Autoxidation of Fatty Materials in Emulsions. I. Pro-oxidant Effect of Histidine and Trace Metals on the Oxidation of Linoleate Esters


Abstract

Aqueous emulsions of methyl or ethyl linoleate (sodium dodecyl sulfate as emulsifier) together with such added components as 1-histidine, metal chlorides, buffers, and acid or alkali, were oxidized in the dark with shaking in an oxygen atmosphere. Under optimum conditions (pH 6.5), the linoleate peroxide content, after 2 hr autoxidation at 20°C, was increased more than 3-fold by the addition of 1 ppm of ferric ions, approximately 20-fold by a 0.01 M concentration of histidine, and more than 60-fold by the addition of both histidine and ionic iron. The pro-oxidative effect of other transition metal ions (Cu++, Co++, Cr++, Mn++, and Ni++) also was investigated. None of these ions had a significant effect alone. Combined with 0.01 M histidine, only Mn++ increased peroxidation over that when histidine alone was added.

The pro-oxidative action of histidine was retarded approximately 60% by 0.1 N acetate buffer and completely repressed by 0.05 M phosphate, nonionic emulsifiers, and low and high pH. The threshold concentration of histidine necessary for pro-oxidative action was greater than 0.0001 M.
The pro-oxidative activity of histidine in linolate emulsions is thought to be due to the formation of pro-oxidant complexes with trace quantities of ionic iron. The solvation of transition metal ions with water or chelation with histidine either enhances or reduces their pro-oxidative efficiency depending on the electronic configuration of the metal ion.

Introduction

Autoxidation of fatty materials is a complicated phenomenon (1) even when it involves only the oxidation of an anhydrous polyunsaturated fatty acid ester under carefully controlled conditions. Autoxidation of fats in food is further complicated because fat is present as a diverse mixture of many lipids. The fat is often in finely divided or emulsified state, intimately associated with a mixture of solid components and a complex aqueous phase containing a variety of both soluble and coagulated dispersed materials. In such a system, numerous reactions, both oxidative and otherwise, occur simultaneously, and the rate, extent, and possibly the course of reactions with oxygen are influenced by numerous compounds (8, 9, 10) which promote or inhibit oxidation. Considerable work has been published on the influence of amino acids on the autoxidation of fats.

Nearly all of the known amino acids have been reported to have pro-oxidative and/or antioxidative activity. Cysteine (2, 7, 13, 22) was found to be strongly pro-oxidative over a wide range of conditions but antioxidative (13) at pH 9.5. Franke (2) reported a number of amino acids to be strong pro-oxidants in linoleic acid emulsion. The most effective, in decreasing order, were: histidine, arginine, cysteine, lysine, and tryptophane. He also found proline to be a highly effective pro-oxidant in anhydrous linoleic acid while Janicki et al. (7) found valine to be second only to cysteine in promoting the autoxidation of anhydrous lard. Marcus (14) found some of these same amino acids to be effective antioxidants in emulsion. The most effective, in decreasing order, were: histidine, tryptophane, threonine, lysine, arginine, phenylalanine, and serine. Kaufmann (10) has found dihydroxyphenylalanine to be an exceptionally effective antioxidant in potassium linoleate emulsions. Janicki et al. (7) reported the following amino acids, in decreasing order of effectiveness, to be strong antioxidant synergists for 

Experimental

Materials. Methyl linolate was prepared by the methanolysis of safflower oil, and purified by a modification of the method of Parker et al. (17) using a ratio of 1:1.6:4, respectively, of safflower oil methyl esters, urea, and methanol. The final product was fractionally distilled through a 75 cm Vigreaux column under 0.8 mm pressure. It was 97.3% linolate with I.V. 172.8, peroxide value 2.6, and 0.5% conjugated diene. Ethyl linolate employed in some early experiments was prepared by a similar procedure. It was 99.8% linolate with I.V. 164.1, peroxide value 1.7, and 0.05% conjugated diene.

1-Histidine was a high purity grade obtained from the Nutritional Biochemicals Corporation. All buffers, salts, acids, and bases were reagent grade chemicals. Metals were used as their chloride salts. Sodium dodecylsulfate (emulsifier) was a highly purified laboratory preparation. Other emulsifiers, used in exploratory experiments, included purified laboratory preparations of sodium myristate, potassium palmitate, and ethenoxylated tetradecanol (averaging 15 ethenox groups per molecule) and two commercial emulsifiers, Tween 20 and Span 20. Redistilled water was used in preparing all emulsion components. It was prepared by redistilling a solution of potassium hydroxide and potassium permanganate in laboratory distilled water in an all-glass still.

Apparatus. Oxidation studies were made in two types of apparatus. Oxygen absorption investigations were made in Warburg apparatus, using the same 90 ml reaction vessels and manometers as described previously (20). In a large proportion of our experimental chemical changes in the autoxidation of emulsi- fied linolate esters were studied using 270 ml glass vessels that we have designated as rocker tubes. These vessels consisted of cylinders with rounded ends and an open-end side-arm attached at right angles. The side-arms were sealed during autoxidation by clamping short pieces of rubber tubing, previously cleaned by boiling first in sodium hydroxide solution and finally in distilled water.

Procedure. All experimental work, wherever feasible, was carried out in all-glass equipment to minimize metal contamination. However, in the preparation of the initial concentrated emulsions, a mixture consisting of 4 ml of methyl linolate, 25 ml of 0.5% sodium dodecylsulfate solution, and 21 ml of water was emulsified for 15 min in a Virtis 45 homogenizer in contact with teflon and stainless steel components of the homogenizer assembly. Ten ml of this emulsion was transferred to each of 4 rocker tubes and diluted to 25 ml with water and/or test solutions. Stable emulsions of comparatively uniform oil particles, averaging less than 1 μ in size were obtained. A typical unbuffered control contained 0.0024 mole of methyl linolate, 0.175 sodium dodecylsulfate, and 24.2 ml of water, and had a pH of 6.5. Test components included buffers, standard solutions of hydrochloric acid and sodium hydroxide, metal chlorides, and 1-histidine. Solutions of these components were added as required in amounts predetermined to give the appropriate pH and concentration in 25 ml of emulsion. Solutions of the pro-oxidant test components (metal salts and histidine) were added last.

As soon as the test mixtures were prepared, the gas in each rocker tube was evacuated and replaced with pure oxygen to a final pressure of 1 atm plus 100 mm Hg and the tube sealed. The rocker tubes were then mounted in a parallel, horizontal position, with the side arm upright, in an Equipoise shaker in a constant temp room at 20°C. The tubes were shaken at 160 strokes per min for 2 hr in the dark. Each emulsion was then extracted once with 75 ml of 2:1 ethyl ether-ethanol and the extract washed with 100 ml of distilled water. The autoxidized methyl linolate was recovered by evaporation in a rotary evaporator under reduced pressure. Tests have shown that this method of recovering the autoxidized methyl linolate resulted in no significant change in the...