Discussion

A tentative molecular formula for gossypurpurin, $C_{30}H_{32}O_7N$, is proposed on the basis of its quantitative elemental composition. The relative ease of conversion of gossypurpurin to gossypol by mineral acid and the identity of the reaction products of both gossypurpurin and gossypol with aniline would seem to indicate that the basic structures of the two pigments are similar. Qualitative tests indicate that gossypurpurin possesses reactive groups similar to those of gossypol, i.e., one or more carbonyl groups, two or more phenolic hydroxyls, and one or more hydroxyls peri or ortho to a carbonyl.

Purification of the isolated native pigment proved rather difficult, owing to the simultaneous extraction from pigment glands by chloroform of materials other than gossypurpurin. Removal of these impurities from the isolated pigment by treatment with dilute acetic acid apparently was not complete since it was not possible to obtain consistent analytical data for the isolated gossypurpurin, and they did not agree exactly with those for the synthetic product. Therefore it cannot be stated with absolute certainty that the two pigments are completely identical. However the native gossypurpurin exhibited an absorption spectrum in chloroform solution with maxima in the same positions as that of the artificial product, and the ratios of the extinction coefficients at 566-568 mμ to those at 530-532 mμ were identical. The similarity of the absorption spectra of the two products indicates that the chromophoric groups are very similar, if not identical, in both native and synthetic gossypurpurin. Native gossypurpurin also gave the same melting point, characteristic blue-green antimony trichloride test, and qualitative tests as the gossypurpurin prepared from gossypol and the conversion to gossypol by acid was identical in both cases. The similarity or identity of the chemical and physical properties of the two compounds would seem to indicate that they possess the same reactive groups and basic nuclei.

Summary

Gossypurpurin was prepared from gossypol via diaminogossypol, and its properties compared with gossypurpurin isolated from cottonseed pigment glands. A tentative molecular formula for synthetic gossypurpurin, $C_{30}H_{32}O_7N$, has been proposed on the basis of its elementary composition. The native pigment could not be obtained in the same degree of purity as the synthetic product and the analytical data could therefore not be brought into exact agreement for the two products. However solutions of both pigments in chloroform exhibit almost identical absorption spectra and identical antimony trichloride tests. Qualitative reactions seem to indicate that the functional groups of both native and synthetic gossypurpurin are identical, and the ready conversion of both products to gossypol upon contact with acid seems to indicate that their basic structures are similar.

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Antioxidants in Aqueous Fat Systems[1]

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WITHIN the past few years several highly effective antioxidants have been developed for the stabilization of fats and oils. Standardized, accelerated tests for the evaluation of antioxidants are widely used, and the results of such tests seem to be generally applicable to the practical problem of protecting substantially pure dry fats during handling and storage.

The situation with respect to the protection of foods in which fat is one component of a more complex, polyphasic system is less encouraging. Antioxidants which have been successful in dry fats have in many instances proved unsuitable for the stabilization of meats, fish, dairy products, baked and fried foods, etc. Precise methods for the evaluation of antioxidants in aqueous fat systems are lacking.

The present study is an evaluation of a number of antioxidants in an artificial aqueous fat system. The study includes phenolic as well as synergistic antioxidants. The phenolic inhibitors included are those now being used in edible fats, i.e., tocopherol, the gallates, nordihydroguaiaretic acid (NDGA), and butylated hydroxyanisole (BHA).

Of a number of synergistic antioxidants previously tested in aqueous fat systems, a group of polyphosphates was most effective in contact with lard (plain or containing added tocopherol) (4). Hence the activity of this group has been further investigated in contact with lard containing the other phenolic inhibitors listed above. A few experiments have also been included on ammonium and potassium polyphosphates, not previously investigated. Orthophosphates, citric acid, and ascorbic acid, the three synergists which have probably been most widely studied in food fats, were also compared with the polyphosphates.

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Methods and Materials

Unless otherwise specified, the fat used was fresh lard bought on the local market. Only samples showing no initial peroxide number were used. The primary antioxidants, alpha-tocopherol, NDGA, BHA, and propyl and lauryl gallates, were dissolved directly in the fat in concentrations of .05%. With the exception of NDGA all were soluble in the cold fat in this concentration. Since the NDGA sample required heating to 150°C. for solution, all were so treated. These concentrated solutions were then diluted with plain lard to the final concentration of .005% antioxidant used in all experiments.

The aqueous phase, unless otherwise stated, was a borate buffer, .01 molar, pH 7.5. This buffer was previously shown to have no effect on rancidity (3). The synergists were adjusted to pH 7.5 with NaOH or HCl, then added to the aqueous phase in a final concentration of 0.1%. Of the phosphates included in this study, Maddrell's salt $(NaPO_3)x$ and potassium metaphosphate $(KPO_3)x$ are insoluble and of unknown structure. Hexametaphosphate $(NaPO_3)x$ and ammonium metaphosphate $(NH_4PO_3)x$ are very soluble and highly polymerized. The ammonium compound is highly viscous even at low concentrations. Both hydrolyze readily in solution. The tripolyphosphate $Na_5P_3O_{10}$, more definitely characterized than the others, is also water-soluble.

All the sodium compounds were previously shown to act as antioxidants in aqueous fat systems. The tripolyphosphate, like pyrophosphate, was less effective in pure solutions than Maddrell's salt or hexametaphosphate. On the other hand, its activity was not so drastically reduced by the activity of other ions (4), and it is slightly more resistant to hydrolysis than hexametaphosphate (1).

The method of obtaining contact between aqueous and fat phases has been previously described (3). The aqueous phase was absorbed on one filter paper, the melted lard containing .01% carotene on another. The paper containing the aqueous phase was placed in a Petri dish with the fat paper on top of it. Diameters of the papers used were 7½ cm. for the aqueous phase and 7 cm. for the fat, instead of two 9-inch circles as previously reported. With the smaller circles, air bubbles could be more easily eliminated, and there was less danger of oxidation initiated at the edges through lack of contact between fat and water soluble synergist. Several drops of a preservative, ethylene chloride, were added. The Petri dishes were sealed with paraffin and stored in an air oven at 45°C. The course of the oxidation was followed by visual inspection of the yellow color. The samples were considered rancid when the papers were approximately half bleached (4).

The method allows the rapid evaluation of the effect of a large number of factors on rancidity. The composition of either the fat or the aqueous phase may be varied at will. On the other hand, the method is somewhat less precise than stability tests on pure fats. There is some uncertainty in judging the time of half bleaching. When the aqueous phase contained an oxidation catalyst, such as ascorbic acid, the bleaching was more or less uniform over the entire paper. When the aqueous phase contained an effective antioxidant, bleaching was initiated at several spots, probably of less perfect contact between fat and synergist, subsequently spreading over the entire paper.

Another source of error occurred particularly in experiments of long duration. Although the Petri dishes were sealed to prevent loss of moisture, some condensation occurred on the covers of the dishes when they were removed for inspection. After some weeks sufficient moisture had left the papers and collected on the inside of the dishes to be visible as large droplets, thus disturbing the moisture relationships of the system. This might be prevented by eliminating changes of temperature during observation of samples, i.e., by use of a constant-temperature room.

Where agreement between duplicate samples was within 20%, an average value was reported. When the results did not agree within 20%, the range was reported. Lack of agreement occurred more frequently in experiments which continued for several weeks or months.

Results and Discussion

Comparison of phenolic inhibitors in the presence and absence of an aqueous phase. Table I summarizes the results of a typical experiment comparing the effect of various primary antioxidants added to lard on rancidity in the dry lard versus a lard-buffer system. In this experiment some of the filter papers containing the fat were placed in Petri dishes alone, others on top of papers containing the buffer solution. All were stored under the same conditions.

TABLE I

Effect of Various Primary Antioxidants on Lard in the Presence and Absence of an Aqueous Phase

Antioxidant added (.005%)	Days to turn rancid at 45°C.[a]	
	Dry lard	Lard and buffer (pH 7.5)
Plain lard	2	3
Alpha-tocopherol	8	8
NDGA	38	17
BHA	28	33
Propyl gallate	12	15
Lauryl gallate	11	12

[a] Time required for half bleaching of carotene (3, 4).

In the dry fat at this concentration NDGA was the most effective antioxidant used. The antioxidants fell into the same order of effectiveness when tested as discs of solid fat, prepared as previously described (4), at room temperature. In the presence of the buffer the effectiveness of the NDGA was greatly decreased, and BHA became the best of the antioxidants studied. This loss of activity of NDGA when brought into contact with the buffer was consistently obtained with several samples of lard.

The most obvious explanation for such shifts in the antioxidant activity of phenolic inhibitors when the fat is brought into contact with water would appear to lie in solubility relations. Unfortunately there seems to be no published data on partition co-efficients of this group of antioxidants between fat and water. Propyl and lauryl gallate were not significantly different in their order of effectiveness in aqueous as compared to dry fat systems although the lauryl gallate would be expected to show the greater fat solubility and has been shown to be somewhat more effective in baking (2). Observations at other pH values would be desirable; the pH may affect both solubility and stability of phenolic inhibitors.

Comparison of synergists in aqueous solution in contact with lard containing various phenolic inhibitors. In previous reports from this laboratory a number of synergists have been evaluated in solutions in contact with plain lard, or with lard containing added tocopherol. Ascorbic acid accelerated rancidity under these conditions (3) whereas citric acid and a group of condensed phosphates protected the fat (4). The activity of the synergists in the presence of water did not parallel their activity when suspended in dry fats.

TABLE II

Some Synergistic Effects with Various Primary Antioxidants in Aqueous Lard Systems

Synergist used[b]	Plain lard	Lard and .005% tocopherol	Lard and .005% NDGA	Lard and .005% BHA	Lard and .005% propyl gallate	Lard and .005% lauryl gallate
Buffer	2	4.5	4-6	22-29	11	6
Ascorbic acid	<1	<1	23	1	<1	<2
Citric acid	3	12	20	66	15-27	16
Orthophosphate (Na_2HPO_4)	2	6-8	11	43	7-14	6
Triphosphate	3	12-16	21	47-77	18	16
Hexametaphosphate	7	.....	34	56-93	28	21
Maddrell's salt	8	.....	31	64	28-41	27

[a] Time required for half bleaching of carotene (3, 4).
[b] Conc. = 0.1% in borate buffer pH 7.5.

Table II summarizes the effect of these synergists with other phenolic inhibitors. The same sample of lard was used for all comparisons. Within the rather wide limits of experimental error it will be oberved that, with the exception of ascorbic acid, the synergists tried fell in approximately the same order of effectiveness irrespective of the primary antioxidant used. Furthermore the protective factors obtained with any one synergist are within the same range with plain lard and with lard plus the several phenolic inhibitors.

In general, orthophosphate gave little protection, citrate and tripolyphosphate slight protection, and hexametaphosphate and Maddrell's salt gave the greatest protection obtained. The magnitude of the protective factors obtained with this sample of lard was smaller for all of the synergists than that previously reported (4). The factor can vary considerably with different samples of lard.

Ascorbate markedly accelerated rancidity with all primary antioxidants except NDGA in the experiment reported. Here and in three other experiments with NDGA at the .005% level, ascorbic acid gave protection. It has consistently accelerated with the other phenolic inhibitors at this level, with the exception of one isolated experiment with tocopherol and one with BHA, where slight protection was obtained. A fairly wide range of activity of ascorbic acid might be expected with different samples of natural fats since the accelerating effect has been shown to be confined to fatty acids more unsaturated than oleic (5), which may vary considerably in pork fat.

The effect of ascorbic acid in combination with polyphosphates. Early in the present study it was observed that addition of a polyphosphate to ascorbic acid solutions frequently eliminated their accelerating effect on rancidity. Thus in five experiments on different samples of plain lard in contact with borate buffers at pH 7.5, control samples turned rancid in 2.5 to 4 days, the addition of 0.1% ascorbic acid accelerated the rate in all cases to a few hours, hexametaphosphate alone increased keeping time to 7 to 17 days, and the combination of ascorbic acid and hexametaphosphate gave variable results ranging from acceleration of the control rate in one case to protections ranging up to 54 days. In all experiments in which a primary inhibitor (tocopherol, NDGA, or BHA) was added to the fat, the combination of ascorbic acid and hexametaphosphate gave prolonged protection.

These results were obtained at concentrations of 0.1% of both polyphosphate and ascorbate. Increasing the concentration of polyphosphate to 1% did not further retard rancidity, either when the phosphate was used alone or when combined with ascorbic acid. At an ascorbate concentration of 1%, acceleration was obtained with the ascorbate alone, but consistent prolonged protection resulted when this amount of ascorbate was combined with 0.1% or more of hexametaphosphate or tripolyphosphate in contact with plain lard.

Prolonged protective effects have also been obtained when ethylene diamine tetra acetic acid was combined with ascorbate in small concentrations either with or without primary inhibitors (5). The mechanism of the protective action of such combinations is unknown. Since both the polyphosphate and ethylene diamine tetra acetic acid form soluble, nonionized complexes with metals, it is possible that removal of traces of copper or other metallic impurity is responsible for the observed effects.

Summary

A number of phenolic inhibitors were tested in pure dry hard lard and in the same lard in contact with an aqueous solution at pH 7.5. In dry lard, at a concentration of .005%, the order of effectiveness of the antioxidants tested was NDGA (best), BHA, propyl gallate, lauryl gallate, and alpha-tocopherol. In contact with aqueous solutions at pH 7.5 NDGA was much less effective, and BHA was the best antioxidant tried.

Of a group of synergistic antioxidants in aqueous solution in contact either with plain lard or with lard containing .005% of the phenolic inhibitors mentioned above, hexametaphosphate and Maddrell's salt were the most effective. Ascorbate generally accelerated rancidity with all of the primary antioxidants except NDGA. Polyphosphates counteracted the accelerating effect of ascorbate. Combinations of polyphosphate with ascorbate and primary inhibitors gave prolonged protection.

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