THE GOSSYPOL CONTENT AND CHEMICAL COMPOSITION OF COTTONSEEDS DURING CERTAIN PERIODS OF DEVELOPMENT

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INTRODUCTION

The recent findings of Schwartze and Alsberg (7), showing that the gossypol content of cottonseeds tends to vary with the locality in which the seeds are grown, and is increased in those seeds which have a high oil content, has made it desirable to determine at what stage of maturity the maximum amount of gossypol is present. The writer desired to know if the gossypol accumulated in the seeds at the same time that there was an accumulation of other materials such as oil and protein, or if it formed after the seeds had fully matured and had separated from the plant. Ever since the isolation of gossypol as a toxic constituent of cottonseeds (10) the writer and coworkers at this station have been interested in the factors which determine the gossypol content of cottonseed meal and have recently published some of the results of this work (4, 5). For obvious reasons, the gossypol content will depend primarily upon the gossypol contained in the original seeds. It is the writer's opinion that climatic or weather conditions are important factors to be considered and that such conditions might be more or less effective, depending upon the time of planting and perhaps of gathering the cotton. The amount of rainfall during the growing period of the cotton plant governs to some extent the amount of oil in the seeds, and if it can be demonstrated that the gossypol content is regulated by the formation of oil, then these same conditions will in turn affect the gossypol content. Present knowledge of the gossypol content of cottonseeds as dependent upon variety, oil content, and geographical region of growth of the seeds is confined to the publication cited above (7).

Balls (2) working with Egyptian cotton No. 77 finds that the cottonseed has reached its full size between 18 and 21 days after the flowers have opened, the boll reaching its full size soon after, but that the seed continues to develop for some time. After 45 days the boll begins to crack and the cotton is usually ready to be picked within 5 or 6 days thereafter. Garner, Allard, and Foubert (6) studying the oil content of cottonseeds at different stages of maturity, found that the increase of oil proceeded somewhat more rapidly than the growth of the seed. In experiments with soy beans these investigators found that during the final stages of ripening there

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3 Reference is made by number (italic) to "Literature cited," p. 991.

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was a slight decrease both in the size of the seed and in the oil content. This phenomenon they attributed to continued respiratory activity after assimilation had ceased. Referring to the transformation of carbohydrate to fat they state that "under proper conditions this transformation takes place in unripe seeds detached from the mother plant, further indicating that the oil is derived from the carbohydrates."

Since gossypol contains only carbon, hydrogen, and oxygen, and is closely associated with the oil in cottonseeds in other ways, it appeared possible that it might also be formed at the same time and that it might be associated in some way with the formation of the oil. The practical value of any such finding is perhaps obscure until our very limited knowledge of the formation of gossypol and its properties, except as these properties are manifested in the feeding of cottonseed products to livestock, is considered. Concerning the rôle of gossypol in the life of the cotton plant little is known. Carruth (3) speaks of gossypol as being formed by the disintegration of adjacent cells. In the light of the histological studies upon the glands of the cotton plant by Stanford and Viehoever (9), this statement is well founded.

EXPERIMENTAL MATERIAL AND METHODS

COLLECTING SAMPLES

Cottonseeds of a selected variety, Oklahoma Triumph 44, were used in this study. They were obtained from the field plots in which cotton variety tests were being made by the department of agronomy. The cotton crop, grown on upland soil, was planted May 10, and picking began October 15. There was a moderate amount of rainfall throughout the growing season, and the season was favorable for a large cotton crop. On October 1 bolls at different stages of development as determined by their appearance and condition, were collected in the field and brought to the laboratory for an analysis of the seeds. In this collection were full-size bolls approximately 50 days old, and showing signs of cracking, bolls which had just opened; bolls which had been open for 2 or 3 days; those which had been open for 5 or 6 days; and finally, bolls which had been open for longer periods and from which the cotton was hanging down, the locks having lost much of their firmness.

On February 1 another collection of matured seeds was made from the unpicked cotton left in the field, and at the same time a representative sample of seeds from the whole field was obtained from the cotton gin. All the seeds were removed from the bolls by hand and delinted with sulphuric acid. The immature seeds were dried at a temperature sufficiently low to prevent the destruction of gossypol or its change to a less soluble form. The seeds were then ground separately and sampled for chemical analysis, including gossypol determinations. Representative samples of the unground seeds were reserved for determinations of their total dry matter.

The methods of analysis used by the Association of Official Agricultural Chemists (1) were employed for the chemical determinations, anhydrous diethyl ether being used as an extractive for the fat determinations. The gossypol was determined by Carruth's method as modified by Schwartz and Alsberg (7). Fifty-gram samples were used and the seeds were extracted for 24 hours in a Soxhlet extraction
apparatus. Calculation of the gossypol in the aniline gossypol precipitates was made by use of the conversion factor .74, since the composition of this precipitate approaches $C_{30}H_{28}O_92C_6H_5NH_2$, and this factor has been used by previous investigators (8).

**DISCUSSION AND RESULTS**

**PROXIMATE ANALYSIS**

The results of the several analyses are given in Table 1.

**Table 1.—Chemical composition and gossypol content of cottonseeds at different stages of maturity**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Condition of boll or seeds</th>
<th>Ash (Per cent)</th>
<th>Crude protein (Per cent)</th>
<th>Crude fiber (Per cent)</th>
<th>Nitrogen-free extract (Per cent)</th>
<th>Ether extract (Per cent)</th>
<th>Gossypol (Per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>About to open</td>
<td>6.20</td>
<td>28.46</td>
<td>15.56</td>
<td>32.81</td>
<td>13.97</td>
<td>0.048</td>
</tr>
<tr>
<td>2</td>
<td>Just open</td>
<td>3.86</td>
<td>31.11</td>
<td>14.46</td>
<td>28.49</td>
<td>24.91</td>
<td>0.438</td>
</tr>
<tr>
<td>3</td>
<td>Open 2 to 3 days</td>
<td>3.16</td>
<td>28.46</td>
<td>15.19</td>
<td>25.43</td>
<td>24.52</td>
<td>0.461</td>
</tr>
<tr>
<td>4</td>
<td>Open 5 to 6 days</td>
<td>3.15</td>
<td>29.86</td>
<td>15.33</td>
<td>27.93</td>
<td>23.73</td>
<td>0.538</td>
</tr>
<tr>
<td>5</td>
<td>Open over 6 days</td>
<td>3.33</td>
<td>28.37</td>
<td>15.20</td>
<td>28.69</td>
<td>24.41</td>
<td>0.546</td>
</tr>
<tr>
<td>6</td>
<td>Seeds obtained from gin</td>
<td>3.34</td>
<td>29.01</td>
<td>15.78</td>
<td>28.46</td>
<td>23.41</td>
<td>0.452</td>
</tr>
</tbody>
</table>

From these results it would seem that there is a period of intense oil formation occurring about the time the boll begins to crack and just previous to its opening. This wide difference in the oil content of the seeds picked at these two intervals appeared questionable, and another picking of the unopened bolls was made. The oil content of the seeds obtained in this second picking, 15.05 per cent, was only slightly higher than the one shown in Table 1, which satisfied the writer that the first figures obtained were approximately correct. Other factors not considered here may influence the oil content of the seeds at this stage; but as this study was concerned primarily with gossypol determinations, the influence of these factors was not investigated further. However, it is interesting to note that there is a slight but variable increase in the oil content of the more mature seeds and that this amount did not show a decrease even when the seeds were allowed to remain in the field for several months after they had matured. The relative amount of oil in the seeds was nearly doubled between the first and last stages of development.

The protein content of the immature and mature seeds does not show any marked increase as the seeds develop. This indicates what might be expected, namely, an early deposition of nitrogenous material. There are, however, fluctuations between the protein content of seeds picked at different times which may be accounted for by the variable size of the seeds and by errors introduced in obtaining samples. The same conditions hold true for the results of the fat determinations, although an attempt was made to eliminate these errors as far as possible by obtaining samples from a selected area of the field. At a later date the writer hopes to be able to make a more thorough study of the accumulation of both oil and protein during the successive stages of development of the cottonseed.

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The ash, crude fiber, and nitrogen-free extract of seeds from opened bolls were considerably less than from unopened bolls, but remained quite constant for the seeds picked during the later stages of development. These general results are in harmony with the findings of previous investigators working with other kinds of seeds as well as with the results of some unpublished work in this laboratory by N. B. Guerrant.

GOSSYPOL DETERMINATIONS

The gossypol determinations on seeds at various stages of maturity given in Table 1 showed some very interesting results. The gossypol content of the mature seeds was much higher than the writer has found for other samples grown in this locality, which were of no particular variety and had been in storage for some time. The formation of gossypol proceeded much more rapidly than that of oil, the gossypol making its greatest increase during the first two stages and continuing to increase at a very slow rate during the later stages. Although there are some indications that gossypol formation is concurrent with the formation of oil, there seems to be no direct relationship between the two; and in the case of sample No. 4, which showed a relatively lower oil content, the gossypol content was increased. The high gossypol content of those seeds which had matured and were left standing in the field is perhaps more indicative of the lysigenous origin of gossypol.

WEIGHT OF COTTONSEED IN RELATION TO ITS CHEMICAL COMPOSITION AND GOSSYPOL CONTENT

In order to observe the absolute as well as the relative changes in the oil content of cottonseeds collected at two stages of maturity, Garner, Allard, and Foubert (6) determined the weight of the seed and from the weight calculated the grams of oil in 1,000 seeds. Although the changes occurring in the weight of the seeds do not necessarily represent the exact growth made by the seeds between the stated intervals, it was felt that to proceed on such an assumption might aid in interpreting the data obtained in this study.

The dry weight of 500 seeds from several pickings was determined and the analysis of these calculated from the figures in Table 1. From these results, which are given in Table 2, it is evident that there was considerable growth between the first two stages, resulting in increased amounts of all the constituents.

Table 2.—Chemical composition and gossypol content of 500 cottonseeds at different stages of development

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Condition of boll or seeds</th>
<th>Weight of dry seeds</th>
<th>Ash</th>
<th>Crude protein</th>
<th>Crude fiber</th>
<th>Nitrogen-free extract</th>
<th>Ether extract</th>
<th>Gossypol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>About to open</td>
<td>20.09</td>
<td>1.25</td>
<td>5.72</td>
<td>3.73</td>
<td>6.39</td>
<td>2.81</td>
<td>0.010</td>
</tr>
<tr>
<td>2</td>
<td>Just open</td>
<td>38.46</td>
<td>1.51</td>
<td>11.96</td>
<td>5.56</td>
<td>10.20</td>
<td>9.23</td>
<td>.165</td>
</tr>
<tr>
<td>3</td>
<td>Open 2 to 3 days</td>
<td>43.27</td>
<td>1.37</td>
<td>12.32</td>
<td>6.55</td>
<td>12.30</td>
<td>10.74</td>
<td>.200</td>
</tr>
<tr>
<td>4</td>
<td>Open 5 to 6 days</td>
<td>44.77</td>
<td>1.41</td>
<td>13.36</td>
<td>6.96</td>
<td>12.51</td>
<td>10.62</td>
<td>.241</td>
</tr>
<tr>
<td>5</td>
<td>Open over 6 days</td>
<td>40.63</td>
<td>1.35</td>
<td>11.53</td>
<td>6.18</td>
<td>11.65</td>
<td>9.92</td>
<td>.222</td>
</tr>
<tr>
<td>6</td>
<td>Mature seeds picked Feb. 1</td>
<td>40.32</td>
<td>1.31</td>
<td>12.25</td>
<td>5.80</td>
<td>10.58</td>
<td>10.38</td>
<td>.222</td>
</tr>
<tr>
<td>7</td>
<td>Seeds obtained from gin</td>
<td>40.96</td>
<td>1.36</td>
<td>11.88</td>
<td>6.46</td>
<td>11.66</td>
<td>9.60</td>
<td>.185</td>
</tr>
</tbody>
</table>
A comparison of Tables 1 and 2 serves to emphasize some important differences in the manner of presenting data of this kind.

From Table 1 it appears that there is a decrease in the crude fiber, ash, and nitrogen-free extract of cottonseeds as they mature, while Table 2 shows an increase in the constituents. Both tables show an increase in the amounts of the other constituents which represent the greatest changes taking place in the seeds as they develop up to the time that the boll has been open for several days. Following this period the results are more or less irregular. The writer believes Table 2 to be a more correct interpretation of what actually happens in the seeds and has used the figures given in this table as a basis for drawing his conclusions. No explanation is offered for the large difference in weight between the seeds in samples Nos. 4 and 5 at this time, although a slight decrease in weight may be expected, due possibly to respiration. The gossypol content of the seeds is shown to be quite independent of their weight, but is slightly increased with the age of the seeds; and these facts lead the writer to think that storage conditions may also have an effect upon this amount.

SUMMARY

The gossypol content and chemical analyses of cottonseeds of the variety Oklahoma Triumph 44, picked at different stages of development, are reported. The seeds were obtained at the different stages from the time the boll was ready to open until it had fully opened, and also included those seeds which had remained in the field unpicked for several months. The greatest change in the composition of the seeds over the range studied occurred at the time the boll was mature and about to crack, from which time until the boll opened the gossypol content increased rapidly and continued to increase for some time thereafter. This increase in gossypol was greater than the increase of any of the other constituents, all of which showed only small increases after the boll had opened. No correlation was found between the formation of oil and the formation of gossypol.

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