beginning of experimentation.

Both methods used for quantitative analysis of linoleic acid autoxidation rate exhibited a prooxidant effect of α-tocopherol that took place during the first 4 days of experimentation. At the end of this period, α-tocopherol was oxidized for the most part and we noted a decreased autoxidation rate of linoleic acid.

GC measurement of unoxidized linoleic acid was the best method for quantitative analysis of fatty acid autoxidation rate in the presence and in the absence of α-tocopherol. In addition, during the prooxidant effect of α-tocopherol (first 4 days), conjugated diene measurement permitted an available evaluation of fatty acid autoxidation rate because the rate of conjugated dienes degradation was negligible compared to the rate of conjugated dienes formation. This method has the advantage of being easy and rapid. After the first 4 days, the conjugated diene levels rapidly reached a maximum while gas chromatographic measurement of linoleic acid indicated a further oxidation of the fatty acid.

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# Effect of Experimental Factors on the Prooxidant Behavior of α-Tocopherol

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## ABSTRACT

We have investigated the effect of experimental factors on the prooxidant effect of α-tocopherol during the autoxidation of linoleic acid. The prooxidant effect depended on two factors: the concentration of α-tocopherol ($\geq 5 \times 10^{-3}$ mol α-tocopherol/1 mol linoleic acid) and the solvent, an aqueous system in which the prooxidant effect occurred more easily. On the other hand, the prooxidant behavior of α-tocopherol was unaffected by the type of surfactant used in water as well as by the presence of different salts. The initial content of hydroperoxides affected the intensity of the prooxidant effect which varied in an inverse ratio to the initial hydroperoxide level.

## INTRODUCTION

Most of the observations on the prooxidant effect of α-tocopherol have been established in oils (1-3). It is a general fact that some phenols used as antioxidants can promote a prooxidant reaction, especially beyond a certain limit of concentration (1-4). Loury et al. (2) reported a limit of 0.1% α-tocopherol in soybean oil. Witting (5) showed that during the autoxidation of methyl or ethyl linoleate, the addition of α-tocopherol as antioxidant was ineffective when the ratio linoleate: α-tocopherol was inferior to $10^3$. He founded that, in this case, the concentration of hydroperoxides increased. At high concentrations, these phenols did not act as free radical scavengers but as free radical chain breakers (4):

Antioxidant $\quad ROO^{\bullet} + AOH \rightarrow ROOH + AO^{\bullet} \quad [I]$

Prooxidant $\quad ROOH + A^{\bullet} \rightarrow ROO^{\bullet} + AH \quad [II]$

or

$\quad RH + A^{\bullet} \rightarrow R^{\bullet} + AH \quad [III]$

Thus, the solvent system has significant effects on lipid autoxidation rate; it affected the metal catalysis in lipid autoxidation. The solvent could inhibit metal catalysis either by formation of a solvent-metal complex (6) or by formation of hydrogen bond between solvent and hydroperoxide preventing hydroperoxide decomposition (4,7). This inhibition was observed with polar solvents such as ethanol (7,8), ethyl acetate (8), acetic acid (7) and water at low moisture content (4). On the other hand, a solvent such as water at high moisture content (4) could enhance the metal catalysis by increasing the mobility of the metal catalyst. Moreover, some solvents such as ethanol, formate and benzoate can act as hydroxyl scavengers (9). During the autoxidation of fatty acids, hydroxyl radicals were formed by the decomposition of hydroperoxides. This work studied the influence of the solvent and of the concentration of α-tocopherol on the advent of the prooxidant effect of α-tocopherol. The effect of water was largely investigated because of its important role in food oxidation. Inherent factors in the use of water were thereby studied; with emphasis on the effect of the surfactant which is required to disperse linoleic acid and α-tocopherol, as well as the effect of the presence of different salts.

## EXPERIMENTAL

### Material

*Solvents and surfactants.* Linoleic acid autoxidation with and without α-tocopherol has been carried out in different solvents such as polar protic solvents (deionized water or ethanol: Normapur, Prolabo-France), polar aprotic solvents

(acetonitrile or dimethylsulfoxide: both Normapur from Prolabo-France) and nonpolar solvents (hexane: Normapur, Prolabo-France or petroleum ether 40-60 C: Merck). With water, we used either distilled water or buffered salt solutions such as disodium phosphate, sodium acetate or potassium borate, all purchased from Prolabo-France. The use of water necessitated the addition of surfactant (0.5 to 5%) to disperse linoleic acid and α-tocopherol. Different surfactants were tested: nonionic surfactants (Tween 20, Merck and Triton X-100, BDH Chemicals-England), anionic surfactant (sodium lauryl sulfate, Prolabo-France) and amphoteric surfactant (Dehyton AB 30 from Henkel, Dusseldorf).

*Preparation of stock solutions and stock dispersion of linoleic acid.* Linoleic acid (9,12-octadecadienoic acid) was purchased from Koch Light Laboratories Ltd., Colnbrook, England (≥ 99% pure). It was dissolved directly in the different solvents, except for water. In water solvent, the following procedure was employed: linoleic acid was dispersed with 0.5 to 5% surfactant in water (buffered or not buffered), at pH 9 under a nitrogen atmosphere. This aqueous dispersion was adjusted to pH 6.9 with chlorhydric acid just before use.

The stock solutions and the stock dispersion of linoleic acid were at a concentration of $10^{-2}$ M linoleic acid; they were stored at 4 C.

*Preparation of stock solutions and stock dispersion of α-tocopherol.* dl α-Tocopherol was supplied by Hoffmann LaRoche-France. α-Tocopherol was further purified by high performance liquid chromatography (HPLC) fitted with a LDC Constametric II G Pump and a Spectrophotometer II as detector. The column (200 mm x 6.35 mm) was packed with Lichrosorb Si 60, 5 μm. α-Tocopherol was eluted with heptane (2 ml/mn) as solvent. Stock solutions of α-tocopherol were prepared by dissolving α-tocopherol directly in the different solvents, except for water. In that case, α-tocopherol was dispersed with a surfactant in water at pH 7 according to the same procedure described for linoleic acid. α-Tocopherol concentration was $5 \times 10^{-4}$ M for all the stock solutions as well as for the stock dispersion. These stock preparations were stored at 4 C.

### Procedure

Samples (100 ml) were prepared by mixing aliquots of the previous stock solutions or stock dispersions of linoleic acid and α-tocopherol, respectively, at time zero. These were placed in glass tubes and left in the dark and under air at room temperature. Controls without α-tocopherol were placed in similar conditions.

### Conjugated Dienes Measurement at 234 nm

The autoxidation of linoleic acid was accompanied in the early stages by an increase of ultraviolet (UV) absorption at 234 nm, which was ascribed to conjugated diene formation. Measurement of the optical density was performed using a Pye Unicam SP 800 Spectrophotometer. Readings were made at 2-hr intervals during the 10 first hr and subsequently every 24 hr.

### Measurement of Unoxidized Linoleic Acid by Gas Chromatography

Gas chromatography (GC) using a Pye Unicam 104 Model gas chromatograph fitted with a flame ionization detector and a 5 ft x 1/8 in. column packed with 10% DEGS on 80/100 mesh Chromosorb W HP. The operating conditions were: column temperature, 180 C; injector-detector tem-

TABLE I

Distribution of α-Tocopherol Concentrations

| | Samples | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Linoleic acid[a] (mol) | $2.5 \times 10^{-3}$ | $2.5 \times 10^{-3}$ | $2.5 \times 10^{-3}$ | $2.5 \times 10^{-3}$ | $2.5 \times 10^{-3}$ | $2.5 \times 10^{-3}$ | $2.5 \times 10^{-3}$ | $2.5 \times 10^{-3}$ | $2.5 \times 10^{-3}$ | $2.5 \times 10^{-3}$ |
| α-Tocopherol[a] (mol) | 0 | $1.25 \times 10^{-4}$ | $0.625 \times 10^{-4}$ | $0.312 \times 10^{-4}$ | $1.25 \times 10^{-5}$ | $0.625 \times 10^{-5}$ | $0.312 \times 10^{-5}$ | $1.25 \times 10^{-6}$ | $0.625 \times 10^{-6}$ | $0.312 \times 10^{-6}$ |
| Mol α-T/mol linoleic acid | 0 | $5 \times 10^{-2}$ | $2.5 \times 10^{-2}$ | $1.25 \times 10^{-2}$ | $5 \times 10^{-3}$ | $2.5 \times 10^{-3}$ | $1.25 \times 10^{-3}$ | $5 \times 10^{-4}$ | $2.5 \times 10^{-4}$ | $1.25 \times 10^{-4}$ |
| α-T(%)[b] | 0 | 7.6 | 3.8 | 1.9 | 0.76 | 0.38 | 0.19 | 0.076 | 0.038 | 0.019 |

[a]Linoleic acid and α-tocopherol were dispersed in phosphate buffered aqueous solution 0.025 M, pH 6.9 with 0.5% Tween 20 as surfactant.
[b]Weight (g) α-T/100 g linoleic acid.

perature, 240 C; carrier gas, nitrogen of high purity; flow rate, 60 ml/min.

Linoleic acid was methylated according to the Rogstad and Reinton method (10) before GC. In the aqueous system, linoleic acid was first extracted from water using a mixture of chloroform-methanol (1:1, v/v).

### Thin Layer Chromatography of α-Tocopherol

Aliquots (5 ml) of the samples were evaporated to dryness under reduced pressure on a rotary evaporator. The dry residue was dissolved with 0.5 ml chloroform and 20 μl of this solution was applied to a Silica Gel $F_{254}$ plate (0.25 mm thick). The chromatogram was developed with chloroform in a saturated chamber. α-Tocopherol and its oxidation products were revealed by spraying the plate with a sulfomolybdic reagent and after heating at 120 C for 5 min the compounds appeared as blue spots.

## RESULTS AND DISCUSSION

### Effect of α-Tocopherol Concentration

The relationship between the concentration of α-tocopherol and the prooxidant effect was investigated in an aqueous media at pH 6.9, with Tween 20 as surfactant. The distribution of the different concentrations of α-tocopherol is reported Table I.

Conjugated dienes measurement at 234 nm demonstrated 2 opposite behavior patterns of α-tocopherol as a function of its concentration. α-Tocopherol exhibited a prooxidant behavior when it was at a concentration equal to or greater than $5 \times 10^{-3}$ mol of α-tocopherol/1 mol linoleic acid. This prooxidant effect was observed in samples 1, 2, 3 and 4 (Fig. 1A). The magnitude of the phenomenon increased with the concentration of α-tocopherol and reached a maximum for a ratio equal to or greater than $1.25 \times 10^{-2}$ mol of α-tocopherol/1 mol of linoleic acid (samples 1, 2 and 3). α-Tocopherol was antioxidant at a concentration equal to or less than $2.5 \times 10^{-3}$ mol α-tocopherol/1 mol linoleic acid (samples 5, 6, 7, 8 and 9, Fig. 1B). The magnitude of the antioxidant effect was in inverse ratio to the α-tocopherol level, it reached a maximum (od at 234 nm minimum) for a value equal to or less than $2.5 \times 10^{-4}$ mol α-tocopherol/1 mol linoleic acid (samples 8 and 9, Fig. 1B).

### Effect of the Solvent

The previous prooxidant behavior of α-tocopherol was observed in aqueous media. Similar investigations were undertaken in nonaqueous media.

(a) Polar protic solvents other than water, such as ethanol, exhibited low autoxidation rates of linoleic acid in the absence and presence of α-tocopherol at high concentrations ($\geq 5.10^{-3}$ mol α-T/mol of linoleic acid). Conjugated dienes (od 234 nm) were maintained at a very low level (Table II). The low autoxidation rate of linoleic acid in ethanol was confirmed by GC measurement of unoxidized linoleic acid. This assay exhibited no significant change in the concentration of linoleic acid with and without α-tocopherol with respect to the initial concentration. Prooxidant behavior of α-tocopherol could not take place in ethanol. Ethanol is a known hydroxyl radical scavenger (9).

(b) Polar aprotic solvents such as dimethylsulfoxide (DMSO) and acetonitrile were tested in the same manner as ethanol. In these solvents, as in ethanol, no prooxidant effect of α-tocopherol was observed. The autoxidation rate

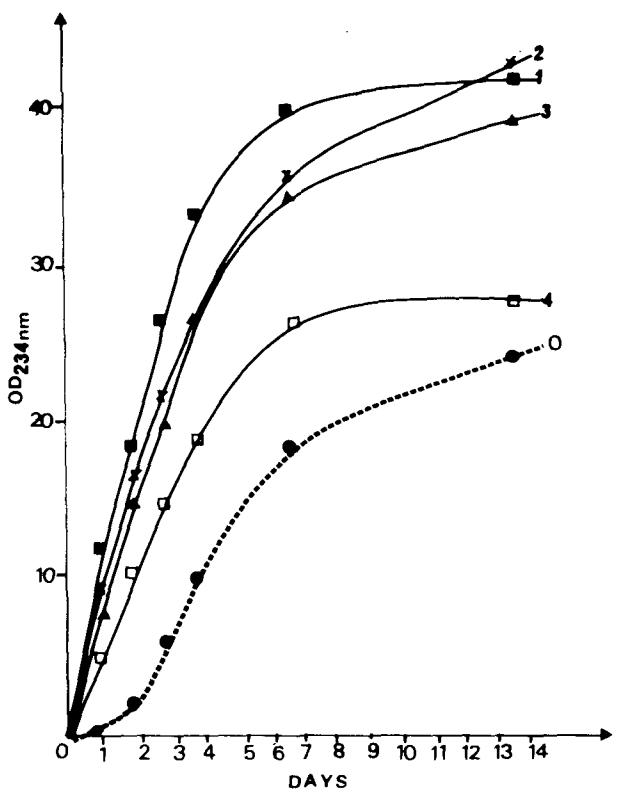


FIG. 1A. Effect of high concentrations of α-tocopherol on the autoxidation of linoleic acid in aqueous media. 0 ●: Linoleic acid; 1 ■: linoleic acid + α-T $5 \times 10^{-2}$ M; 2 X: linoleic acid + α-T $2.5 \times 10^{-2}$ M; 3 ▲ linoleic acid + α-T $1.25 \times 10^{-2}$ M; 4 □: linoleic acid + α-T $5 \times 10^{-3}$ M.

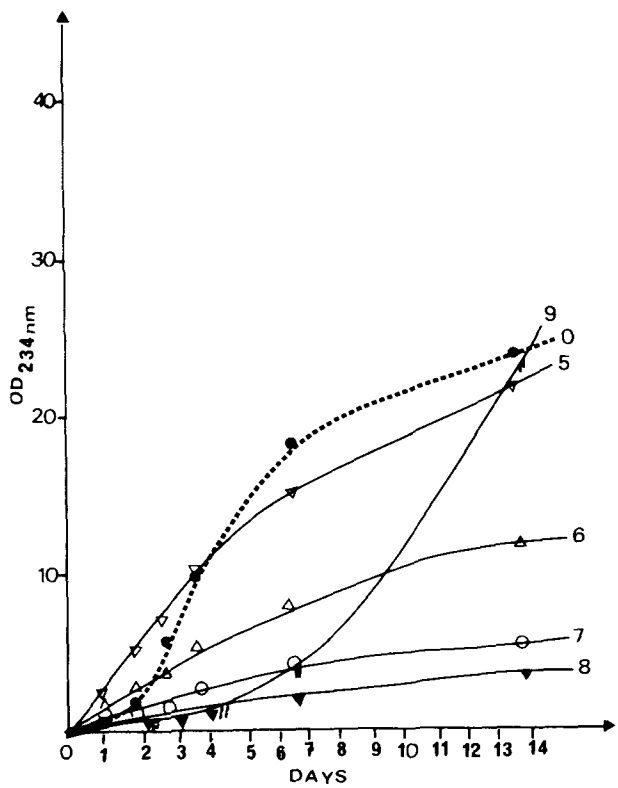


FIG. 1B. Effect of low concentrations of α-tocopherol on the autoxidation of linoleic acid in aqueous media. 0 ●: Linoleic acid; 5 ▽: linoleic acid + α-T $2.5 \times 10^{-3}$ M; 6 △: linoleic acid + α-T $1.25 \times 10^{-3}$ M; 7 ○: linoleic acid + α-T $5 \times 10^{-4}$ M; 8 ▼: linoleic acid + α-T $2.5 \times 10^{-4}$ M, 9 //: linoleic acid + α-T $1.25 \times 10^{-4}$ M.

TABLE II

Conjugated Diene Measurement (od 234 nm) during Linoleic Acid Autoxidation in Absence and in Presence of High Levels of α-Tocopherol in Different Solvents

| Solvents | Days | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Water[a] (100%) | | | | | | | |
| $E_o$[b] | 0.38 | 1.68 | 8.10 | – | – | 16.82 | 19.32 |
| Eα-T[c] | 5.06 | 10.94 | 17.28 | – | – | 34.18 | 36.03 |
| Ethanol (100%) | | | | | | | |
| $E_o$ | 0 | 0 | 0 | 0.02 | 0.02 | – | – |
| Eα-T | 0 | 0.02 | 0.02 | 0.02 | 0.02 | – | – |
| Dimethylsulfoxide (100%) | | | | | | | |
| $E_o$ | −0.01 | +0.01 | +0.01 | – | – | +0.01 | +0.03 |
| Eα-T | −0.01 | −0.01 | −0.02 | – | – | +0.02 | +0.04 |
| Acetonitrile (100%) | | | | | | | |
| $E_o$ | +0.02 | −0.02 | 0 | −0.03 | – | −0.04 | −0.04 |
| Eα-T | −0.01 | −0.01 | −0.01 | +0.01 | – | 0 | +0.01 |
| Dimethylsulfoxide (50%) + water (50%) | | | | | | | |
| $E_o$ | 0.110 | 0.180 | 0.220 | – | – | 0.19 | 0.25 |
| Eα-T | 0.180 | 0.370 | 0.560 | – | – | 0.78 | 0.88 |
| Dimethylsulfoxide (25%) + water (75%) | | | | | | | |
| $E_o$ | 0.060 | 0.120 | 0.140 | – | – | 0.24 | 0.24 |
| Eα-T | 0.700 | 5.180 | 9.540 | – | – | 20.24 | 21.44 |
| Acetonitrile (50%) + water (50%) | | | | | | | |
| $E_o$ | −0.05 | −0.01 | −0.02 | −0.03 | – | −0.01 | −0.01 |
| Eα-T | −0.01 | −0.01 | −0.01 | −0.01 | – | −0.04 | −0.04 |
| Hexane | | | | | | | |
| $E_o$ | 0.02 | 0.06 | 0.05 | 0.07 | – | – | 0.11 |
| Eα-T | −0.04 | −0.08 | −0.03 | −0.01 | – | – | +0.03 |
| Petroleum ether (40-60 C) | | | | | | | |
| $E_o$ | 0 | 0 | 0 | +0.02 | – | – | – |
| Eα-T | −0.09 | −0.07 | −0.05 | +0.03 | | | |

[a]Phosphate buffered aqueous solution 0.025 M, pH 6.9.

[b]Linoleic acid in absence of α-tocopherol.

[c]Linoleic acid in presence of high level of α-tocopherol.

of linoleic acid with and without α-tocopherol was very low in every case. No significant increase of the conjugated dienes was noted (Table II).

The addition of either 50% or 75% water to dimethylsulfoxide and acetonitrile involved an increase of the conjugated dienes as a function of the percentage of water for dimethylsulfoxide only. The addition of water induced a prooxidant effect with α-tocopherol (Table II).

(c) Nonpolar solvents such as hexane and petroleum ether (40-60 C) gave results similar to those previously obtained. The prooxidant effect of α-tocopherol did not take place in these solvents. The autoxidation rate of linoleic acid with and without α-tocopherol was very low. The conjugated diene level did not increase with time (Table II).

The investigation of the effect of the solvent on the prooxidant behavior of α-tocopherol showed that in our model system, the prooxidant effect only took place in an aqueous media. In solvents other than water, the autoxidation rate of linoleic acid was considerably slowed even in the presence of high levels of α-tocopherol.

## Effect of Surfactant

The use of surfactant was compulsory in order to disperse α-tocopherol and linoleic acid in the aqueous media. Surfactants are distributed into 4 groups: nonionic surfactants, such as Tween 20 and Triton X-100; anionic surfactant, such as sodium lauryl sulfate, cationic surfactant, such as cetyl pyridinium bromide and amphoteric surfactant, such as Dehyton AB 30 (sulfobetaine). All these surfactants were employed at a concentration of 0.5% except Dehyton AB 30, which should be used at a concentration of 5% in order to have a suitable dispersion. The prooxidant effect of α-tocopherol took place with all the surfactants tested except for the amphoteric surfactant, which exhibited the antioxidant activity of α-tocopherol (Fig. 2B). According to the surfactant used, we noted differences in the autoxidation rate of linoleic acid with and without α-tocopherol depending on the degree of oil dispersion. In our experiments, the most effective was Tween 20, which gave the most important increase in conjugated dienes, especially with α-tocopherol (Fig. 2A).

## Effect of Salts

The prooxidant effect of α-tocopherol was first studied in a 0.025 M borate buffer at pH 6.9. We examined the effect of different buffers, all at a standard pH 6.9, as well as the effect of distilled water on the prooxidant behavior patterns.

*Distilled water.* Traces of heavy metals, especially $Fe^{++}$ and $Cu^{++}$ were strong prooxidative catalysts in lipid oxidation when they were present at a concentration < 5 ppm (11). The amount of $Fe^{++}$ and $Cu^{++}$ present in distilled water was measured with an atomic absorption type IL 251. Less than 0.01 ppm Fe and Cu was found in distilled water.

Linoleic acid and α-tocopherol were emulsified in distilled water with Tween 20 as surfactant. As soon as α-tocopherol was added to linoleic acid, we noted an important increase of the conjugated dienes without any induction period (Fig. 3). On the other hand, in absence of α-tocopherol, the conjugated diene measurement showed an induction period of 3 days (Fig. 3). These results agreed

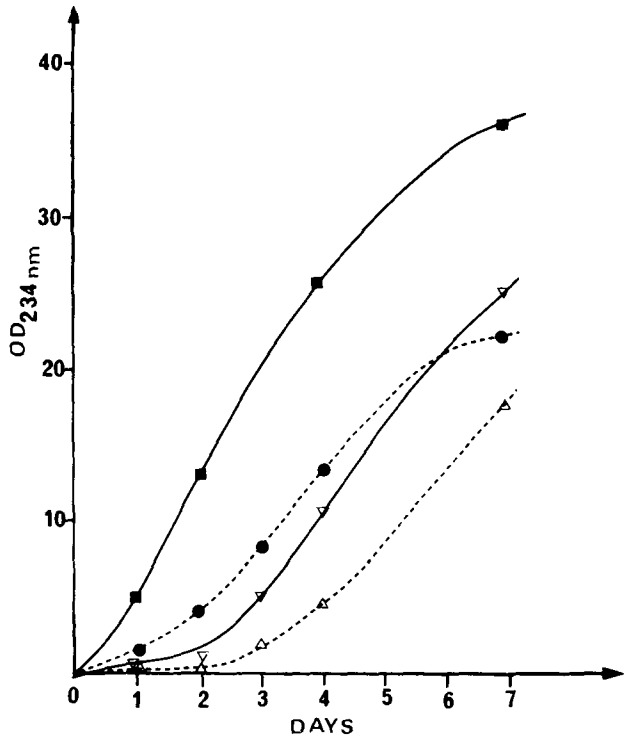


FIG. 2A. Effect of nonionic surfactants on the prooxidant activity of α-tocopherol. ●■: Tween 20, 0.5% (●: linoleic acid without α-T; ■: linoleic acid with α-T). △▽: Triton X-100, 0.5% (△: linoleic acid without α-T; ▽: linoleic acid with α-T).

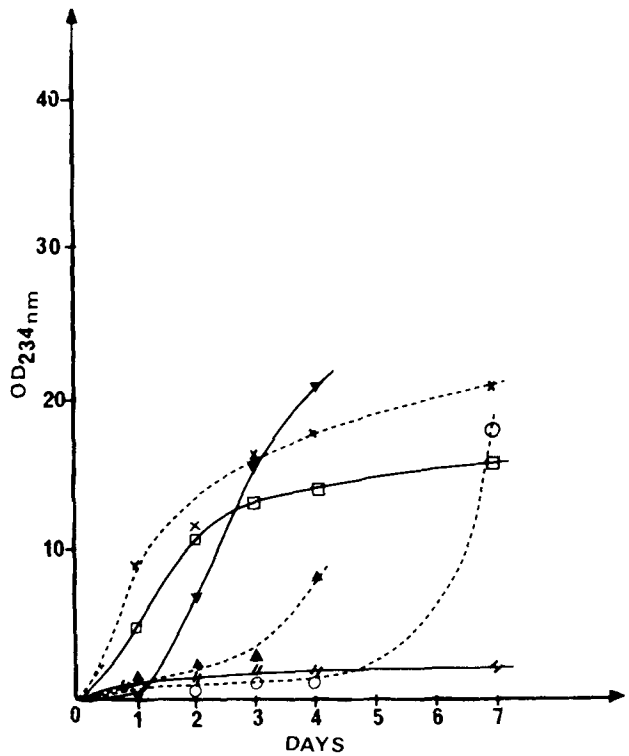


FIG. 2B. Effect of anionic, cationic and amphoteric surfactants on the prooxidant activity of α-tocopherol. ▲▼: sodium lauryl sulfate (anionic) 0.5%, ○□: cetyl pyridinium bromide (cationic) 0.5%; X //: Dehyton AB 30 (amphoteric) 5%, ▲○X: linoleic acid without α-T; ▼□//: linoleic acid with α-T.

with those of Farag and Osman (12) who reported an induction period of 45 hr for linoleic acid ($10^{-2}$ M) emulsified in deionized water with 0.02% Tween 20.

*Phosphate buffer.* Linoleic acid was dispersed with Tween 20 in a 0.025 M phosphate buffer at pH 6.9. The conjugated dienes rapidly increased after the addition of a high level of α-tocopherol (Fig. 3).

*Acetate buffer.* This was used in conditions similar to those of the phosphate buffer (0.025 M, pH 6.9). The addition of α-tocopherol involved an increase of the conjugated dienes (Fig. 3). The prooxidant effect of α-tocopherol has the same magnitude in acetate buffer, in phosphate buffer and in distilled water. On the other hand, linoleic acid without α-tocopherol exhibited an increase in conjugated dienes which was inhibited by the acetate buffer (Fig. 3).

### Effect of Initial Level of Hydroperoxides

The effectiveness of an antioxidant is affected by the initial concentration of peroxides. When the initial peroxide content is too high, the addition of an antioxidant is ineffective. The same holds true for a prooxidant. In our model system, most of the hydroperoxides present in the sample at time zero were formed during the dispersion of linoleic acid in the aqueous solvent in spite of the nitrogen atmosphere. In this experiment, one lot of linoleic acid was divided into 2 parts.

One part of the linoleic acid was dispersed in water according to the procedure described in Experimental. At time zero, the aqueous solution of linoleic acid which contained ca. 1.5%. initial hydroperoxides was mixed with an aqueous solution of α-tocopherol (high concentration). A control without α-tocopherol was placed in similar conditions.

The second part of the linoleic acid was first mixed with α-tocopherol and then dispersed in water as previously

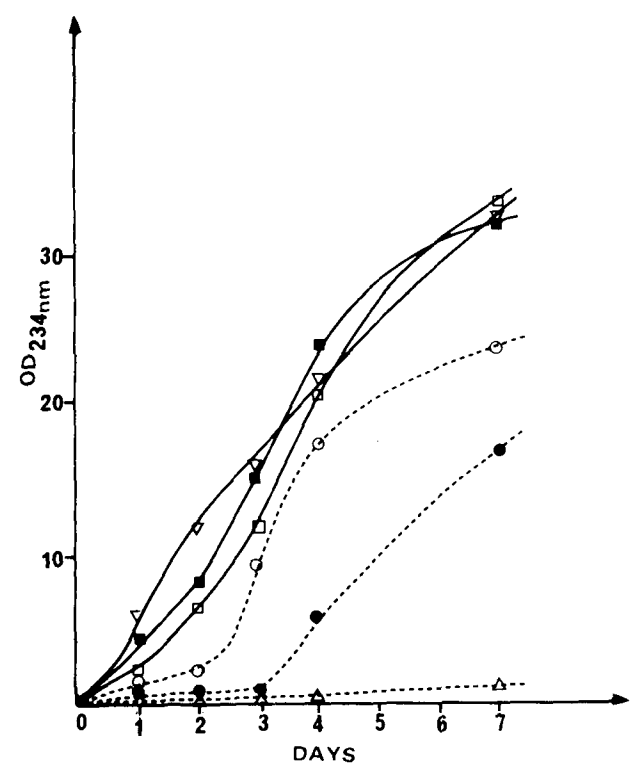


FIG. 3. Effect of the salts on the prooxidant activity of α-tocopherol. ●■: Distilled water; ○□: phosphate buffer 0.025 M, pH 6.9; △▽: acetate buffer 0.025 M, pH 6.9; ●○△: linoleic acid without α-T; ■□▽: linoleic acid with α-T.

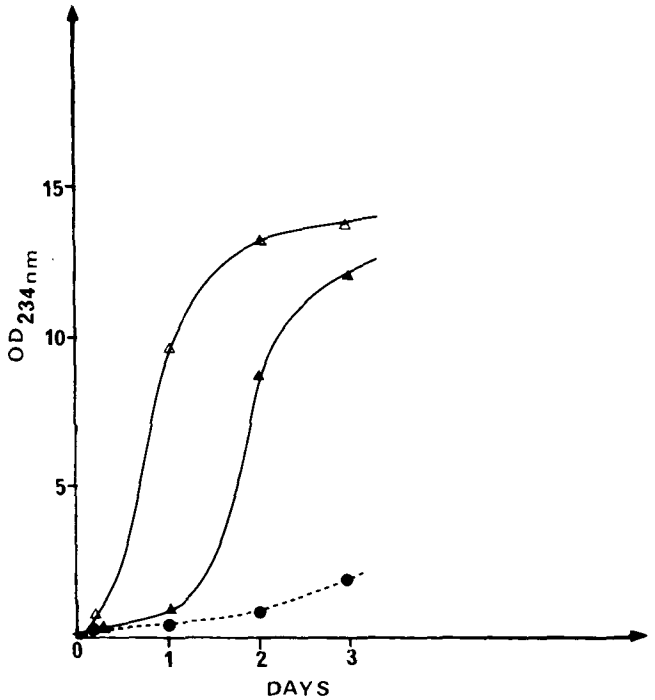


FIG. 4. Effect of the initial level of hydroperoxides on the prooxidant effect of α-tocopherol. ●▲: Hydroperoxides ≅ 1.5% (●: linoleic acid without α-T; ▲: linoleic acid with α-T). △: hydroperoxides < 1.5% (△: linoleic acid with α-T).

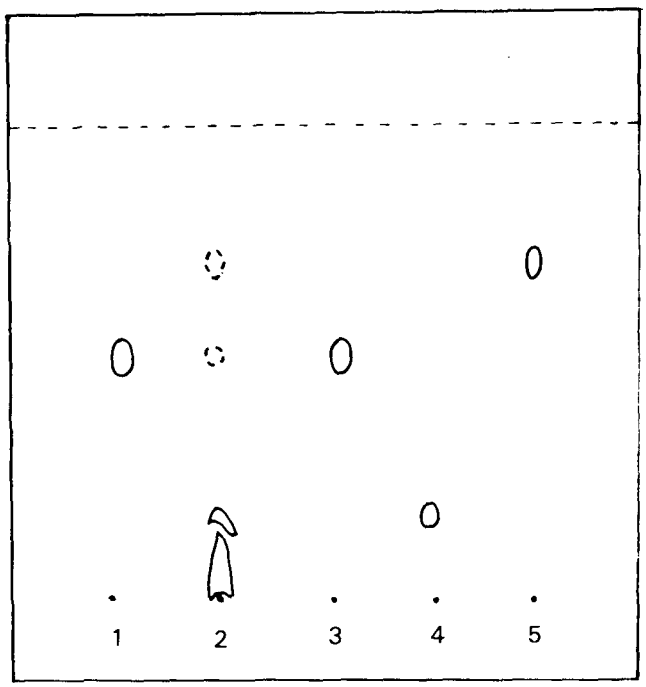


FIG. 5. Thin layer chromatogram of α-tocopherol during linoleic acid autoxidation (4 days). 1: Control α-tocopherol in aqueous solution; 2: sample α-tocopherol with linoleic acid in aqueous solution; 3: reference α-tocopherol; 4: reference α-tocopherylquinone; 5: reference dimer of α-tocopherol.

described. The initial level of hydroperoxides was significantly less than 1.5%.

The conjugated dienes measured vs time exhibited the greatest increase when linoleic acid was added to α-tocopherol before the dispersion and when the initial hydroperoxide content was less than 1.5% (Fig. 4).

These experiments have demonstrated that according to our model system, the prooxidant effect of α-tocopherol depended on the concentration of α-tocopherol and on the solvent.

The prooxidant effect occurred only in a aqueous system when the concentration of α-tocopherol was equal to or greater than $5 \times 10^{-3}$ mol α-T/1 mol linoleic acid. Phenols (α-tocopherol, butylhydroxytoluene) which are antioxidant at low concentrations become prooxidant beyond a certain limit. This reverse activity was accounted for by the action of phenols as free radical chain breakers. The increased autoxidation rate of linoleic acid by the presence of water could be attributed to an increased mobility of catalysts such as heavy metals (4). Moreover, in our model system, linoleic acid and α-tocopherol occurred as micelles in which the molecules were oriented according to their hydrophobic and hydrophilic groups which could influence the autoxidation. The fact that the prooxidant activity was in inverse ratio to the initial hydroperoxides content could probably be related to the oxidation of α-tocopherol by the hydroperoxides according to the mechanism proposed by Gruger and Tappel (13). This oxidation involved a decrease in the initial concentration of α-tocopherol in such a way that α-tocopherol always acted as a prooxidant, but the effect observed was less important. This oxidation of α-tocopherol by the hydroperoxides was confirmed by a thin layer chromatography (TLC) which showed a rapid oxidation of α-tocopherol mainly into α-tocopherylquinone and traces of a dimer (Fig. 5). On the other hand, the prooxidant activity of α-tocopherol was not affected by the composition of the aqueous media. This prooxidant effect exhibited the same magnitude in 0.025 M buffered solutions such as borate, phosphate or acetate buffers as well as in distilled water. These results illustrate the participation of heavy metals in the prooxidant reaction. The prooxidant effect of α-tocopherol was unaffected by distilled water which contains less than 0.01 ppm Fe or Cu. Nevertheless metals were not entirely absent since linoleic acid and α-tocopherol always contain traces of metals (8). Results obtained in acetate buffer were more ambiguous. The autoxidation of linoleic acid was strongly inhibited in acetate buffer. Hendry and al. (7) reported the same observation in acetic acid. According to these authors, acetic acid hydrogen bonding with the hydroperoxide and therefore the decomposition of hydroperoxide was slowed, preventing the propagation of the autoxidation. In our experimentation, the prooxidant effect of α-tocopherol was unaffected by the acetate buffer, likewise by acetic acid $10^{-2}$ M (14). This result was opposite to the prooxidant action of α-tocopherol acting as a hydroperoxide decomposer (1,15).

The effect of surfactant has shown that, according to the type of the surfactant used, the autoxidation rate of linoleic acid was more or less important, since α-tocopherol always exhibited a prooxidant effect except with one of the amphoteric surfactants (Dehyton AB 30). The behavior of other amphoteric surfactants must be further studied.

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# ⁂Fatty Acids: XX[1]. Location of the Position of the Furan Ring in 2,5-Disubstituted Furan-containing Fatty Acids by GLC Analysis of Oxidation Products

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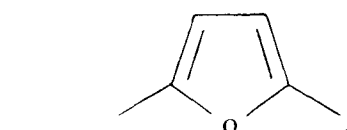
## ABSTRACT

Oxidation of 2,5-disubstituted furan-containing fatty acids by the von Rudloff or Brown procedures results in the production of monoacid and diacid fragments, which can be readily identified by gas liquid chromatography (GLC) analysis. Cleavages occur at the double bonds of the furan ring system with von Rudloff's reagent. A mixture of 2 monoacid and 2 diacid fragments are obtained instead when the furan-containing fatty acid is treated with chromic acid according to Brown's procedure. These chemical methods are suitable for locating the position of the furan system in the fatty acid chain.

## INTRODUCTION

Furan-containing fatty acids have been isolated from seed oil (1) and fish oils (2-6), and were more recently found in high proportion (90%) in the triglyceride fraction of the latex of the rubber tree *(Heavea brasiliensis)* (7). The role of these fatty acids in nature remains vague, but they are believed to be associated with sexual maturity in the fish as these compounds occur in high concentrations in the testes during spawning time.

The author has reported the synthesis of a complete series of 2,5-disubstituted furan fatty acids (8). Some of the chromatographic properties have already been reported (9,10), whereas the spectrometric behavior is currently being assessed (Lie Ken Jie, M.S.F., unpublished results). Mass spectrometric analyses of the various furan fatty acid isomers have clearly shown a characteristic mass fragmentation pattern, which permits the location of the furan ring system in the fatty acid chain (1,8,11,12). We now report a ready chemical method for the determination of the furan ring position by gas liquid chromatography (GLC) analysis of the oxidation products when the furan-containing fatty acid is treated either with von Rudloff's oxidant or chromic acid. The following 4 positional isomers of 2,5-disubstituted $C_{18}$ furan fatty esters were selected for this study:




$CH_3(CH_2)_n$ $(CH_2)_mCOOCH_3$

| n | m |
|---|---|
| 8 | 4 |
| 7 | 5 |
| 3 | 9 |
| 1 | 11 |

## EXPERIMENTAL PROCEDURE

### Modified von Rudloff Oxidative Cleavage Procedure (13)

A mixture of *tert*-butanol (40 ml), 2% aqueous potassium carbonate (7 ml), oxidant (14 ml, prepared by dissolving 0.2 g potassium permanganate and 10.4 g sodium periodate in 500 ml of water) and furan fatty ester (25 mg) contained in a 100-ml stoppered round-bottomed flask was mechanically stirred at room temperature for 18 hr. The light-purple-colored mixture faded only slightly after this reaction period. Sulfur dioxide gas was bubbled through the solution until a persistent light-yellow solution was obtained. Potassium hydroxide pellets (15 pellets = ∿2 g) were added and the mixture swirled until all potassium hydroxide pellets were dissolved. A 2-phase mixture was obtained and a drop of this basic mixture was removed and tested with litmus or universal indicator paper. If the mixture was found to be acidic, more potassium hydroxide was added until a strongly basic solution/mixture was obtained. The mixture was then carefully evaporated under reduced pressure (∿40 mm Hg) using a rotary evaporator at a bath temperature of 70 C until no solvent remained. A white powder was left in the flask, which was acidified with 2 M HCl (30 ml). A 30% sodium chloride solution (10 ml) was added and the resulting mixture extracted with diethyl ether (3 x 20 ml). The ethereal extract was washed with 30% sodium chloride solution (2 x 20 ml) and dried over anhydrous sodium sulfate. The ether was carefully distilled on a water bath (50 C) and the residue treated with

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