Cottonseed Oil Oxidation Catalyzed by Amino Acids and Albumin in Aqueous and Nonaqueous Media

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ABSTRACT

Oxidation of refined cottonseed oils catalyzed by various α-amino acids and albumin has been studied in aqueous and nonaqueous media. Cysteine, phenylalanine, and albumin possessed prooxidant effect in cottonseed oil in aqueous and nonaqueous media. Serine exhibited prooxidant activity in aqueous media and minor antioxidant activity in nonaqueous media. Effectiveness of the amino acids on cottonseed oil oxidation was in the following descending order in both aqueous and nonaqueous media: cysteine > phenylalanine > serine. The prooxidant effect in aqueous media might be due to the predominant presence of the protonated amino nitrogen, whereas amino acid-metal complex might be responsible for the prooxidant effect in nonaqueous media.

INTRODUCTION

Much work has been carried out by many researchers dealing with the oxidation of lipid materials using simple model systems in aqueous media. For instance, Haase and Dunkely (1), Natake (2), Tsai and Smith (3) and Allen and Farag (4) used, in general, linoleic acid catalyzed by ascorbic acid, Cu^{2+}, amino acids and bases, respectively. Few attempts were undertaken to elucidate the course of lipid oxidation in anhydrous sources. For example, Labuza et al. (5) investigated the oxidation of linoleic acid, methyl linoleate, and trilinolein in model systems based on various solid supports and at varying relative humidity.

From the above consideration and the growing need to increase the stability of lipids toward rancidity, our main interest was focused on designing model systems analogous to the lipids occurring in biological materials. These models consisted of oil catalyzed by amino acids or albumin. The lipids concentration in the present investigation was 10^{-2}M; therefore, a comparison can easily be made between the oxidation of oil and linoleic acids alone or catalyzed by various amino acids or albumin.

MATERIALS AND METHODS

Cottonseed Oil

Refined cottonseed oil was obtained from Cairo Company for Oil and Soap. The oil was nearly free from peroxides (peroxide number 0.1) as estimated by the method outlined by the AOAC (6), and the digested oil contained Cu, Fe, Co, Ni, and Mn not more than 0.007 ppm. The iodine value of the refined oil was also measured as reported by the AOAC (6).

Amino Acids and Albumin

Pure amino acids and albumin used in the present work were "PROLABO" grade (serine and albumin) and "BDH" grade (cysteine and phenylalanine). The amino acids and albumin gave one spot by thin layer chromatography and were practically free from heavy metals (0.006-0.008 ppm) after they had been acid digested. The cysteine content of albumin was determined by the method described by Chinard and Hellerman (7).

Other Reagents

Sulfuric and nitric acids were "ANALAR" grade, Tween 20 and ethylene diamine tetraacetic acid disodium salt (EDTA) were "BDH" grade.

Deionized Water

Deionized water with conductivity less than 1 megohm-cm^{-1} was used for the preparation of all aqueous solutions and cleaning all glassware.

Apparatus

Trace metals analyses were performed using a Pye Unicam model 1900 atomic absorption spectrophotometer, and CHRIST Delta Lyophilizer apparatus was used for the preparation of nonaqueous cottonseed oil model systems. Warburg manometric apparatus was used for the measurement of oxygen uptake.

Prevention of Contamination by Heavy Metals

Extreme care was taken to avoid contamination by heavy metals. All experimental work was carried out in all-glass equipment to minimize metal contamination. All glassware were immersed for 24 hr in a 0.5% solution of EDTA, rinsed five times with deionized water, and dried at 120 C before use.

Preparation of Refined Cottonseed Oil Aqueous and Nonaqueous Model Systems

A mixture consisting of refined cottonseed oil (0.738 g) and Tween 20 (0.5 ml) was made up to 25 ml with deionized water. Emulsification was achieved by agitation using a Vortex shaker for 15 min. Aliquots (0.2 ml) of this emulsion were quantitatively transferred into Warburg flasks, diluted to 2 ml with deionized water, and amino acid or albumin was added. Amino acids and albumin were added to emulsified oil in 0.2 ml quantities of appropriate stock solutions to give final amino acid or albumin concentrations of 10^{-3}M. Therefore, the lipid emulsion was composed of cottonseed oil (10^{-2}M), Tween 20 (0.2%), and amino acid or albumin (10^{-3}M). In the case of aqueous model systems, the Warburg flasks were directly attached to manometers for the determination of oxygen uptake. For nonaqueous model systems, the contents of each Warburg flask were freeze-dried in lyophilizer apparatus, and the water was removed under high vacuum and -30 C. Thereafter, the flasks were directly attached to manometers.

Measurement of Oxidation

Warburg apparatus was used to measure the oxygen absorbed by cottonseed oil in aqueous and nonaqueous media. Experiments were conducted at 40 C with a constant shaking rate of 80 oscillations per min. Three replicates of each experiment were carried out against two cottonseed oil controls, and the average value was taken, provided that the three figures did not differ by not more than 5%. Also, controls with the individual amino acids and albumin were run simultaneously with the corresponding experiment. No appreciable oxygen uptake was recorded for the last controls. No appreciable oxygen uptake was

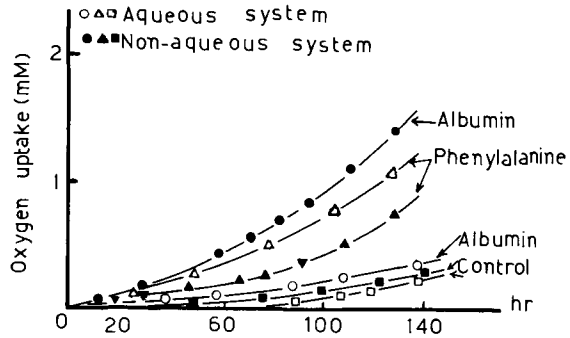

FIG. 1. The effect of albumin or phenylalanine on the oxygen absorbed by cottonseed oil in aqueous and nonaqueous systems.

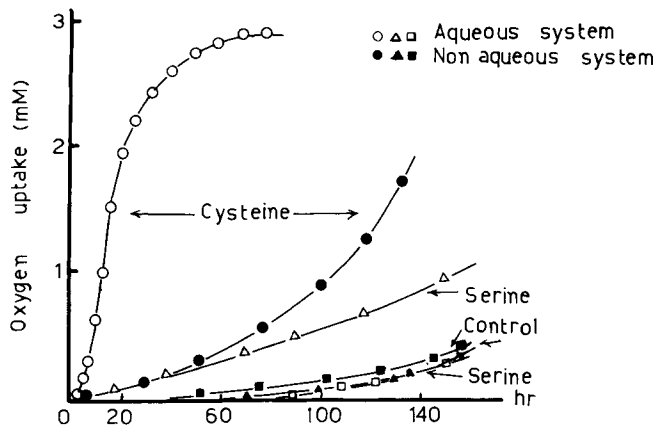

FIG. 2. The effect of cysteine or serine on the oxygen absorbed by cottonseed oil in aqueous and nonaqueous systems.

recorded for the last controls. The central well of the vessel contained 0.2 ml of 20% (w/v) potassium hydroxide solution to absorb gases evolved by the model systems.

RESULTS AND DISCUSSION

Oxidation of lipids in aqueous emulsion and in dehydrated sources is of great importance in handling and storing of these materials. Model systems were designed to approximate conditions of lipids as they occur in nature. For a better understanding of the lipid oxidation process, simple model systems consisting of cottonseed oil and amino acids were first studied. A model system analyogous to the occurrence of lipids in nature consisted of intimate association of lipids and proteins.

Catalysis of Refined Cottonseed Oil by Amino Acids in Aqueous and Nonaqueous Media

The present experiments were conducted to investigate the role of various amino acids on cottonseed oil oxidation. It was found that the six amino acids used as catalysts for linoleic acid (Farag et al., 8 and 9) played an important role in the oxidation. In this set of experiments only three amino acids (serine, cysteine, and phenylalanine) which exhibited minor, moderate, and highly prooxidant activity on linoleic acid oxidation, respectively, were used. Therefore, a comparison can be easily made between the effect of various amino acids on linoleic acid and cottonseed oil oxidation.

The iodine value for cottonseed oil used in the present investigation was 115. Therefore, cottonseed oil contained oleic and linoleic acids as major fatty acid constituents. Oleic acid was used in calculating the molarity of the oil. In comparing the oxidation rate of linoleic acid and cottonseed oil, one would expect to find that linoleic acid has a much faster oxidation rate than cottonseed oil. Indeed the induction periods for linoleic acid in aqueous and nonaqueous media were 46 hr and 15 hr, respectively, whereas, cottonseed oil started to oxidize after 75 hr and 45 hr in aqueous and nonaqueous media, respectively. In this respect, Kkan (10) studied the effect of adding methyl linoleate to pure methyl oleate on the autoxidation induction period of methyl oleate at 75 C. Addition of 1% methyl linoleate eliminated the induction period of methyl oleate.

Figures 1 and 2 show oxygen absorption curves for cottonseed oil catalyzed by serine, phenylalanine, cysteine, and albumin in aqueous and nonaqueous media. The oxidation rate of cottonseed oil catalyzed by amino acids increased as a function of time even in the cysteine model system. The autocatalysis of cottonseed oil in aqueous and nonaqueous systems was nil up to 75 hr and 45 hr, respectively, at 40 C.

In order to compare the pro- or antioxidative behavior of the three amino acids in both aqueous and nonaqueous systems, a value of 0.25 mM oxygen was chosen since some of these model systems were still at the induction period after 45 hr and 75 hr. The catalytic effects of serine, cysteine, and phenylalanine on the stability of cottonseed oil in aqueous and nonaqueous media are shown in Table I. The effectiveness of the amino acids on cottonseed oil oxidation was in the following descending order in both aqueous and nonaqueous media: cysteine > phenylalanine > serine.

In this respect, Siechowski (11) reported that the antioxidant activities of the sulfur amino acids on olive oil oxidation were in the following order: cysteine > methionine > cystine.

In dealing with the effect of serine on cottonseed oil oxidation, a marked faster oxidation reaction had taken place in aqueous than in nonaqueous system. These results were in accordance with the results obtained from linoleic acid experiments (Farag et al., 8 and 9). The prooxidant activity of cysteine as a catalyst for cottonseed oil oxidation was the highest in these experiments. Cysteine in aqueous media was a more effective catalyst for cottonseed oil oxidation at 40 C than in nonaqueous media. In aqueous media, the oxygen absorption leveled off when 3 mM of oxygen has been absorbed in about 60 hr by cottonseed oil. Phenylalanine was shown to be prooxidant (Fig. 1) in model systems composed of cottonseed oil in aqueous and nonaqueous media. Once again, the reaction was faster in aqueous than in nonaqueous media.

One could interpret the more prooxidant effect of the tested amino acids in aqueous media to the predominant presence of protonated amino nitrogen as reported by Farag et al. (8). It has been found that some trace metals such as Cu, Mn, Fe, and Co, and Mg are present in refined cottonseed oil (0.007 ppm) as well as in the amino acids (0.006-0.008 ppm). Trace metals can be easily bound to the unprotonated amino nitrogen of the amino acids, and these complexes decrease the stability of cottonseed oil.

Catalysis of Cottonseed Oil with Albumin in Aqueous and Nonaqueous Media

This work is mainly aimed to throw more light on the oxidation deterioration in milk and milk products and freeze-dried foods. According to the previously reported results using various amino acids as catalysts for lipid oxidation, one would expect that if a protein contains a high level of cysteine, it will indeed oxidize the lipid material much faster than that catalyzed with protein containing low levels of cysteine. The only protein used in the present investigation was albumin in which cysteine constitutes 2.7% of the total amino acid composition. Albumin contains the amino acids which possess mild, minor, and highly

TABLE I

Time (hr) of Oxygen Uptake at 0.25 mM Oxygen for Model Systems

Model system Cottonseed oil (10^{-2}M) + amino acid (10^{-3}M)	Time (hr)	
	Aqueous	Nonaqueous
Cottonseed oil	145	137
Cottonseed oil + cysteine	8	43
Cottonseed oil + serine	43	144
Cottonseed oil + phenylalanine	40	75
Cottonseed oil + albumin	116	39

prooxidant activity. One might also expect that the organization of amino acids in the structure of the protein molecule may hinder the reactive groups of amino acids and subsequently lower the prooxidant activity.

The oxidation rate of cottonseed oil catalyzed by albumin is increased with time, i.e., the reaction possessed autocatalytic behavior as shown in Figure 1. Since the reaction proceeded with time and up to 150 hr, the reaction is still at the induction period. A value of 0.25 mM oxygen has been taken to illustrate the effect of albumin on cottonseed oil oxidation and the results are shown in Table I. The addition of albumin to cottonseed oil showed a prooxidant effect, and it was more pronounced in nonaqueous media than in aqeous media. Similar findings were mentioned by Chipault and Hawkins (12) who found that the autoxidation of lipids was a serious cause of deterioration in freeze-dried meat. The prooxidant effect of albumin on cottonseed oil might be due to the formation of albumin metals complexes. Trace metals were present in cottonseed oil and albumin, and their concentrations lie between 0.006 to 0.008 ppm. In this respect, Tappel (13) reported that albumin showed a marked affinity for copper, and copper-protein complexes were effective catalysts for linoleate oxidation. In dealing with the effect of protein concentration on lipid oxidation, Yukami (14) showed that oxygen uptake was very dependent on the protein concentration. As the protein concentration increased, more effect was obtained.

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