

The Coloring Matter of Cottonseed Hulls

Reporting the Isolation of a New Substance from the Hulls

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WHILE it is not difficult to remove the color from cottonseed oil, it is far from an easy matter to identify it; it was thought therefore that a study of the coloring matter in the seed hulls might throw some light on the colors in the oil itself.

Little is known of these coloring matters even though considerable work has been done on them. Palmer¹ says that the coloring matter of the crude oil consists of carotin and related pigments, and his statement has some proof in the work carried on by Priest² of the Bureau of Standards. However, this is in opposition to work carried on by Gill³ who has shown that the Crampton-Simons test for palm oil is caused by the carotin present in the oil. If carotin be added to cottonseed oil the Crampton-Simons test will be given. As the oil does not give this test without the addition of carotin it is fairly evident that carotin is not present. Further work by the Bureau of Standards⁴ has shown that crude oil contains resins which will combine with caustic soda to increase the refining loss. However, the same article states that no gossypol is found, even though Marchlewski⁵ first isolated it from the soap stock produced by the refining. This is

probably due to the difference in manner of obtaining the oil from the seed. For, according to Withers and Carruth⁶, gossypol is held in the kernels from which the oil is pressed, if the kernels be first heated as in the case of hot pressed oil. However, in the case of cold pressed oil, gossypol comes out with the oil.

Marchlewski appears to be the only investigator who has done much work on the foots. The fatty acids from the foots when distilled under diminished pressure leave a residue in the still from which no coloring matter has been isolated.

No references to work on hulls were found in the literature although a thorough search was made. In fact, all the important work seems to have been, with the exception of Marchlewski's work, on the cottonseed meal. In this material the amount of oil present is not great enough to cause serious difficulties. The most important work has been done by Withers and Carruth.

Coloring Matter

A. The most common coloring matters found in plants are as follows:

1. Carotin ($C_{40}H_{56}$) a red crystalline solid found in carrots, orange peel and palm oil⁷, butter fat, some leaves, etc. Melting point of this substance is

¹ "Carotin and Related Pigments." L. S. Palmer.

² Unpublished work.

³ Color Test for Oils. Gill.

⁴ Cotton Oil Press (No. 5) 29.

⁵ J. Prakt. Chem, 60 89-90 (1899).

⁶ J. Agri. Research 5 (261) (1915).

⁷ Color Test for Oils. J. Ind. and Eng. Chem, 10 612 (1918).

168°C., it is unsaturated and is characterized by the blue color which it gives when dissolved in concentrated sulfuric acid and by its absorption spectra. The structural formula for carotin is unknown even though considerable work has been done on it. It absorbs Iodine forming $C_{40}H_{56}I_2$. It crystallizes from carbon disulfide solution in which it has a deep red color.

2. Lycopin ($C_{40}H_{56}$) is an isomer of carotin and can be separated from it by its removal from petrolic ether solution by methyl alcohol. It also gives characteristic absorption spectra. As in the case of carotin its structural formula is unknown and it forms an iodine addition product.
3. Xanthophyll is a closely related pigment with a yellow color. It is best dissolved by methyl alcohol from which it can be readily crystallized. It is precipitated from alcoholic solution by adding water.
4. Fucoxanthin is another related pigment and is separable by its solubility in methyl alcohol.
5. Chlorophyll is the common green pigment found in green leaves, grass and many green plants. It can be separated from the other coloring materials by precipitation from ether solution with alcoholic caustic soda or potash.
6. Gossypol. This is the yellow coloring material isolated from cottonseed foots by Marchlewski⁸. It was also found in cottonseed meal by Withers and Carruth⁹ who proved it to be the toxic substance present.

They made an alcoholic extract of the meal, evaporated and dissolved it in ether. From this they precipitated it by adding aniline.

Analysis of Foots

B. Method of analysis of the foots and its result:

1. The soap stock was completely saponified to remove the excess of oil by adding alcoholic caustic soda solution and heating. The completely saponified material was dissolved in alcohol and thoroughly dried in an oven at 100°.
2. The dried material was extracted with ether for several hours. The ether extract, when washed with water and acidified slightly, proved to be colorless.
3. The residue from the extraction was dissolved in water and 36° Be. salt solution was added. The soap was salted out and the dark aqueous solution below was acidified. This was then evaporated to dryness and the residue dissolved in alcohol. However the material was in the form of a gum from which nothing could be crystallized. Aniline when added formed no crystalline material so there was evidently no gossypol in this portion of the foots.
4. The salted out soap was then dried and extracted with ether, carbon disulfide, petrolic ether, benzene and acetone successively, but no coloring material was present in the extract.
5. The residue was dissolved in water, acidified and treated with lead acetate. The resulting lead soaps were extracted with ether. This removed the soaps of oleic and linolic acid

⁸ J. Prakt. Chem. 60 89-90 (1899).

⁹ J. Agri. Research 5 261 (1915).

and all the coloring matter as the residue was colorless. This extract was dried and in turn extracted with benzene and carbon disulfide, but the coloring matter was not removed.

6. The residue was again dissolved in water acidified and calcium hydroxide added in excess. The resulting calcium soaps were extracted with acetone, benzene, ether and carbon disulfide, but, as before, no coloring matter was extracted even after extraction lasting as long as forty-eight hours.
7. The fatty acids were again split out by dissolving the soap from the above procedure in water, acidifying, and boiling. The fatty acids were then agitated with decolorizing carbon but an insufficient amount of the color was removed in this manner to warrant continuation of this method of procedure.

From this work it appears that separation of the coloring matter from the soap stock by either plain extraction with organic solvents or by extraction of the lead or calcium salts of the fatty acids is not feasible, owing to the greater solubility of the coloring matter in the soap itself or in the fatty acids split out by acidifying the soap.

Hull Coloring Matter

C. Analysis of the coloring matter from cottonseed hulls.

In this analysis the complications due to the presence of oil or fatty acids encountered in the work on foots or oil were negligible, owing to the very small amount of oil present in the hulls.

The hulls were extracted with various solvents in periods ranging

from a few hours to two weeks. The apparatus consisted of a large flask containing the solvent, inside of which were suspended the hulls enclosed in a white cloth bag. This was heated under a return condenser and afterwards the extract was filtered. The following solvents were used:

(a). alcohol; (b) ether; (c) carbon disulfide; (d) water. The water extract was by far the most colored and the carbon disulfide next. The alcohol and ether extracts were only faintly colored yellow and were treated as follows: (According to Palmer¹⁰.)

- (a) The first part was evaporated under diminished pressure and dissolved in ether. It was then shaken with alcoholic caustic potash which saponified the small amount of oil which was present. The ether extract was then evaporated and dissolved in petrolic ether. This was washed with 100cc. of 80%, 100cc. of 85%, and two 50cc. portions of 90% methyl alcohol. According to Palmer the carotin would be in the petrolic ether solution and xanthophyll in alcohol solution. Both of these were transferred to carbon disulfide and examined spectroscopically according to the method of Formanek¹¹. The alcohol solution gave no absorption spectrum and the petrolic ether solution which was in carbon disulfide when compared to carotin extracted from carrots did not resemble it at all.

The results of this method were checked by using a tube tightly packed with dried calcium

¹⁰ "Carotin and related Pigments." L. S. Palmer.

¹¹ "Spektral Analytische Noichweis Kunstliche Organische Farbstoffe."

carbonate and running the solutions, which had previously been combined, through it according to the method recommended by Palmer. The results given were the same, as no different colored bands were found by this method.

(b) The second portion was transferred to ether and aniline added to the amount of one fifth of the bulk of the small ether solution. This was warmed and set aside. After three days crystals were observed. These crystals according to the method of Withers and Carruth¹², were heated with alcoholic potash, and dissolved in water. The solution was acidified and the precipitate dried. Its melting point was 136°. This was acetylated by heating with acetic anhydride and allowing the acetylated product to crystallize from the acetic anhydride. The crystals so obtained were needle shaped and had a melting point of 220°. This (a and b) proves the absence of carotin and xanthophyll and the presence of gossypol in the hulls. However, the quantity of gossypol present is exceedingly small as about fifteen pounds of hulls were extracted and the amount of the aniline product obtained was less than one gram. The toxic effect of this small amount would be negligible.

D. Analysis of the water extract from cottonseed hulls.

This extract was made in the same manner as the previous extracts, the extraction being continued for four days. At the end of this time it was filtered

and evaporated to small bulk. A small portion was dried completely but it absorbed oxygen and darkened with this process. The remaining portion was poured into a large volume of alcohol from which it precipitated in a reddish brown flocculent form. Repeated extraction with alcohol and ether gave a reddish brown powder. All efforts to crystallize this substance from alcohol, ether, carbon disulfide, benzene, petroleic ether, acids and bases were vain. The only solvents in which it was soluble were water and dilute acids and bases. Glacial acetic acid would not dissolve the substance. It was impossible to crystallize this substance from water for the amorphous form always separated out when the solution was evaporated to small bulk and allowed to stand.

A systematic analysis of the substance according to Mulliken¹³ was made and the results would not fit any compound mentioned by him; also no compound was found in the literature which would fit these reactions. The analysis with its results was as follows:

1. A melting point determination on the material was made. The result of this determination showed that the substance would not melt but instead it charred at 320°.
2. Tests for elements present gave the following elements as those present: (a) Carbon, (b) Hydrogen, (c) Oxygen (by difference). No other elements were present and the substance when ignited left no residue.
3. The material was treated with both concentrated and fuming sulfuric acid. In each case the only effect was that of charring.
4. Nitric acid was added to the

¹² J. Agri. Research 5 261 (1915).

¹³ Organic Analysis, S. P. Mulliken.

substance and as no action was noticed it was heated. No effect was observed.

5. The substance was heated with the following reagents in turn: acetic anhydride, acetic acid, acetyl chloride, picryl chloride, hydroxyl amine. In no case was any change in the substance observed.
6. The material was heated with bromine. When it was redissolved in water and reprecipitated by adding an excess of alcohol it was unchanged.
7. Boiling with dilute hydrochloric acid had no effect on the substance.
8. Boiling with concentrated sodium hydroxide solution gave a dark black tar which would not crystallize from any solvent.
9. Tests for class reactions of the carbon, hydrogen and oxygen compounds were made. The substance was neutral and gave a reddish brown precipitate with Fehling's solution, which later was shown to be caused by the copper sulphate in the solution. A faint Molisch reaction for carbohydrates was shown. However, no carbohydrates listed in the literature were similar to the substance.
10. Silver nitrate was added to a solution of the material and a dark precipitate was observed. The solution was heated and filtered. The filtrate was evaporated to small bulk and cooled. No crystals were obtained by this procedure. It was then filtered and the filtrate found to contain only silver nitrate. The precipitate was then treated with ether, alcohol, carbon disulfide, carbon tetrachloride, petrolic ether and amyl alcohol but it was not soluble in any of these.

Copper Reaction Product

11. Copper nitrate was added to the aqueous solution of the material and a dark brown precipitate was observed. This was heated, filtered, and the filtrate concentrated as with the silver precipitate. Nothing was found in the filtrate except copper nitrate. The precipitate was treated with ether, methyl and ethyl alcohols, carbon disulfide, carbon tetrachloride, petrolic ether, amyl alcohol and glacial acetic acid but was not dissolved.

The copper nitrate product was carefully prepared by adding copper nitrate solution to a solution of the material. The mixture was heated and allowed to stand over night. The resulting compound formed settled to the bottom of the beaker and was removed by filtration. It was then washed with alcohol and ether and dried in the electric oven at 40°C. The melting point determination was made but the substance seemed to have no definite melting point.

The amount of copper present was determined in the following manner: approximately two-tenths of a gram of the substance was carefully weighed out into a porcelain crucible and ignited. When the ignition was complete the crucible was cooled and the residue dissolved in nitric acid. This was again ignited and when the ignition was complete the crucible was cooled in a dessicator. On weighing, the two analyses of the material checked rather well indicating practically uniform composition. The results of the copper analysis showed 10 per cent copper oxide.

Converting this copper oxide

into copper nitrate and taking the basis of one molecular weight of copper nitrate adding directly on to one molecular weight of the substance the material was calculated to have molecular weight of 612. This agrees fairly closely with the molecular weight obtained later by calculation from the combustions made to determine the carbon, hydrogen and oxygen, calculated to be 597.

The substance was carefully prepared and all the copper found was combined with the organic substance. As the material is so resistant to the action of the various reagents present, it is assumed to be added onto the copper nitrate and to not have the molecule affected in any other way. The copper may be recovered by suspending the copper nitrate product in water and passing hydrogen sulfide through the suspension. The copper is precipitated as copper sulfide and on filtering the reddish brown color of the original material is observed in the filtrate and it can be recovered by adding sufficient quantities of ethyl alcohol.

This evidence led to the belief that the material may be classed as a resin or possibly a pectin.

12. A quantity of the material was prepared in as pure a state as possible by repeated dissolving in water, precipitating by adding an excess of ethyl alcohol, washing with alcohol and ether.

An ultimate analysis of the material was made to determine the carbon, hydrogen and oxygen content. The results checked well in practically every case and were as follows:

	1	2	Average
Carbon ..	37.9%	38.0%	38.0%
Hydrogen	5.4	5.5	5.4
Oxygen ..	56.7	56.6	56.6

The simplest possible formula which would fit these results would be approximately $C_{19}H_{28}O_{21}$. Molecular weight determinations by the freezing point lowering method were made. As it was impracticable to analyze the solution, the method was to make a solution containing four grams per liter of water. This solution was placed in a test tube inside of a larger test tube containing alcohol to prevent supercooling. This was placed in a Dewar flask containing ether through which air was bubbled. The results obtained by four trials of this method were in no way satisfactory as they varied from over two degrees lowering to a fraction of the smallest division on the scale. The prevention of supercooling was hindered by the fact that one gram of the substance in 250 cc. of water gave so dark a solution that it was impossible to tell if ice were present. Also the method of adding small crystals of ice when the mixture was near the freezing point and making a cooling curve as ordinarily made in this kind of determination was tried but the results were still as unsatisfactory as ever.

Thus it was not possible to check the molecular weight determinations found by the copper nitrate method and the least possible formula method with the freezing point lowering method. The results of this work were as follows:

1. The coloring matter is not extracted from the soap stock or foots of cottonseed oil by any common organic or inorganic solvent which will not dissolve the soap stock. This causes the failure of any plan of isolation of the coloring matter which will

- give results with other material containing vegetable coloring material. Possibly some of the coloring matter from the spent liquor in the manufacture of yellow soap from cottonseed foots could be isolated. This spent liquor is dark brown to black in color and contains very little soap.
2. The extracts of cottonseed hulls were made with ether, carbon disulfide, alcohol, acetone and benzene. When those extracts were analyzed with the absorption method they failed to show the presence of carotin, xanthophyll or closely related pigments. This result was checked by the spectroscopic examination and agreed with the work done by Gill in his work on the color tests for oils.
 3. The extracts of cottonseed hulls made with the above solvents when combined showed very small amounts of gossypol as identified by the aniline and acetyl derivatives. However, the amount found present was exceedingly small, amounting to a fraction of a gram in fifteen pounds of hulls. This amount

would have no toxic effect or at least the toxic effect would be so slight as to be negligible.

4. The water extract of cottonseed hulls was made and from this a substance was obtained which consisted of carbon, hydrogen, and oxygen in the proportions of carbon, 38.1%; hydrogen 5.4%; oxygen 56.5%. This substance was reddish brown and could not be decolorized by animal charcoals, etc. It gave no class reactions and was remarkably inactive towards most reagents. It gave a compound with copper nitrate and was acted on by caustic soda to some extent, giving a black, tarry mass.

Molecular weight determinations by the freezing point lowering method were not successful as they did not give reasonable results. The molecular weights calculated from the combustion and from the determination of copper in the copper nitrate were 597 and 612 respectively. The best formula fitting these molecular weights would be $C_{19}H_{33}O_{21}$. This compound is not listed in the literature and seems to belong in resin group.

Babassu Nut Oil

THE New York Journal of Commerce recently stated that, "The importance of the export of Babassu kernels in Brazil is steadily increasing, reaching last year the value of approximately \$3,000,000, according to Commercial Attache Carlton Jackson, Rio de Janeiro. Europe receives almost all of it, only one ton being shipped to the United States. There is practically no shipment of oil from these kernels, the oil being made in the countries of destination where the residue, after extraction, is used as cattle feed and fertilizer. The oil is used for various purposes; in Denmark it is used for the manufacture

of oleomargarine. A new Danish company has recently been formed for the purpose of trade with Brazil, the principle article desired being the Babassu kernels. Other countries that purchase quantities of the nut are Germany, Holland, France, Belgium, Portugal and Great Britain." Attempts to commercially introduce the Babassu nut into the United States have not been lacking, but so far, apparently, the results have been negligible.

It is claimed that Babassu nut oil is equal in quality to the oils for which it would substitute, but very little work with it has been done in this country, although Brazilian firms are anxious to do so.