The Role of Amino Acids in the Autoxidation of Milk Fat

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The effects of amino acids and their analogs on milk fat oxidation were examined under various conditions by measuring oxygen consumption and total unsaturated fatty acids. All the amino acids tested acted as antioxidants, characteristically extending the induction period (IP). Not only primary amino groups are responsible for the antioxidative activities of amino acids, but also the side-chain groups contribute, at least partially, to the protective effects of L-cysteine, L-tryptophan and L-tyrosine. In aqueous and HCL solutions, the antioxidative effects of L-alanine were significantly reduced. The freeze-dried L-lysine-HCL and L-alanine-HCL accelerated, while the corresponding control amino acids inhibited, milk fat oxidation.

KEY WORDS: Amino acids, antioxidant, autoxidation, milk fat.

Amino acids have been reported to act as antioxidants, prooxidants and/or to have no effect on the oxidation of lipids (1,2). The mechanisms reported for the antioxidant effects include the following: (i) they act as primary antioxidants mainly due to their primary groups (3); (ii) chelation of prooxidative trace metals (4); (iii) generation of primary antioxidants (5); (iv) synergism with other food constituents (3). However, fewer explanations have been proposed for the prooxidative effects of amino acids. Farag and co-workers (2) suggested that protonated amino groups (NH_3^+) accelerate lipid oxidation, while non-protonated amino groups (NH_2) inhibit the lipid oxidation. Riisom *et al.* (6) reported that in emulsion the antioxidant/prooxidant effects of amino acids depend on the pH. The prooxidant activity of amino acids was enhanced with decreasing pH.

In the present work, the effects of amino acids on milk fat oxidation were studied further.

MATERIALS AND METHODS

Materials. All amino acids (99%), Nα-acetyllysine (99%), Nε-acetyllysine (99%) and Nα-acetyllysine methyl ester (99%) were purchased from Sigma Chemical Co. (St. Louis, MO). Succinic acid (99%), and indoleacetic acid (99%) were obtained from Aldrich Chemical Company (Milwaukee, WI).

Preparation of milk fat. Fresh milk was obtained from the University of Massachusetts farm (Amherst, MA). After pasteurization, the cream was separated by centrifugation, churned at 4°C, melted at 45°C and centrifuged at 2,000 rpm (Size Z, Model K, International Equipment Co., Boston, MA) for 40 min. The milk fat layer was pipetted and saved.

Measurement of oxygen uptake. The reactive vessel described by Bunick (7) was used to monitor the milk fat oxidation. Ten milligrams of amino acids or their corresponding analogs were weighed into the reactive vessels. Two milliliters of hexane containing 200 mg of milk fat was placed into each reaction vessel. The components were mixed thoroughly, and the hexane was removed under a gentle stream of nitrogen. The reaction vessel was then flushed with air and sealed tightly. In some cases, 300 μL of distilled water or 0.1 N HCL solution was added. Oxidation was conducted at 50°C and 95°C with constant stirring. The headspace oxygen was sampled periodically with a gas-tight syringe and analyzed in a Varian 90-P gas chromatograph fitted with a ⅛" × 3' stainless steel column packed with Molecular Sieve 5A (60/80 mesh) (supelco, Bellefonte, PA), and a thermal conductivity detector was used. The percent oxygen in the headspace was calculated from the ratio of the oxygen to nitrogen peaks.

Fatty acid analysis. Fatty acids were methylated according to Glass (8), analyzed in an HP 5890 A instrument equipped with a 30 m × .32 mm I.D. Supelcowax 10 capillary column. Triheptadecanoin was used as an internal standard for quantitation.

RESULTS AND DISCUSSION

All of the amino acids tested exhibited considerable protection of milk fat against oxidation at 95°C, as indicated by oxygen uptake (Fig. 1-3). This action was typically demonstrated by prolongation of the induction period (IP). As seen in Table 1 the degree of protection varied among the amino acids with cysteine, tryptophan and lysine showing the strongest inhibition. When each of these three amino acids was added, the milk fat remained highly stable throughout the period examined, i.e. 150 hr. Fatty acid analysis data were consistent with the above results. While total unsaturated fatty acids dropped by 50% in 48 hr in the control milk fat, they remained

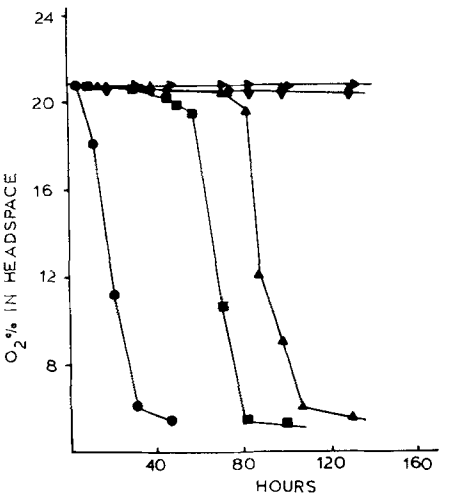

FIG. 1. Effects of amino acids on the oxidation of milk fat at 95°C in the absence of water: ▶ — instrumental control, ● — milk fat, ◆ — milk fat + L-cysteine, ▲ — milk fat + L-serine, ■ — milk fat + L-tyrosine.

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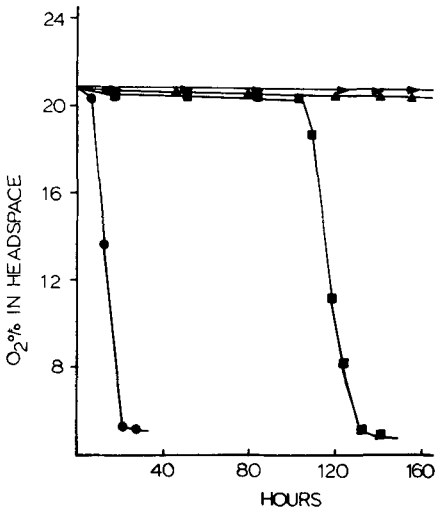

FIG. 2. Effects of amino acids on the oxidation of milk fat at 95°C in the absence of water: ▶ — instrumental control, ● — milk fat, ■ — milk fat + L-alanine, ▲ — milk fat + L-tryptophan.

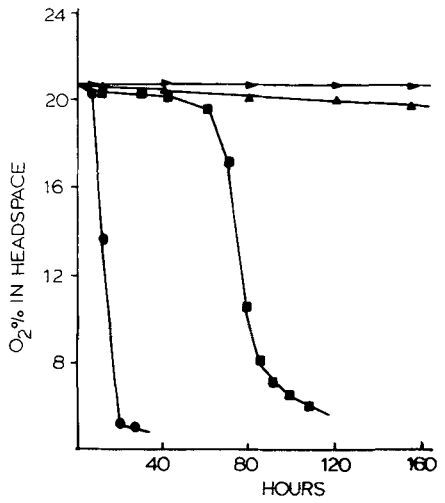

FIG. 3. Effects of amino acids on the oxidation of milk fat at 95°C in the absence of water: ▶ — instrumental control, ● — milk fat, ▲ — milk fat + L-lysine, ■ — milk fat + L-histidine.

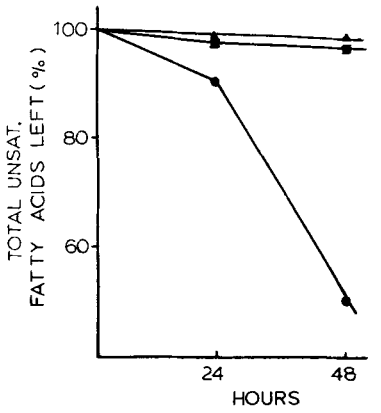

FIG. 4. Effects of amino acids on change in total unsaturated fatty acids of milk fat at 95°C in the absence of water: ● — milk fat, ■ — milk fat + L-alanine, ▲ — milk fat + L-lysine.

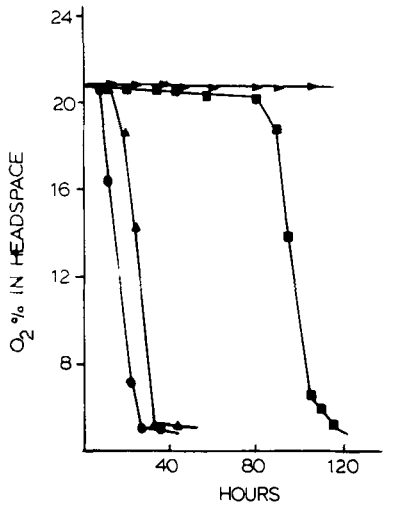

FIG. 5. Effects of L-aspartic acid and its analog succinic acid on the oxidation of milk fat at 95°C in the absence of water: ▶ — instrumental control, ● — milk fat, ■ — milk fat + L-aspartic, ▲ — milk fat + succinic acid.

TABLE 1

Induction periods (IP) of Milk Fat with the Addition of Amino Acids at 95°C

Samples	IP (hr)
Milk fat	<10
Milk fat + cysteine	>150
Milk fat + tryptophan	>150
Milk fat + lysine	>150
Milk fat + alanine	120
Milk fat + serine	90
Milk fat + histidine	70
Milk fat + tyrosine	65

unchanged when any of the amino acids tested was added. Only the effects of alanine and tryptophan are shown here (Fig. 4).

To investigate the role of the amino groups in antioxidation, the inhibiting effects of amino acids were compared with analogs which are structurally similar but where the amino group was absent or blocked. As shown in Figure 5, aspartic acid significantly prolonged the IP of milk fat (85 hr), while the sample containing succinic acid oxidized almost as fast as the control (10 hr). This clearly indicates that the primary amino group plays a major role in the inhibiting activity of amino acids. Figure 6 shows that an amino group on the side chain of an amino acid is also protective although to a lesser degree than that of the alpha amino group. When the epsilon amino group of lysine was blocked, it exhibited less protection than when the primary group was inactivated. Obviously, the strongest effect was demonstrated when both groups were present.

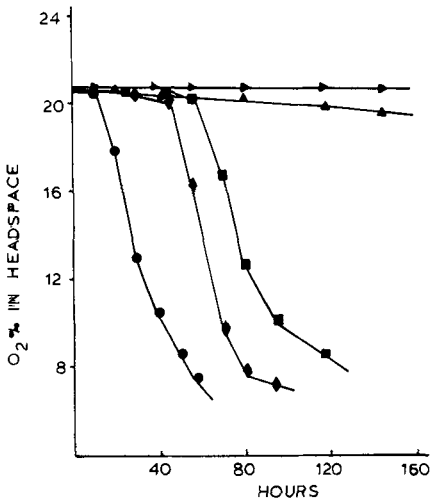

FIG. 6. Effects of L-lysine and its analogs on the oxidation of milk fat at 95°C in the absence of water: ▶ — instrumental control, ● — milk fat, ▲ — milk fat + L-lysine, ◆ — milk fat + Nα-acetyllysine, ■ — milk fat + N$_{\alpha}$-acetyllysine.

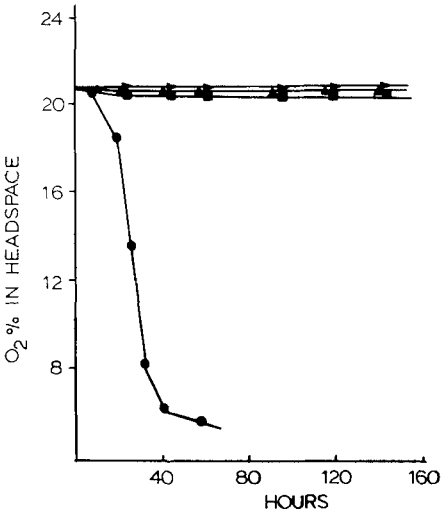

FIG. 7. Effects of L-tryptophan and its analog indoleacetic acid on the oxidation of milk fat at 95°C in the absence of water: ▶ — instrumental control, ● — milk fat, ▲ — milk fat + L-tryptophan, ■ — milk fat + indoleacetic acid.

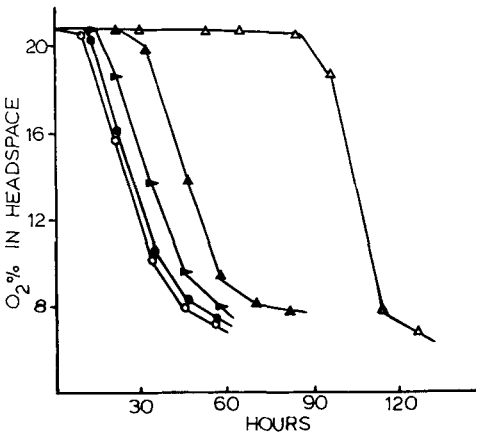

FIG. 8. Effects of L-alanine in 0.1 N HCL on the oxidation of milk fat at 95°C: ○ — milk fat, ● — milk fat + water, △ — milk fat + L-alanine, ▲ — milk fat + L-alanine + water, ▶ — milk fat + L-alanine + 0.1 N HCL solution.

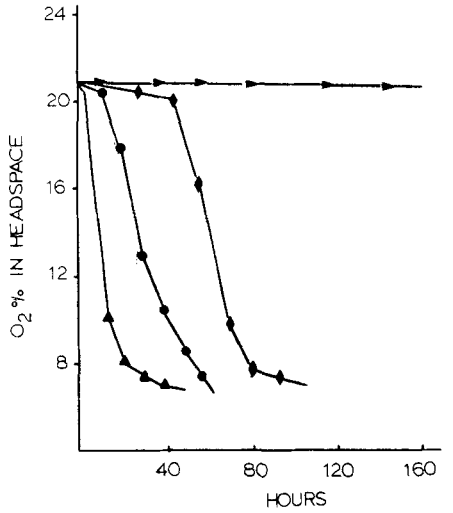

FIG. 9. Effects of the carboxyl group of L-lysine on the oxidation of milk fat at 95°C in the absence of water: ▶ — instrumental control, ● — milk fat, ◆ — milk fat + Nα-acetyllysine, ▲ — milk fat + Nα-acetyllysine methyl ester.

The remarkable antioxidant effect of the indolyl group is demonstrated in Figure 7. Even in the absence of the primary amino group, as when indoleacetic acid was used, strong stability of milk fat could be observed beyond 150 hr.

To study the effect of the medium in which the substrate, i.e. the lipid, and the amino acid co-exist, the effects of some amino acids in dry, aqueous and acidified media were compared.

At 95°C the presence of water significantly reduced the antioxidative effect of alanine (Fig. 8). The ineffectiveness of the primary amino group in the presence of water may be due to a lesser contact with the substrate, to hydrogen bonding between the amino group and the water molecules, or to a higher degree of protonation in the aqueous system (2). In 0.1 N HCL solution, the protective influence of alanine was further reduced, the induction period approaching that of the control. In this case a change in the state of the carboxyl group may be involved. The protonated form (-COOH), more abundant in the acidified system, may be less protective than the negatively charged group (COO-) at higher pH. The fact that carboxyl groups of amino acids may exhibit some antioxidative effect is evident from the data in Figure 9. When the carboxyl group of Nα-acetyllysine, which exhibited an inhibiting effect, was blocked by methylation, the oxidation of milk fat was accelerated.

When the alanine-HCL or lysine-HCL solutions were freeze-dried, their antioxidative effects at 95°C were reversed. They accelerated oxidation of milk fat (Fig. 10). The same behavior was also observed at 50°C (Fig. 11).

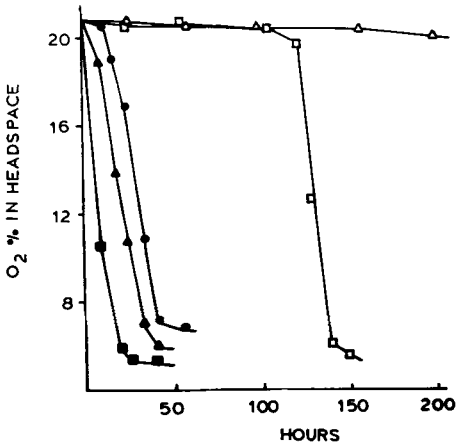

FIG. 10. Effects of freeze-dried amino acid-HCL solution on the oxidation of milk fat at 95°C: ● — milk fat, △ — milk fat + L-lysine, ▲ — milk fat + L-lysine.HCL, □ — milk fat + L-alanine, ■ — milk fat + L-alanine.HCL.

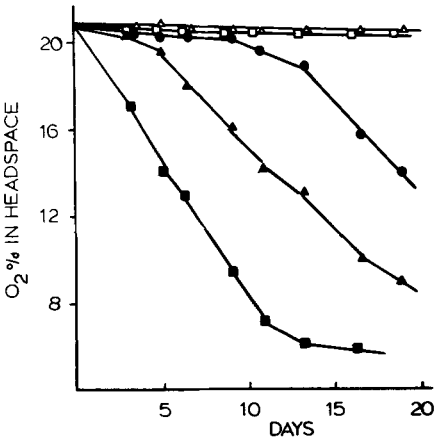

FIG. 11. Effects of freeze-dried amino acid-HCL solution on the oxidation of milk fat at 50°C: ● — milk fat, △ — milk fat + L-lysine, ▲ — milk fat + L-lysine.HCL, □ — milk fat + L-alanine, ■ — milk fat + L-alanine.HCL.

It can be seen that HCL appears to negate the antioxidative effects of amino acids, both in the presence and the absence of water. Whether this behavior is due to an effect on the amino or the carboxyl groups, to an effect of the chlorine moiety, or to some other physical phenomenon, is not clear at this time. The antioxidative effects of lysine and alanine were also markedly reduced when H_2SO_4 was used instead of HCL, although no acceleration was observed.

The primary amino group of amino acids plays a major role in their antioxidative properties. However, carboxyl groups, as well as various functional groups on the side chains, contribute to varying degrees to the protective effects of amino acids. The presence of water and addition of acids reduce, partially or completely, the ability of amino acids to act as antioxidants. Under some conditions amino acids may exhibit prooxidative activities. It is more than likely that the effect of any single parameter reflects the net balance or interaction between opposing influences occurring simultaneously.

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