CONSTITUENTS OF FATS AND OILS AFFECTING THE DEVELOPMENT OF RANCIDITY[1]

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I. INTRODUCTION

Like all organic materials, the fats are subject to deterioration. Most important of the degradative changes which may occur are those which lead to the condition known as rancidity. A fat may be described as rancid when it contains enough oxidation products or free short-chain fatty acids to provide organoleptic evidence of their presence. In those natural fats containing appreciable quantities of the lower fatty acids, hydrolysis induced by lipases derived from tissues or microörganisms is, in itself, sufficient to produce rancidity; oxidation may accompany such hydrolysis. However, spontaneous atmospheric oxidation, non-enzymatic in character, is by far the most common cause of rancidity. The "off" flavors and odors in various natural foods have been called rancid, pun-

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gent, tallowy, soapy, oily, ester-like (perfume or ketonic rancidity), metallic, musty, fishy, bitter, cardboard, and burnt. These descriptive terms are evidence of the complexity of the processes of rancidity.

The subject of oxidative rancidity in fats is obviously too extensive to be covered in its entirety here. No attempt will be made to discuss the proposed mechanisms, means of detection or measurement, or end products of the reactions resulting in rancidity. Several recent reviews covering various aspects of these fields of investigation are available (51, 54, 63, 80). Furthermore, the very broad subject of accelerators and inhibitors of these reactions has been limited to include only what is known about the natural constituents of fats and oils which affect their stability.

Since ethylenic linkages in the fatty acids are the points of attack by oxygen, it is obvious that the amount of unsaturation is among the factors which determine the susceptibility of fats to oxidation. Linoleic acid is oxidized more quickly than oleic acid (40, 79), and linolenic acid still more rapidly (47). A difference in the rate of oxidation was also observed between oleic acid and a mixture of oleic and linoleic acids (5), but oleic acid gave organoleptic evidence of rancidity sooner than the mixture, despite the higher peroxide value of the latter.

The molecular structure of the fatty acids also determines the ease with which a fat is oxidized (47). In polyethenoid acids the relative positions of the double bonds with respect to each other and in the chain are critical factors; thus, pseudo-eleostearic acid (conjugated) is more susceptible to oxidation than its isomer linolenic acid (non-conjugated) (44). The absorption spectra of edible oils indicate (56) that these contain traces of fatty acids with conjugate double bonds not accounted for by the amounts of recognized fatty acids present. The importance of this observation lies in the fact that these minute amounts of more reactive fatty acids may immediately give rise to active peroxides capable of initiating reaction chains. Morrell and his coworkers (57) and others (26) have made extensive studies leading to a differentiation between fat peroxides of differing reactivities. Little is known about the stability of synthetic simple or mixed glycerides.

It has long been recognized, however, that the stability of many fats and oils is *not* dependent primarily upon their relative saturation. Thus, sweet almond and sesame oils remain stable for much longer periods than other fats and oils of similar or higher iodine number (42). The extraordinary stability of wheat bran oil has lately been described (19). The first intimation that such stability could be attributed to naturally occurring antioxidants was suggested by Mattill (52), who confirmed the observation (2) that the addition of wheat germ oil to rat diets containing lard stabilized them against rancidity. Mattill and Crawford (55) later

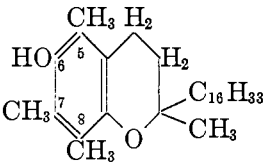
showed that the induction period of corn oil was markedly decreased by the various steps taken in its commercial production. Hilditch and Sleightholme (39) prepared purified fatty acids and glycerides from olive oil and found that they were very much more susceptible to oxidation than was the original oil. Thus, vegetable oils were shown to contain antioxidants which were progressively removed in attempts to obtain purified products. Even the most highly refined vegetable oils, however, still contained substances having demonstrable antioxygenic activity. Some of the constituents responsible for these reactions have been identified in the past few years, and an attempt will be made here to review the available information. Clear-cut conclusions are rare. The field is complicated by difficulties in assaying and in interpreting results. Substances which appear to be effective antioxidants under one set of conditions prove to be inactive in a different environment. Thus, for example, Evans (21) reported that potassium cyanide was an effective stabilizer for oleic acid when the oxidation was accelerated by the presence of cobalt oleate. However, in the absence of metallic catalysts, the cyanides are ineffective (61). Similarly, compounds effective in fats whose oxidation is accelerated by exposure to light may be inactive in the dark, and those suitable for use at room temperature may be useless in tests at elevated temperatures. Most unsatisfactory of all is the difficulty in duplicating antioxidant assays even under the most standardized conditions, due in all likelihood to a lack of appreciation of all of the factors involved in such measurements.

II. INHIBITOLS

Early attempts (9, 65) to concentrate the stabilizers occurring in various vegetable fats and oils utilized the methods employed in the preparation of vitamin E concentrates. Sterol-free unsaponifiable fractions obtained from wheat germ, cottonseed, and palm oils were effective antioxidants for animal fats and purified fatty acids and esters. No means could be found to separate the antioxidant from the vitamin, but since the two activities were not parallel in concentrates from different sources, it was assumed that the substances responsible for them were not identical. Those responsible for the stabilizing action were called inhibitols (67) to indicate that they were effective by virtue of hydroxyl groups which, as in the phenolic antioxidants, became ineffective on esterification. With the isolation of vitamin E and the demonstration that there were several tocopherols (22), the earlier confusion was resolved; α-tocopherol is more effective as vitamin E than β- or γ-tocopherol, whereas the latter are the more effective antioxidants (64). Although the tocopherols appear to be the principal antioxidants in certain vegetable oils, the existence of other

inhibitols, some of which may not appear in the unsaponifiable fraction, is by no means excluded. Among these are certain vitamin K compounds (28), especially in their reduced form, as well as sesamol, gossypol, and others.

The tocopherols contain the aromatic ring and a free hydroxyl group



α-Tocopherol or 5,7,8-trimethyltocol
(β-tocopherol is 5,8-dimethyltocol; γ-tocopherol is 7,8-dimethyltocol (20))

characteristic of known phenolic inhibitors (62). Ethers and esters, except for the allophanates (64), are inactive as antioxidants. A study of the parent chromans and related substances has shown (28) that the heterocyclic oxygen is as essential to the antioxygenic action of the tocopherols as is the second hydroxyl group in hydroquinone or catechol. The introduction of successive methyl groups decreases the stabilizing action of chromans (and tocopherols), just as it does that of hydroquinone.

The tocopherols can be concentrated in the residue obtained from the distillation of methyl or ethyl esters prepared from vegetable oils (68), but are found in the first fractions when the oils themselves are subjected to molecular distillation (16). Riemenschneider *et al.* (70) have described an unusual stability in the first fraction from such a distillation of *refined* cottonseed oil, while Fawcett (23) has shown that antioxidants are concentrated in both the first fraction and the residue during the molecular distillation of *crude* cottonseed oil.

Although inhibitol fractions and tocopherols were found to be effective stabilizers for animal fats, they had little, if any, antioxygenic value when added to the vegetable fats from which they were obtained (68). This paradoxical result rests only in part on the use of too small amounts, proportionately, for test purposes; no adequate explanation for the phenomenon will be forthcoming until more is known about the mechanism by which tocopherols inhibit oxidation. Preliminary observations (27) indicate that the oxidation of tocopherol during the induction period follows slightly different paths in animal and in vegetable fats. Nakamura (58) has reported that relatively large amounts (0.5 to 1.0 per cent) of the unsaponifiable fraction from soybean oil stabilize soybean oil slightly.

Various other substances, however, do act as effective stabilizers for vegetable fats, among them certain inorganic and organic acids (17, 33, 68) most of which have little if any stabilizing effect on animal fats. They

are relatively insoluble in fats and are not fat constituents as such, but they may and do occur in association with fats under natural conditions, perhaps in amounts undetectable by ordinary chemical means. Citric and ascorbic acids are present in milk, and phosphoric acid, in the form of phospholipid, is widely distributed in fats of animal and plant origin. Since these acids stabilize vegetable oils containing inhibitols but do not stabilize animal fats (68), their action is described as synergistic with the inhibitols.

The chemical mechanism of this synergism has not been investigated in any detail except with ascorbic acid. The effect of this acid in reducing the susceptibility of milk fat to oxidative deterioration was first indicated by experiments in which it was fed to cows with satisfactory results (10), but even before that time its capacity to act as a synergist with inhibitols had been observed (61). Its value in increasing the keeping quality of milk fat has been the subject of several recent inquiries (45), and its use as a stabilizer in mayonnaise has been described (31). Ascorbic acid is an excellent stabilizer of tocopherol (41). A recent study (30) has indicated the nature of this action. Successive determinations of tocopherol in autoxidizing fat substrates during the progress of the induction period showed that it disappeared rapidly at first, and later more slowly, and that by the end of the period the tocopherol had all been oxidized to tocoquinone and beyond. This was also true when ascorbic acid was present, but the induction period was greatly prolonged and, again, tocopherol had disappeared by the end of the induction period. Ascorbic acid also slowly disappeared, but animal fat substrates containing ascorbic acid without tocopherol became rancid while most of the ascorbic acid was still present (27). The oxidation of tocopherol appears to be a two-step oxidation, the first (reversible) step being the formation of a phenoxyl radical (29). The apparent oxidation potential of tocopherol is 0.656–0.597 volt; that of ascorbic acid is much lower, $E_0 = 0.390$ volt. It would appear, therefore, that ascorbic acid is not appreciably oxidized by fat peroxides and does not prevent their formation, but it retards the oxidation of tocopherol, which in turn temporarily prevents the oxidation of fat and the initiation of reaction chains.

The stabilizing action of phosphoric acid is also confined to those fats and oils which contain tocopherol or related inhibitols; by itself phosphoric acid has little antioxygenic effect on animal fat substrates (68). Studies by Golumbic (27) on the oxidation of tocopherol in the presence of phosphoric acid indicate that phosphoric acid favors ring closure of toco(hydro)-quinone with re-formation of tocopherol, a cyclization that has become well known in the chemistry of vitamins E and K.

Obviously, the intimate chemistry of the synergism between inhibitors

offers many interesting problems of fundamental nature and practical importance.

III. PHOSPHOLIPIDS

The antioxidant properties of crude lecithin preparations were discovered by Bollman (8) and described briefly by several other investigators (21, 46). Olcott and Mattill (66) showed that the various commercial preparations were more effective in vegetable oils than in animal oils or purified fatty acids or esters, although marked activity could be demonstrated in the latter substrates, if tocopherols or other phenolic inhibitors were added (68). Purified lecithin was inactive, but the activity appeared quantitatively in the cephalin fraction. Hilditch and Paul (38) found that highly purified soybean phosphatides were inactive in olive oil esters. Similarly, Diemair and Fox (14) found that purified oat oil phosphatides were not antioxygenic, although the complex system from which the phosphatide had been separated (a protein–phosphatide complex (15)) was supposedly the most active factor in producing the antioxidant effect of oat oil. The reported inactivation of lecithin by heating above 65°C. (21, 73) has not been confirmed (61).

In view of the somewhat conflicting results and the recent suggestion that a considerable part of the cephalin fraction may contain serine rather than colamine (24), a reinvestigation of the problem would be timely. If the acid reaction of cephalin is due to the carboxyl group of serine, the hypothesis that the antioxidant activity can be ascribed solely to an ionizable phosphoric acid (66) may be subject to revision.

IV. CAROTENOIDS

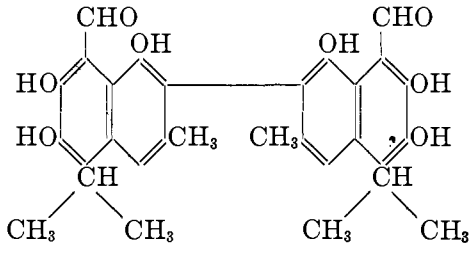
The effects of carotenoids on the oxidation of fats and oils have been studied in several laboratories. With the oxygen absorption technique applied to lard or mixtures of lard and cod-liver oil, it was found that carotene, xanthophyll, and lycopene acted as proöxidants in decreasing the length of the induction period (9, 62). Franke (25) found that carotene and xanthophyll were proöxidants for oleic and linoleic acids but were inactive in linseed or olive oil. Newton (59) concluded that carotene was an antioxidant, because fatty extracts of carotene-containing sources such as paprika, alfalfa, and palm oil acted as antioxidants in lard. Inasmuch as the effect was enhanced by bleaching the carotene, the activity of the extracts has been interpreted as indicating the presence of non-carotenoid antioxidants, presumably of the inhibitol type (67). Thus, the presence of easily oxidizable carotenoids in fats and oils might appear to be presumptive evidence for the presence of protecting antioxidants. Täufel and Müller (77) recently recorded that xanthophyll possesses only slight proöxygenic activity in chicken fat and butterfat.

The occurrence of synergistic effects is suggested by Franke's (25) observations that carotene and xanthophyll in the presence of hemin were proöxygenic for olive and linseed oils, whereas, in the presence of pyridine, marked antioxidant activity was noted.

The action of vitamin A may be assumed to be similar to that of the carotenoids, although the effect of the pure compound on the induction period of fats has not been described. Esters of vitamin A are more stable toward oxidation than is the free vitamin (37) and would presumably be less likely to act as proöxygens.

V. GOSSYPOL

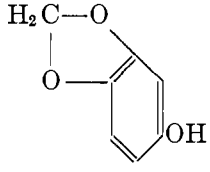
Crude cottonseed oil may contain from traces to 1.5 per cent (expeller oil) of gossypol. Mattill (53) first recorded and Royce (71, 72) confirmed the marked antioxidant activity of this substance. Royce concluded that gossypol was the most effective antioxidant in crude cottonseed oil and was responsible for the better keeping quality of crude over refined cottonseed oil. The multiple phenolic groups of gossypol are sufficient to account for its activity.



Gossypol (1)

VI. SESAMOL

Sesame oil, like other vegetable oils (36, 52, 60), has the property of imparting stability to other fats when added in small amounts. The unsaponifiable fraction contains a compound, sesamolin, which Böeseken, Cohen, and Kip (7) consider a glucoside of sesamol, the phenolic sub-



Sesamol

stance responsible for the Villavecchia and Baudouin color tests for sesame oil. Sesamol is an effective inhibitor in fats and oils (61). Perhaps the gradual liberation of sesamol from sesamolin creates a particularly favor-

able environment for preventing oxidation. The distillate secured during the deodorization of hydrogenated sesame oil is antioxygenic (35) and possibly contains sesamol. Sesamin (7), a closely related compound and one more easily isolated, has no antioxidant properties (61).

VII. CHLOROPHYLL

The importance of chlorophyll in accelerating the oxidation of oils exposed to light has been emphasized by Coe (11) and Täufel and Müller (77). The addition of chlorophyll is said to reduce the induction period markedly, and rancidity occurs at very low peroxide values (11). Pure chlorophyll is a more effective proöxidant than crude preparations (77). Although most crude oils contain chlorophyll, refined oils contain only traces, if any (6), and it has been pointed out that the oils from which the chlorophyll has been removed are less stable than those containing maximum amounts (6). Presumably, the effect of antioxidants is more pronounced than that of the proöxidant chlorophyll in these reactions. Under conditions of ordinary in-door illumination, added chlorophyll does not affect the induction period of lard (27, 61).

Coe (13) has devised a method for determining relative stability, based upon the ability of fresh oils to quench the red fluorescence of magnesium-chlorophyll. As the oils become oxidized, the quenching effect decreases. Bickford, Anderson, and Markley (6) have suggested that dissolved oxygen in the oil under test is the responsible agent. However, the quenching action is not altered by degassing in a high vacuum, and the substance or substances responsible for the blue fluorescence and quenching can be concentrated in the unsaponifiable fractions (lard and cottonseed oil) (61). Hence, it appears more likely that the fluorescence is connected in some way with the antioxidants or other oxidizable constituents in the unsaponifiable fractions, the concentration of which decreases during the induction period. Pure α-tocopherol fluoresces with a somewhat darker blue than the crude unsaponifiable fractions, but both possess the property of quenching the red fluorescence of chlorophyll.

VIII. WATER

The presence of water in fats may modify the reactions leading toward rancidity in several ways. The evidence as to the effect of moisture on the rate of oxidation of pure fats leads to the conclusion that water probably has a slight retarding effect (51).

The presence of moisture in fats, however, permits the growth of organisms, some of which possess powerful enzyme systems capable of accelerating glyceride hydrolysis (lipases) and oxidation (oxidases or lipoxidases). Jensen and Grettie (43) have shown that as little as 0.3 per cent

of water in fats is sufficient to permit the growth of bacteria and molds. Insofar as fats or oils are susceptible to this type of spoilage, substances which inhibit such growth might be classed as antioxidants. Added phenols in such systems would thus play a double rôle, that of inhibiting bacterial growth as well as protecting the fats from oxidative deterioration.

Lipoxidases are known to be fairly widely distributed in the animal and vegetable kingdoms, having been identified in soybean, wheat germ, peanut, etc., and in adipose and muscle tissue (50, 51). Presumably, these are effective only in the presence of water. Soybean lipoxidase also appears to be indirectly responsible for the bleaching of carotene in unsaturated fats (76), a reaction which had been assumed to be the function of a separate enzyme (75).

Lea (49) studied the effect of numerous water-soluble compounds on the oxidation of lard in contact with an aqueous phase and found that citric and related acids and several amino acids were the most effective inhibitors. The obvious complexity of such systems makes interpretation difficult.

Hilditch and his coworkers (3, 39) found that the induction period of olive and linseed oils was markedly reduced by treatment with boiling water for 3 hr. Carbon dioxide was bubbled through the mixtures to prevent oxidation. Inasmuch as no activity could be recovered from the aqueous layer, the antioxidants involved were destroyed by such treatment. These observations have been duplicated for cottonseed and sesame oils (61). There is as yet no definite clue as to the nature of the compounds affected. In the case of cottonseed and sesame oils, the treatment not only decreased the length of the induction period, but also changed the reactions of the oils toward the two types of inhibitors. The acid inhibitors were no longer as effective, while some activity could be demonstrated with inhibitol concentrates.

Elder (19) has described a decrease in the induction period of wheat germ oil following a cook with brine, presumably due to the leaching out of antioxidant.

IX. MISCELLANEOUS SUBSTANCES

The antioxidant activity of unsaponifiable fractions of oils and fats is found in the non-sterol constituents. Purified sterols from a number of sources were found to be uniformly inactive (53, 58). The occasional references to antioxygenic sterols usually involve impure preparations of sterols.

The concentration of metal salts in oils and fats is almost vanishingly small, yet, since these substances have been shown to be such powerful proöxidants, some mention of their effects should be included. For ex-

ample, the processing of fats or fatty foods in contact with metal is known in some cases markedly to accelerate oxidative decomposition. Copper and iron are the common offenders. Lea (51) has summarized the information available on this subject.

Aside from the natural constituents of fats and oils which affect their susceptibility toward oxidative rancidity, there occur in nature numerous compounds and materials which, upon addition to fats and oils, act as antioxidants. Gum guaiacum (34) and gallic acid (68) are examples. Cereal and oil-seed flours have been found to act as antioxidants (69), but only part of the antioxidant activity can be extracted with fat solvents (61). Hilditch *et al.* (32, 38) have described antioxidant fractions extractable from oil-seed meals after digestion with organic acids. These possessed marked reducing power and contained small amounts of phosphorus and nitrogen, and their activity could be destroyed by exposure to traces of hydrogen chloride. Greater yields were obtained from the meals than from the oils themselves.

Coe (11, 12) has claimed that catalase preparations are more effective antioxidants in the light-catalyzed oxidation of vegetable oils than any others except pyrogallol. However, Täufel and Müller (78) found that the antioxidant activity of liver catalase preparations not only did not parallel the enzymic activity but persisted after the removal of the catalase. The antioxidant activity of cottonseed meal has been found not to depend on the catalase present (61), and highly purified catalase preparations were inactive as antioxidants in lard (not light-catalyzed) (27). The effect of catalase and the rôle of hydrogen peroxide in rancidity reactions thus require further investigation. Coe (11) was unable to detect catalase activity in oils or fats.

The "soft" fat of animals given oil-containing diets is more susceptible to oxidation than the fat laid down on rations not containing such oils (4, 48). Butterfat from summer pasturage cows is more unsaturated than that obtained during the winter, but of two samples of equal iodine number, the summer butter is the more stable (74). Much remains to be learned about the influence of ration constituents upon the susceptibility of body and milk fats to oxidation.

Elder (17) has found that the oil of roasted coffee has three times the induction period of that obtained from the original green beans, presumably owing to the synthesis of an antioxidant, possibly pyrrole, during roasting.

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