TABLE VI

Carbonyl Content in Aged Lard[a]

Carbonyl	Days at 62.5°C.				
	0	0.8	9	14	23
Saturated	6.4	6.7	9.4	9.9	37.7
Unsaturated	1.9	2.2	6.4	6.3	18.3
Total	8.3	8.9	15.8	16.2	56.0

[a] Micromole/gram.

unsaturated carbonyl exceeds that of the saturated while the opposite effect is noticed in the lard. The increase in the carbonyl of these aging vegetable oils parallels the increase in the peroxide, but at lower values. The appearance of rancid flavor in both oils accompanied by similar peroxide and carbonyl values suggests that a relationship exists. A marked increase in the rate of formation of saturated carbonyl after the rancid flavor appears is observed in both oils. Further work is in progress to explain these observations.

Summary

A convenient method for the quantitative determination of the carbonyl compounds in fats and oils is described. The procedure is based upon the formation of the 2,4-dinitrophenyl hydrazones of the carbonyl compounds in the presence of trichloroacetic acid catalyst and the colorimetric determination of the hydrazone compounds in alkaline solution. Standardizations are presented for the simultaneous determination of saturated and allenic carbonyl content from the absorbences at 430 and 460 mμ. Applications of these procedures to edible oils are described, and data are presented showing the carbonyl values in relation to other criteria of change. The problem of correlating changes in the carbonyl content of oils with flavor deterioration is now under investigation.

REFERENCES

1. Dean, W. J., and Dickson, R. B., Anal. Chem., *23*, 636 (1951).
2. Kawahara, F. K., and Dutton, H. J., J. Am. Oil Chem. Soc., *29*, 372 (1952).
3. Lappin, G. R., and Clark, L. C., Anal. Chem., *23*, 541 (1951).
4. Martin, C. H., Schepartz, A. I., and Daubert, B. F., J. Am. Oil Chem. Soc., *25*, 113 (1948).
5. Pool, M. F., and Klose, A. A., J. Am. Oil Chem. Soc., *28*, 215 (1951).
6. Schepartz, A. I., and Daubert, B. F., J. Am. Oil Chem. Soc., *27*, 367 (1950).

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The Antioxidant and Antipolymerization Properties of Gossypol, Dianilinogossypol, and Related Materials

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THE availability from certain other investigations in this laboratory of preparations of gossypol and several related materials led the authors to evaluate these materials for their antioxidant and antipolymerization properties. Mattill (15) and others (11, 18) have reported the antioxidant properties of gossypol, and more recently Hove and Hove (13) have investigated the antioxidant activity of dianilinogossypol. The potential availability of gossypol in amounts exceeding 40,000 tons annually (4, 17) with no current commercial outlet makes gossypol a particularly attractive raw material for investigation.

It was realized at the outset that the toxicological properties of certain of the materials tested were unestablished. However the concentrations at which gossypol has been found to be effective are below reported deleterious physiological levels (3, 9, 12). Dianilinogossypol moreover is said to be physiologically inert (12, 13). Similarly the fact that gossypol and related materials are highly colored even in dilute solution was recognized as a probable deterrent to their use as either antioxidant or antipolymerization agents except in those cases where color is unimportant.

Experimental

Preparation of Materials. The polyphenolic binaphthalene compound, gossypol, was obtained from acetone extracts of cottonseed pigment glands. This pigment was purified by crystallization from diethyl ether-light petroleum naphtha mixture. The yellow dog-toothed prisms of gossypol (1, 6) melted at 184°C.

Dianilinogossypol was prepared according to the method of Clark (8). It was recrystallized from chloroform and yielded orange rectangular plates (2) m.p. 300°C.

The combination products of gossypol-glycine and gossypol-urea were prepared by combining equal parts by weight of gossypol and urea and nine parts by weight of gossypol with one part by weight of glycine. The ingredients were combined in aqueous sodium hydroxide solution of pH 11. The pH of the mixtures was lowered to 7 by addition of hydrochloric acid, and the solutions were lyophilized. The yellow, dried combination products were water-soluble and contained sodium chloride (5, 7).

Gossypolaminobenzenethiol was prepared by dissolving one g. of gossypol in 20 ml. of diethyl ether. To this was added 4 ml. of 2-aminobenzenethiol. The product was washed with diethyl ether and dried. It was repeatedly recrystallized from hot benzene until further recrystallization did not increase the melting point. The orange boat-shaped crystals melted at 270° C. The formation of gossypolaminobenzenethiol might be expected to result from the reaction of 2 moles of 2-aminobenzenethiol with one mole of gossypol, producing a Schiff's base similar to dianilinogossypol. But the analytical data for gossypolaminobenzenethiol do not support this assumption.

Anal. Calc'd for $C_{30}H_{40}O_6S_2N_2$: C, 68.9; H, 5.46; N, 3.83; S, 8.74.

Found: C, 72.5; H, 5.8; N, 4.02; S, 3.31; ash, 0.21.

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The absorption spectrum of gossypolaminobenzenethiol in ethanol solution exhibited maxima at 249.5, 258, 309-311, and 442 mμ. The spectrum of this compound in ethanol or chloroform is similar to the absorption spectrum of dianilinogossypol in either solvent. Furthermore the absorption spectrum of the gossypolaminobenzenethiol-antimony trichloride reaction product possesses spectral characteristics which are similar to those of the dianilinogossypol-antimony trichloride reaction product. Dianilinogossypol and gossypolaminobenzenethiol react similarly with ferric chloride, potassium permanganate, and Fehling's solution. Both are insoluble in aqueous basic solution and are readily hydrolyzed to gossypol by strong acids.

Antioxidant Activity. The antioxidant activity of the several materials was evaluated at a concentration of 0.05% in two substrates, a) refined, bleached, and deodorized cottonseed oil having an initial peroxide value of zero and b) fresh, commercial prime, steam-rendered lard, free of added antioxidants and peroxides.

Each antioxidant to be tested was incorporated in each of the substrates at a concentration of 0.05% with the aid of 1-3 ml. of purified absolute ethanol. An equivalent amount of ethanol was added to the control sample. Twenty g. of the fortified substrates as well as their controls were placed in test tubes previously cleaned with a detergent (10) and were aerated with purified air at the rate of 2.33 ml./sec. (14, 19) at 97.7°C. At regular intervals a 1-g. sample was removed from each tube, and the peroxide value was determined according to the method described by Moore and Bickford (16). All of the stability measurements were made in duplicate.

Antipolymerization Activity. The effectiveness or lack thereof, at selected concentration levels of the various inhibitors acting within the several monomers, was measured as change in viscosity. Viscosity was determined by means of the Gardner-Holdt bubble tube standards. The viscosity values thus obtained, of course, lack accuracy from the absolute standpoint. For the purpose of comparative evaluation and from the standpoint of ease of experimental manipulation, the bubble tube technique is ideal. Each monomer, freed of stabilizer by vacuum distillation, was added to a series of viscometer sample tubes. Each sample tube of the series contained a selected concentration level of the particular material under investigation. The bubble tubes containing monomer and inhibitor were subjected to the polymerizing treatment (ultraviolet light or heat). The sample tubes were then compared, at intervals with the Gardner-Holdt bubble tube standards, and the extent of polymer formation as indicated by increase in viscosity was recorded. Controls (monomers free of inhibitor) were set up for each series. Each treatment (stabilizing material and individual concentration level thereof) was replicated. Heat polymerization was carried out in a thermostatically controlled water bath. Polymerization by ultraviolet light was carried out at room temperature with the samples placed equidistant from the light source.

Results and Discussion

The antioxidant activity of gossypol and related substances in lard and cottonseed oil is shown in Table I.

TABLE I

Comparison of Antioxidant Activities of Gossypol and Various Other Inhibitors in Lard and Cottonseed Oil

Antioxidant[a]	Lard		Cottonseed oil	
	AOM hours[b]	Antioxidant index[c]	AOM hours[b]	Antioxidant index[c]
Control	9.0	1.0	8.9	1.0
Hydroquinone	.....	....	35.0	3.9
Gossypol	75.0	8.3	14.5	1.6
Dianilinogossypol	52.0	5.8	12.2	1.4
Gossypol-urea	55.5	6.1	13.3	1.5
Gossypol-aminobenzene-thiol	66.0	7.3	12.5	1.4
Gossypol-glycine	49.0	5.4	12.4	1.4

[a] The antioxidants were used in a concentration of 0.05%.
[b] Number of hours required by the sample to acquire a peroxide content of 100 milli-equivalents per kilogram of fat during aeration at 97.7°C. with an air flow of 2.33 ml./sec.
[c] Ratio of AOM hours of stabilized to unstabilized substrate.

Hydroquinone was included in both the antioxidant and antipolymerization tests for purposes of comparison. Table I indicates the antioxidant performance of gossypol and the gossypol-related materials. The effectiveness of the related materials is roughly equivalent to gossypol on a molar basis. Such a relationship has been commented on earlier in the case of dianilinogossypol and gossypol by Hove and Hove (13).

The antipolymerization activity of gossypol, dianilinogossypol, and hydroquinone in styrene, vinyl acetate, and methyl acrylate are shown in Table II.

TABLE II

Comparison of Antipolymerization Activities of Gossypol, Dianilinogossypol and Hydroquinone in Various Monomers

Inhibitor	%	Styrene[a,c]	Vinyl acetate[b,c]	Methyl acrylate[b,c]
Control[d]	.....	68	60	19
Gossypol	0.01	68	66	23
Gossypol	0.05	142	>160[e]	72
Dianilinogossypol	0.01	114	90	43
Dianilinogossypol	0.05	>164[f]	>160[e]	96
Hydroquinone	0.01	90	60	19
Hydroquinone	0.10	142	160	19

[a] Expressed as the time in hours required at 70°C. to attain a viscosity of 148 poises measured at 25°C.
[b] Expressed as the number of hours required under ultraviolet irradiation for the samples to attain a viscosity of 148 poises measured at 25°C.
[c] All viscosities were measured by means of a Gardner-Holdt Bubble Viscometer.
[d] Substrate without added inhibitor.
[e] Viscosity still less than 0.50 poises after 160 hours.
[f] Viscosity measured 80.4 poises at 164 hours.

Dianilinogossypol at the 0.05% level is a very effective polymerization inhibitor for each of the monomers studied. Dianilinogossypol at the 0.01% level is very nearly as effective as a polymerization inhibitor as is hydroquinone at ten times that concentration. At both the 0.01% and 0.05% level dianilinogossypol is superior as a polymerization inhibitor to gossypol at equivalent concentrations. It is noteworthy that in the role of antipolymerization agents, the relative effectiveness of gossypol and dianilinogossypol is reversed with respect to their antioxidant activity.

Monomers protected with gossypol or kindred products are highly colored and range from light yellow to bright orange even at low levels of concentration. This fact imposes certain obvious limitations on the use of these materials except in those cases where color is unimportant or where ultimate removal of the inhibitor is contemplated.

REFERENCES

1. Adams, R., Morris, R. C., Geissman, T. A., Butterbaugh, D. J., and Kirkpatrick, E. C., J. Am. Chem. Soc., *60*, 2193 (1938).
2. Adams, R., Price, C. C., and Dial, W. R., J. Am. Chem. Soc., *60*, 2158 (1938).
3. Ambrose, A. M., and Robbins, D. J., J. Nutrition, *43*, 357 (1951).
4. Bailey, A. E., "Cottonseed," Interscience Publishers Inc., New York, 215 (1948).
5. Castillon, L. E., and Altschul, A. M., Proc. Soc. Exptl. Biol. Med., *74*, 623 (1950).
6. Castillon, L. E., Hall, C. M., and Boatner, C. H., J. Am. Oil Chem. Soc., *7*, 233 (1948).
7. Castillon, L. E., Karon, M. L., Altschul, A. M., and Martin, F. N., Arch. Biochem. Biophys., in press.
8. Clark, E. P., J. Biol. Chem., *75*, 725 (1927).
9. Eagle, E., Castillon, L. E., Hall, C. M., and Boatner, C. H., Arch. Biochem., *18*, 271 (1948).
10. Fore, S. P., Moore, R. N., and Bickford, W. G., J. Am. Oil Chem. Soc., *28*, 73 (1951).
11. Hove, E. L., J. Biol. Chem., *156*, 633 (1944).
12. Hove, E. L., and Hove, Z., J. Biol. Chem., *156*, 611 (1944).
13. *Ibid.*, *156*, 623 (1944).
14. Mattil, K. F., Filer, L. J. Jr., and Longenecker, H. E., Oil and Soap, *21*, 160 (1944).
15. Mattill, H. A., J. Biol. Chem., *90*, 141 (1931).
16. Moore, R. N., and Bickford, W. G., J. Am. Oil Chem. Soc., *29*, 1 (1952).
17. Olcott, H. S., Cotton and Cotton Oil Press, *43*, No. 7, 22 (1942).
18. Royce, H. D., Oil and Soap, *10*, 123 (1933).
19. Swift, C. E., Mann, G. E., and Fisher, G. S., Oil and Soap, *21*, 317 (1944).

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Reactions of Fatty Materials With Oxygen. XV.[1] Formation of 9,10-Dihydroxystearic Acid and Cleavage Products in the Oxidation of Oleic Acid and Methyl Oleate in Acetic Acid[2]

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THE prolonged autoxidation of unsaturated fatty materials in the absence of solvents leads eventually to highly viscous products through which efficient dispersion of oxygen is extremely difficult (11). The obvious solution, namely, use of a diluent, is complicated by the problem of finding an "inert" material. Consideration of possible "inert" solvents, as well as examination of the literature, indicated that glacial acetic acid most nearly meets the requirements and, in addition, it possesses the characteristic of accelerating autoxidation reactions (1, 5, 13).

This paper describes the results obtained in the autoxidation of oleic acid and methyl oleate at 25-30°, 65°, and 115-120°C. in acetic acid solution with cobalt acetate (or occasionally cobalt oleate) as the catalyst. Samples were withdrawn at suitable time intervals, the acetic acid was recovered, and the non-volatile residue was worked up to isolate 9,10-dihydroxystearic acid, short-chain cleavage products (mono- and dibasic), unoxidized and monohydroxy materials, and polymers.

Experimental

Materials Used. The oleic acid and methyl oleate (composition: oleic, 94-98%; linoleic, 0.3%; saturates, 2-6%) were prepared from olive oil (7). Cobaltous acetate and glacial acetic acid were analytical reagent grade. Cobaltous oleate was prepared from purified oleic acid as described previously (11).

Autoxidation Procedure. A typical experiment at 65° is described in detail. (Tables I, II, and III summarize the results obtained at 25-30°, 65°, and 115-120°). A solution consisting of 1,000 g. of oleic acid (or methyl oleate), 10.1 g. of cobaltous acetate (or 33 g. of cobaltous oleate), and 2,000 ml. of glacial acetic acid was prepared in a 5-l., three-neck flask equipped with a thermometer, a reflux condenser, and two fritted discs immersed in the solution. A vigorous stream of air or cylinder oxygen was passed through the solution while the temperature was maintained at 65°. Samples of the solution (ca. 450-500 ml.) were withdrawn at suitable time intervals for isolation and analysis of oxidation products.

The acetic acid was recovered under reduced pressure and the residue analyzed. The oxidation was stopped when the iodine number became substantially constant (5-10); this usually required 150-250 hours at 65°. The peroxide values were negligible throughout.

The residue was refluxed for six hours with an excess of 6 *N* aqueous sodium hydroxide, acidified while hot with 6 *N* sulfuric acid, and stirred vigorously. A dark brown viscous oil separated. After the mixture had cooled to room temperature, it was treated with ether and the aqueous layer was reextracted. The combined ether solutions, in which a considerable quantity of insoluble white solid was suspended, was washed three times with small quantities of water to remove excess sulfuric acid and salt. These washings were reextracted with ether, and all ether solutions were then combined. The ether:solute ratio was approximately 3:1 at this point. The ether solution was cooled to 0 to 5° and filtered, and the solid was washed once with a small amount of cold ether. The precipitate was substantially pure high-melting 9,10-dihydroxystearic acid, m.p. 129-130° and neutralization equivalent, 317-323. A mixed melting point with an authentic sample, m.p. 130-131°, showed no depression. The yield was 12-17%, depending on the oxidation time (Table II). [An additional 1-2% of slightly impure high melting 9,10-dihydroxystearic acid, m.p. 120-123°, could be obtained by evaporation of the ether, solution of the residue in acetone (5 ml. per g. of solute) and cooling to −20°, followed by recrystallization of the precipitate from 95% ethanol at 0°.]

The dark-brown viscous residue obtained after recovery of the ether was converted to methyl esters by refluxing for eight hours with a large excess of anhydrous methanol (sulfuric acid catalyst). These esters were distilled from a Claisen flask to a maximum pot

[1] Paper XIV is reference 10.
[2] Presented at the Fall Meeting of the American Oil Chemists' Society, Chicago, Ill., Nov. 2-4, 1953.
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