Anti-oxidants and Synergists[1]

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SINCE the subject of antioxidants for fats was last reviewed (1), some new stabilizers have been discovered and some new combinations of inhibitors have been proposed but not a great deal has been learned as to their manner of action. Our knowledge of the mechanism whereby they accomplish the desired stabilization is limited. We must still work mostly along empirical lines. As a result, correlation is not easy and unfortunately further investigation is sometimes not properly oriented. If one sets up an unorganized inquiry, he is likely to get an unorganized answer. Generalizations are always hazardous when they are based on incomplete data: the exception does not always prove the rule. Nevertheless attempts to generalize are worth while if they stimulate analysis of the exceptions.

In one of the early papers from our laboratory (2) nitrogen-free inhibitors of fat oxidation were classified into three groups, acid type, inhibitols and hydroquinone, and other phenolic inhibitors. This classification may now be tentatively simplified under two propositions: 1. With few exceptions the only substances which have primary anti-oxygenic action on fatty acids are ortho and para di- and poly-phenolic compounds or substances having similar electronic configuration. 2. All other substances which delay or inhibit the autoxidation of fats should properly be called synergists because they merely reinforce the effect of the phenolic compounds present and have little if any activity apart from them.

The phenolic inhibitors include simple and well-recognized chemical compounds as well as naturally occurring substances whose chemistry is more complex. Probably the first mention of naturally occurring antioxidants was the observation of Deschamps (3) over 100 years ago that lard containing gum benzoin or populin did not become rancid as quickly as ordinary lard. The wide range in stability of natural oils and fats is as dependent upon the presence of natural anti- and pro-oxidants as it is on the kind and degree of unsaturation of the fatty acids.

The stability of sesame oil is probably due to the gradual release of sesamol from sesamolin (1). Sesamol has a hydroxyl group para to oxygen. Crude cottonseed oil owes its stability partly to gossypol (4) which has two pairs of ortho hydroxyls. The first of the naturally occurring inhibitors to be studied extensively was probably the group of tocopherols, vitamin E (5). When the constitution of these substances was established (6), the manner of their action was entirely consistent with the theory which explains why di- and poly-phenols are antioxygenic.

Associated with the tocopherols in vegetable oils is another inhibitor which, although similar to the tocopherols in structure, has no vitamin E action. It is responsible for the red or orange color which appears in some vegetable fats toward the close of the induction period. By a series of very clever procedures Golumbic (7) found this substance to be identical with the red chromane-5, 6-quinone produced from tocopherols under the oxidizing action of nitric acid. According to Smith and his coworkers (8) it has the structure of an orthoquinone. Golumbic showed that this compound was not an oxidation product of α-tocopherol during the autoxidation of fat, that it must have originated from a quinol precursor, perhaps 5-hydroxy tocopherol.* Such a structure might explain its destruction by saponification; it does not appear in the nonsaponifiable fraction.

More recently another naturally occurring phenolic inhibitor has been described, nordihydroguaiaretic acid (9), whose phenolic properties adequately explain its inhibitory action. Favorably mentioned, recently, were also 1, 5-dihydroxy naphthalene (10) and hematoxylin (11); the latter contains two benzene rings each of which carries a pair of ortho hydroxyls.

THE activity of all these substances apparently depends upon their oxidation to quinones, whereby hydrogen or electrons or both are released or donated to a fat molecule in process of being oxidized. The reaction chain is broken at the expense of the oxidation of the more readily oxidizable inhibitor. Because it is a chain reaction, a little inhibitor goes a long way. The oxidation of phenols has long been accepted as a 2-step process (12); the first step produces a phenoxyl radical, or, in the case of quinol, a quinol or semiquinone ion, the second step the fully oxidized quinone. Also it is usually accepted that the first step is relatively easily reversible whereas the second step is so only under special conditions.

By the end of the induction period a phenolic inhibitor like tocopherol has been completely oxidized to the quinone and beyond (13). In vegetable fats containing the precursor of the red quinone oxidation product as well as tocopherols, the situation is somewhat different. The stabilizing action of tocopherol is supported by the gradually increasing amounts of red quinone, not all of which has disappeared even by the end of the induction period (organoleptic). The association of these two kinds of inhibitors accounts for the absence of a sharply marked induction period with vegetable fats.

The great variability in effectiveness between phenolic inhibitors is due in large measure to the extent and character of nuclear alkylation and to the length of the side chains and their configuration (14) (15). Among the active substances having a suitable electronic configuration, but without a free hydroxyl group is 3-phenyl isocoumaranone. Its effectiveness is due to enolization and when this can not occur, as in the di-phenyl derivative, the substance is inactive (15).

These differences should be related to oxidation potentials. A beginning was made some years ago in connection with inhibitors for cracked gasoline (16). The fact that there is an optimum concentration for tocopherols, above which the effectiveness decreases

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* Very recently it has been shown (Swift, E. C., Mann, G. E., and Fisher, G. S., Oil and Soap *21*, 317-20) that γ-tocopherol is a precursor of the red chromane-5,6-quinone.

(13) (17), indicates the complicated character of these relations. The long side chain probably makes tocopherols autoxidizable like the fats, and their nuclear alkylation does not favor the loss of hydrogen.

The acid type inhibitors seem to be of at least three kinds. Most commonly used are the dibasic organic acids, such as malonic, maleic, malic, citric, and others. All of these are typical acid inhibitors; they are synergists and can only reinforce the action of phenolic antioxidants that occur naturally in vegetable oils or which may be added to animal fats. The effect of these synergists was recently described (18) as stabilizing the vegetable oil solvent, such that added phenolic inhibitors could become effective. Such a statement not only begs the question but introduces confusion.

OXALIC acid, the simplest dibasic acid, differs from the other active acids in being a stabilizer by itself, without the presence of phenolic inhibitors; it stabilizes animal as well as vegetable fats. This may possibly be due to the nature of the bond between the two carbons in oxalic acid which, we are told (19), partakes of the nature of a double bond; at least the distance between the two carbons is shorter than in the aliphatic carbon to carbon bond. Even without the additional hydroxyls due to hydration (two molecules of water) oxalic acid thus provides the equivalent of two hydroxyls in the ortho position.

Ascorbic acid also belongs to this group of acid inhibitors and its action has been studied rather extensively in our laboratory by Golumbic (20) and by Calkins (21). Fat peroxides readily oxidize phenolic inhibitors, hence the oxidation potentials of the fat peroxides must be higher than those of phenolic inhibitors. The oxidation potential of ascorbic acid is below that of phenolic inhibitors but despite the large difference between fat peroxides and ascorbic acid, the latter is not appreciably oxidized during the induction period of fats. Present explanation is that the oxidation of ascorbic acid is a sluggish two-step process which requires an intermediary (22). When a phenolic inhibitor donates hydrogen to a fat peroxide and thereby becomes a phenoxyl radical, the lost hydrogen is restored by ascorbic acid which thereby becomes dehydro ascorbic acid. As long as ascorbic acid or any of its oxidation products (perhaps even oxalic acid) remains to restore hydrogen to the phenoxyl group, fat peroxides do not accumulate. That is, ascorbic acid is a potential reservoir of hydrogen for the maintenance of the status quo in the fat, but ascorbic acid can not supply hydrogen directly to the nascent fat peroxide; it requires an intermediate.

The nature of the synergistic action of the di- and poly-basic organic acids is somewhat more obscure. In all of the active acids there is, adjacent to the carboxyl group, a functional group such as keto, hydroxyl, halogen, amino, or there is proximal unsaturation. Many substances can be recognized as synergists on the basis of such composition.

The activating group or structure at the alpha carbon enhances the hydrogen binding power of the hydroxyl groups and tends to repel their hydrogen toward the phenoxyl radical or semi-quinone ion which are avid hydrogen acceptors. Some information as to the nature of the oxidation products of the active dibasic acids is needed to explain their synergistic action, but their effect appears to be entirely like that of ascorbic acid in donating hydrogen.

Of still greater interest is the remarkable synergistic effect of certain inorganic acids, especially sulphuric and phosphoric acids. Calkins (23) has made a careful study of the action of phosphoric acid. His results have not yet been completely interpreted, but the effectiveness of phosphoric acid seems to be due to the ease with which it provides hydrogen. He believes the explanation resides in hydrogen bonding with the semi-quinone ion of the phenolic inhibitor. The effectiveness of phosphoric acid with tocopherol and quinol has already been described (24). Perhaps two hydroxyls are necessary, one for ionization, the other for hydrogen bonding. Phosphoric and sulfuric acids have them, nitric acid and the halogen acids do not.

ADSORPTION must also play a role because these acids are not soluble in fat; they are more likely distributed throughout the fat in particles that may be of almost molecular size. The evidence suggests that on occasion they also have a dehydrating action. Golumbic (24) showed that tocopheryl quinone, which is inactive, remains inactive in the presence of ascorbic acid, but becomes an active antioxidant in the presence of phosphoric acid. This can come about only by re-closing of the ring with loss of a molecule of water. Phosphoric can do this but ascorbic can not. This ring closure was also demonstrated by the biological test for vitamin E, formed from tocohydroquinone by the action of phosphoric acid. Perhaps the tocopheryl hydroquinone radical, having become more soluble in water with loss of hydrogen, is adsorbed on a particle of phosphoric acid, which gives it another hydrogen, and, after binding up its broken ring by abstracting a molecule of water, lets it go again; it loses its hydrogen to the first potential fat peroxide molecule it meets and comes back again for repair and supply. Adsorption would thus be fundamental to the synergistic action of phosphoric acid.

Cephalin is a synergist with phenolic inhibitors; pure lecithin is not (25). Cephalin has a free hydroxyl group for hydrogen bonding whereas lecithin does not because it is internally neutralized. Perhaps cephalin is less effective as a synergist than phosphoric acid because it dissolves in the fat. Whether this consideration applies to ascorbic acid also is not known but if it does, the development of fatty acid esters of ascorbic acid for stabilization purposes is an unnecessary project (26). Further study should reveal whether the esters of ascorbic acid are more effective than the free acid, if this is thoroughly distributed throughout the fat.

There is a final group of acid inhibitors comprising di- and poly-phenolic acids. Gallic acid is the best example (27), and it is effective in animal fats devoid of phenolic inhibitors by virtue of the fact that it is itself phenolic. In vegetable oils it is also effective because of its acid character. When the phenolic hydroxyls are esterified or converted to methoxy groups, gallic acid is no longer effective in animal fats. Its esters, such as ethyl and propyl gallate (11) (28), have lately received favorable mention for general use. Doubtless some hydrolysis of the ester takes place in fats.

In most instances it is possible to identify the nature of an unknown inhibitor by the nature of the

fats in which it is effective. The converse proposition also holds; if an acid like ascorbic, citric or phosphoric is effective in a natural fat one may be fairly certain that the fat also has a naturally occurring phenolic inhibitor in it. Much work remains to be done to identify the characteristics of some of the more obscure antioxidants, like those released from seed cakes by acetic acid (29), those found in various flours (30), the polymerization products in heated sugars, perhaps di-enols (31), or those found in rice bran extracts (32).

WE MUST admit that empirical facts are more abundant than logical explanations, but the facts must be obtained with care and must be closely scrutinized. Needless to say the manner of preparation of the fat substrates and the relative purity of the various components of the system may greatly modify the results obtained. For example, the successful stabilization of lard by soy bean phospholipids (26) is not valid evidence against the thesis here proposed; the soy bean phospholipids contained traces of admixed tocopherols and the combined effectiveness was increased by addition of more tocopherol, of ascorbyl esters, or of both.

Even though we know only in part, it is possible to set up a few fundamental principles in stabilization. If the fat in question naturally has an optimum content of some phenolic antioxidant, the addition of further amounts may be useless; it may even be detrimental as in the case of tocopherols because they are also vulnerable to oxidation; the quality of the fat may be depreciated by the oxidation products of phenolic inhibitors. Such fats can be benefited by the addition of synergists which prolong the action of the phenolic stabilizer and make a little of it go farther.

By the same token, the addition of a phenolic inhibitor to an animal fat should not be overdone. It is wise to add as little as necessary and to reinforce what is added by the simultaneous addition of synergists alone or in combination. Intensive study of the action of synergists on the phenolic inhibitors and upon each other promises to be highly interesting in theory and fruitful in practice.

LITERATURE REFERENCES

(1) Olcott, H. S., Oil and Soap, *18*, 77-80 (1941); Olcott, H. S., and Mattill, H. A., Chem. Reviews, *29*, 257-68 (1941).
(2) Olcott, H. S., and Mattill, H. A., J. Am. Chem. Soc., *58*, 2204-8 (1936).
(3) Deschamps, J. pharm, et chim., *4*, 201 (1843).
(4) Mattill, H. A., J. Biol. Chem., *90*, 141-51 (1931); Royce, H. D., Oil and Soap, *10*, 123-5 (1933).
(5) Olcott, H. S., and Emerson, O. H., J. Am. Chem. Soc., *59*, 1008-9 (1937).
(6) Fernholz, E., J. Am. Chem. Soc., *60*, 700-5 (1938).
(7) Golumbic, Calvin, J. Am. Chem. Soc., *64*, 2337-40 (1942).
(8) Smith, L. I., Irwin, W. B., and Ungnade, H. E., J. Am. Chem. Soc., *61*, 2424-9 (1939).
(9) Lundberg, W. O., Halvorson, H. O., and Burr, G. O., Oil and Soap *21*, 33-6 (1944).
(10) Lea, C. H., J. Soc. Chem. Ind., *63*, 55-7 (44).
(11) Lea, C. H., J. Soc. Chem. Ind., *63*, 107-12 (44).
(12) Conant, J. B., Chem. Reviews, *3*, 1-40 (1926).
(13) Golumbic, Calvin, Oil and Soap, *20*, 105-7 (1943).
(14) Olcott, H. S., J. Am. Chem. Soc., *56*, 2492-3 (1934).
(15) Golumbic, Calvin, J. Am. Chem. Soc., 63, 1142 (1941).
(16) Lowry, C. D., Jr., Egloff, G., Morrell, J. C., and Dryer, C. G., Ind. Eng. Chem., *25*, 804-8 (1933).
(17) Swift, C. E., Rose, W. G., and Jamieson, G. S., Oil and Soap, *19*, 176-80 (1942); Oliver, G. D., Singleton, W. S., and Bailey, A. E., *ibid.*, *21*, 188-93 (1944).
(18) Williams, K. T., Bickoff, E., and Lowrimore, B., Oil and Soap, *21*, 161-4 (1944).
(19) Robertson, J. M., and Woodward, Ida, J. Chem. Soc., 1936, 1817-24.
(20) Golumbic, Calvin, and Mattill, H. A., J. Am. Chem. Soc., *63*, 1279-80 (1941).
(21) Calkins, V. P., and Mattill, H. A., J. Am. Chem. Soc., *66*, 239 42 (1944).
(22) Michaelis, L., Chem. Reviews, *16*, 243-86 (1935).
(23) Calkins, V. P., Unpublished.
(24) Golumbic, Calvin, Oil and Soap *19*, 181-2 (1942).
(25) Olcott, H. S., and Mattill, H. A., Oil and Soap, *13*, 98-100 (1936).
(26) Riemenschneider, R. W., Turer, J., Wells, P. A., and Ault, W. C., Oil and Soap, *21*, 47-50 (1944).
(27) Golumbic, Calvin, and Mattill, H. A , Oil and Soap, *19*, 144-5 (1942).
(28) Hilditch, T. P., Chem. and Ind., 1944, 67-71; Boehm, E., and Williams, R., Pharm. J., *151*, 163-4 (1943).
(29) Green, T. G., and Hilditch, T. P., J. Soc. Chem. Ind., *56*, 23-6 (1937); Hilditch, T. P., and Paul, S., *ibid.*, *58*, 21-4 (1939).
(30) Dahle, C. D., and Nelson, D. H., J. Dairy Science, *24*, 29-39 (1941).
(31) Bergel, F., Chem. and Ind., 1944, 127-8.
(32) György, P., and Tomarelli, R. M., J. Biol. Chem., *154*, 317-24 (1944).

Detergency Studies at Low Solution Concentrations[1]

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PREVIOUS studies of the detersive efficiencies of soap and alkaline soap builders (1, 2) have been made in both soft and hard water, but at solution concentrations varying from 0.10-0.20% in 50 p.p.m. soft water and from 0.27-0.32% in 300 p.p.m. hard water. Other studies with synthetic detergents and builders have been made in water of varying hardness and in sea water, but generally at rather high levels of solution concentration (3, 4). Several investigators have made tests of soap at 0.1% concentration to which varying concentrations of alkaline builders have been added, one using an especially constructed small laboratory washer (5) and another (6) utilizing both a Launderometer and a small (24 x 40 inch) monel wash wheel.

General power laundry practice is to reduce the concentration of the soap and alkali combination as the clothes become cleaner, i.e., to use the largest amounts of detergent at the periods when the greatest amounts of soil are present, thus varying the concentration throughout the wash formula. Consequently, soap solution concentrations may vary (7) from approximately 0.15% to 0.01%, and the builder concentrations from 0.09% to 0.012%. Detergent results under such conditions have been fairly well established, but similar work with synthetic detergents has not been undertaken. Other than the data published for Santomerse combined with certain alkaline builders (4) the little information which has been given regarding the detersive efficiency of synthetic agents combined with alkalies deals with relatively high solution concentrations. Tests were therefore un-

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