Linalyl Acetate and Methyl Silicone Effects on Cholesterol and Triglyceride Oxidation in Heated Lard[1]

Pearlly S. Yan[2] and Pamela J. White*
Iowa State University, Food Science and Human Nutrition Department, Iowa State University, Ames, Iowa 50011

The effectiveness of linalyl acetate (LA) at 0.02 and 0.04% as a high-temperature antioxidant was tested in lard with two levels (2 times and 10 times) of added cholesterol. Lard samples with and without added LA or methyl silicone (MS, 0.2 and 1.0 ppm) and a control with no additives were heated at 180°C for 10 hr/day for up to 240 hr. The loss of cholesterol, the formation of cholesterol oxidation products (COP) and the percentage fatty acid retention were measured during the heating periods. Addition of 1.0 ppm MS was significantly effective in reducing the loss of fatty acids ($p<0.0005$) in lard with 2 times the added cholesterol, but MS at 0.2 ppm was not effective in lard with 10 times the added cholesterol. Addition of LA at 0.02 and 0.04% had little effect at reducing changes in cholesterol loss, COP formation and fatty acid loss in lard samples with both cholesterol levels. But there was a tendency for MS and 0.04% LA to reduce formation of some COP in lard containing 2 times the added cholesterol and to reduce the loss of cholesterol.

KEY WORDS: Antioxidants, cholesterol oxidation, linalyl acetate, methyl silicone, polydimethyl siloxane.

Use of antioxidants in the food industry and their functional mechanisms have been reviewed extensively (1-7). Because of consumer demands, the food industry is using less saturated animal fats and more polyunsaturated vegetable oils. These changes prompted the introduction and the approval of monotertiary butyl hydroquinone (TBHQ) for food uses in 1972. A recent survey (8) reported that the fast-food industry has taken steps to switch from animal to vegetable fats in its frying operations. But some fast-food restaurants continue to fry french fries in shortenings containing some tallow to produce a beefy flavor. Thus, reduction in thermally induced changes in fats and oils from both vegetable and animal sources is desirable.

Most phenolic antioxidants undergo steam distillation or destruction at deep-frying conditions (9). To date, methyl silicone (MS) remains the only compound used by the industry to protect fats and oils during frying. Because it is synthetic, many processors avoid adding MS to bottled salad oil, so that they may label the oil "all natural."

Antioxidants isolated from plant sources may provide alternatives to the current choices of effective, high-temperature antioxidants. A number of plant sterols, including Δ^5- and Δ^7-avenasterol, vernosterol and citrostadienol, have been found to reduce the chemical changes that occur in vegetable oils during frying (10-13). Gordon and Magos (12) theorized that the ethylidene side chain present on these sterols (shown in Fig. 1) reacts rapidly with lipid free radicals to form stable allylic tertiary free radicals that interrupt the oxidation chain. The ethylidene side chain forms free radicals rapidly because of the presence of unhindered hydrogen atoms on an allylic carbon atom. This theory was further tested by examining the antioxidative effect of compounds containing an ethylidene-like group, but without the sterol moiety (14). Compounds fitting these requirements include a group of monoterpene alcohols. The high-temperature antioxidant effects of linalyl acetate (LA) and undecylenic acid (not a terpene) on soybean oil heated at 180°C were reported in previous studies (14,15). (See Figure 1 for their structures). LA at 0.04% was as effective as MS at 0.3 ppm, but LA at 0.02% was only slightly effective.

Attempts have been made to arrest cholesterol oxidation by incorporating known antioxidants. Park and Addis (16) studied the effects of adding 500 ppm ascorbyl palmitate and 100 ppm dL-α-tocopherol to tallow heated at 135°C. They reported that no cholesterol oxidation products (COP) were detected for up to 70 hr of heating in tallow protected with the antioxidants. But, after 70 hr, the inhibitory effect of the antioxidants was no longer observed. Morgan and Armstrong (17) added butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallates (PG) either alone or in combination

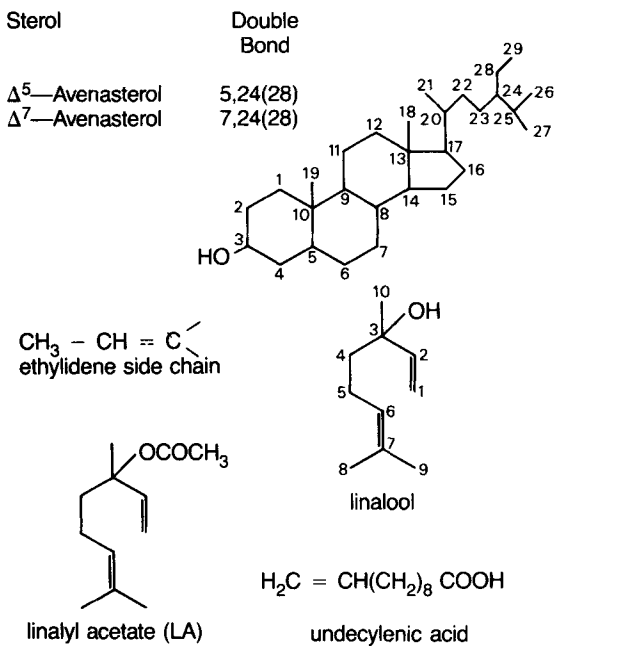

FIG. 1. Chemical structures of compounds containing an ethylidene group.

[1] Presented in part at the 80th AOCS Annual Meeting, Cincinnati, OH, in May, 1989.

[2] Present address: University of Missouri-Columbia, Department of Human Nutrition and Foods, 217 Gwynn Hall, Columbia, MO 65211.

*To whom correspondence should be addressed at Iowa State University, 2312 Dairy Industry Bldg., Ames, Iowa 50011.

to liquid egg products before spray-drying. The antioxidants did not significantly affect the levels of COP found in the final products. Tsai and Hudson (18) evaluated scrambled egg mixes containing a substantial amount of soybean oil or corn oil that was protected with BHA, BHT, PG and citric acid (CA). The egg mixes that had been stored for 5 years had substantially more epoxides than those stored for 3 years.

Nourooz-Zadeh and Appelqvist (19) studied Swedish dehydrated egg products. One dehydrated egg mix and two types of egg yolks contained traces of COP in freshly dried products and those stored for only 2 months. Another egg mix (Petit-Choux mix) was free from oxidation products at 2 and 6 months of storage, presumably because of the antioxidants in the mix. But when Maerker and Unruh (20) carried out hot alkaline saponification of cholesterol in the presence of 0.3% BHT, there was no substantial reduction of COP formation.

In the current study, the antioxidant effect of linalyl acetate (LA) at 0.02 and 0.04% and of methyl siloxane (MS) at 1.0 or 0.2 ppm was tested in lard heated to 180°C with two levels (2 times and 10 times) of added cholesterol. The level of MS added in test 1 (0.02 ppm) was based on the suggested effective range of Freeman *et al.* (21). They tested MS in sunflower seed oil heated at 180°C. They found that MS added at 0.02 to 1.00 ppm significantly protected the oil in comparison with the control, and the protective effects were similar throughout the range tested. They concluded that MS gave its full protective effects at 0.02 ppm. For test 2, a higher level of MS (1.0 ppm) was tested because levels higher than 0.02 ppm were used by other researchers. Sims *et al.* (10) tested MS at 0.5 ppm in heated safflower oil. Ishikawa *et al.* (22) reported that processors regularly add up to 2.5 ppm MS to corn oil. Drew (23) patented the use of 1–25 ppm MS in hydrogenated vegetable oils to increase the smoke point. To further examine the protective effects of LA and MS, the loss of fatty acids in lard with two times the original amount of added cholesterol was followed.

EXPERIMENTAL PROCEDURES

Lard. Distilled lard was purchased from the Meat Laboratory (Iowa State University, Ames, IA), and a distilled and deodorized lard was obtained from Hormel (Austin, MN) for use as the heating medium. The original cholesterol content of each lard type was determined by using the colorimetric method of Searcy and Bergquist (24) as modified by Reitmeier and Prusa (25). The lard from the Meat Laboratory, which was used in test 1, was found to have 95 mg cholesterol/100 g lard, whereas that from Hormel (used in test 2) had 70 mg/100 g lard.

Antioxidants. Linalyl acetate (LA) was purchased from Aldrich Chemical Company (Milwaukee, WI). Nonfood grade polydimethyl siloxane or methyl silicone (MS, 0.93 g/mL; viscosity, 5 centistokes at 25°C; mol. wt. approx. 15,000; stock no. DMPS-V) was purchased from Sigma Chemical Company (St. Louis, MO).

Chromatographic standards. Cholesterol (99%+) was purchased from Sigma Chemical Company. Duplicate gas-liquid chromatograph (GLC) determinations verified its purity. The cholesterol was used as a standard in GLC analyses and also was added to the lard to provide additional substrate so that an increased amount of COP could accumulate in the heated lard system. The resultant COP peaks produced better integration, smaller relative error and, hence, greater accuracy in detecting and quantitating these minor compounds. The following cholesterol oxide standards were purchased from Sigma Chemical Company: 5α, 6α-epoxy-5α-cholestan-3β-ol (α-epoxide); 3β-hydroxycholest-5-en-7-one (7-keto); and 5α-cholestane-3β, 5, 6β-triol (triol). These additional standards were purchased from Steraloids, Inc. (Wilton, NH): cholest-5-ene-3β, 7α-diol (7α-OH); cholest-5-ene-3β, 7β-diol (7β-OH); and cholest-5-ene-3β, 25-diol (25-OH). To quantitate and account for losses due to sample preparation and chromatographic analysis, 5α-cholestane (Sigma Chemical Company) was added ahead of saponification as an internal standard.

Heating studies: Test 1. Lard samples (600 g) spiked with 10 times (10X; *i.e.*, 950 mg cholesterol/100 g lard) the amount of cholesterol originally present in the lard were heated in Fry Baby containers at 180 ± 5°C for 10 hr/day for 24 days. The temperature of each fryer was controlled by a rheostat. Four treatments were tested, including a control (lard with no additives), lard with 0.2 ppm MS and lard with 0.02 and 0.04% LA. The additive was dissolved in hexane and added to the lard. An equal amount of hexane was added to the control. Sample aliquots were taken at time zero and at the end of each day. They were stored under N_2 in teflon-capped tubes at −10°C until analyzed.

Test 2. Deodorized lard samples (500 g) spiked with two times (2X; *i.e.*, 140 mg/100 g lard) the amount of cholesterol originally present in the lard were heated at 180 ± 5°C for 10 hr/day for 16 days. The levels of compounds tested were MS at 1.0 ppm, LA at 0.02 and 0.04%, and a control sample (lard with no additives). The regulation of fryer temperature, the addition of antioxidants to the oils and oil sampling were the same as described in test 1.

Cholesterol oxidation analysis. The method of Park and Addis (16) for analysis of cholesterol oxidation products was followed with some modifications, as previously reported (26), and briefly described as follows. The amount of internal standard used was reduced to 30 μg to match the level of COP found in the heated lard. More washing steps were necessary to remove residual methanol, which would inactivate the trimethylsilylating (TMS) reagent to be added in the following step. Instead of specifying the total number of washing steps, checking for the neutrality of the washing water indicated the completion of this process. The amount of TMS reagent (Sylon BTZ, Supelco, Bellefonte, PA) was increased to 150 μL to properly derivatize the sample and to minimize baseline shifts. A DB-1 capillary column with dimensions of 0.25 mm × 15 m × 0.1 micron film thickness (J&W Scientific, Inc., Rancho Cordova, CA) was used in a Varian Aerograph series 3300 gas chromatograph (GLC) (Varian Associates, Palo Alto, CA) equipped with a flame ionization detector and a split/splitless injector. Chromatographic parameters were as follows: injector temperature, 270°C; detector temperature, 300°C; column temperature programming, 180 to 250°C at 3°C/min with 10 min holding time at the end; carrier gas, He at 1.1 mL/min; and split ratio, 100 to 1. All test results are the average of duplicate samples.

Triglyceride oxidation analysis. A Varian Aerograph series 3700 GLC equipped with a flame ionization detector was used. The method of Metcalfe *et al.* (27) was followed for the preparation of fatty acid methyl esters

(FAME). The GLC contained a stainless-steel packed column (100/120 Gas Chrom Q II with 10% Silar 10C coating; Alltech Associates, Deerfield, IL) of 6.0 ft. × 1/8 in. O.D. Peak areas were measured with the internal standard procedure of an HP3390A reporting integrator. Triheptadecanoin was added to all the samples as an internal standard. This method of measurement was suggested by Waltking and Zmachinski (28) as the preferred method in determining total polyunsaturated fatty acids. The fatty acid data reported in this study list the percentage retention for each fatty acid during the heating time, based on the amount at time zero. All test results are the average of duplicate analyses and duplicate injections.

Statistical analysis. The rate constants of cholesterol disappearance and COP formation were calculated from the first-order rate plots by using the linear regresion model. Data were tested by the analysis of variance (ANOVA) by means of the general linear models procedure of the Statistical Analysis System (SAS) computer program (29). The treatment effects on cholesterol and triglyceride disappearances, as well as on COP formation in tests 1 and 2 (cholesterol added at 10X and 2X, respectively), were compared by the Student's *t*-test.

RESULTS AND DISCUSSION

Loss of cholesterol during heating of lard. In both heating tests, the cholesterol content decreased steadily with heating time. In test 1, in which the lard contained 10X the original amount of cholesterol, no significant protective effect of LA at 0.02 and 0.04% or MS at 0.2 ppm was seen when compared with the control (Fig. 2A). All lard samples lost about 1500 ppm over the 240 hr of heating. In test 2, in which the lard contained 2X the original amount of cholesterol, the cholesterol content in all treatments dropped from an average of 1900 ppm to a final level of about 494 ppm during 160 hr of heating (Fig. 2B). In this test, LA was tested at 0.02 and 0.04%, while MS was added at 1.0 ppm. Throughout the heating, the lard sample containing MS had a slightly smaller drop (by about 10%) in cholesterol than did all other samples. But this drop was not significant when compared with the control.

When first-order rate constants were calculated from the cholesterol data from test 2, coefficients of variance (R^2) ranged from 0.96 to 0.98. Lard with no additives and lard with 0.02% LA had first-order rate constants of -2.6×10^{-6} sec^{-1}. Lard protected with 0.04% LA and 1.0 ppm MS had rates of -2.4 and -2.1×10^{-6} sec^{-1}, respectively. These values show that MS at 1.0 ppm (and maybe LA at 0.04%) helped to retain slightly more cholesterol than the control and than 0.02% LA in the heated lard. In test 1, the correlation coefficients were not as high as in test 1 (0.75–0.93), possibly because of the small amount of internal standard (IS) present in the samples. The quantity of IS used was more suitable for analyzing the level of cholesterol in test 2 than in test 1. The first-order rate constants in this test were -3.0×10^{-7} sec^{-1} for the control and for lard protected with 0.02 and 0.04% LA, and -4.0×10^{-7} sec^{-1} for lard with MS. When the first-order slopes from the two tests were compared (*i.e.*, treatments at 2X *vs.* treatments at 10X added cholesterol) the tests were significantly different from one another at $p < 0.05$, indicating that the differences in the amounts of

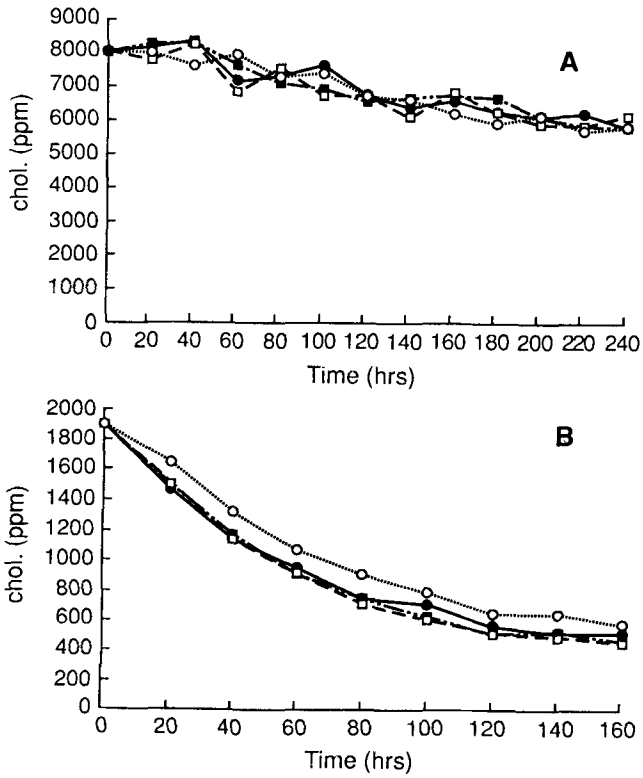

FIG. 2. The amount of cholesterol loss in lard with 10X added cholesterol (A) and 2X added cholesterol (B) protected with: (■) LA at 0.02%, (●) LA at 0.04%, (○) MS at 0.2 ppm in A and 1.0 ppm in B, (□) Control.

cholesterol lost (1900 ppm and 1500 ppm) were real. A detailed discussion of the destruction of cholesterol and of the accumulation of COP in a heated lard system appears in a related paper (26).

Formation of COP during heating of lard. Figures 3A and 3B illustrate the levels of 7α-OH accumulation in tests 1 and 2. In test 1, the large excess of cholesterol (10X the original amount) covered up the 7α-OH peak that elutes very close to it. As a result, the 7α-OH peak was not resolved from the cholesterol peak until 100 hr of heating, when the total amount of cholesterol had dropped. There was no evident effect on 7α-OH formation by any of the additives. About five times more 7α-OH was observed in test 1 (Fig. 3A) when compared with test 2 (Fig. 3B). This is the only COP that reflected the five-fold difference in added cholesterol between tests 1 and 2. First-order rate calculations could not be made on 7α-OH because its formation was initially masked by the cholesterol peak.

The levels of α-epoxide accumulation are shown in Figures 4A and 4B. The first-order rate constants for test 1 ranged from 1.6 to 2.5×10^{-6} sec^{-1}, whereas those for test 2 ranged from 1.6 to 2.2×10^{-6} sec^{-1}. None of the additives showed a significant antioxidant effect on α-epoxide formation when compared with the control; however, there was a tendency in test 2 for the lard samples protected with 0.04% LA and MS to form less α-epoxide than the control.

The formation of 7β-OH is shown in Figures 5A and 5B. The 7β-OH was more prevalent than that of 7α-OH

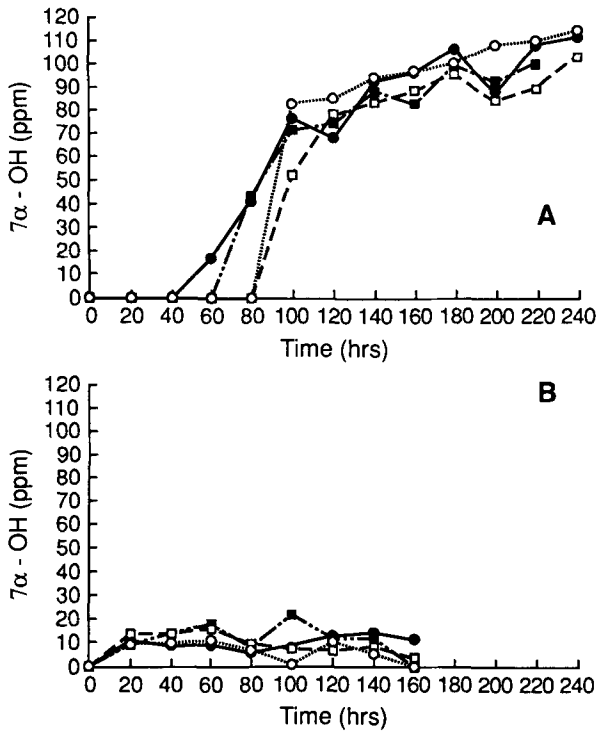

FIG. 3. The formation of cholest-5-ene-3β, 7α-diol (7α-OH) in lard with (A) 10X added cholesterol and (B) 2X added cholesterol protected with: (■) LA at 0.02%, (●) LA at 0.04%, (○) MS at 0.2 ppm in A and 1.0 ppm in B, (□) Control.

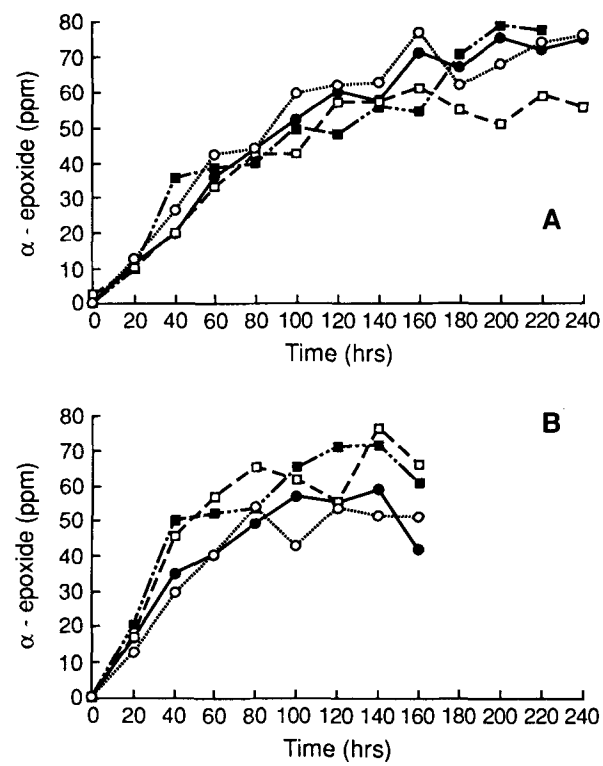

FIG. 4. The formation of 5α, 6α-epoxy-5α-cholestan-3β-ol (α-epoxide) in lard with (A) 10X added cholesterol and (B) 2X added cholesterol protected with: (■) LA at 0.02%, (●) LA at 0.04%, (○) MS at 0.2 ppm in A and 1.0 ppm in B, (□) Control.

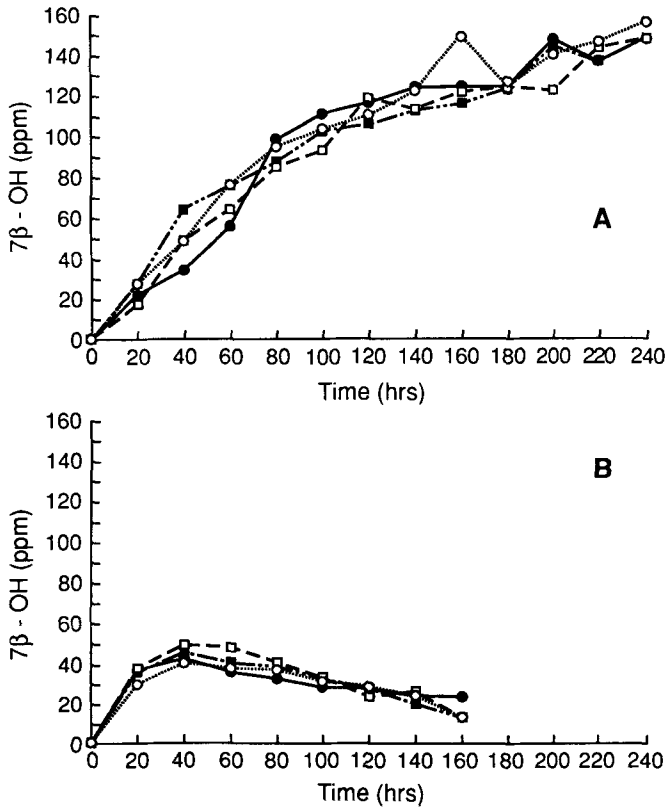

FIG. 5. The formation of cholest-5-ene-3β, 7β-diol (7β-OH) in lard with (A) 10X added cholesterol and (B) 2X added cholesterol protected with: (■) LA at 0.02%, (●) LA at 0.04%, (○) MS at 0.2 ppm in A and 1.0 ppm in B, (□) Control.

(Figs. 3A and 3B). The preferential formation of 7β-OH over 7α-OH was observed by other researchers (19,20) and has been attributed to the presence of less steric hindrance in the formation of the β-epimer. The plots in Figure 5B showed a gradual decline of 7β-OH for all treatments after a peak was reached; no such trend was observed in Figure 5A. Because of this decline in test 2, the first-order rate constants were negative. This suggests that degradation of 7β-OH occurred faster than its accumulation, possibly because of the low levels formed in this test. The first-order rate constants for test 1 ranged from 1.6 to 2.0×10^{-6} sec^{-1}, with no significant differences between treatments. For test 2, the first-order rate constants ranged from -1.0 to -2.0×10^{-6} sec^{-1}. The lard with 0.04% LA had significantly less drop in 7β-OH (slope = -1.0×10^{-6} sec^{-1}) than the control and lard with 0.02% LA (both slopes = -2.0×10^{-6} sec^{-1}). Because the slopes were negative, it is difficult to explain why there was a treatment effect.

Figure 6 represents the level of triol measured during test 2. Again, no significant evidence of antioxidant protection by MS and LA was seen, but the MS-treated sample tended to have less triol. The amounts of triol produced were so low that it was difficult to obtain accurate numbers. Formation of triol in test 1 was so erratic that we chose not to include those data and, in test 2, triol was not found before 60 hr of heating. For these reasons, first-order rate constants are not reported. Triols form from the hydrolysis of the epoxy ring of the epimeric epoxides

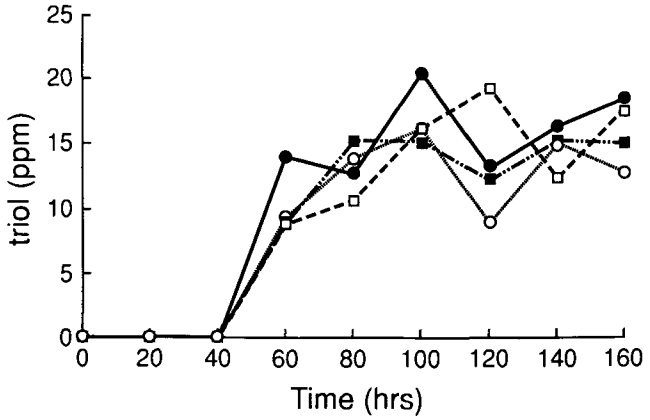

FIG. 6. The formation of 5α-cholestane-3β,5,6β-triol (triol) in lard with 2X added cholesterol protected with: (■) LA at 0.02%, (●) LA at 0.04%, (○) MS at 1.0 ppm, (□) Control.

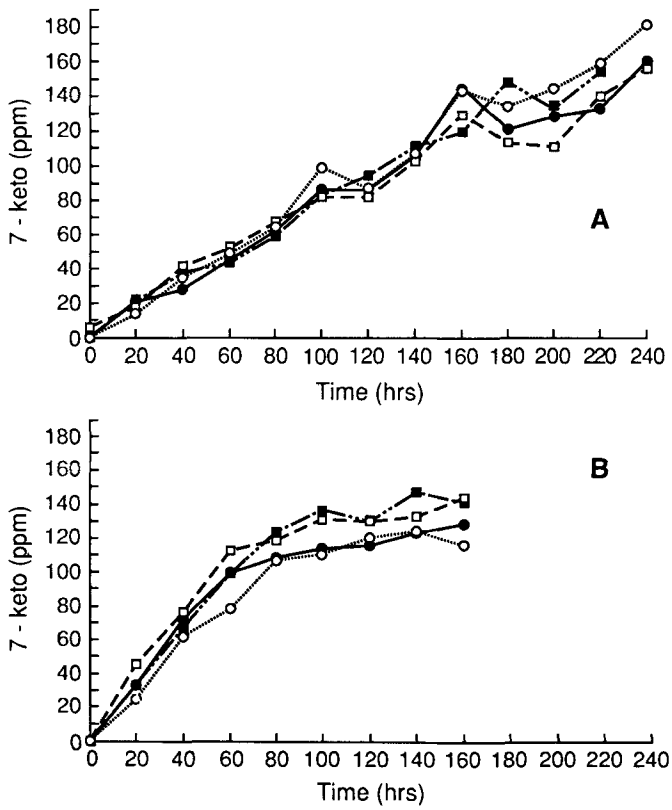

FIG. 7. The formation of 3β-hydroxycholest-5-en-7-one (7-keto) in lard with (A) 10X added cholesterol and (B) 2X added cholesterol protected with: (■) LA at 0.02%, (●) LA at 0.04%, (○) MS at 0.2 ppm in A and 1.0 ppm in B, (□) Control.

(30,31), and it is sometimes included in the total amount of epoxide formed (32). Also, the triol may not be formed until enough free fatty acids accumulate in the lard to cause hydrolysis of the epoxy ring.

Figures 7A and 7B present the levels of 7-keto formed in the heated lards. Nearly straight-line relationships between 7-keto accumulation and the heating time were seen in test 1, but not in test 2. Park and Addis (33,34) reported that the formation of 7-keto was proportional to heating time and suggested that its formation may follow zero-order kinetics. However, they did not report reaction rates. In our study, the first-order reaction rates for test 1 ranged from 2.3 to 2.9 $\times 10^{-6}$ sec^{-1}, with R^2 from 0.74 to 0.89 and, for test 2, from 1.8 to 2.5 $\times 10^{-6}$ sec^{-1}, with R^2 from 0.67 to 0.72. None of the differences between treatments was significant at $p < 0.05$, but there was a slight tendency for 0.04% LA and MS to lower the amount of 7-keto formed in test 2. Zero-order reaction rates ranged from 1.6 to 2.1 $\times 10^{-4}$ sec^{-1}, with R^2 from 0.94 to 0.97 in test 1, and from 2.1 to 2.5 $\times 10^{-4}$ sec^{-1}, with R^2 from 0.82 to 0.86 in test 2. The seemingly linear relationship between 7-keto formation and heating time in test 1 suggests that 7-keto was still accumulating and had not plateaued, as observed in test 2. The R^2s for zero-order rates were higher than those for first-order for 7-keto formation; however, because the formation of COP involves cholesterol in the lard and excess amounts of oxygen from the air, it is likely that pseudo-first-order reaction kinetics was followed for 7-keto rather than second or zero order. Also, the formation of the other COP in this study more closely followed first-order, rather than zero-order, kinetics.

Triglyceride oxidation during heating of lard. In previous studies, LA and MS protected the fatty acids in triglycerides of soybean oil (14,15). In this study, fatty acid analyses were run on the lard samples from test 2 in which MS, and perhaps 0.04% LA, slowed the disappearance of cholesterol and, possibly, the formation of some COP during heating. The amounts of palmitic and stearic acid were not affected by treatment; therefore, the data are not shown. The percentages of retention of oleate (18:1) and linoleate (18:2) from all treatments in test 2 are plotted in Figures 8A and 8B, respectively. The 18:1 disappearance rate did not show a treatment effect and all samples followed a first-order reaction rate with an R^2 of 0.95 and above, ranging from -1.1 to -1.3×10^{-6} sec^{-1}. Lard with added MS showed a significantly greater ($p=0.0005$) retention of 18:2 compared with the other treatments. The disappearance of 18:2 in the sample containing MS followed a first-order reaction rate ($R^2=0.99$, $k=-4.9 \times 10^{-6}$ sec^{-1}). The disappearance of 18:2 in the control and the LA-treated samples also followed a first-order reaction rate with an R^2 of 0.99 and k values ranging from -6.0 to -6.1×10^{-6} sec^{-1}.

Data from the retention of fatty acids and, to a smaller extent, the disappearance of cholesterol, showed that there was some antioxidant effect of MS on lipid oxidation in heated lard with 2X added cholesterol. Little or no antioxidant effect was seen with the addition of LA. In this test, the MS and 0.04% LA had a tendency to reduce COP formation (α-epoxide and 7-keto), but it was difficult to detect COP changes because of high variability and low accumulation of all COP. In test 1, the large excess of cholesterol (10X the original amount) increased the pool of oxidizable substrates in the heated lard. Since cholesterol oxidation is a free radical process, the added cholesterol may have overwhelmed the ability of LA to react rapidly with the large amount of lipid free radicals. This may have shortened the initiation step, whereby no treatment effects were observed.

In 1989, Willemse (35) received a patent on the use of silicone (at 0.02 to 50 ppm), linalool and linalyl acetate or their mixtures. The mixture was effective at lowering

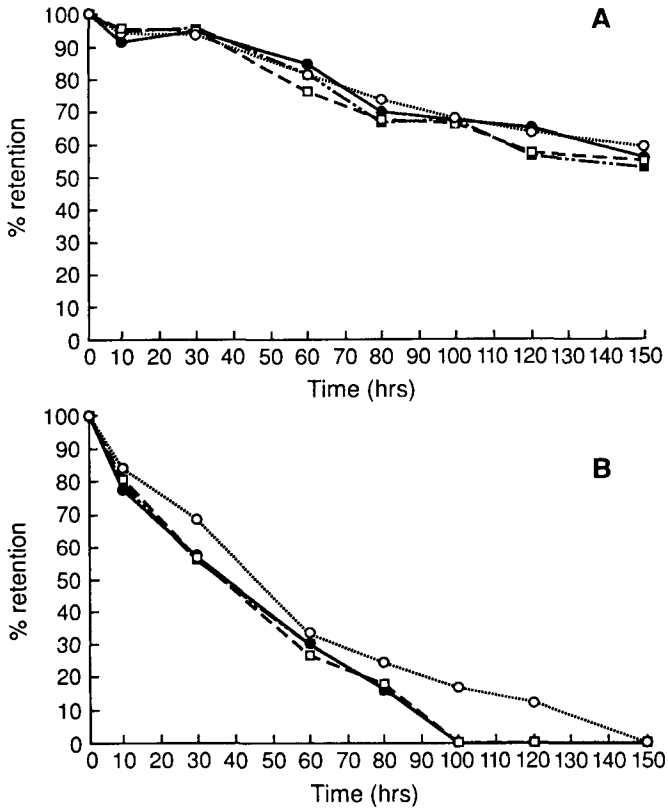

FIG. 8. Percentage retention of 18:1 (A) and 18:2 (B) in lard with 2X added cholesterol protected with: (■) LA at 0.02%, (●) LA at 0.04%, (○) MS at 1.0 ppm, (□) Control.

the development of unpleasant flavors in the presence of a small amount of MS when fats and oils were subjected to prolonged heating above 120°C. Essential oils with weight ratios of linalool:LA between 1:2 and 1:10 at a total of 4–40 ppm were effective antioxidants.

The high-temperature antioxidant effect of the essential oils described in the patent of Willemse was likely because of the synergism between LA or linalool and MS. Synergism between primary antioxidants and MS also was observed by Frankel *et al.* (36). In their study, after 19 hr of heating and intermittent frying of bread cubes at 190°C, hydrogenated SBO with the addition of TBHQ, CA and MS had the lowest (best) room odor score among all other treatments. The same compounds were less effective antioxidants when used singly. In our study, the lard samples were free of additives such as CA; however, their presence may have enhanced the effects of LA and MS.

Among compounds containing an ethylidene group, Gordon and Magos (12) observed that the antioxidative activity of vernosterol was higher than that of Δ^7-avenasterol and fucosterol, possibly because of the presence (on the former) of one or more endocyclic double bonds (in addition to the ethylidene group). The endocyclic bonds create other sites for free radical formation and delocalization. Recently, Burton and Ingold (4) demonstrated the effectiveness of β-carotene as an antioxidant at low oxygen pressures (*e.g.*, 15 torr). They theorized that the antioxidant effect of β-carotene was based on the stability of a carbon-centered radical. This stable radical subdues further oxidative chain reactions by trapping the peroxy radicals on the conjugated double-bond system of β-carotene and other effective carotenoids (37). There are similarities between the theories for antioxidant effectiveness of β-carotene and of compounds containing an ethylidene group, although the compounds work at different temperatures.

Although LA was not particularly useful at reducing oxidation of cholesterol or even of triglycerides in heated lard under the present conditions, it has been shown to be effective in other systems (14,15,35). It may be helpful to study other structure-reactivity relationships, such as altered polarity of LA effectiveness in different food matrices or even in the autoxidation of membranes, as suggested by Simic and Hunter (5) and Porter (3).

ACKNOWLEDGMENT

This work was supported by a research grant from the Iowa Pork Producers Association. This is Journal Paper No. J-14177 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA (Project No. 2568).

REFERENCES

1. Sherwin, E.R., *J. Am. Oil Chem. Soc. 53*:430 (1976).
2. Sherwin, E.R., *Ibid. 55*:809 (1978).
3. Porter, W.L., in *Autoxidation in Food and Biological Systems*, edited by M.G. Simic and M. Karel, Plenum Press, New York, 1980, pp. 295–389.
4. Burton, G.W., and K.V. Ingold, *Science 224*:569 (1984).
5. Simic, M.G., and E.P.L. Hunter, in *Chemical Changes in Food During Processing*, edited by D.E. Schwass, AVI Publishing Company, CT, 1985, pp. 107–119.
6. Houlihan, C.H., and C.T. Ho, in *Flavor Chemistry in Fats and Oils*, edited by D.B. Min and T.H. Smouse, American Oil Chemists' Society, Champaign, IL, 1985, pp. 117–134.
7. Dziezak, J.D., *Food Technol. 40*:94 (1986).
8. Haumann, B.F., *J. Am. Oil Chem. Soc. 64*:789 (1987).
9. Peled, M., T. Gutfinger and A. Letan, *J. Sci. Food Agric. 26*:1655 (1975).
10. Sims, R.J., J.A. Fioriti and M.J. Kanuk, *J. Am. Oil Chem. Soc. 49*:298 (1972).
11. Boskou, D., and I.D. Morton, *J. Sci. Food Agric. 26*:1149 (1975).
12. Gordon, M.H., and P. Magos, *Food Chem. 10*:141 (1983).
13. White, P.J., and L.S. Armstrong, *J. Am. Oil Chem. Soc. 63*:525 (1986).
14. Yan, P.S., and P.J. White, *Ibid. 64*:556 (1987).
15. Yan, P.S., and P.J. White, *J. Agric. Food Chem. 38*:1904 (1990).
16. Park, W.S., and P.B. Addis, *Anal. Chem. 149*:275 (1985).
17. Morgan, J.N., and D.J. Armstrong, *J. Food Sci. 52*:1224 (1987).
18. Tsai, L.S., and C.A. Hudson, *Ibid. 50*:229 (1985).
19. Nourooz-Zadeh, J., and L.Å. Appelqvist, *Ibid. 52*:57 (1987).
20. Maerker, G., and J. Unruh, Jr., *J. Am. Oil Chem. Soc. 63*:767 (1986).
21. Freeman, I.P., F.B. Padley and W.L. Sheppard, *Ibid. 50*:101 (1973).
22. Ishikawa, Y., K. Morimoto and T. Hamasaki, *Ibid. 61*:1864 (1984).
23. Drew, E.F., U.S. Patent 2,998,319 (1961).
24. Searcy, R.L., and L.M. Bergquist, *Clin. Chim. Acta 30*:192 (1960).
25. Reitmeier, C.A., and K.J. Prusa, *J. Food Sci. 52*:916 (1987).
26. Yan, P.S., and P.J. White, *J. Am. Oil Chem. Soc. 67*:927 (1990).
27. Metcalfe, L.D., A.A. Schmitz and J.R. Pelka, *Anal. Chem. 38*:514 (1966).
28. Waltking, A.E., and H. Zmachinski, *J. Am. Oil Chem. Soc. 47*:530 (1970).
29. SAS Institute, Inc., Cary, N.C., 1985.
30. Smith, L.L., in *Cholesterol Autoxidation*, Plenum Press, New York, 1980.
31. Maerker, G., *J. Am. Oil Chem. Soc. 64*:388 (1987).
32. Zulak, I.M., and G. Maerker, *Ibid. 66*:1499 (1989).
33. Park, W.S., and P.B. Addis, *J. Agric. Food Chem. 34*:653 (1986).
34. Park, W.S., and P.B. Addis, *J. Food Sci. 51*:1380 (1986).
35. Willemse, J.M., U.S. Patent 4,806,374 (1989).
36. Frankel, N.F., K. Warner and K.J. Moulton, Sr., *J. Am. Oil Chem. Soc. 62*:1354 (1985).
37. Terao, J., *Lipids 24*:759 (1989).

[Received September 5, 1990; accepted August 5, 1991]