different candied zucca melon samples by each method (Table II). In addition to the correlation coefficient, r, the average difference of paired values, was calculated and a Student's t test made to determine if the average difference of the paired values was significantly different from zero. Values of r and t are given at the foot of Table II.

Data presented in Table II indicate that correlation between the methods is significant at the 99% significance level for determination of both total and reducing sugars. In both determinations the mean difference of the paired values was shown to be not significantly different from zero—i.e., the accuracy of the rapid method was equal to that of the official method.

Further experiments of identical design were made to compare the rapid method with the modified AOAC procedure in analysis of 80 samples of assorted candied fruits and jams. It was found that the methods are equivalent in precision and accuracy when applied to analysis of candied fruits. However, as shown in Table III, the rapid method gave 0.24% higher total sugars in jams than the modified AOAC procedure. No significant difference was found between the methods in the determination of reducing sugars in jams.

Application of the rapid method to analysis of fresh-frozen strawberries, with no added sugar, was satisfactory because, under the conditions of the test, no significant difference in accuracy or precision of the methods was noted. For fresh-frozen apricots and prunes the rapid extraction method had a significantly higher precision than the modified AOAC procedure, while the accuracy of the methods was equal.

The rapid method has the important advantage of requiring less time and involving fewer manipulations than the modified AOAC extraction procedure. Extraction requires only 15 minutes compared with 90 minutes by the conventional procedure of neutralization, boiling extraction, and clarification. A complete determination of total and reducing sugars may be made in less than 1 hour by the rapid method, as compared with 2.5 hours required for the same operation by the modified AOAC method.

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COTTONSEED MEAL IN POULTRY FEED

Inactivation of Gossypol by Treatment with Phloroglucinol

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NUMEROUS ATTEMPTS have been made to destroy the gossypol present in raw cottonseed kernels, or make it unavailable to animals. Most of the methods involve cooking in the presence of water to rupture the pigment glands that contain the gossypol, and to enhance the reaction of the gossypol with various meal components. One process utilizes reaction with organic amines to decrease the available gossypol (*6*). Altschul (*1*) and Curtin (*2*) have ably summarized this subject.

The amount of a characteristic yellow component [believed to be gossypol-cephalin (*9*)] present in the eggs of hens fed gossypol is an extremely sensitive measure of the availability of dietary gossypol (*3*). The exact relationship between the yolk discoloration observed during egg storage and the amount of the yellow component have not been established, but it is known that gossypol is the principal constituent of cottonseed meals that is responsible for such discoloration (*8*). Thus, any process that reduces available gossypol to a very low level should prevent yolk discoloration during storage.

Storherr and Holley (*7*) have proposed phloroglucinol as a reagent for determining gossypol content of mixed feeds containing cottonseed meal. In the present work, this reaction has been studied as a means of decreasing the level of available gossypol in a cottonseed meal.

Methods and Results

In preliminary experiments, a diet containing a commercial cottonseed meal known to contain free gossypol was compared with others to which 0.1 or 1.0% phloroglucinol was added, and with control diets containing phloroglucinol without cottonseed meal. Egg production with all of these diets was normal, and gossypol availability (estimated by the ammonia test) was not affected by the added phloroglucinol; all the eggs were discolored in ammonia.

During conventional processing much of the gossypol of cottonseed becomes bound in an unavailable form. Not enough is bound, however, to eliminate the possibility of gossypol-caused discoloration of yolks of eggs laid by hens fed cottonseed meal. Cottonseed meal was treated by a modification of an analytical procedure that utilized phloroglucinol, hydrochloric acid, and ethyl alcohol as the reagents and diets containing the treated meal were fed to hens. A sensitive biological test detected no available gossypol in the meal preparation, as measured by the gossypol-cephalin content of eggs from hens fed the meal.

The ammonia test consisted of comparing visually the discoloration produced when broken-out "gossypol eggs" or normal eggs were put into an ammonia-containing atmosphere for 2 hours. Eggs from hens fed 1% phloroglucinol had shell membranes of a salmon color, in the presence or absence of gossypol.

The next experiments consisted of reaction of the phloroglucinol with a sample of fat-free cottonseed kernels. Four hundred grams of hexane-extracted meats [free and total gossypol 0.86%, as determined by the method of Pons and others (*4*, *5*)] were made to react with 20 grams of phloroglucinol (Eastman) dissolved in 1600 ml. of 95% ethyl alcohol plus 50 ml. of concentrated hydrochloric acid. After heating on a steam bath in a covered vessel for 4 hours, the cover was removed, and the mixture was dried on the steam bath. The meal thus produced was magenta in color; its gossypol content could not be determined by the *p*-anisidine method (*4*) because of color interference. A control reaction without phloroglucinol produced a meal with a free and total gossypol content of 0.16%.

In biological tests, the first comparison was made between dietary levels of 2% of the original hexane-extracted meats and 2% of the reacted product. Eggs laid 9 to 13 days after the diets were started were analyzed in the same manner as before (*3*), with the results shown in Table I. In the second comparison, a diet containing 20% of the reacted meal—i.e., 20 grams of meal plus 80 grams of laying mash—was compared to one containing 2% of the original fat-free meats, or to diets containing 2 or 10% of the meal, using reagents and methods identical except for omission of phloroglucinol.

The phloroglucinol-reacted meal, when fed at a level of 2% (Experiment 1) or 20% (Experiment 2) could not be differentiated from the diets in which no source of gossypol was present; in contrast, 2% of the hexane-extracted meats resulted in eggs containing high levels of gossypol-cephalin. Meals treated with ethyl alcohol and hydrochloric acid contained much less available gossypol than the original meats, but enough to be easily detected.

Table I. Effects of Phloroglucinol Treatment of Cottonseed Meats on Availability of Gossypol to Hens

[Absorbance difference (absorbance at 400 mμ minus absorbance at 445 mμ) is a measure of the gossypol present in the egg as gossypol-cephalin]

	Gossypol Source			Absorbance Difference, Av. of All Eggs per Hen	
	Material for	Level in diet, %	No. Eggs per Hen		Av. of Hens
Expt. 1	None		1	0.011	0.011
			1	0.012	
			1	0.006	
			2	0.014	
	Hexane-extracted meats	2	2	0.423	0.373
			2	0.323	
	Hexane-extracted meats reacted with phloroglucinol + ethyl alcohol + HCl	2	2	0.009	0.008
			2	0.008	
			1	0.008	
Expt. 2	None		1	0.010	0.009
			1	0.010	
			1	0.008	
	Hexane-extracted meats	2	2	0.420	0.425
			2	0.430	
	Hexane-extracted meats reacted with phloroglucinol + ethyl alcohol + HCl	20	3	0.009	0.009
			3	0.009	
	Hexane-extracted meats reacted with ethyl alcohol + HCl	2	3	0.038	0.035
			3	0.032	
	Hexane-extracted meats reacted with ethyl alcohol + HCl	10	3	0.085	0.082
			2	0.079	

Discussion

The phloroglucinol-reacted product is the only cottonseed meal found thus far that contains no detectable available gossypol, as measured by the gossypol-cephalin contents of eggs from hens fed the meal. The practicality of this or similar reactions as steps in the manufacture of cottonseed meals for poultry feeding must await further study. Furthermore, no data are available on the effects of the treatment on total nutritive value. No attempt has been made to determine the conditions for maximum gossypol reaction; neither has the possibility of combining this treatment with such operations as cooking and screw-pressing been studied.

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