A Simplified Process for the Elimination of the Halphen-Test Response in Cottonseed Oils[1]

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Abstract

Since certain biological effects have been ascribed to the cyclopropenoids that give a positive response to the Halphen test, processes were explored to eliminate this response from cottonseed oils. Heating alkali-refined, Halphen-positive cottonseed oils in a modified laboratory deodorizer in the presence of cottonseed oil fatty acids, capric acid, citric acid, or phosphoric acid was found to be an effective method of eliminating this response.

These treated, Halphen-negative cottonseed oils, the untreated Halphen-positive cottonseed oil, and a corn oil control were incorporated into rations fed to different groups of laying hens. Hens that ingested either the Halphen-negative cottonseed oil or the corn control produced normal eggs, whereas hens fed the Halphen-positive cottonseed oil produced eggs with pink whites, decreased pH of whites, and increased pH of yolks on storage, and lower ratios of oleic acid to stearic acid in the yolk lipids.

The simple processes presented—particularly the use of cottonseed oil fatty acids—appear to offer a practical means of inactivating the cyclopropenoids in cottonseed oil and thus eliminating the biological effects attributed to them.

Introduction

In two recent reviews on cyclopropenoids (1,2), it was pointed out that a number of investigators have shown that many oilseeds such as cottonseed contain cyclopropenoid (malvalic and sterculic) acids in their triglycerides. Although the concentration of these cyclopropenoids in cottonseed oil is relatively small (3–6), the incorporation of low levels of cottonseed oil into the diet of laying hens results in certain biological effects, such as production of eggs having pink whites, decreased pH of whites, increased pH of yolks, and enlarged, mottled yolks upon storage (1,2), and lower ratios of oleic acid to stearic acid in yolk lipids or tissue lipids (7–10).

However, if these cyclopropenoids are inactivated before being ingested by laying hens, the biological effects are no longer observed (10,11). Therefore, the cottonseed industry has become interested in the development of an economical method of producing cottonseed oils that are essentially free of cyclopropenoids. This paper reports a simplified process for the inactivation of cyclopropenoids in alkali-refined cottonseed oils.

Materials and Equipment

Commercial, alkali-refined, Halphen-positive cottonseed oil containing about 0.66% cyclopropenoids (calculated as malvalic acid); commercial corn salad oil (Corn Products Company, Mazola); crystallized distilled cottonseed oil super grade alkyd acids (Armour and Company, Neo Fat 140); distilled capric acid (Armour and Company, Neo Fat 10); anhydrous powder citric acid (Baker analyzed reagent grade); 85% phosphoric acid (Baker analyzed reagent grade); chromatographic alumina (Alcoa F-20 80–200 mesh); bleaching earth (AOCS Natural Bleaching Earth); and glass wool fiber (Pyrex No. 3950) were used.

The laboratory deodorizer for fats and oils described by Bailey and Feuge (12) was modified by removing the spray trap, inserting a thermometer well into the Claisen-type distilling flask, enlarging the base of the first dry-ice condenser, and installing the inlet near the base of the first dry-ice condenser and the outlet near the base of the second dry-ice condenser.

The fatty acid methyl esters from egg yolk lipids were analyzed in a Jarrell-Ash gas-liquid chromatograph, model 28-700, equipped with a flame-ionization detector and ¼ in. × 6 ft. stainless steel column packed with 18% ethylene glycol succinate on acid-washed Chromosorb W (80–100 mesh). The instrument was calibrated with a KD (lot 568-38) reference standard from Applied Science Laboratories and also with GLC reference mixture No. 1 from Hormel Institute.

Experimental Procedures and Results

Partial Inactivation of the Cyclopropenoids in Cottonseed Oil by Heat Treatment

In a 1-liter laboratory deodorizer (12) 370 g of commercial cottonseed oil containing 0.66% cyclopropenoids were heated at 235C for 1 hr with a small flow of nitrogen sparging through the mixture. The flow of nitrogen was discontinued, and the cottonseed oil was steam deodorized (12) at 235C for 1 hr at a pressure of 1 mm to eliminate the volatile substances. The oil had a positive response to the Halphen test (13). When the oil was analyzed by the stepwise HBr titration method (6), the titration value for cyclopropenoids was 0.15% (calculated as malvalic acid). The concentration of free fatty acids obtained for the oil was 0.02% (13) calculated as oleic acid.

When the oil was heated for 2 hr at 235C followed by deodorization at 235C for 1 hr, the titration value for cyclopropenoids was reduced to 0.04%. However, since the oil still had a positive response to the Halphen test, it was not assayed for biological effects with laying hens.

Elimination of the Halphen-Test Response in Cottonseed Oil by Treatment with Acids

Cottonseed Oil Fatty Acids and Capric Acid. In a modified 5-liter laboratory deodorizer, 1850 g (2.14 moles) of the commercial cottonseed oil containing 0.66% cyclopropenoids and either 49 g of cottonseed oil fatty acids (4 equivalents based on cyclopropenoid concentration) or 46 g of capric acid (6 equivalents based on cyclopropenoid concentration) were heated at 235C for 2 hr with a small flow of nitrogen sparg-

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TABLE I

Elimination of the Response of the Halphen Test in Cottonseed Oil by Heat Treatment [a] in the Presence of Cottonseed Oil Fatty Acids or Capric Acid

Acid equivalent [b]	Percent of cyclopropenoids [c]		Response of Halphen test	
	C/S acids	Capric acid	C/S acids	Capric acid
2	0.02	0.03	+	+
4	0.01	0.02	−	+[d]
6	0.01	0.01	−	−
8	0.01	0.01	−	−

[a] Heated for 2 hr at 235C followed by deodorization at 235C for 1 hr.
[b] Based on the equivalent concentration of cyclopropenoids.
[c] Determined by HBr titration (6) and calculated as malvalic acid.
[d] This response was only slightly positive, if at all.

ing through the mixture. The flow of nitrogen was discontinued, and the treated cottonseed oil was steam deodorized (12) at 235C for 1 hr at a pressure of 1 mm to eliminate the volatile substances, including excess free fatty acids. Oil treated with either cottonseed oil fatty acids or capric acid had a negative response to the Halphen test (13). When oil treated by either acid was analyzed by the stepwise HBr titration method (6), the titration value for cyclopropenoids was 0.01% (calculated as malvalic acid). This value is within the sensitivity limits of the method. The concentration of free fatty acids obtained for both oils was 0.02% (13) calculated as oleic acid. The results of additional experiments with varying equivalents of both acids show that in comparison with cottonseed oil fatty acids, a higher molar concentration of capric acid was required to achieve the same effect, perhaps because of its greater volatility (Table I).

The salient features to be noted, however, are that either the higher molecular weight cottonseed oil fatty acids or the lower molecular weight capric acid completely inactivates the cyclopropenoids in alkali-refined cottonseed oil, and that the resulting oil is light in color.

Citric Acid. In a 5-liter round-bottom flask equipped with stirrer, thermometer, and inert gas inlet, 1850 g (2.14 moles) of the commercial cottonseed oil containing 0.66% cyclopropenoids and 37 g of citric acid (13 acid equivalents based on cyclopropenoid concentration) were stirred and heated at 235C for 2 hr under a nitrogen atmosphere. The treated oil was cooled to room temperature and filtered through a thin layer of glass wool to remove dispersed particles. After steam deodorization as described above, the oil had a negative response to the Halphen test. The titration value for cyclopropenoids was 0.01%.

Although the citric acid treatment is effective and the treated oil is light in color, the monofunctional fatty acid treatments appear to be more promising in that these fatty acids are readily soluble in the oil; they do not tend to char in the oil; and they do not promote the formation of dispersed particles requiring filtration.

Phosphoric Acid. In a 5-liter round-bottom flask equipped with stirrer, thermometer, and inert gas inlet, 3000 g (3.5 moles) of the commercial cottonseed oil containing 0.66% cyclopropenoids was bleached with 166 g of AOCS Official Natural Bleaching Earth (13).

In a 5-liter round-bottom flask equipped as above, 2820 g (3.26 moles) of the bleached cottonseed oil and 25 g of 85% phosphoric acid (10 acid equivalents based on cyclopropenoid concentration) were stirred and heated at 180C for 1 hr under a nitrogen atmosphere. The treated cottonseed oil was cooled to room temperature and percolated through an activated alumina column (1400 g alumina gravity packed in a 10 cm I.D. column) to remove excess phosphoric acid, fatty acids, and colored material. This treatment produces an oil which is unusually light in color. After steam deodorization as described above, the oil had a negative response to the Halphen test. The titration value for cyclopropenoids was 0.01%.

Although this process would not provide a practical approach for the inactivation of the undesirable cyclopropenoids in cottonseed oil, preliminary evidence indicates that it does eliminate the cyclopropenoid-phosphoric acid reaction products from the treated oil (14). Since the previously mentioned processes utilizing cottonseed oil fatty acids, capric acid, or citric acid do not eliminate the cyclopropenoid reaction products from the oil, treatment of cottonseed oil with phosphoric acid provides an excellent control oil, relatively free of such reaction products (14), for use in biological studies.

TABLE II

pH of Stored Eggs from Hens Fed Basal Ration Supplemented with 5% Oil

Oil		pH after 3 months		pH after 6 months	
Type	Treatment	White	Yolk	White	Yolk
Corn	(Negative control)	8.82	6.58	8.79	6.73
Cottonseed	(Postive control)	8.46	8.32	8.40	8.32
Cottonseed	Cottonseed acids	8.99	6.52	8.83	6.56
Cottonseed	Capric acid	8.97	6.55	8.79	6.58
Cottonseed	Citric acid	8.90	6.63	8.81	6.52
Cottonseed	Phosphoric acid	8.95	6.65	8.71	6.63

TABLE III

Fatty Acid Analysis of Eggs from Hens Fed Basal Ration Supplemented with 5% Oil

Oil		Fatty Acids in Yolk Lipids, %					
Type	Treatment	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
Corn	(Negative control)	Trace	25.4	2.03	10.2	36.9	26 1
Cottonseed	(Positive control)	0.63	28.4	Trace	20.7	23.6	26.7
Cottonseed	Cottonseed acids	Trace	26.2	1.92	10.7	34.9	26.8
Cottonseed	Capric acid	Trace	25.8	1.96	10.3	34.1	28.1
Cottonseed	Citric acid	Trace	25.3	1.56	10.7	36.8	26.2
Cottonseed	Phosphoric acid	Trace	25.9	1.90	9.9	36.1	26.7

Biological Assay of Treated-Halphen-Negative Cottonseed Oils

Six groups of 5 young-adult pedigreed White Leghorn hens were caged individually in a sanitary air-conditioned animal room. They were fed ad libitum for 10 days on a basal ration with the following percentage composition: ground corn, 60; soybean meal (50% protein), 30; alfalfa leaf meal (17% protein), 5; ground limestone, 2; bone meal, 1; corn distillers solubles, 1; and iodized salt, 0.5. To each pound of the mixture, the following compounds were added: manganese sulfate, 55 mg; vitamin B_2, 850 μg; vitamin B_{12}, 15 μg; choline, 120 μg; vitamin A, 2000 IU; and vitamin D_3, 495 ICU.

Each group of hens was then fed different experimental rations prepared by supplementing the basal ration with 5% of one of the following oils: 1) corn oil, 2) commercial alkali-refined cottonseed oil containing 0.66% cyclopropenoids, 3) Halphen-negative cottonseed oil prepared from the commercial cottonseed oil by heat treatment with cottonseed oil fatty acids, 4) capric acid, 5) citric acid, and 6) phosphoric acid, respectively.

After each group of hens had been fed its respective experimental ration for a period of 2 weeks, collection of eggs was begun. Twelve eggs were collected from each hen and stored at 1–5C. After 3 months' storage, each odd-numbered egg was analyzed for pH of the white, pH of the yolk, and discoloration of the

white. After being stored for 6 months, each even-numbered egg was analyzed for pH of the white, pH of the yolk, and discoloration of the white.

As shown in Table II, the pH of the white of the stored eggs produced by hens fed the ration supplemented with one of the treated Halphen-negative cottonseed oils was almost comparable to the pH of the white of the stored eggs produced by hens fed the ration supplemented with corn oil, whereas the pH of the white of the stored eggs produced by hens fed the ration supplemented with Halphen-positive cottonseed oil was decreased about 0.5 pH unit. In addition, the pH of the yolk of the eggs produced by hens fed the basal ration supplemented with one of the treated Halphen-negative cottonseed oils was comparable to the pH of the yolk of the eggs produced by the hens fed the basal ration supplemented with corn oil, but the pH of the yolk of the eggs produced by hens fed the basal ration supplemented with the Halphen-positive cottonseed oil was increased almost 2 pH units. In other words, the difference of about 2 pH units which is normally found between the white and yolk of eggs was reduced to about 0.1 pH unit in the eggs produced by the hens fed the basal ration supplemented with the Halphen-positive cottonseed oil. The marked decrease of pH difference between the white and yolk of eggs results from diffusional processes which occur in disordered eggs (15).

In appearance, the white and the yolk of the eggs produced by hens that ingested corn oil do not differ markedly from the white and yolk of eggs produced by hens that ingested the various treated Halphen-negative cottonseed oils. However, the white and yolk of the eggs produced by hens that ingested Halphen-positive cottonseed oil are characterized by pink discoloration of the whites, and enlarged, mottled yolks, caused respectively by diffusion of material from the yolk into the white and from the white into the yolk (2).

Steinke (16), of the Ralston Purina Company, has developed an excellent technique to illustrate the latter type of diffusion. His technique was used to prepare the boiled eggs shown in Figure 1. The mass diffusion of material from the white into the yolk is apparent in the quarter-sectioned egg on the left. This egg was produced by a hen whose ration was supplemented with Halphen-positive cottonseed oil. The quarter-sectioned egg on the right, which does not show diffusion of egg-white material into the yolk, was produced by a hen whose ration was supplemented with cottonseed oil treated with cottonseed oil fatty acids.

In addition, the yolks of the odd-numbered eggs from each hen were pooled and fatty acid methyl esters were prepared by interesterification of representative samples of the pooled yolk lipids (17). Although the fatty acid patterns in the oils were comparable for the untreated alkali-refined cottonseed oil, which contained about 0.66% cyclopropenoids, and the four treated cottonseed oils, the patterns in the lipids of the egg yolks differed. As shown in Table III, hens that ingested one of the treated cottonseed oils produced eggs with a fatty acid pattern in the yolk lipids similar to that of eggs produced by the hens that ingested corn oil. However, hens whose ration was supplemented with Halphen-positive cottonseed oil produced eggs that had a much lower oleic acid to stearic acid ratio in the yolk lipids than that of the hens whose ration was supplemented with one of the treated Halphen-negative cottonseed oils. These results are in agreement with the findings of Evans, et al. (7–9).

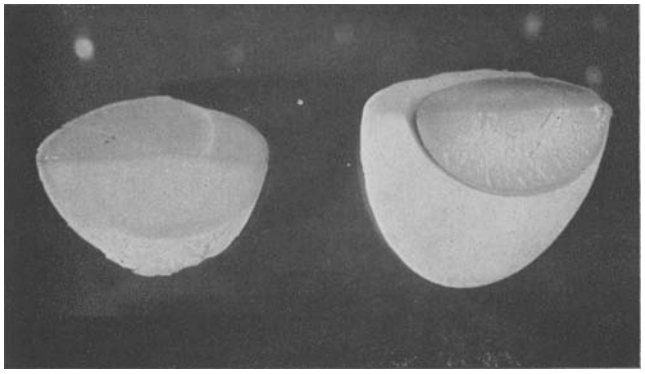

FIG. 1. Two quarter-sectioned boiled eggs prepared by the method of Steinke.

The simple processes for the reduction or elimination of the cyclopropenoids in cottonseed oil by heat treatment or heat treatment in the presence of small concentrations of monofunctional fatty acids, such as cottonseed oil fatty acids, appear to be of considerable industrial interest. Therefore, additional laboratory and pilot-plant investigations and biological assays are planned.

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