Linoleic Acid Oxidation Catalyzed by Various Amino Acids and Cupric Ions in Freeze-Dried Model Systems

R.S. FARAG, S.A. OSMAN, Biochemistry Department, Faculty of Agriculture, Cairo University, **S.A.S. HALLABO,** Food Science and Technology Department Faculty of Agriculture, Cairo University, **A.N. GIRGIS,** and **A.A. NASR,** National Orbanization for Drug Control and Research, Cairo, Egypt

ABSTRACT

The present investigation into the effect of amino acids on linoleic acid oxidation in freeze-dried model systems illustrates the existence of an autocatalytic chain reaction, in which all amino acids, except cysteine, exhibited minor antioxidant behavior. The antioxidant effect might be attributed to the absence of protonated amino nitrogen. Linoleic acid alone had an induction period of 15 hr, and on the addition of various α-amino acids, the systems had induction periods ranging from 16-19 hr. This increase did not exhibit any specific function for the studied amino acids. Cysteine exhibited an exceptional prooxidant effect due to the role of the HS-group. The addition of copper at concentrations of 10^{-5}M and 10^{-3}M to the model systems composed of linoleic acid and various α-amino acids exhibited minor and highly prooxidant effects, respectively, The prooxidant effect of these amino acids in the presence of copper might be due to amino acids-copper complexes.

INTRODUCTION

Unsaturated fatty acids form peroxides in reaction with oxygen under appropriate conditions. Autoxidation is a problem in a wider sense in the industries dealing with fats and oils because of the development of rancidity or undesirable flavors. Lipids are often present in an emulsified state or with a mixture of solid mixtures. Very little work has been carried out using freeze-dried and intermediate moisture model systems, e.g., Karel et al. (1), Labuza et al. (2), and Chou et al. (3). Therefore, simple model systems were designed to approximate conditions in freeze-dried foods consisting of linoleic acid catalyzed with amino acids, cupric ions, and the combined addition of amino acids and cupric ions. Hence, the oxidation behavior and the relation between the rate of linoleic acid oxidation and the chemical structure of various α-amino acids were investigated.

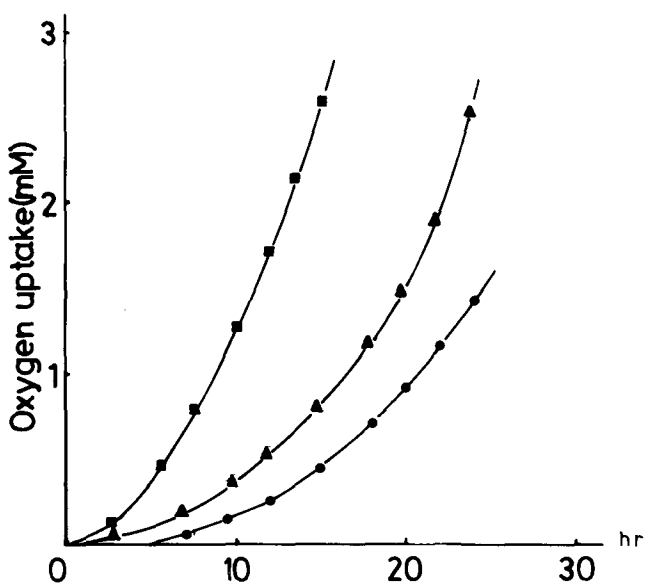

FIG. 1. The effect of cupric ions on the oxygen absorbed by linoleic acid in freeze-dried systems. ● = Control; ▲ = Cu(10^{-5}M); ■ = Cu(10^{-3}M).

MATERIALS AND METHODS

The materials used in the present work, prevention of contamination by heavy metals, and measurement of oxidation were exactly the same as mentioned by Farag et al. (4).

Apparatus

Trace metals were analyzed using a Pye Unicam model Sp 1900 atomic absorption spectrophotometer, and a CHRIST Delta Lyophilizer apparatus was used for the preparation of nonfreeze-dried model systems. Warburg manometric apparatus was used for the measurement of oxygen uptake.

Preparation of Freeze-Dried Model Systems

A mixture consisting of linoleic acid (0.706 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 the aqueous emulsions were pipetted into Warburg flasks, diluted to 2 ml with deionized water with or without amino acid and with or without Cu^{2+}. Amino acids and cupric ions were added to emulsified linoleic acid in 0.2 ml quantities of appropriate stock solutions to give final amino acid and metal concentrations of 10^{-3}M and 10^{-5}M, respectively. The contents of each flask were freeze-dried in lyophilizer apparatus, and the water was removed under high vacuum and -30 C. Thereafter, the flasks were attached directly to manometers for the determination of oxygen uptake.

RESULTS AND DISCUSSION

Lipid materials naturally occur in dehydrated and aqueous sources. The oxidation of anhydrous lipids is a complex process as mentioned by Lundberg (5). Simple model systems composed of linoleic acid and Tween 20 without recourse to solid supports were designed to approximate conditions in the freeze-dried foods to study their oxidation behavior in the presence or absence of α-amino acids and cupric ions.

TABLE I

Stability and Relative Stability of Linoleic Acid Uncatalyzed and Catalyzed with Cupric Ions in Freeze-Dried Systems

Parameter	Linoleic acid	Linoleic acid + Cu^{2+} (10^{-5}M)	Linoleic acid + Cu^{2+}(10^{-3}M)
Stability (hr)	15	8	3
Relative stability[a]	1.00	0.53	0.20

[a]Relative stability for linoleic acid (induction period = 15 hr) is given as 1.00.

Stability of Linoleic Acid in Freeze-Dried Systems

The model system consisting of linoleic acid (10^{-2}M) and Tween 20 (0.02%) had an induction period of 15 hr (Fig. 1). The results showed that even exceedingly small amounts of metals, as previously mentioned, can be catalytically active. In comparing the oxidation rate with the aqueous system [Farag et al. (4)], it was found that the freeze-dried model system of linoleic acid was much more susceptible to oxidation. One could explain this effect by the adsorption of the accompanied trace metals at the surface of the clustered lipids. The concentration of trace metals in these model systems is much higher in comparison with that in the aqueous sytems due to severe dilution. Hence, the rate of linoleic acid oxidation in the freeze-dried system is much faster than that in the aqueous system.

The data obtained in the present work agreed quite well with those of Labuza et al. (6) who investigated the oxidation of linoleic acid, methyl linoleate, and trilinoleate in model systems based on various solid supports and at various relative humidities. Their results indicated that at 40 C, an increasing water content had an inhibitory effect on the oxidation of trilinoleate as it did on methyl linoleate, and the effectiveness of antioxidatns increased with increasing humidity.

On the deliberate addition of cupric ions at concentrations of 10^{-5}M and 10^{-3}M, the induction periods for linoleic acid became 8 hr and 3 hr, respectively. The stability and relative stability of linoleic acid catalyzed and uncatalyzed with cupric ions are shown in Table I.

It is evident from data in Table I that the deliberate addition of cupric ions cuts down the time required for determination of linoleic acid stability to about one-half and one-fifth that of its original value. Copper had a marked prooxidant effect at concentrations of 10^{-5}M and 10^{-3}M. At the higher copper concentration, the rate of linoleic acid oxidation was more pronounced. Similar results have been obtained by several investigators. For instance, Allen and Farag (7) and Haase and Dunkely (8) showed that as copper concentration increased, a higher rate of reaction was observed.

Catalysis of Linoleic Acid by Amino Acids in Freeze-Dried Systems

Freeze-dried model systems consisting of linoleic acid (10^{-2}M), Tween 20 (0.02%), and amino acid (10^{-3}M) were used in this set of experiments. The results of these experiments showed a variety of effects on the rates of oxidation in comparison with the control experiments (containing no amino acid). The stability and relative stability (the latter meaning the stability in hours of the lipid materials relative to that of linoleic acid) of linoleic acid catalyzed with amino acids were calculated from Figures 2, 3, and 4, and the obtained results are shown in Table II. A relative induction period value of < 1 indicates a prooxidant effect, whereas a value of > 1 indicates an antioxidant effect.

The effect of amino acids under investigation in all model systems showed features of an autocatalytic chain reaction, i.e., the rate of hydroperoxide formation increased with time, and the secondary products are necessary to catalyze the linoleate oxidation. The results indicate that all amino acids tested except cysteine exhibit minor antioxidant effect. The antioxidant activities of the amino acids for linoleic acid oxidation were in the following order: alanine = serine > phenylalanine > tryptophan > histidine. Our results (except for cysteine) agree with the work of Karel et al. (1) who studied the oxidation of methyl linoleate in freeze-dried model systems consisting of microcrystalline cellulose and methyl linoleate with respect to the effect of various amino acids.

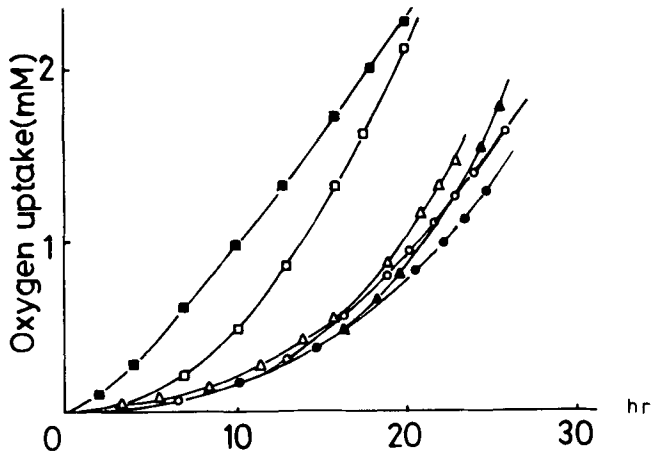

FIG. 2. The effect of alanine and serine with or without cupric ions on the oxygen absorbed by linoleic acid in freeze-dried systems. ● ▲ ■ = Alanine; ○ △ □ = serine; △ ▲ = Cu(10^{-5}M); □ ■ = Cu (10^{-3}M).

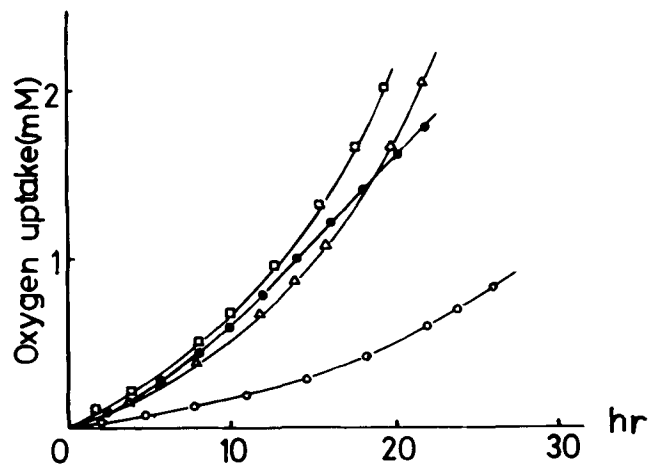

FIG. 3. The effect of cysteine or phenylalanine with or without cupric ions on the oxygen uptake by linoleic acid in freeze-dried systems. ● = Cysteine, ○ △ □ = phenylalanine, △ = Cu(10^{-5}M); □ = Cu(10^{-3}M).

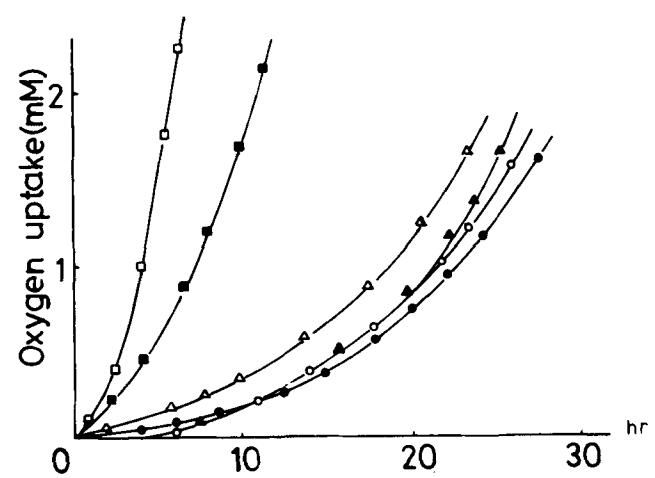

FIG. 4. The effect of histidine or tryptophan with or without cupric ions on the oxygen uptake by linoleic acid in freeze-dried systems. ○ △ □ = Histidine; ● ▲ ■ = tryptophan; △▲ = Cu(10^{-5}M); □ ■ = Cu(10^{-3}M).

The amino acids used in the present study have been shown to exhibit a potential prooxidant effect in aqueous media [Farag et al. (4)] thought to be due to the role of

TABLE II

Catalytic Effects of Various Amino Acids, Cupric Ions Together with Amino Acids (10^{-3}M) on the Stability of Linoleic Acid in Freeze-Dried Model Systems

Model system	Induction period (hr) Without added metal	Induction period (hr) Cu^{2+} 10^{-5}M	Induction period (hr) Cu^{2+} 10^{-3}M	Relative induction period[a] Without added metal	Relative induction period[a] Cu^{2+} 10^{-5}M	Relative induction period[a] Cu^{2+} 10^{-3}M
18:2 + alanine	19	17.4	4.0	1.27	1.16	0.27
18:2 + serine	19	17.0	10.0	1.27	1.13	0.67
18:2 + cysteine	4.0	---[b]	---[b]	0.27	---	---
18:2 + phenylalanine	18	12	10	1.20	0.80	0.67
18:2 + tryptophan	17	11	7	1.13	0.73	0.47
18:2 + histidine	15.8	16	3	1.05	1.06	0.20

[a]Relative induction period for linoleic acid (induction period = 15 hr) is given as 1.00.
[b]No manometric readings were recorded due to evolution of hydrogen sulfide.

protonated amino nitrogen of the amino acids. The antagonistic effect which was found in the freeze-dried systems might be explained as follows. It is well known that ionization takes place in an aqueous media. Freeze-dried model systems expected to contain trace quantities of water were not sufficient to produce pronounced quantities of the protonated amino nitrogen which exhibit the prooxidant effect. The minor antioxidant behavior of alanine, serine, phenylalanine, tryptophan, and histidine may be attributed to insufficient amounts of the protonated amino nitrogen. Also, in the presence of very small amounts of water, the micelle structure for linoleic acid may be excluded and one may expect that the clustered structure would be predominant under the experimental conditions. The clustered structure can stand against the trace metals in the systems and preclude the attack by oxygen. Further studies using an electron microscope are quite necessary to support these theories. The control experiments had induction periods of 15 hr, and the test experiments (except cysteine) had induction periods ranging from 16-19 hr. The small increase in the induction period cannot be used as an index to relate the linoleic acid oxidation to the presence of certain functional groups that may have a specific function in the amino acids used in this work.

Cysteine,in particular, exhibited a prooxidant effect. The mechanism of linoleic acid oxidation catalyzed by other amino acids largely depends upon their protonated amino nitrogen, whereas in cysteine the reaction is basically dependent upon the function of the HS-group as mentioned by Lewis and Wills (9).

Catalysis of Linoleic Acid by Amino Acids and Copper Combined in Freeze-Dried Model Systems

The effect of amino acids together with cupric ions on linoleic acid oxidation was studied, and the results are shown in Table II. The relative induction period of linoleic acid catalyzed by amino acid with or without added Cu^{2+} was used as an index to show the prooxidative or antioxidative behavior of amino acids. Alanine combined with Cu^{2+} (10^{-5}M) had very little effect in decreasing the stability of linoleic acid. At a high copper concentration (10^{-3}M), linoleic acid stability was enormously decreased. The model system consisting of serine, Cu^{2+} (10^{-5}M), and linoleic acid had a relative induction period slightly longer than that of the model system that contained no added metal, whereas, copper at the higher concentration, markedly decreased the induction period.

In a freeze-dried system composed of cysteine, Cu^{2+} (10^{-5}M or 10^{-3}M), and linoleic acid, a peculiar reaction took place, No manometric readings were recorded in this model system because the system itself evolved hydrogen sulfide, which was easily detected by its characteristic odor after 24 hr from the beginning of the experiment. This indicates that homolytic scission has taken place somehow between the β-carbon atom of cysteine moiety and sulfur of the sulf-hydryl group. In this respect much work is needed to reveal the mechanism by which the scission occurred, and in order to follow up the course of oxidation in this model system, other procedures which do not depend on the oxygen uptake should be used (for example, the absorption at 234 nm or a TBA-test).

Amino acids having an aromatic side chain together with Cu^{2+} (10^{-5}M) showed a minor prooxidant effect. In general, copper at a concentration of 10^{-3}M apparently increased the rate of linoleic acid oxidation especially in the presence of histidine and Cu^{2+} (10^{-3}M).

Results of this work illustrate that all investigated amino acids (except cysteine) and copper at concentrations of 10^{-5}M and 10^{-3}M exhibit minor and highly prooxidant effects, respectively. The prooxidant effect of these amino acids in the presence of copper might be interpreted as follows. In freeze-dried systems the unprotonated amino nitrogen predominates, hence copper could easily bind with the amino groups to form some sort of complex. The amino acid copper complex is probably responsible for rapid oxidation. In this respect, Scaife (10) indicated that the binding of copper to amino acids had a strong catalytic effect on the oxidation of ascorbic acid and linoleate.

REFERENCES

1. Karel, G., S.R. Tannenbaum, D.H. Wallace, and H. Maloney, J. Food Sci. 31:892 (1966).
2. Labuza, T.P., L. McNally, D. Gallagher, J. Hawkes, and F. Hurtado, Ibid. 37:154 (1972).
3. Chou, H.E., K. Acott, and T.P. Labuza, Ibid. 38:316 (1973).
4. Farag, R.S., S.A. Osman, S.A.S. Hallabo, and A.A. Nasr, JAOCS (In press).
5. Lundberg, W.O., "Autoxidation and Antioxidation," Vol. 1, John Wiley & Sons, Inc., New York, 1961.
6. Labuza, T.P., H. Tsuyuli, and M. Karel, JAOCS 409 (1969).
7. Allen, J.C., and R.S. Farag, III International Symposium on Metal-Catalysed Lipid Oxidation, Institut des Corps Gras, Paris, 1973.
8. Haase, G., and W.L. Dunkely, J. Lipid Res. 10:561 (1969).
9. Lewis, S.E., and E.D. Wills, Biochem. Pharmacol. 11:901 (1962).
10. Scaife, J.F., Can. J. Biochem. Physiol. 37:1049 (1959).

[Received February 3, 1978]