Processing of Cottonseed

1. Pigment Distribution in Oils and Meals Produced by Hydraulic and Screw Press Methods

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Introduction

COTTONSEED is processed for oil in the United States by the hydraulic and continuous screw press methods. In both processes the seed is usually cooked for periods ranging from 30 minutes to one hour after which it is subjected to high pressures to express the oil. The amount of moisture added to the seed before or during cooking and the duration of cooking may vary greatly in different mills because they are based upon the conditions which have been found in practice to produce the maximum amount of oil from the type and quality of seed usually received at a given mill. In addition to these variations, processing of cottonseed by the hydraulic and continuous screw press methods may differ with respect to the amount of hulls left with the meats and the temperatures and pressures attained during expression of the oil. Hulls are frequently not removed prior to processing by the screw press method, and the temperatures and pressures during expression of the oil are usually higher than in the hydraulic press method.

In one of the earliest scientific investigations of the effect of processing on the pigmentation of cottonseed (1) it was observed that the amount of gossypol extractable with diethyl ether decreased markedly upon prolonged cooking of the kernels. Correlation of this observation with the absence of toxicity in processed cottonseed meal led to the theory that the gossypol in raw cottonseed reacts with the free amino groups of the protein of the surrounding tissue under the influence of heat to form "bound" gossypol. Subsequently, support for the theory of "bound" gossypol was found in the observation of Clark (2) that the compound, which forms when cooked cottonseed is extracted with hot aniline, is identical with dianilino-gossypol formed by the reaction of pure gossypol with aniline. In more recent investigations, Gallup (3) showed that the "bound" gossypol of cooked cottonseed does not account for all of the gossypol of the original uncooked seed. This investigator (4) also showed that cooked cottonseed of the same "free" or "bound" gossypol content could be produced by different conditions of cooking, and that these cooked seed did not necessarily produce the same physiological effects when fed to experimental animals. Gallup concluded from these observations that, during cooking of cottonseed, gossypol undergoes more complex changes than can be accounted for by its combination with the free amino groups of the protein of the seed.

Investigations of the pigmentation of cottonseed oils have likewise been limited to determinations of their gossypol content. Oils obtained by continuous screw pressing are reported to contain more gossypol than hydraulic-pressed oils (1, 5, 6), and the more compact foots and lower refining losses of the former oils have been attributed (6) to their higher gossypol content.

Nevertheless, it is quite apparent that the dark colors of crude cottonseed oils cannot be due entirely to the presence of the pale yellow gossypol. Moreover, the colors of the glands, in which the pigments of the raw seed are largely localized, range from a pale yellow to a deep purple color, and the more deeply colored glands are found to predominate in most samples of cottonseed. These observations may be presumed to indicate the presence of pigments other than gossypol. Recent investigations of cottonseed pigments have resulted in the isolation of three pigments in addition to gossypol. These are gossyfulvin (7), an orange-colored pigment; gossypurpurin (8), a purple cottonseed pigment; and gossyaeurulin (9), a blue pigment which has been found only in cooked cottonseed. Solutions of these pigments exhibit characteristic and specific absorption in the visible and ultraviolet wavelength regions. Characteristic absorption bands occur at different wavelengths so that it is possible to measure the relative concentration of each pigment in any given solution or extract of the pigments in terms of the absorption at the wavelength of maximum absorption of each pigment. The instability of gossypol and the fact that the more recently isolated cottonseed pigments are derivatives of gossypol seemed to indicate that the latter might be converted to other pigments during the processing of cottonseed and that these conversion products might be responsible for the pigmentation of cottonseed products.

Recent investigations of the structure of cottonseed pigment glands demonstrated the existence of a wall enclosing the pigments. It was found that the gland wall is highly resistant to a variety of physical and chemical forces but is readily ruptured by the action of water (10, 11). Since the reactivity of the gland wall determines the behavior of pigments in stored seed (11), it seemed probable that it would also affect their behavior during cooking of the seed and expression of the oil.

The present investigation was undertaken with the objective of determining the nature of the changes which occur in gossypol and other pigments during the cooking and subsequent pressing of cottonseed by the hydraulic and continuous screw press methods. Because of the instability of the cottonseed pigments and the difficulty of reproducing actual processing conditions on a small scale the investigation was presented at the 37th annual meeting of the American Oil Chemists Society, New Orleans, Louisiana, May 15, 1946.
divided into two parts: (1) laboratory-scale experiments on the effect of cooking; (2) mill-scale experiments on the effect of cooking and pressing. The laboratory-scale cooking experiments were designed to simulate two extremes of moisture obtaining during mill-scale cooking of seed. Mill-scale experiments were run at two mills, at one mill by both the hydraulic and screw press methods and at the other by the hydraulic method only. Although the seed processed at the two mills were of similar origin and variety and the processing conditions by the two hydraulic press methods were approximately the same, the crude oils produced at the more southern mill were very much darker than those produced at the more northern mill. At the mill where seed was processed by both the hydraulic and continuous screw press methods the screw-pressed oils were more deeply colored than the hydraulic-pressed oils.

**Methods**

**Absorption spectra of pure pigments.** The absorption spectra of chloroform solutions of the known gossypol pigments are shown in Figure 1. Gossypol exhibits two principal absorption bands (12) in the wavelength region studied, one in the ultraviolet with maximum at 288 to 289 nm, \( E_{1\%}^{1\text{cm}} = 674 \); and the other in the near ultraviolet with maximum at 363.5 nm, \( E_{1\%}^{1\text{cm}} = 386 \). Gossyfulvin has two principal absorption bands (12), one in the ultraviolet, with maximum at 312 to 313 nm, \( E_{1\%}^{1\text{cm}} = 370.9 \); and the other in the visible with maximum at 439 to 440 nm, \( E_{1\%}^{1\text{cm}} = 649.4 \). Gossycaculin has a single broad absorption band in the visible wavelength region (9) with maximum at 605 nm, \( E_{1\%}^{1\text{cm}} = 315.4 \).

Since the absorption spectrum of gossypurpurin had previously been determined (8) only in extracts of cottonseed, this pigment was prepared and its absorption spectrum determined after adequate purification. An etheral extract of cottonseed was re-extracted with \( \frac{1}{2} \) ammonium hydroxide containing 10% sodium dithionite (\( \text{Na}_2\text{S}_2\text{O}_4 \)), and the separated alkaline extract allowed to stand for one hour, whereupon an amber-colored solid separated. The alkaline suspension was treated with enough concentrated hydrochloric acid to reduce the pH to 8.4, and was then extracted with diethyl ether. Fifty ml. of glacial acetic acid was added to the ether extract, the mixture was heated on a steam bath for 30 minutes and allowed to stand overnight. The purple precipitate which formed was separated and washed by decantation with petroleum naphtha (Skellysolve \( F \)), transferred to a Buchner funnel and dried. In order to remove acetic acid a slurry of the compound in water was heated on a steam bath for two hours. The mixture was then freed of gossypol by repeated extraction with 55% ethanol, in which gossypol is very soluble and gossypurpurin only slightly soluble. Chloroform solutions of this purified preparation of gossypurpurin exhibited two absorption bands in the ultraviolet wavelength region, with maxima at 326 to 327 nm, \( E_{1\%}^{1\text{cm}} = 184.6 \), and 370 to 371 nm, \( E_{1\%}^{1\text{cm}} = 195.7 \); and two absorption bands in the visible wavelength region with absorption maxima at 530 nm, \( E_{1\%}^{1\text{cm}} = 186.7 \), and at 565 to 566 nm, \( E_{1\%}^{1\text{cm}} = 225.7 \).

**Preparation of samples.** In order to accomplish contact of all the pigment glands of the seed with the solvent all seed samples were very thoroughly comminuted. Large samples of seed were de-hulled

![Fig. 1. Absorption spectra of pure cottonseed pigments.](image_url)

A. Gossypol
B. Gossyfulvin
C. Gossypurpurin
D. Gossycaculin