SHORT COMMUNICATION

Antioxidant Efficacy of Amino Acids in Methyl Linoleate at Different Relative Humidities

A.G. Gopala Krishna and **J.V. Prabhakar***
Department of Lipid Technology, Central Food Technological Research Institute, Mysore - 570 013, India

Antioxidant efficacy of the amino acids methionine, tryptophan, aspartic acid, serine, alanine and arginine in methyl linoleate were compared to a methyl linoleate control at 2, 50 or 79% relative humidity (RH) at 37°C. Antioxidant efficacy varied with RH and the individual amino acids. Arginine had the highest antioxidant efficacy at all RH values compared to the control. The efficacy of alanine was equal to that of arginine at RHs of 50 and 79% but was lower at 2% RH. The presence of aliphatic, alkaline amino, hydroxyl or thiol groups in the side chain of the amino acids increased the antioxidant efficacy at high RHs.

KEY WORDS: Amino acids, antioxidants, autoxidation, methyl linoleate, relative humidity.

Amino acids exhibit antioxidant properties in oils and fats (1–5). Marcuse (1) reported tryptophan and histidine to have good antioxidant effects in methyl linoleate emulsions. Karel *et al.* (2) observed that histidine, lysine, β-amino butyric acid and cysteine have an antioxidant effect in freeze-dried model systems of methyl linoleate supported on cellulose and in the absence of water. Methionine, threonine, lysine and histidine have antioxidative effects in freeze-dried emulsions of safflower oil (3). Seher and Löschner (4) found a mixture of amino acids to have good antioxidant activity in lard. Recently, Chen and Nawar (5) studied the positive antioxidant effects of amino acids on milk fat oxidation. However, the antioxidant efficacy of amino acids as a function of relative humidity in lipid systems is less well known. Because free amino acids are present in foods, a study of their antioxidant effects is of practical interest. The antioxidant activities of aliphatic, aromatic, basic, dicarboxylic, hydroxylic and sulfur amino acids in methyl linoleate at 37°C and at 2, 50 and 79% relative humidities (RHs) are reported.

MATERIALS AND METHODS

Materials. Methyl linoleate was prepared by methanolysis of safflower oil and purified by silica-gel column chromatography, followed by high-vacuum distillation, as described earlier (6). Amino acids included L-methionine (L. Light & Co., Ltd., Colnbrook, Bucks, England), DL-tryptophan and L-arginine (E. Merck, Darmstadt, Germany), DL-aspartic acid, DL-α(-alanine and DL-serine (BDH Chemicals Ltd., Poole, England).

Autoxidation. Duplicate methyl linoleate (2.5 mL) samples with and without amino acids were placed in the main vessel of Warburg flasks. Finely powdered amino acids were dispersed in methyl linoleate at 500 ppm prior to transfer to the flasks. Appropriate RH was maintained in the Warburg flask by 0.5 mL of a saturated solution of magnesium nitrate for 50% RH, 0.5 mL of a saturated solution of ammonium sulfate for 79% RH and 0.75 g (equivalent to 0.5 mL of salt solution) of fused calcium chloride for 2% RH. The saturated salt solution or fused calcium chloride was placed in the side arm of the Warburg flask. The flasks were connected to manometers and flushed with air of the respective RH. The entire assembly was placed in a shaker water bath at 37 ± 0.1°C. After equilibrating 2 h, the system was sealed, and the samples were heated for up to 182 h.

Methods. The Warburg flasks for determining oxygen uptake were calibrated with the procedure of Umbreit *et al.* (7). The monomolecular rate constant was calculated from oxygen uptake data according to procedure of Labuza *et al.* (8) and analyzed statistically by one-way analysis of variance and by Duncan's multiple range test for segregating differences between mean values of rate constants (9). Antioxidant efficacy (AE) of the amino acids was calculated as the ratio of rates of oxygen uptake of the methyl linoleate control to that of amino acid-treated methyl linoleate samples.

RESULTS AND DISCUSSION

The oxygen uptake values of methyl linoleate oxidized at 37°C with 500 ppm of methionine, tryptophan, aspartic acid, serine, alanine or arginine are shown in Figure 1. At 2 and 79% RH, control methyl linoleate showed higher oxygen uptake than at 50% RH, thus exhibiting the usual high-low-high pattern of oxidation of lipids with increasing RH of the system (10). All samples treated with amino acids showed decreasing oxygen absorption with increasing RH, from 2 to 50%. Alanine and arginine showed the lowest oxygen absorption at both 50 and 79% RH.

The monomolecular rate constant (K_m) calculated from the oxygen uptake values are given in Table 1. The K_m values of methyl linoleate dropped significantly when treated with any of the amino acids at all three RHs 2, 50 and 79%). Alanine and arginine had zero K_m values at 50 and 79% RH.

The ratios of K_m of control methyl linoleate to that of linoleate treated with amino acids, expressed as AE for individual amino acids, are shown in Figure 2. The results showed that while AE of all amino acids was high as compared to the control, that of arginine was the highest at all RHs. Alanine had an AE close to that of arginine at RHs of 50 and 79%; however, at RH of 2% it was less effective. The amino acids showed the following order (=, equal to; >, higher than; >>, much higher than) of decreasing antioxidant efficacy at indicated RHs. At 2% RH, arginine > alanine = serine = methionine > tryptophan > aspartic acid; at 50% RH, arginine = alanine >> methionine = serine = aspartic acid > tryptophan; and at 79% RH, arginine = alanine >> methionine > serine > aspartic acid = tryptophan.

The reason for the variation in AE for different amino acids with RH is not clear; however, this study indicated that the functional groups in the side chain of individual

*To whom correspondence should be addressed.

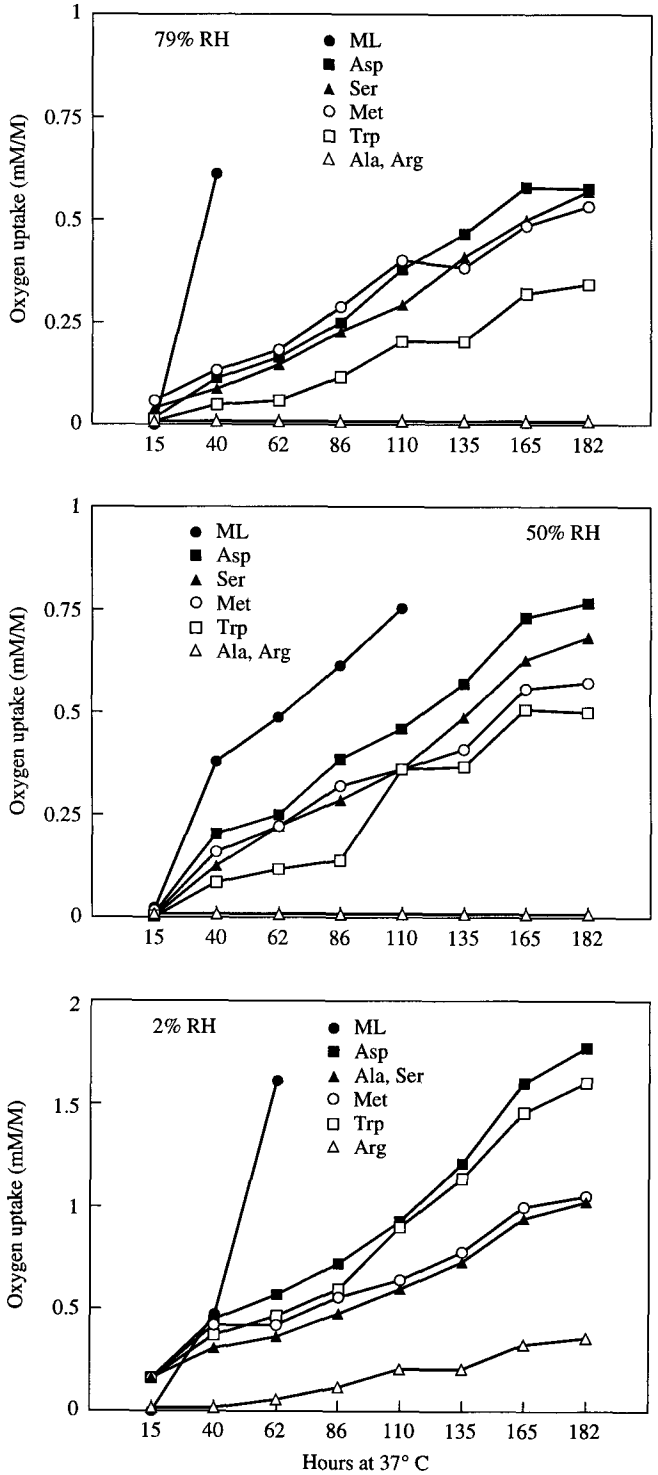

FIG. 1. Oxygen uptake of amino acids treated methyl linoleate at 37°C and at three levels of relative humidity (RH) (ML, methyl linoleate; Met, ML + methionine; Trp, ML + tryptophan; Asp, ML + aspartic acid; Ser, ML + serine; Ala, ML + alanine; Arg, ML + arginine).

amino acids may be important. Alanine has a methyl group in the side chain, arginine has a guanidino ethyl group attached to the methyl group of alanine and aspartic acid has a carboxyl group attached to the methyl group

TABLE 1

Monomolecular Rate Constants of Methyl Linoleate Autoxidized at Different Relative Humidities with and without Added Amino Acids at 37°C

Sample	$K_m \times 10^3$ (mM O_2/Mole)$^{1/2}$/h[a] at relative humidity (%) 2	50	79
1. Methyl linoleate (ML) (control)	20.5[g]	12.4[f]	17.6[g]
2. ML + methionine	3.3[d]	2.8[d]	2.4[d]
3. ML + tryptophan	4.8[e]	3.2[e]	3.3[f]
4. ML + aspartic acid	5.2[f]	2.9[d,e]	3.2[e,f]
5. ML + serine	3.1[d]	2.8[d]	2.9[d,e]
6. ML + alanine	3.1[d]	0.0[c]	0.0[c]
7. ML + arginine	2.1[c]	0.0[c]	0.0[c]
SE_m (df)	±0.10 (14)	±0.11 (14)	±0.09 (14)

[a]Rate constants with different superscript letters in a column are significantly different from each other ($P \leq 0.05$). SE_m = standard error; df = degrees of freedom.

of alanine (Table 2). Substitution of one hydrogen atom of the methyl group of alanine by a guanidino ethyl group enhanced the antioxidant efficacy, while a carboxyl group substitution lowered the AE (Table 2). Substitution altered the isoelectric pH of alanine (11) and also brought in the factor of pK value of the side chain. All the amino acids studied showed considerable AE at 2% RH in the absence of moisture. This indicates that the amino acids can exhibit considerable antioxidant activity in the undissociated form. Dissociation of the amino acid (or the side chain) therefore may not be a factor for antioxidant activity of amino acids. At 50 and 79% RH, some dissociation of the amino acids could exist, but such dissociation appears to be important only with arginine. Increase in isoelectric pH of the aliphatic amino acids (except the aromatic tryptophan) seems to correlate with the AE; however, with the limited data available, no definite conclusion can be made.

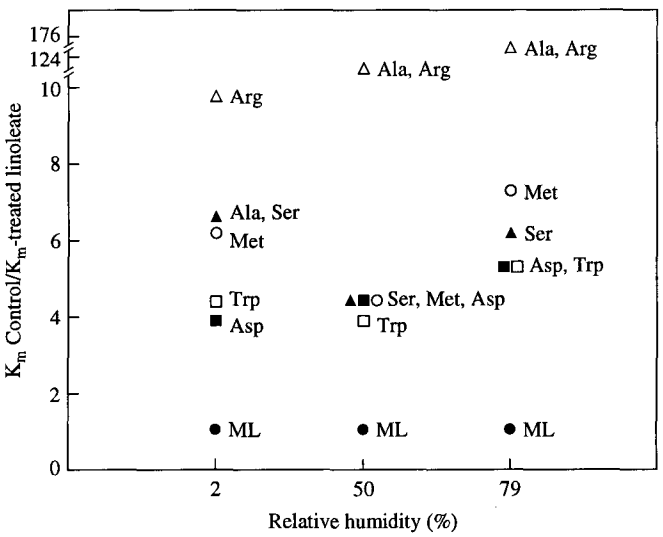

FIG. 2. Antioxidant efficacy of amino acids to oxidizing methyl linoleate at different RHs. Antioxidant efficacy equals the rate of autoxidation of control methyl linoleate/rate of autoxidation of amino acid-treated methyl linoleate. For samples Ala and Arg at 50 and 79% RH, the rate of autoxidation has been taken as 0.1 (K_m = 0.0) for calculation of their antioxidant efficacy. Abbreviations as in Figure 1.

TABLE 2

Comparison of Observed Antioxidant Efficacy of Amino Acids with Isoelectric pH and pK Values from the Literature

	$\begin{matrix}COOH\\CH_2\\CHNH_2\\COOH\end{matrix}$	$\begin{matrix}OH\\CH_2\\CHNH_2\\COOH\end{matrix}$	$\begin{matrix}CH_2SCH_3\\CH_2\\CHNH_2\\COOH\end{matrix}$	$\begin{matrix}Indolyl\\CH_2\\CHNH_2\\COOH\end{matrix}$	$\begin{matrix}H\\CH_2\\CHNH_2\\COOH\end{matrix}$	$\begin{matrix}Guanidinoethyl\\CH_2\\CHNH_2\\COOH\end{matrix}$	
	Aspartic	Serine	Methionine	Tryptophan	Alanine	Arginine	Reference
ipH[a]	2.77	5.68	5.74	5.89	6.01	10.76	11
pK[b]	3.65	—	—	—	—	12.84	11
AE[c]	3.9	6.6	6.2	4.3	6.6	9.8	—

[a]ipH, isoelectric pH.
[b]pK, pK of side chain.
[c]AE, antioxidant efficacy at 2% relative humidity.

At high RHs of 50 and 79%, AE of all amino acids increased in comparison to the control methyl linoleate, irrespective of the functional groups in their side chain. Water molecules have been implicated in the radical recombination reactions (12), and perhaps the reactions are more effective in the presence of amino acids.

These results indicate that free amino acids significantly protected methyl linoleate against oxidation. Shelf life of foods can be improved by regulation of the equilibrium RH to control lipid oxidation reactions (10). Therefore, improved antioxidant activity in foods may be possible under specific RH conditions and with amino acids naturally present in such foods.

ACKNOWLEDGMENTS

The authors thank S.R. Bhowmik, Director, Central Food Technological Research Institute, Mysore, and B.L. Amla, former Director of the Institute, for encouragement during this investigation; and S. Dhanaraj, Scientist of the Institute, for statistical analysis.

REFERENCES

1. Marcuse, R., *J. Am. Oil Chem. Soc. 39*:97 (1962).
2. Karel, M., S.R. Tannenbaum, D.H. Wallace and H. Maloney, *J. Food Sci. 31*:892 (1966).
3. Riisom, T., R.J. Sims and J.A. Fioriti, *J. Am. Oil Chem. Soc. 57*:354 (1980).
4. Seher, A., and D. Löschner, *Fette Seifen Anstrichm. 88*:1 (1986).
5. Chen, Z.Y., and W.W. Nawar, *J. Am. Oil Chem. Soc. 68*:47 (1991).
6. Gopala Krishna, A.G., and J.V. Prabhakar, *Ibid. 69*:178 (1992).
7. Umbreit, W.W., R.H. Burris and J.F. Staufer, (eds.) *Manometric Techniques*, Burgress Publishing Co., Minnesota, 1959.
8. Labuza, T.P., H. Tsuyuki and M. Karel, *J. Am. Oil Chem. Soc. 46*:409 (1969).
9. Amerine, M.A., R.M. Pangborn and E.B. Roessler, (eds.) *Principles of Sensory Evaluation of Food*, Academic Press, New York and London, 1965, Chapter 10, p. 437.
10. Labuza, T.P., *CRC Crit. Rev. Food Technol. 2*:355 (1971).
11. Barrett, G.C., (ed.) *Chemistry and Biochemistry of the Amino Acids*, London and New York, Chapman and Hall, 1985, p. 9.
12. Rockland, L.B., *Food Technol. 23*:1241 (1969).

[Received April 7, 1993; accepted March 29, 1994]