

Linalyl Acetate and Other Compounds with Related Structures as Antioxidants in Heated Soybean Oil[†]

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Linalyl acetate and undecylenic acid were studied to determine their abilities to reduce oxidative changes in soybean oil held at frying temperature. All compounds to be tested were added to soybean oil and heated to 180 °C for 56-70 h. Fatty acid changes and conjugated diene formation were monitored. Acetylation of linalool to linalyl acetate (LA) caused the formation of many byproducts, which were separated by thin-layer chromatography into three bands. The materials isolated from the bands were tested and found to be equally effective antioxidants. Purchased LA had an antioxidant effect similar to that of the bands. The LA materials from the bands were further purified and identified by GC-MS. All the effective compounds were similar in structure to LA. Undecylenic acid provided some protective effect but less than that of LA, which had less antioxidant effect than Δ^7 -avenasterol and poly(dimethylsiloxane).

INTRODUCTION

A large portion of fats and oils consumed in the United States each year is used in the preparation of fried foods. During deep-fat frying, the fat is exposed to light, elevated temperature, and atmospheric oxygen. Fritsch (1981) described the complex decomposition pattern that is formed as a result of superimposing both thermolytic and oxidative reactions. Sherwin (1978) reported many of the aspects of thermal oxidation of lipids. It is widely accepted that unsaturated fatty acids are much more susceptible to oxidation than their saturated analogues as summarized by Gere (1982). The principal reactions are via the formation and decomposition of hydroperoxide intermediates through a free-radical process. Certain phenolic compounds are able to inhibit such free-radical chain reactions and thus lengthen the oxidation-induction period. Sherwin (1976) and Buck (1981) described antioxidants used by the food industry. A few of the phenolic compounds such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), propyl gallates, and tocopherols are permitted for use alone or in combination with each other as antioxidants in foods. These antioxidants are very effective at room temperature; however, at deep-frying conditions, they can be steam-distilled or destroyed and have little carrythrough effect (Peled et al., 1975). Martin (1953) and Freeman et al. (1973) studied the antioxidative effects of poly(dimethylsiloxane) (MS). It is known to suppress foaming in aqueous solutions. During frying, MS is thought to indirectly inhibit oxidation by suppressing the accumulation of foam-promoting oxidation products such as free fatty acids and food exudates.

Most approved antioxidants added to foods are synthetic. The intense interest of consumers in natural food products has broadened the market for naturally derived antioxidants. Houlihan and Ho (1985) recently prepared an extensive summary on the identification and application of natural antioxidants, but most of those mentioned are useful mainly at room temperature. Sims et al. (1972), Gordon and Magos (1983), and White and Armstrong (1986) demonstrated the effects at frying temperatures of plant sterol antioxidants such as Δ^5 - and Δ^7 -avenasterol. Gor-

don and Magos (1983) proposed that an ethylidene side chain (Figure 1) on the effective sterols 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 due to the presence of unhindered hydrogen atoms on an allylic carbon atom.

The purpose of the present study was to determine whether the high-temperature antioxidative activity of the sterols is caused by the presence of the ethylidene side chain. Compounds containing a double-bond structure similar to the ethylidene group of the effective plant sterols (linalool, products of its acetylation, and undecylenic acid) were tested for antioxidant activity in heated soybean oils.

EXPERIMENTAL PROCEDURES

Materials. *Oils.* Two separate batches of refined, bleached, and deodorized soybean oil (SBO) were obtained from a commercial refining operation. Citric acid (CA) was used during the processing, but no additives were included.

Before the heating tests, peroxide values (PVs) for all the batches of oil were determined according to AOCS Method Cd 8-53 (Walker, 1983). PVs of 0.0-0.3 were obtained.

Antioxidants. Linalool and MS were purchased from Sigma Chemical Co., St. Louis, MO. Linalyl acetate (LA, Figure 2) was purchased from Aldrich Chemical Co., Milwaukee, WI. The Δ^7 -avenasterol (Figure 1) was a gift from Dr. J. Fioriti and had been prepared according to his reported procedure (Fioriti et al., 1971).

Linalyl acetate also was synthesized in the laboratory by adapting the acetylation procedure from AOCS Method Cd 4-40 (Walker, 1983). Thin-layer chromatography (TLC) revealed four major spots with some minor spots after the completion of the acetylation step. The labile nature of linalool and LA under acidic conditions and elevated temperatures as reported by Morin and Richard (1985) likely contributed to the number of spots. The material later was chromatographed by preparative TLC (0.5 mm silica G Uniplates, Alltech Associates, Newark, DE) by using hexane/diethyl ether (95:5) as the developing solvent. The spots were designated the lower, middle, and upper bands. The fourth major spot was very close to the origin and had an R_f similar to that of linalool. Undecylenic acid was purchased (Sigma) and used without additional purification.

Heating Tests. SBO samples (60 g), with and without the various additives, were heated in 100-mL Pyrex beakers at 180 ± 5 °C for 7 h each day for 5-10 days. This fairly severe heat treatment was chosen to enhance possible differences between treatments for easier analyses. The oil was not replenished, as is common in actual deep-fat frying, because the amount removed each day was so small. Samples were cooled to room

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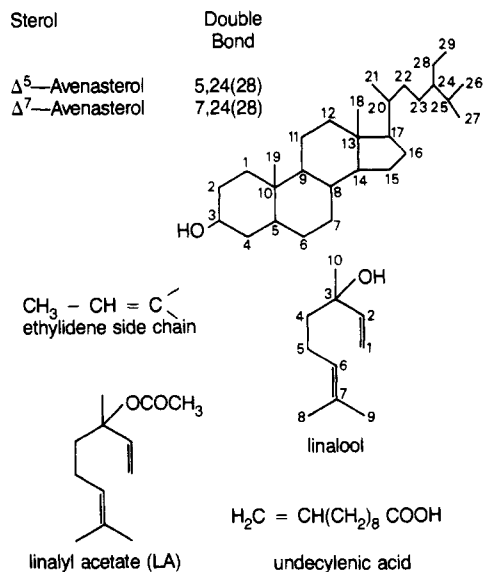


Figure 1. Chemical structures of compounds containing an ethylidene group.

temperature between days. Aliquots (1 mL) were removed at 4 h on day 1 and then at the end of each day of heating and stored under nitrogen at -18°C until analyzed.

In test I, the materials isolated from the lower, middle, and upper bands from TLC of the laboratory-prepared linalyl acetate (LA-LP) were heated in SBO at a level of 0.05% each. Linalool alone (97% pure), added to SBO at 0.05%, and a control SBO containing no additives also were tested. The breakdown of LA was followed by monitoring the GC-MS and GC of a commercial source of LA (LA-C) heated in naphthalene. In test II, undecylenic acid was heated in SBO at levels of 0.0 (control), 0.5, 1.0, 2.0, and 4.0%. The same batch of oil was used in tests I and II.

Several different antioxidants were added to SBO, heated, and compared in test III. A known antioxidant (Δ^7 -avenasterol) was tested at 0.02%, MS was tested at 0.3 ppm, and LA-C was tested at 0.02 and 0.04%. A control SBO with no additives was heated for comparison. The lower band from LA-LP of test I was further purified by gravity-flow column chromatography (CC) (silica gel 60–100 mesh, Davisil, Aldrich), and the resulting material was tested at 0.02%. Its purity was determined by using analytical TLC. A second batch of refined, bleached, and deodorized SBO from the same source as in tests I and II was used in test III.

Analysis of Heated Oils. The heated oils were analyzed for changes in fatty acid composition and conjugated dienoic acids during heating. These methods were previously shown to parallel that of polymer formation in heated oils (White and Armstrong, 1986; White and Wang, 1986). We focused on changes in the fatty acids because of recent interest on the nutritional impact of the heated oil as related to the loss of polyunsaturated fatty acids.

Gas-Liquid Chromatography. A Varian Aerograph Series 3700 gas-liquid chromatograph (GC) equipped with a flame ionization detector was used. The method of Metcalfe et al. (1966) was followed for the preparation of fatty acid methyl esters (FAMES). The GC 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 \times 0.085 in. Peak areas were measured with the internal standard procedure of a Hewlett-Packard (HP) 3390A reporting integrator. Triheptadecanoin was added to all the samples as an internal standard (IS). This method of measurement was suggested by Waltking and Zmachinski (1970) to be the preferred method in determining total polyunsaturated fatty acids. The fatty acid data reported in the current study list the percentage retention for each fatty acid over the heating time, on the basis of the amount at time zero. All test results are the average of duplicate samples.

The same GC also was used for following the degradation of LA-C heated in naphthalene. A DB-5 capillary column (30 m

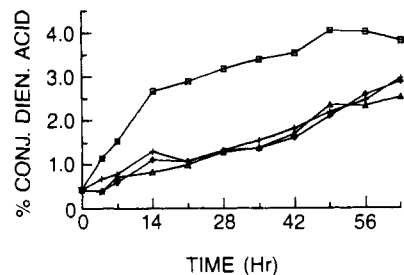


Figure 2. Percentages of conjugated dienoic acid in SBO protected with 0.05% of LA-LP fractions: (□) control; (⊕) lower; (◇) middle; (Δ) upper. (Test I.)

\times 0.25 mm, 1.0- μm film thickness; J&W Scientific, Inc., Rancho Cordova, CA) was used. The column was temperature programmed at $10^\circ\text{C}/\text{min}$ from 80 to 250°C . The injection port temperature was set at 200°C and later at 250°C to compare LA degradation at two injection port temperatures.

Ultraviolet Spectrometry. Conjugated dienoic acids (CD) were measured by using AOCS Method Ti la-64 (Walker, 1983). All test results are the average of duplicate samples. A Gilford Model 240 spectrophotometer (Gilford Instrument Laboratories Inc., Oberlin, OH) was used.

Gas Chromatography-Mass Spectrometry (GC-MS). A Finnigan 4500 GC-MS with a DB-1 fused silica capillary column (30 m \times 0.25 mm, 1.0- μm film thickness) was used to identify the chemical components of LA-LP fractions. The mass spectrometer was linked to a 1984 Revision A NBS library of more than 38 000 chemical compounds. The injection port temperature was set at 250°C . The column was temperature programmed at $10^\circ\text{C}/\text{min}$ from 80 to 250°C , with helium as the carrier gas.

RESULTS AND DISCUSSION

Test I. Linalool is a nonsterol compound containing a double-bond structure similar to that found in plant sterols such as Δ^5 - and Δ^7 -avenasterol, whose high-temperature antioxidant activity was described by Sims et al. (1972), Gordon and Magos (1983), and White and Armstrong (1986) (see Figure 1). Linalool is found in large amounts in herbs such as basil and coriander (Heath, 1981). Many herbs and spices are known sources of natural antioxidants. In linalool, the allylic proton at C5, like that in effective plant sterols, is relatively unhindered and, thus, accessible for oxidation. The resultant free radical (after isomerization) is tertiary allylic in nature and, thus, is very stable and has the potential to terminate oxidative chain reactions. However, when linalool was tested at 0.05% in heated SBO, it was shown to be slightly prooxidative when compared with the control. The data are not presented here. This prooxidative effect possibly was caused by the reactive tertiary alcohol group at C3. To reduce the reactivity at C3, a protective group (acetate) was added to produce LA by using the AOCS Method Cd 4-40 (Walker, 1983). The acetyl form of linalool has a bp of 220°C , compared with a bp of 196°C for linalool, which could have an advantage in a frying oil.

The products resulting from acetylation of linalool were separated by TLC into four major spots with some closely eluting minor spots. The intensity of the spots varied according to the reaction conditions. By preparative TLC, the mixture eluted into four major bands, and they were referred to as the upper, middle, lower, and lower-lower (LL) bands. The materials eluted from the top three bands were tested at 0.05% in SBO heated to $180 \pm 5^\circ\text{C}$ for 56–70 h. The LL band was not tested because its R_f was similar to that of linalool and it was presumed to be such.

The percentage of CD and the percentage retention of 18:1, 18:2, and 18:3 determined in the heated oils over the heating period are shown in Figures 2 and 3, respectively.

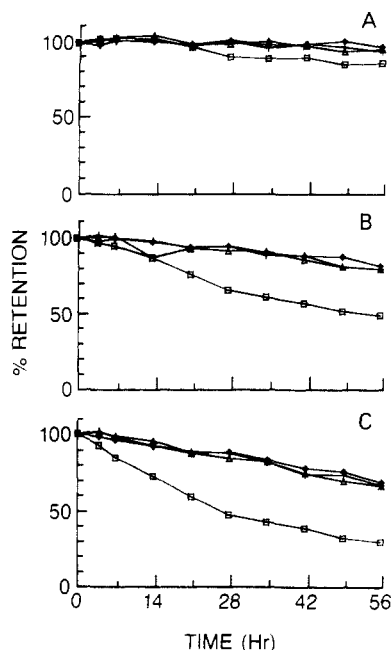


Figure 3. Percentage retention of C18:1 (A), C18:2 (B), and C18:3 (C) in SBO protected with 0.05% of LA-LP fractions: (□) control; (+) lower; (◇) middle; (Δ) upper. (Test I.)

The three fractions were similarly effective in minimizing the deterioration processes of SBO at frying temperature. The SBOs containing the LA fractions produced considerably less % CD and retained much more 18:2 and 18:3 over the heating period when compared with the control SBO. Even retention of 18:1 was better in the treated SBOs.

Attempts were made to separate the LA bands into purer compounds for testing in oils by using additional preparative TLC, but closely eluting spots still were not separated. In addition, purification of the compounds was tried by using high-vacuum fractional distillation, but the compounds were too close to each other in bp and their viscous nature caused cross contamination.

GC-MS Identification. By repeatedly rechromatographing the LA bands on preparative plates (0.50 mm in thickness), the purity of the bands was somewhat improved. These were used in the GC-MS determination of LA-LP fractions. The fragmentation pattern of the lower band material came closest to that of geranyl acetate with a fit of 0.73. Geranyl acetate and LA are allylic isomers. In geranyl acetate, the double bond is shifted to C2 and the alcohol group is moved from C3 to C1. It is likely that such allylic rearrangement occurs during GC-MS analysis. The library search on the middle band material did not reveal any closely fitting compounds. The upper band material was not pure enough for interpretation of GC-MS results. The MS spectra of the eluting peaks from both the middle and upper bands had molecular ions higher than that of LA. That they also eluted higher on the TLC plates suggests that the compounds may be dehydrated and/or dimerized.

Morin and Richard (1985), Valenzuela and Cori (1967), and von Rudloff (1961) reported that terpene alcohols and esters undergo elimination and rearrangement reactions when they are subjected to intense heat, steam distillation, and/or acidic conditions. To test the stability of LA at deep-frying temperatures and at GC injection port temperatures, 5 g of LA-C was added to 60 g of naphthalene. Naphthalene was chosen as the heating medium because it has a high bp and because the LA-C/naphthalene mixture could be directly injected onto the

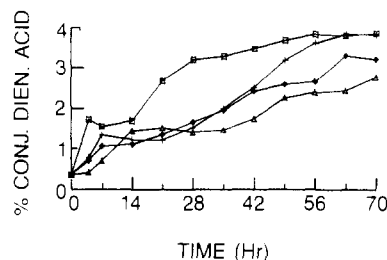


Figure 4. Percentages of conjugated dienoic acid in SBO protected with different levels of undecylenic acid: (□) control; (+) 0.5%; (◇) 1.0%; (Δ) 2.0%. (Test II.)

GC column. The mixture of LA-C and naphthalene was heated to 180 ± 5 °C. Samples were collected at 0, 4, and 7 h of heating. These heated samples plus unheated LA-C were chromatographed by GC on a DB-5 capillary column as described earlier. The unheated LA-C sample was run at injection port temperatures of 200 and 250 °C. At 200 °C, there were one major peak (LA) and six minor peaks. At 250 °C, the major peak decreased in size, the six minor peaks increased in size, and two new ones were observed. This shows that even a slight increase in injection port temperature causes an increase in LA degradation. For LA heated in naphthalene, the injection port temperature was kept at 200 °C to reduce degradation during GC analysis. As heating time increased, the LA peak decreased while the minor peaks grew steadily. By 7 h of heating, the major peak was no longer LA. From these results, it is evident that LA does degrade with time and temperature.

To gain some insight into the nature of the compounds formed from LA during heating, the unheated LA-C and the 7-h sample were subjected to GC-MS in the Finnigan 4500 under operating conditions described previously. The unheated LA-C was not matched to LA by a library search. The first-choice fit for LA-C was geranyl acetate with a fit of 0.73, again indicating that LA isomerizes to form geranyl acetate in GC injection ports. The heated LA-C sample yielded eight peaks (not including the naphthalene peak), and six of these were library searched. Three peaks were matched to compounds that had MWs that were one acetate group less than LA and their fits ranged from 0.88 to 0.89. The LA peak and two other peaks were all matched by the search to geranyl acetate. Their fits ranged from 0.73 to 0.81. Finally, all the compounds that were matched to the peaks resulting from LA breakdown had a double-bond system similar to that of the ethylidene side chain of the effective sterols and at least one other double bond. Therefore, one can postulate that although LA is not stable at the heating conditions described here, its beginning degradation products may still possess high-temperature antioxidant activity.

Test II. Undecylenic acid has a terminal double-bond system which is easily assessable for possible proton abstraction (see Figure 2). It differs from LA in that the carbon involved is a secondary carbon, which in theory should be less effective in free-radical dispersal during oxidative processes. The free-radical forms should be less stable; thus, undecylenic acid should show less effect than LA in SBO heated to frying temperatures. Undecylenic acid was heated in SBO at levels of 0.0 (control), 0.5, 1.0, 2.0, and 4.0%. The CD values and data on the percentage retention of 18:1, 18:2, and 18:3 are shown in Figures 4 and 5, respectively. Clearly, the antioxidant effect was concentration dependent within the range tested. The SBO had the highest % CD and least retention of 18:1, 18:2, and 18:3 when compared with SBO containing undecylenic acid. Undecylenic acid was more effective at 2.0% than at 1.0 or 0.5%. When it was tested at 4.0%, the effect

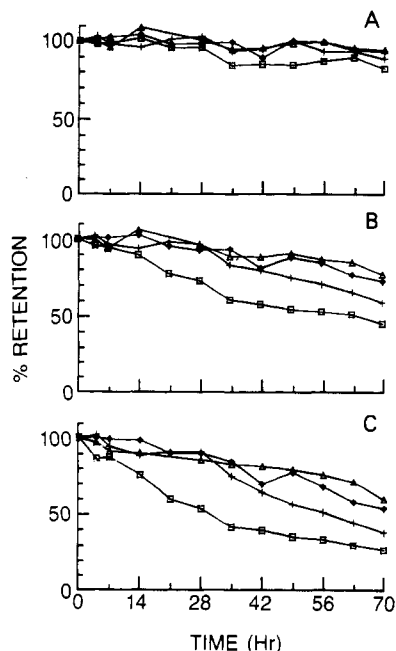


Figure 5. Percentage retention of C18:1 (A), C18:2 (B), and C18:3 (C) in SBO protected with different levels of undecylenic acid: (□) control; (+) 0.5%; (◇) 1.0%; (△) 2.0%. (Test II.)

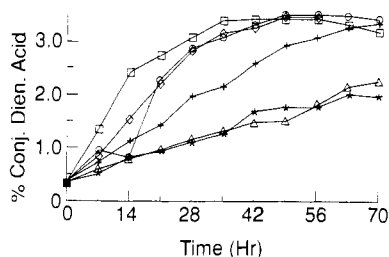


Figure 6. Percentages of conjugated dienoic acid in SBO protected with 0.02% Δ^7 -avenasterol (+), 0.02% and 0.04% of LA-C (◇ and △), 0.02% of the material from the LA-LP lower band (○), 0.3 ppm MS (*), and a control (□). (Test III.)

was similar to that of 2.0%, so those data are not shown. At 0.5% undecylenic acid was not as effective as the LA-LP fractions from test I. At 56 h of heating, only 50% of the 18:3 was left in oil protected with 0.5% of undecylenic acid, whereas 70% of the 18:3 was left in oils containing only 0.05% of any one of LA-LP fractions. The effectiveness of undecylenic acid at frying temperature and the observed differences in effectiveness between it and the LA-LP fractions further point to the feasibility of the ethylidene theory in plant sterol high-temperature antioxidants.

Test III. The LA-C was tested in SBO at 0.02 and 0.04% without further purification. When chromatographed by TLC, the R_f of the major spot in LA-C had the same R_f value as that of the lower band from the LA-LP. The less intense spot of LA-C had the same R_f value as that of the upper band of LA-LP. A column-purified fraction from the lower band of LA-LP was tested at 0.02%. The purification procedure was described earlier. One SBO sample containing 0.02% Δ^7 -avenasterol and one containing 0.3 ppm MS also were tested. The CD data and the percentage retention of 18:1, 18:2, and 18:3 are shown in Figures 6 and 7, respectively. After 70 h of heating, LA-C at 0.04% and MS were still protecting the oil, whereas the LA-LP, Δ^7 -avenasterol, and 0.02% LA-C had lost their protective effect. The retention of the fatty acids showed similar effects. Only about 20% of the 18:3 in the control remained at the end of the heating period. The LA-LP showed some protective effect up to 35 h of

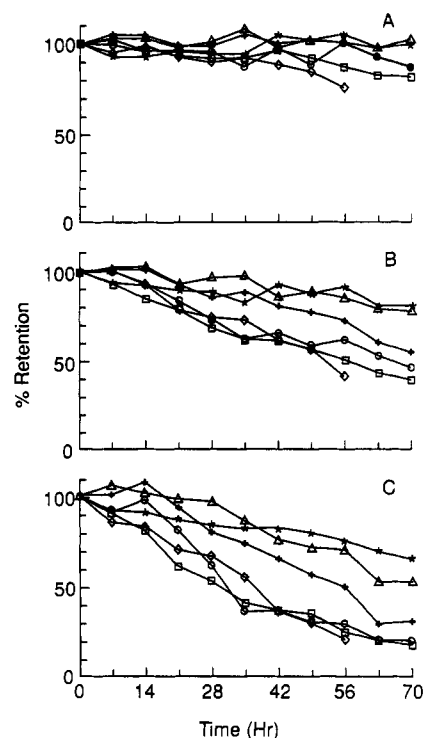


Figure 7. Percentage retention of C18:1 (A), C18:2 (B), and C18:3 (C) in SBO protected with 0.02% Δ^7 -avenasterol (+), 0.02% and 0.04% of LA-C (◇ and △), 0.02% of the material from the LA-LP lower band (○), 0.3 ppm MS (*), and a control (□). (Test III.)

heating, whereas Δ^7 -avenasterol was still exerting some effect on 18:3 at 70 h of heating. The samples containing MS and 0.04% LA-C retained the most unsaturated fatty acids over the heating period. A level of 0.02% LA-C was slightly effective up to about 42 h.

The LA-C did exhibit antioxidant activity in SBO at deep-fat frying temperature. However, LA-C at 0.02% was less effective than Δ^7 -avenasterol at the same concentration. The molar concentrations of Δ^7 -avenasterol and LA in the SBO were about 3.0×10^{-5} and 5.5×10^{-5} M, respectively. Although a higher molar concentration of LA was present, its MW of 196 and bp 220 °C could have caused it to be volatile at frying temperature, whereas Δ^7 -avenasterol (MW 412) might have remained in the SBO longer.

It is likely that the ethylidene group that is found in certain plant sterols and the similar double-bond system found in LA are at least partly responsible for the high-temperature antioxidant activity of these compounds. Boskou and Morton (1976) also suggested that the double bonds within the rings of effective plant sterols contribute toward their total antioxidant activity. The lack of a ring structure with a double bond in LA could help account for the difference in activity between Δ^7 -avenasterol and LA.

ACKNOWLEDGMENT

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Registry No. Undecylenic acid, 112-38-9; linalyl acetate, 115-95-7; Δ^7 -avenasterol, 23290-26-8; linalool, 78-70-6; geranyl acetate, 105-87-3.