Some Effects of Amino Acids and Certain Other Substances On Lard Containing Phenolic Antioxidants*

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THE phenomenon of synergism has practical importance in the stabilization of both vegetable and animal fats. In the former, appreciable quantities of natural antioxidants are present whose effectiveness may be greatly increased by the addition of synergists. In animal fats, the practical importance lies in the possibility of effecting a reduction in the costs and the amounts of added inhibitors that are necessary to achieve a desired stability. In many cases it has been found, for example, that 0.01% of a costly phenolic antioxidant together with 0.01% of a relatively inexpensive synergist is as effective in stabilizing lard as 0.1% of the phenolic antioxidant alone.

The present investigation was undertaken to find synergists for three phenolic antioxidants: alpha tocopherol, hydroquinone, and nordihydroguaiaretic acid. The amino acids, because of their low toxicity and their availability in mixed forms in a wide variety of preparations, were selected as synergists or secondary antioxidants. Other compounds that had been selected for specific reasons were also tested.

Experimental

The lards used as substrates were rendered at low temperature under vacuum. Each antioxidant and each synergist were added in amounts of 0.01 gram per cent (10 mgm. per 100 ml. melted lard). Each lard was tested without added antioxidant or synergist, with antioxidant alone added, with synergist alone added, and with antioxidant and synergist both added. The test used was the active oxygen method at 98.6°C. (1).

The data are reported in three sections, each section representing the data for one phenolic antioxidant. The protective index is here defined as the ratio obtained when the keeping time in hours of the substrate plus 0.01% of primary phenolic antioxidant plus 0.01% of synergist is divided by the keeping time in hours of the substrate plus 0.01% of primary phenolic antioxidant. This empirical definition is used because the present lack of knowledge concerning the mechanisms involved makes any definition on a theoretical basis unwarranted.

In all, three substrates were used, having keeping times of 5, 11, and 15 hours. In some cases more than one substrate was used, and it was found that the protective index was independent of the substrate within experimental error.

In each section synergists have been listed in 5 groups corresponding to protective indices of 4.0 to 3.1, 3.0 to 2.1, 2.0 to 1.6, 1.5 to 1.1, and 1.0 or less. Within each group the synergists are listed in decreasing order of effectiveness.

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Summary of Results

SECTION I.

PRIMARY PHENOLIC ANTIOXIDANT: ALPHA-TOCOPHEROL

Group A. *Synergists with protective indices from 4.0 to 3.1*
None.

Group B. *3.0 to 2.1*
Ascorbic acid, methionine.

Group C. *2.0 to 1.6*
Threonine, leucine, milk protein hydrolysate No. 2, norvaline, ascorbyl, palmitate, phenylalanine, milk protein hydrolysate No. 1, cysteine.

Group D. *1.5 to 1.1*
Tryptophane, isoleucine, proline, alanine, peptic digest of pancreas protein, milk protein hydrolysate No. 000319, alpha amino iso-butyric acid, amidol, asparagin, acid precipitate from alkali treated beef serum protein, casein hydrolysate made with sulfuric acid, trypsin, arginine, barbituric acid, arsphenamine, valine, trypsin hydrolysate of beef serum globulin, ninhydrin, glutamic acid, trypsin hydrolysate of beef serum, alkali treated beef serum protein, trypsin hydrolysate of beef serum albumin, propamidine, histidine, sulfuric acid hydrolysate of pancreas protein, pepsin hydrolysate of beef serum albumin, peptone, glycerophosphoric acid, hydroxyproline, sulfuric acid hydrolysate of beef serum protein.

Group E. *1.0 or less*
Pepsin hydrolysate of beef serum globulin, pepsin hydrolysate of beef serum, erepsin hydrolysate of beef serum, diiodotyrosine, dioxyphenylalanine, nicotinic acid, serine, lysine, norleucine, citrulline, glycolic acid, erepsin hydrolysate of beef serum globulin, melanin, choline, cystine, casein, hydrolysate made with HCl, aspartic acid, tyrosine, erepsin hydrolysate of beef serum albumin, egg albumin, casein, pepsin, gluconic acid, uric acid, succinimide, urea, peptic-tryptic digest of pancreas, creatinine, levulinic acid, bovine alpha pseudoglobulin, casein hydrolysate made with barium hydroxide, oxamide, hydroxylamine.

SECTION II.

PRIMARY PHENOLIC ANTIOXIDANT: HYDROQUINONE

Group A. *Synergists with protective indices from 4.0 to 3.1*
Methionine.

Group B. *3.0 to 2.1*
Ascorbic acid, milk protein hydrolysate No. 1, tryptophane.

Group C. *2.0 to 1.6*
Leucine, milk protein hydrolysate No. 2, ascorbyl palmitate, proline, phenylalanine, cysteine, alanine, glutamic acid, valine, peptic digest of pancreas protein, asparagin, arginine, barbituric acid, arsphenamine, ninhydrin, propamidine, histidine, norleucine, glycerophosphoric acid, trypsin, casein hydrolysate made with hydrochloric acid.

Group D. *1.5 to 1.1*
Trypsin hydrolysate of beef serum globulin, trypsin hydrolysate of beef serum albumin, threonine, isoleucine, alpha-amino-isobutyric acid, acid precipitate from alkali treated beef serum protein, casein hydrolysate made with sulfuric acid, alkali treated beef serum protein, sulfuric acid hydrolysate of pancreas protein, sulfuric acid hydrolysate of beef serum protein, norvaline, milk protein hydrolysate No. 000319, levulinic acid, diiodotyrosine, amidol, hydroxyproline, erepsin hydrolysate of beef serum globulin, casein hydrolysate made with barium hydroxide.

Group E. *1.0 or less*

Trypsin hydrolysate of beef serum, pepsin hydrolysate of beef serum albumin, nicotinic acid, melanin, dioxyphenylalanine, pepsin hydrolysate of beef serum, serine, lysine, cystine, egg albumin, casein, pepsin, urea, peptic-tryptic digest of pancreas, pepsin hydrolysate of beef serum globulin, glycollic acid, aspartic acid, erepsin hydrolysate of beef serum albumin, creatinine, oxamide, choline, bovine alpha pseudoglobulin, hydroxylamine, peptone, erepsin hydrolysate of beef serum, tyrosine, citrulline, succinimide, gluconic acid, uric acid.

SECTION III.

PRIMARY PHENOLIC ANTIOXIDANT: NORDIHYDROGUAIARETIC ACID

Group A. *Synergists with protective indices from 4.0 to 3.1*

None.

Group B. *3.0 to 2.1*

Methionine.

Group C. *2.0 to 1.6*

Phenylalanine, leucine, tryptophane, alanine, norleucine, milk protein hydrolysate No. 2, norvaline, valine, ascorbic acid.

Group D. *1.5 to 1.1*

Milk protein hydrolysate No. 1, threonine, isoleucine, proline, alpha-amino-isobutyric acid, dioxyphenylalanine, ninhydrin, glutamic acid, propamidine, cystine, casein, milk protein hydrolysate No. 000319, cysteine, ascorbyl palmitate, arginine, histidine, choline, levulinic acid, bovine alpha pseudo-globulin, serine, asparagin, glycerophosphoric acid, hydroxyproline, diiodotyrosine, citruline, uric acid, urea, creatinine.

Group E. *1.0 or less*

Trypsin hydrolysate of beef serum, amidol, casein hydrolysate made with sulfuric acid, trypsin hydrolysate of beef serum albumin, peptone, tyrosine, erepsin hydrolysate of beef serum globulin, melanin, aspartic acid, egg albumin, pepsin, gluconic acid, peptic-tryptic digest of pancreas, peptic digest of pancreas protein, acid precipitate from alkali treated beef serum protein, trypsin, nicotinic acid, lysine, erepsin hydrolysate of beef serum albumin, succinimide, casein hydrolysate made with barium hydroxide, oxamide, barbituric acid, sulfuric acid hydrolysate of beef serum protein, glycollic acid, trypsin hydrolysate of beef serum globulin, alkali treated beef serum protein, sulfuric acid hydrolysate of pancreas protein, arsphenamine, casein hydrolysate made with hydrochloric acid, erepsin hydrolysate of beef serum, pepsin hydrolysate of beef serum albumin, pepsin hydrolysate of beef serum globulin, pepsin hydrolysate of beef serum, hydroxylamine.

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Antioxidant Properties of Carrot Oil[1]

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WHILE purified carotene is quite susceptible to oxidation, carotene in carrots is remarkably stable and quite unaffected by autoclaving and drying on air. Occurrence of fat-soluble antioxidants in carrots has been described by Bradway and Mattill (1), but they excluded the presence of tocopherols on the following grounds: 1) Bioassays of carrots gave low results. 2) Wheat germ antioxidants were preferably soluble in petroleum ether while carrot antioxidants were not. 3) The distillation ranges under reduced pressure of the two factors were different.

In the following paper evidence will be presented which indicates that tocopherols are chiefly responsible for the antioxidant properties of the fat-soluble portion of carrots. Molecular distillation was used for the purpose of concentrating and characterizing the antioxidants. This technique has frequently been successful in dealing with labile fat-soluble substances. Robeson and Baxter (13) for example identified the antioxidant of shark liver oil as α-tocopherol by molecular distillation. For a discussion of the "analytical" technique of molecular distillation used in this work the original articles of Hickman (3) and Embree (2) should be consulted.

Determination of Antioxidant Effect

The antioxidant effect of different materials was compared by addition to a substrate of distilled methyl esters of olive oil fatty acids and determination of peroxide concentrations by a modification of Wheeler's method (14). Such a substrate is convenient for volumetric and colorimetric work, but quite unstable. It was freshly prepared before use and a sample of substrate was included in every analytical run to account for variations in the susceptibility to autoxidation between different batches of substrate. A typical substrate preparation is described below:

One hundred grams of olive oil were dehydrated by mixing with a small amount of benzene and distilling off the water-benzene azeotrope. The olive oil was refluxed with 300 ml. absolute methanol and 2.5 ml. concentrated sulfuric acid at the boiling point of methyl alcohol for about 16 hours. After cooling, fatty acids and glycerol were removed by adding ether and extracting with sodium carbonate solution. The product was dried overnight with drierite, the ether distilled off, and the crude methyl esters, weighing 91 gm., subjected to vacuum distillation in an asbestos-covered modified Claisen flask with electrically heated Vigreaux column. Most of the material (83 gm.) distilled between 190 and 200° at 10 mm. pressure. The saponification equivalent of this fraction was 293, the iodine value (Wijs' method) was 79.5.

For the estimation of the relative antioxidant effect the substrate and added test materials were incubated in glass-stoppered 125-ml. Erlenmeyer flasks in an ordinary water bath kept at 50 ± .25°C. Constant illumination from a 200-W. light bulb, about one foot above the surface of the water, was used to accelerate autoxidation. Every 24 hours the flasks were mixed by rotation, then a sample was withdrawn by pipette. After having once determined the weight of material delivered by this pipette at 50°, weighing of samples

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