0
Saponification Value	192.2
Acid Value	2.56
Titer Test	19.65° C.

Examination of the foregoing figures shows that from the constants alone it is practically impossible for any chemist to prove that a sample of oil, which is a blend of 75% tea seed and 25% of pure olive oil, is adulterated. The acid value of olive oil varies, the U. S. P. XI permits a maximum acid value of 3.0 and oils are known which have an acid value as low as 0.4.

The authors have developed a test which they believe is positive for as little as 5% of tea seed oil in the presence of 95% olive oil. Results below this value depend upon the olive oil used. While the test will recognize the presence of tea seed oil in pure olive oil, it is not claimed that it will be specific for tea seed oil—as is shown later