Prooxidative and Antioxidative Effects of Phospholipids on Milk Fat

Z.Y. Chen[1] and **W.W. Nawar***
Department of Food Science, University of Massachusetts, Amherst, MA 01003

The effects of dipalmitoylphosphatidylethanolamine (DPE) and dipalmitoylphosphatidylcholine (DPC) on milk fat oxidation was examined at 50°C and 95°C under various conditions by monitoring oxygen uptake and fatty acid composition. DPE strongly inhibited milk fat oxidation both at 50°C and 95°C in the absence of water. DPC was less effective than DPE. In aqueous systems, the reverse was observed. DPE accelerated milk fat oxidation at both 50°C and 95°C. DPC accelerated the oxidation at 50°C, but inhibited it at 95°C. The free amino group in DPE may be responsible for its inhibiting effect in the dry system. The accelerating activity of DPE in the aqueous system is probably due to the formation of a more dispersed structure with better oxygen accessibility.

KEY WORDS: Amino group, antioxidant, milk fat, oxidation, phosphatidylcholine, phosphatidylethanolamine, phospholipids, prooxidant.

Phospholipids have been studied as potential antioxidants because many crude oils containing them are more stable than the refined oils. Addition of milk phospholipids, in the amounts normally present in milk fat, improves the oxidative stability of isolated milk fat triacylglycerols (1). Phospholipids are thought to act synergistically with tocopherol and other antioxidants (2,3). Phosphatidylethanolamine (PE) and phosphatidylinositol (PI) appear to be more effective than phosphatidylcholine (PC) (4). Antioxidative activities of seed PE added to butterfat do not vary with the seed sources, indicating that its effect is independent of fatty acid moieties of the phospholipid molecules (5). However, the synergistic effects of phospholipids with tocopherol have been disputed, and their antioxidative effects are attributed to the browning products formed during the preheating of PE and PC (6).

On the other hand, phospholipids containing polyunsaturated fatty acids have been claimed to be susceptible to lipid oxidation and to cause the deterioration of meat products (7). PE is more easily oxidized than PC (8). Tsai and Smith (9) have studied the roles of base and phosphoryl base moieties of phospholipids in the autoxidation of a methyl linoleate emulsion, and found that they act as both antioxidants and prooxidants, depending on pH. We have observed the same antioxidative or prooxidative activities in milk fat globule membrane phospholipids (10). However, the mechanism by which phospholipids act as both antioxidants and prooxidants is not well understood. Our previous study has shown that the free amino group in amino acids is partly responsible for their antioxidative activity and that blocking of the amino group results in a partial or complete loss of its protective ability (11).

The objective of this study was to examine the effects of the saturated dipalmitoylphosphatidylethanolamine (DPE) and dipalmitoylphosphatidylcholine (DPC) on the oxidation of milk fat under varied conditions.

MATERIALS AND METHODS

Preparation of milk fat. The milk fat was prepared from fresh milk obtained from the University of Massachusetts farm. The milk was pasteurized for 30 min at 63°C and the cream was separated by centrifugation and churned. The churned cream was then melted at 45°C and centrifuged. The milk fat was pipetted and saved in a bottle that had been previously washed with deionized water and thoroughly dried.

Measurement of oxygen uptake. The reaction vessel described by Bunick (12) was used to monitor oxygen absorption. Two hundred milligrams of milk fat containing 5% of DPE or DPC (purity above 98%; Sigma Chemical Co., St. Louis, MO) were loaded into two identical reaction vessels. In some cases, 300 μL of distilled water was added (DPE and DPC function as emulsifiers). Oxidation was conducted at 50°C and 95°C with constant stirring. The headspace oxygen uptake was measured periodically as previously described (11).

Fatty acid analysis. Fatty acids were methylated and analyzed in an HP 5890A gas chromatograph (Hewlett Packard, Palo Alto, CA) on a 30 m × 0.32 mm I.D. Supelcowax 10 column (Supelco, Bellefonte, PA). The methylation procedure described by Glass was used (13). For quantitation, triheptadecanoin (Sigma Chemical Co.) was used as an internal standard.

Statistics. ANOVA followed by Student's *t*-test was used for statistical evaluation of difference between samples.

RESULTS AND DISCUSSION

The results of the headspace oxygen consumption test at 50°C are shown in Figure 1. The following features were observed: i) In the aqueous system, the sample to which DPE was added oxidized faster than the control milk fat, indicating that DPE possesses prooxidant activities; ii) in the dry system, the reverse was observed. DPE and DPC exhibited protective effects; and iii) DPE was more effective than DPC.

The fatty acid analysis showed the pattern illustrated in Figure 2. In the aqueous system the loss of unsaturated fatty acids was faster in the samples containing DPE or DPC than in the control. DPE and DPC exhibited protective effects in the dry system. However, the inhibiting effect of DPE was considerably stronger than that of DPC.

The effects of DPE and DPC on milk fat oxidation were also examined at 95°C (Figs. 3 and 4). As was the case at 50°C, both oxygen consumption and fatty acid analysis indicate that in the dry system DPE and DPC exhibited protection, with the former being much more effective. In the aqueous system DPE accelerated the oxidation

[1] Present Address: Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada.
*To whom correspondence should be addressed.

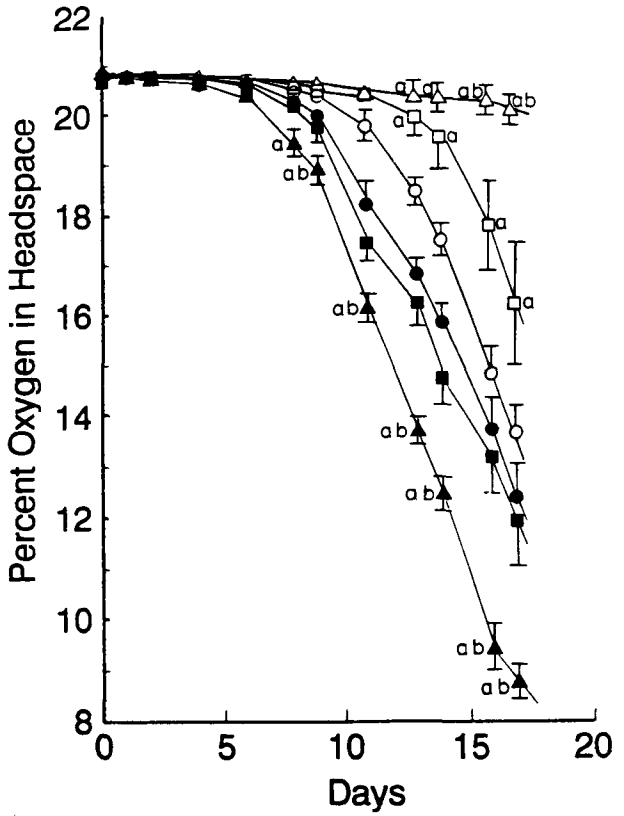

FIG. 1. Effects of phospholipids on oxidation of milk fat at 50°C. Data are expressed as mean ± SD for n=3/time point. ○, Milk fat; ●, milk fat (aqueous); △, milk fat + DPE; ▲, milk fat + DPE (aqueous); □, milk fat + DPC; ■, milk fat + DPC (aqueous). a, The sample with addition of DPE or DPC is significantly different from the corresponding control (milk fat only), if $p<0.05$. b, The sample with addition of DPE is significantly different from the corresponding sample with addition of DPC, if $p<0.05$.

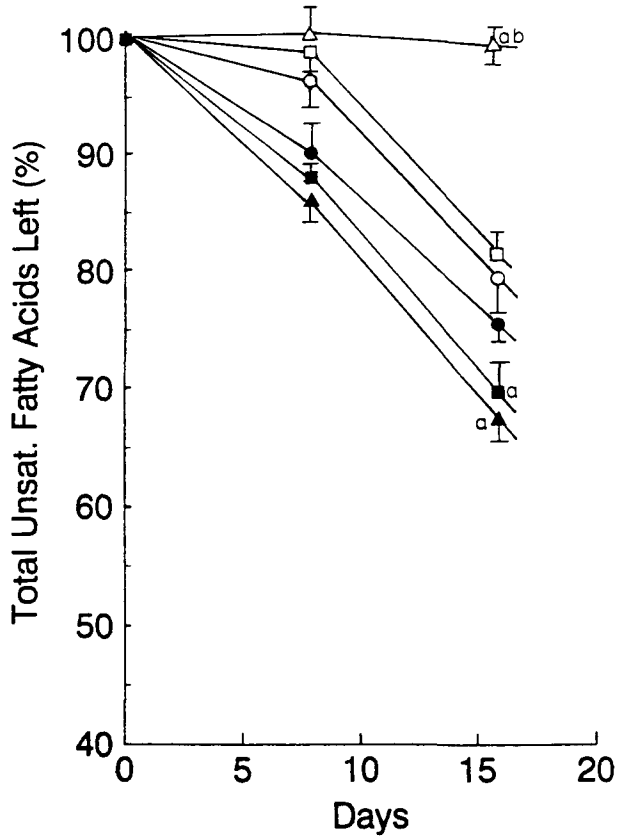

FIG. 2. Effects of phospholipids on change in total unsaturated fatty acids of milk fat at 50°C. Data are expressed as mean ± SD for n=3/time point. ○, Milk fat; ●, milk fat (aqueous); △, milk fat + DPE; ▲, milk fat + DPE (aqueous); □, milk fat + DPC; ■, milk fat + DPC (aqueous). a And b are as in Figure 1.

(Fig. 3), as was also the case at 50°C (Figs. 1 and 2). In contrast, however, DPC was slightly protective at the higher temperature.

In the dry state, the milk fat samples containing DPE remained orange-yellow throughout the period studied, while the control and the sample to which DPC was added were colorless. DPE protected not only the milk fat but also the pigments against oxidation.

The fact that DPE exhibits much stronger inhibition than DPC suggests that the free amino group in PE is involved in the antioxidant activity, as was shown previously with amino acids (11). Tsai and Smith (9) suggested that the protonated amino group (NH_3^+) accelerates lipid oxidation while the non-protonated group (HN_2) inhibits it. According to their hypothesis, the presence of a pair of free electrons on the nitrogen atom may be necessary for the antioxidant activity of the primary amine.

The exact role of the amino group in promoting or inhibiting oxidation is still unclear. The possibility that a higher level of protonation in the aqueous system is responsible for the observed accelerating effect must be studied further. It may also be possible that the free amino group reacts with carbonyl compounds derived from lipid oxidation to form Schiff-base reaction products, which may exhibit antioxidant effects (6). An alternative mechanism may be related to the ability of DPE to form a more dispersed emulsion, allowing better oxygen accessibility in the aqueous system (14).

Phospholipids can accelerate or inhibit oxidation of milk fat depending on the parameters of oxidation. The phospholipids used in this work contained only saturated fatty acid moieties; hence, the accelerating effect of phospholipids observed here cannot be attributed solely to unsaturation. We believe that this is the first evidence that saturated DPE can act as a prooxidant. The involvement of free amino groups in both antioxidation (*e.g.*, in dry systems) and prooxidation (*e.g.*, in aqueous systems) is evidenced by the fact that DPE exhibited stronger antioxidant, as well as stronger prooxidant activities, when compared with DPC (Figs. 1 and 3).

ACKNOWLEDGMENT

This research was supported in part by University of Massachusetts Experiment Station Hatch Project No. 654, and a grant from the Dairy Bureau of Canada.

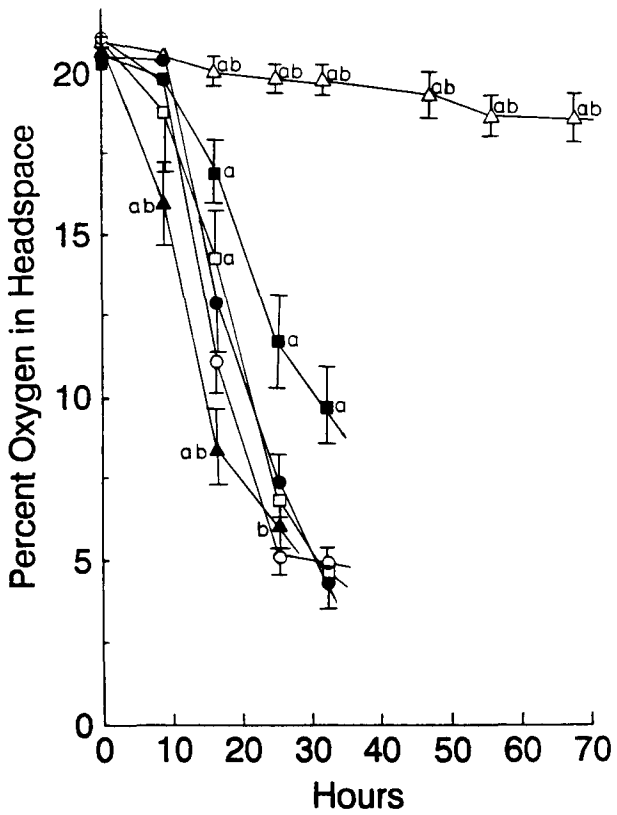

FIG. 3. Effects of phospholipids on oxidation of milk fat at 95°C. Data are expressed as mean ± SD for n=3/time point. ○, Milk fat; ●, milk fat (aqueous); △, milk fat + DPE; ▲, milk fat + DPE (aqueous); □, milk fat + DPC; ■, milk fat + DPC (aqueous); a and b are as in Figure 1.

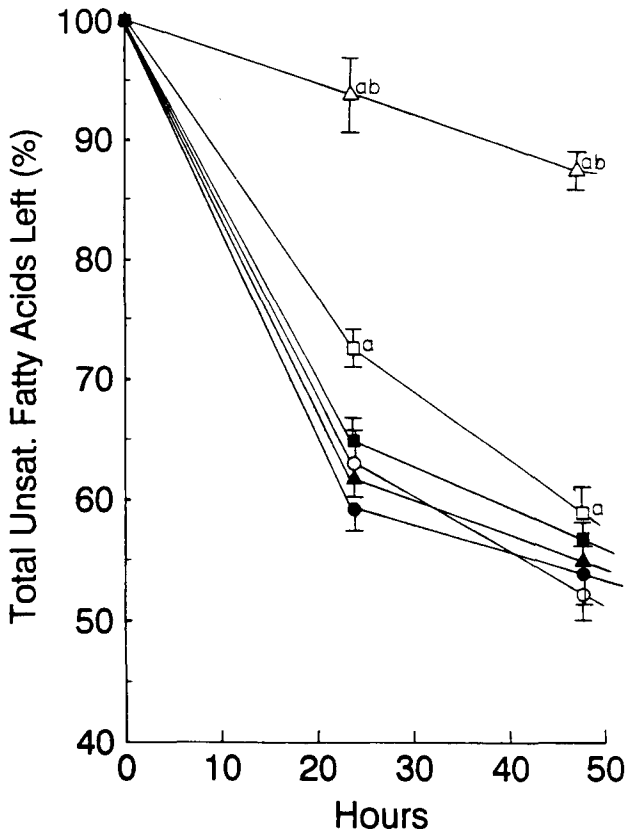

FIG. 4. Effects of phospholipids on change in total unsaturated fatty acids of milk fat at 95°C. Data are expressed as mean ± SD for n=3/time point. ○, Milk fat; ●, milk fat (aqueous); △, milk fat + DPE; ▲, milk fat + DPE (aqueous); □, milk fat + DPC; ■, milk fat + DPC (aqueous), a and b are as in Figure 1.

REFERENCES

1. Nath, B.S., and M.K.R. Murthy, *Indian J. Dairy Sci. 36*:151 (1983).
2. Olcott, H.S., and J. Van Der Veen, *Ibid. 28*:313 (1963).
3. Bertram, J.F., and E.O.M. Salah, *J. Sci. Food Agric. 32*:208 (1981).
4. Hildebrand, D.H., J. Terao and M. Koto, *J. Am. Oil Chem. Soc. 61*:552 (1984).
5. Batia, I.S., N. Kaur and P.S. Sukhija, *J. Sci. Food Agric. 29*:747 (1978).
6. Husain, S.R., J. Terao and S. Matsushita, *J. Am. Oil Chem. Soc. 63*:1457 (1986).
7. Keller, J.D., and J.E. Kinsella, *J. Food Sci. 38*:1200 (1973).
8. Corlis, G.A., and L.R. Dugan, *Lipids 5*:846 (1970).
9. Tsai, L-S., and L.M. Smith. *Ibid 6*:196 (1971).
10. Chen, Z.Y., and W.W. Nawar, *J. Food Sci. 56*:398 (1991).
11. Chen, Z.Y., and W.W. Nawar, *J. Am. Oil Chem. Soc. 68*:47 (1991).
12. Bunick, F.J., Ph.D Dissertation, University of Massachusetts at Amherst, 1984.
13. Glass, R.L., *Lipids 6*:919 (1971).
14. Bidlack, W.R., and A.L. Tappel, *Ibid. 8*:203 (1973).

[Received May 21, 1991; accepted October 22, 1991]