Modified Procedures for Determination of Gossypol Pigments. II. Determination of Free Gossypol in Low Gossypol Meals and of Gossypol in Oils

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Abstract

Much of the "free" gossypol in meals of low free gossypol content consists of "soluble-bound" gossypol. Addition of dilute hydrochloric acid reduces the optical densities of gossypol blanks in free and total gossypol calibrations and assays. The reductions in these optical densities were lower than expected in free gossypol assays on meals of lower free gossypol content. The soluble-bound gossypol in these can be converted into gossypol by heating the extracts with dilute acid at 100C. This does not affect the optical densities of the nongossypol pigments, nor does it affect the optical densities obtained on heating with aniline. The procedure affords more accurate analyses for these meals. A similar modification has been developed for gossypol in oils.

Introduction

Pons et al. (5) point out that the gossypol analytical method is valid, e.g., for crude oils, if the spectrophotometric absorptions because of their gossypol pigments do not differ materially from those of the equivalent amount of pure gossypol since the same technique is used in the calibration.

The addition of a drop of dilute hydrochloric acid has been shown to reduce the optical densities of gossypol blanks in both calibrations and assays for free and total gossypol (2). However the reductions in these optical densities in sample extracts for free gossypol assays on cottonseed meals have been lower than expected.

Martin (4) has shown that most of the free gossypol of cottonseed meals of lower free gossypol contents consists of soluble-bound forms of gossypol. King et al. (3) show that the gossypol-like pigments in an aqueous acetone extract of cottonseed meats that had been cooked for 18 hr could be converted into gossypol by heating for one hour at 65C with 1% of hydrochloric acid. Likewise Pons et al. (5) converted the gossypol-like pigments in cottonseed oil to gossypol by heating with oxalic acid, as used in the official procedure for total gossypol determinations in meals and meats (1).

Accordingly the use of hydrochloric acid has been investigated for the conversion of these substances into gossypol in free gossypol assays on cottonseed meals and in the determination of gossypol in oils.

Experimental Procedures

An aqueous acetone extract of cottonseed meats had an optical density of 0.87. A drop of dilute hydrochloric acid reduced this to 0.247. Heating of duplicate aliquots with a drop of dilute acid at 100C reduced the optical density only slightly more, to 0.230 and 0.222.

Duplicate sets of aliquots of a 70% acetone extract of screw-press cottonseed meal were heated with one drop (0.05 ml) of dilute (1.2N) or of concentrated hydrochloric acid for one hour at 65C, or for 10 minutes at 100C. Ten milliliters of 80% aqueous isopropyl alcohol were also added to the samples to be heated at 100C to prevent evaporation to dryness. One set of aliquots was then heated with aniline, and, after cooling, both sets were diluted to volume with aqueous isopropyl alcohol. The results are shown in Table I. The reduction in the optical density of the unheated aliquot was less than half that of the heated aliquots. The aliquots containing a drop of concentrated acid gave a slightly higher optical density after heating with aniline than did those using the dilute acid.

Cottonseed oil samples were weighed into 50-ml volumetric flasks. Twenty-five milliliters of pure isopropyl alcohol, two drops (0.10 ml) of 10% aqueous thiourea, and five drops (0.25 ml) of dilute (1.2N) hydrochloric acid were added; the mixtures were heated for 30 minutes on the boiling water-bath. The flasks were then cooled, 5 ml of water was added, and the mixtures were diluted to volume with hexane-isopropyl alcohol (794 ml : 206 ml) (5).

Duplicate aliquots were taken. One was heated with aniline and cooled. Both were then diluted to volume with the hexane-isopropyl alcohol. The optical densities were 0.517 and 0.040. The corresponding optical densities of aliquots that were not heated with acid were 0.520 and 0.070.

Discussion

The soluble-bound forms of gossypol are not converted into gossypol by the action of hydrochloric acid at room temperature, but they are at higher temperature. Thus, as seen in the table, a drop of dilute hydrochloric acid reduced the optical density of a gossypol blank from 0.091 to 0.074, but heating reduced it to 0.046 to 0.050. Heating with dilute acid, followed by heating with aniline, gave the same optical density as did heating with aniline alone. This shows

TABLE I

Effect of Heating Low Free Gossypol Extracts with HCl on Gossypol Blanks and on Duplicates Heated with Aniline

	Preliminary Treatment				
	1 drop conc. HCl		1 drop dil. HCl		No acid
	1 hr/ 65C	10 min/ 100C	1 hr/ 65C	10 min/ 100C	
OD 1[a]	0.387	0.395	0.375	0.379	0.379
OD 2[b]	0.049	0.046	0.050	0.048	0.091
Difference	0.338	0.349	0.325	0.331	0.288

[a] Optical densities of aliquots heated with aniline measured at 442 mμ.
[b] Optical densities of gossypol blanks. The OD of the unheated blank, without acid, decreased from 0.091 to 0.074 on the addition of dilute HCl at room temperature.

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that the acid treatment did not affect the optical density of the nongossypol pigments present. When a drop of concentrated hydrochloric acid was used, heating with aniline did give a slightly higher optical density. Hence the use of concentrated acid should be avoided.

It is most convenient to heat with acid at 100C since the same boiling water-bath is used in the heating with aniline. Five or ten milliliters of aqueous isopropyl alcohol are also added, prior to heating with the acid to prevent evaporation to dryness. The optical density of the acidified extract of cottonseed meats was reduced only slightly on heating. Hence it would have a negligible effect in an assay. However heating of the acidified extracts should be employed in assays on cooked meats and in meals.

After heating of the mixture of oil and isopropyl alcohol with dilute hydrochloric acid for 30 min at 100C, dilution with hexane or isopropyl alcohol, or mixtures of these gave slightly turbid solutions. This is eliminated by the addition of 5 ml of water, followed by the dilution with hexane isopropyl alcohol. Some turbidity remained when the hexane-isopropyl alcohol was 400 ml: 600 ml but not when the mixture was 794 ml of hexane to 206 ml of isopropyl alcohol.

A sample of rather old rancid oil was analyzed. The aliquots, heated with aniline in the regular procedure, varied in optical density from 0.056 to 0.130. This may be owing to the oxidation of aniline by the oxidized oil. An aliquot, after heating with aniline plus two drops (0.10 ml) of 10% aqueous thiourea, had an optical density of only 0.036. Because cottonseed oils, especially those of lower free gossypol content, may contain relatively little antioxidant, the presence of thiourea in aliquots to be heated with aniline is desirable.

Crude cottonseed oils do not dissolve completely in pure isopropyl alcohol although the refined oil does. When one sample, containing 0.018% of gossypol, was mixed with isopropyl alcohol, the insoluble portion amounted to 2% of the weight of the oil, but it contained about two-thirds of the gossypol. It was readily soluble in petroleum ether. It was stable since the gossypol could be determined after evaporating the solvent on a steam-bath in an open beaker. It is probably a compound of gossypol with a portion of the phosphatide.

Analyses of oils by the modified method were compared with those determined by the regular method by using aniline as the reagent for both. The regular procedure gave 0.029% and 0.834% of gossypol, and the modified method gave 0.034% and 0.872%.

Proposed Modifications

The gossypol blank aliquots, in free gossypol assays on products of low free gossypol content, such as cooked meats and cottonseed meals, should be heated for 10 minutes on the boiling water-bath in the presence of one drop (approximately 0.05 ml) of dilute (1.2N) hydrochloric acid and of 5 to 10 ml of 80% aqueous isopropyl alcohol.

It would also be desirable to add one drop of dilute hydrochloric acid to the corresponding aliquots to be heated with aniline.

Because some slight darkening of the aniline may occur during heating in the presence of the acid, two drops (approximately 0.10 ml) of 10% aqueous thiourea should also be added to the gossypol blanks and sample aliquots prior to heating.

For the determination of gossypol in oil, the sample, not more than one gram, should be weighed into a 50-ml volumetric flask. Twenty-five milliliters of pure isopropyl alcohol, two drops (approximately 0.10 ml) of 10% aqueous thiourea, and five drops (approximately 0.25 ml) of dilute (1.2N) hydrochloric acid added. The mixture should be heated for 30 minutes on the boiling water-bath. The mixture is cooled; 5 ml of water are added and diluted to volume with hexane-isopropyl alcohol (794 ml: 206 ml). Duplicate aliquots of this solution are taken. One is heated with aniline in the usual manner, then both are diluted to volume with the hexane-isopropyl alcohol. It would be advisable to add an additional two drops of thiourea prior to heating with the aniline.

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