Technical

Inhibitors of the Prooxidant Activity of α-Tocopherol

J. Cillard* and **P. Cillard**
Laboratoire de Botanique & Biologie Cellulaire, UER "Medicament," Avenue Leon-Bernard, 35043 Rennes Cedex France

The prooxidant activity of α-tocopherol (α-T) can be reduced or inverted into antioxidant activity by various compounds.

An important antioxidant activity was obtained by the association of α-T with cysteine, BHT, hydroquinone and ascorbyl palmitate. EDTA 10^{-4}M, phosphoric, malonic and citric acids can partially decrease the prooxidant effect of α-T, although the strong acids exhibited no antioxidant activity without α-T. Other amino acids such as glycine and alanine do little to reduce the prooxidant behavior of α-T while they showed a strong antioxidant activity without α-T.

The distribution of hydroperoxide isomers of linoleic acid formed with and without α-T was not modified by the addition of the various compounds except BHT, which led to the production of only 13 *c,t* and 9 *c,t* isomers as observed with α-T.

The oxidation of α-T in the presence of linoleic acid was completely inhibited by the addition of cysteine, phosphoric, malonic and citric acids, BHT, hydroquinone, ascorbyl palmitate and EDTA 10^{-4}M.

The inhibitors of the prooxidant activity of α-T could act in two different ways: by chelating the prooxidant metals traces (i.e., amino acids, EDTA) and/or by regenerating α-T, thus reducing the concentration of chromanoxy radical which would be involved in the prooxidant activity (i.e. phosphoric, malonic, citric acids, cysteine and the common antioxidants).

The antioxidant effectiveness of primary antioxidants such as tocopherols can be enhanced by the addition of various products usually classified as synergists. Different synergists have been used. They include organic and inorganic acids (1-3), amines (4,5), amino acids (2,6,7), nucleic acids (8) and phospholipids (9). Most of these synergists did not show any antioxidative activity when alone except amino acids, which behaved as antioxidants (10-12) or prooxidants (13).

Furthermore, the association of antioxidants also leads to a synergistic effect (14-16). The mechanism of action of synergists is not fully understood and varied with the type of synergist. It is commonly admitted that they act by (i) chelation of the prooxidant metals; (ii) regeneration or sparing of primary antioxidants, or (iii) inhibition of peroxide decomposition, thus interrupting the autoxidation process.

Disodium ethylene diamine tetraacetate (EDTA), a well known metal chelating agent, is also a good inhibitor of lipid oxidation (15).

Synergism has been investigated with respect to the antioxidant activity of α-tocopherol (α-T). However, α-T can exhibit a prooxidant activity especially in aqueous media (17). This prooxidant effect is not appreciable because it involves a degradation of both fatty acid and α-T (Vitamin E) and an increase in the level of peroxides which are toxic products (18).

The purpose of this work is to investigate the action of different compounds which would inhibit the prooxidant activity of α-T. The compounds have been selected among antioxidant synergists such as phosphoric acid, organic acids, amino acids and antioxidants. The action of the chelating agent EDTA also has been studied.

MATERIALS AND METHODS

Reagents and chemicals. Linoleic acid (≥ 99%) was purchased from Koch Light (Colnbrook Bucks, England); α-tocopherol (> 98%) and ascorbylpalmitate (> 97%) from Hoffmann La Roche (Neuilly/Seine, France); citric acid, glycine, alanine, cysteine, hydroquinone, disodium ethylene diamine tetraacetate (EDTA) and Tween 20 from Merck (Darmstadt, Germany); butylhydroxytoluene (BHT) from Schuchart (München, Germany); acetic acid, malonic acid, phosphoric acid (ortho), disodium phosphate, methanol, chloroform and sulfuric acid from Prolabo (Paris, France), and n-heptane "chromasol" by S.D.S. (France).

Procedure. Linoleic acid, α-tocopherol, phosphoric acid, organic acids, amino acids, EDTA, ascorbyl palmitate, hydroquinone and BHT were dispersed with 0.5% Tween 20 in a phosphate buffer solution at pH 7.0 (17). One series of samples contained linoleic acid at a concentration of 2.5×10^{-3}M with either organic acids (10^{-2}M), phosphoric acid (10^{-2}M), amino acids (10^{-2}M), EDTA (10^{-4}, 10^{-6} and 10^{-7}M respectively), ascorbyl palmitate (5×10^{-4}M), hydroquinone (2.5×10^{-5}M) or BHT (2.5×10^{-6}M). The other series of samples was identical to the previous one but contained in addition α-tocopherol at a concentration of 1.25×10^{-4}M.

All the samples were left in the dark and under air at room temperature. Controls without linoleic acid were placed in the same conditions.

Measurements of linoleic acid autoxidation and hydroperoxide isomers. Linoleic acid autoxidation was estimated by the increase of the absorption at 234 nm ascribed to the conjugated dienes formation. Measurement was achieved by a Pye Unicam SP 8-400 Spectrophotometer.

The hydroperoxide isomers of linoleic acid were extracted from the aqueous media at 10 days of autoxidation and separated by high performance liquid chromatography (HPLC) as previously described (19).

Measurement of α-tocopherol. α-Tocopherol was measured by HPLC as previously described (20). Nevertheless, the eluting solvent was modified slightly. It was composed of methanol/water/sulfuric acid N (94:4:2, v/v/v) at a flow rate of 1.5 ml.min. γ-Tocopherol (Hoffmann La Roche, Neuilly/Seine, France) was used as internal standard.

It was dissolved in methanol at a concentration of 50 μg/ml.

*To whom correspondence should be addressed.

RESULTS

Linoleic acid autoxidation: Effect of phosphoric acid and organic acids. As previously described (17), α-T at the concentration of 0.05 mol/mol of linoleic acid exhibited a prooxidant effect (Fig. 1). Associating α-T with phosphoric acid, malonic acid or citric acid suppressed the prooxidant effect of α-T, and we noted an antioxidant effect with the association α-T-malonic acid and α-T-citric acid (Fig. 1). On the contrary, acetic acid did not modify the prooxidant behavior of α-T (Fig. 1).

The autoxidation rate of linoleic acid without α-T was slightly increased by phosphoric acid and organic acids while malonic acid exhibited a net prooxidant activity (Fig. 2).

Effect of amino acids. The addition of alanine or glycine to α-T did not modify the prooxidant effect of α-T during the first four days of linoleic acid autoxidation (Fig. 1). Nevertheless, the maximum conjugated dienes level was decreased two times with these acids. The association of α-T with cysteine led to a strong antioxidant effect (Fig. 1).

The three amino acids (alanine, glycine and cysteine) tested on the autoxidation of linoleic acid without α-T showed antioxidant activity (Fig. 2).

Effect of EDTA. The addition of EDTA slowed down the prooxidant effect of α-T and the autoxidation of linoleic acid without α-T (Fig. 3). The effectiveness of EDTA at reducing autoxidation increased with its concentration.

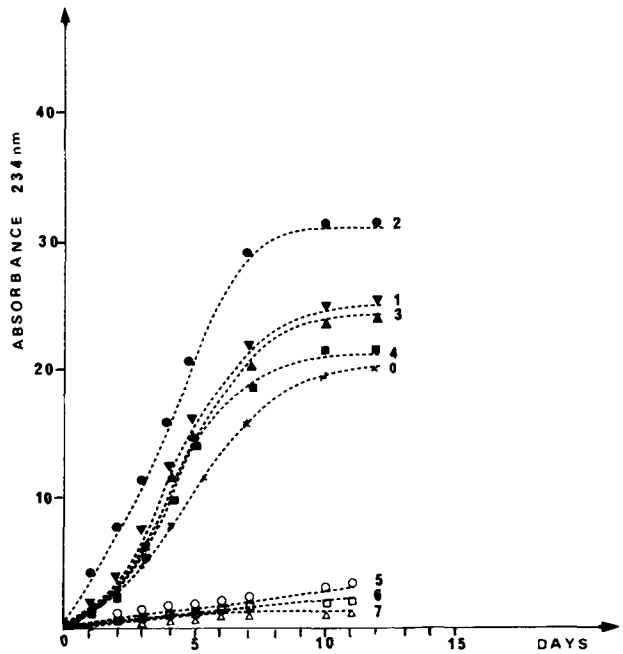

FIG. 2. Conjugated dienes level during linoleic acid (LA) autoxidation. Effect of phosphoric acid, organic acids and amino acids. 0: LA 2.5 × 10^{-1}M alone; 1: LA + phosphoric acid 10^{-2}M; 2: LA + malonic acid 10^{-2}M; 3: LA + citric acid 10^{-2}M; 4: LA + acetic acid 10^{-2}M; 5: LA + alanine 10^{-2}M; 6: LA + glycine 10^{-2}M; 7: LA + cysteine 10^{-2}M.

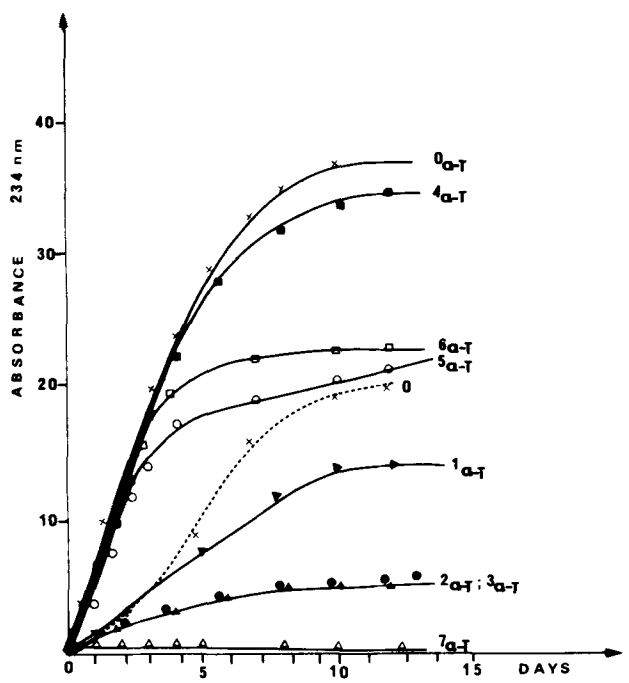

FIG. 1. Conjugated dienes level during the autoxidation of linoleic acid (LA) with α-tocopherol (α-T). Effect of phosphoric acid, organic acids and amino acids. 0 α-T: LA 2.5 × 10^{-1}M + α-T 1.25 × 10^{-4}M; 1 α-T: LA + α-T + phosphoric acid 10^{-2}M; 2 α-T: LA + α-T + malonic acid 10^{-2}M; 3 α-T: LA + α-T + citric acid 10^{-2}M; 4 α-T: LA + α-T + acetic acid 10^{-2}M; 5 α-T: LA + α-T + alanine 10^{-2}M; 6 α-T: LA + α-T + glycine 10^{-2}M; 7 α-T: LA + α-T + cysteine 10^{-2}M. 0: LA 2.5 × 10^{-1}M alone.

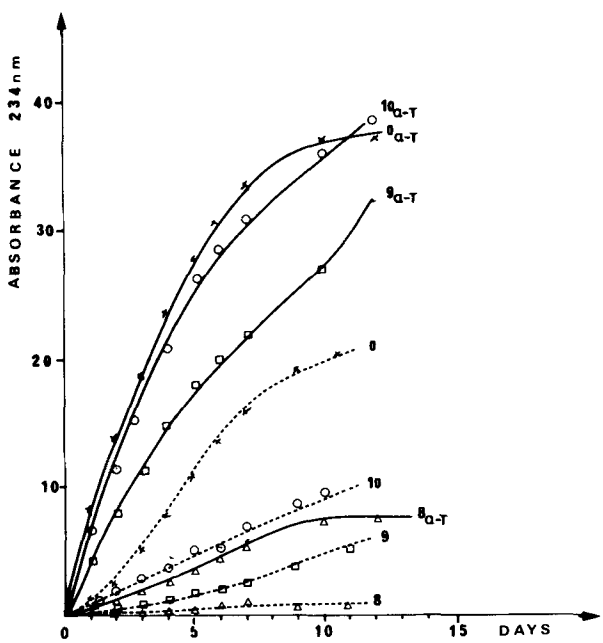

FIG. 3. Conjugated dienes level during linoleic acid (LA) autoxidation. Effect of EDTA. 0: LA 2.5 × 10^{-1}M alone; 8: LA + EDTA 10^{-4}M; 9: LA + EDTA 10^{-6}M; 10: LA + EDTA 10^{-7}M; 0 α-T: LA 2.5 × 10^{-1}M + α-T 1.25 × 10^{-4}M; 8 α-T: LA + α-T + EDTA 10^{-4}M; 9 α-T: LA + α-T + EDTA 10^{-6}M; 10 α-T: LA + α-T + EDTA 10^{-7}M.

For the same concentration, EDTA was less effective when α-T was added to linoleic acid. The minimum concentration of EDTA required to reduce the prooxidant effect of α-T was 10^{-6}M.

Effect of antioxidants. The association of α-T with other antioxidants, such as BHT, hydroquinone and ascorbyl palmitate, suppressed the prooxidant effect of α-T and led to a strong antioxidant effect (Fig. 4). Furthermore, ascorbyl palmitate with α-T involved a rapid decrease of the initial absorbance at 234 nm.

BHT, hydroquinone and ascorbyl palmitate also showed excellent antioxidant activity on the autoxidation of linoleic acid in an aqueous medium (Fig. 4).

Distribution of hydroperoxide isomers. The autoxidation of linoleic acid led to the formation of four hydroperoxide isomers: 13-hydroperoxy-9-*cis*, 11-*trans*-octadecadienoic (13 *c,t*); 13 hydroperoxy-9-*trans*, 11-*trans*-octadecadienoic (13 *t,t*); 9-hydroperoxy-10-*trans*, 12-*cis*-octadecadienoic (9 *t,c*), and 9-hydroperoxy-10-*trans*,12-*trans*-octadecadienoic (9 *t,t*) acids. At 10 days of autoxidation ca. 25% of each isomer was present.

The addition of phosphoric acid, organic acids, amino acids and EDTA did not affect the distribution of the four isomers whatever the antioxidant or prooxidant effect or the lack of effect of these compounds on the autoxidation rate of linoleic acid.

On the contrary, when α-T was added to linoleic acid at the concentration of 0.05 mol/mol of linoleic acid, only the isomers 9 *t,c* (50%) and 13 *c,t* (50%) were formed (18).

The association of α-T with phosphoric acid, organic acid, amino acids and EDTA did not modify the distribution of the hydroperoxide isomers previously noted with α-T. Equal amounts of 9 *t,c* and 13 *c,t* isomers were produced whatever the prooxidant effect or the antioxidant effect induced by the association.

In the same way the addition of BHT to linoleic acid with or without α-T led to the production of only *c,t* isomers. The formation of *t,t* isomers was inhibited by BHT with and without α-T. Paradoxically, in the samples containing hydroquinone or ascorbyl palmitate instead of BHT, no hydroperoxide isomer was detected by HPLC.

α-Tocopherol oxidation. As previously mentioned, α-T in the presence of linoleic acid was completely oxidized within 14 days while α-T alone was almost stable in the same conditions (18).

The association of phosphoric, malonic, citric acids, cysteine, BHT, hydroquinone, ascorbyl palmitate and EDTA 10^{-4}M with α-T in the presence of linoleic acid resulted in a complete protection of α-T from oxidation even when the autoxidation of linoleic acid was substantial as in the sample with phosphoric acid (Fig. 5).

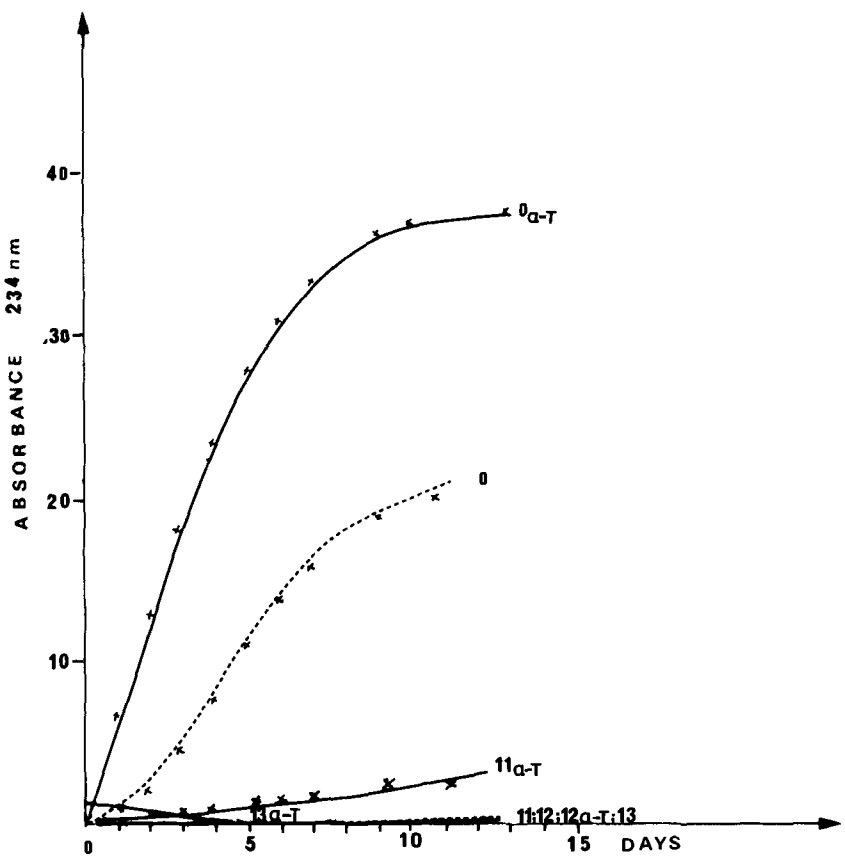

FIG. 4. Conjugated dienes level during linoleic acid (LA) autoxidation. Effect of antioxidants. 0: LA 2.5 × 10^{-3}M alone; 11: LA + BHT 2.5 × 10^{-6}M; 12: LA + hydroquinone 2.5 × 10^{-5}M; 13: LA + ascorbyl palmitate 5 × 10^{-4}M; 0 α-T: LA 2.5 × 10^{-3}M + α-T 1.25 × 10^{-4}M; 11 α-T: LA + α-T + BHT 2.5 × 10^{-6}M; 12 α-T: LA + α-T + hydroquinone 2.5 × 10^{-5}M; 13 α-T: LA + α-T + ascorbyl palmitate 5 × 10^{-4}M.

Alanine, glycine and EDTA 10^{-6}M were less effective in protecting α-T, although only 25% of α-T was oxidized at 14 days (Fig. 5). EDTA 10^{-7}M involved no protection of α-T, while acetic acid slightly delayed the oxidation of α-T (Fig. 5).

DISCUSSION

The best inhibition of the prooxidant effect of α-T was obtained by the association with cysteine, usual antioxidants such as BHT, hydroquinone and ascorbyl palmitate.

A strong antioxidant activity of these compounds was observed with and without α-T. Previously, we noted that cysteine was also a good inhibitor of the autoxidation of arachidonic acid with and without α-T at a prooxidant level (21). A synergistic effect of ascorbic acid on the antioxidant activity of α-T in aqueous media has been reported previously and was ascribed to the regeneration of α-T by ascorbic acid (22).

Strong acids such as phosphoric, citric or malonic acids were also effective in reducing the prooxidant activity of α-T, but the magnitude of their inhibitory effect was less important than the one observed with the previous compounds.

On the contrary, a weak acid such as acetic acid did not modify the prooxidant activity of α-T.

The strong acids showed no antioxidant activity on linoleic acid without α-T.

Loury et al. (1) have also noted a synergistic effect of phosphoric acid on both antioxidant and prooxidant activities of α-T. They suggested that phosphoric acid regenerated α-T by providing protons.

In our experiment α-T does not undergo any oxidation when it is associated with strong acids.

Nevertheless, phosphoric acid, like citric and malonic acids, is also known to complex metals (23).

An increase of the concentration of these strong acids from 10^{-2}M to 0.2 M does not alter the magnitude of their inhibitory effect.

We have noted that hydrochloric acid is also a good inhibitor of the prooxidant activity of α-T.

Although all the samples were prepared with a phosphate buffered solution at pH 7, the addition of strong acids lowered the pH to 2.4-2.8. Increasing the pH to 7 may suppress the inhibitory effect of these acids.

Thus, acidic pH favors the inhibitory activity of these acids.

The metal chelating agent EDTA at the concentration of at least 10^{-4}M also inhibited the prooxidant activity of α-T. The magnitude of its inhibitory effect at 10^{-4}M was practically the same as the one observed with citric and malonic acids at 10^{-2}M. Labuza et al. (24) stated that EDTA was a superior chelating agent compared to citric acid.

Other compounds such as alanine and glycine, which are good antioxidants on linoleic acid alone, were practically ineffective on the prooxidant behavior of α-T, although they chelate the prooxidant metals (23).

The distribution of hydroperoxides isomers of linoleic acid with and without α-T was not modified by strong acids, amino acids and EDTA. Without α-T, four isomers (9 *trans-cis*; 9 *trans-trans*; 13 *cis-trans* and 13 *trans-trans*) were formed, while with α-T only the *cis-trans* isomers were produced independently of the prooxidant or antioxidant activity of the association. The inhibition of the formation of *trans-trans* isomers

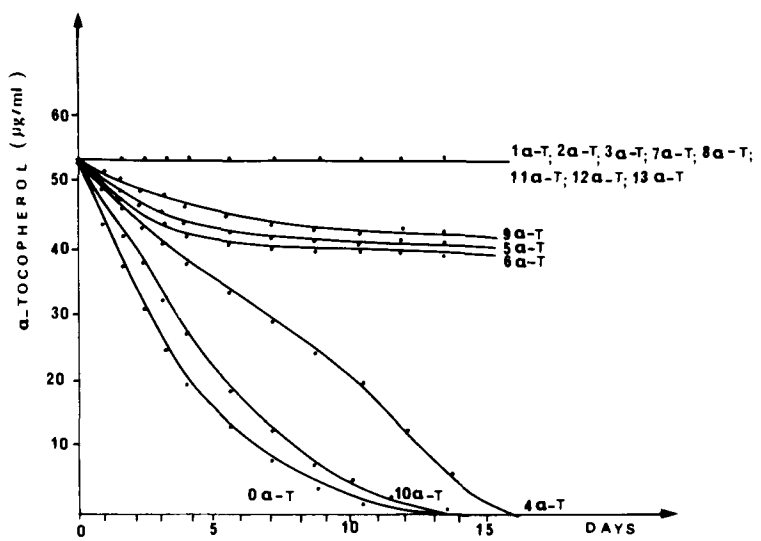

FIG. 5. HPLC measurement of α-tocopherol (α-T) during linoleic acid (LA) autoxidation. Effect of phosphoric acid, organic acids, amino acids, EDTA and antioxidants. 0 α-T: LA 2.5×10^{-3}M + α-T 1.25×10^{-4}M; 1 α-T: LA + α-T + phosphoric acid; 2 α-T: LA + α-T + malonic acid; 3 α-T: LA + α-T + citric acid; 4 α-T: LA + α-T + acetic acid; 5 α-T: LA + α-T + alanine; 6 α-T: LA + α-T + glycine; 7 α-T: LA + α-T + cysteine; 8 α-T: LA + α-T + EDTA 10^{-4}M; 9 α-T: LA + α-T + EDTA 10^{-6}M; 10 α-T: LA + α-T + EDTA 10^{-7}M; 11 α-T: LA + α-T + BHT; 12 α-T: LA + α-T + hydroquinone; 13 α-T: LA + α-T + ascorbyl palmitate.

by tocopherols at high concentration has been discussed earlier (18,25). In the same way, BHT inhibited the production of *trans-trans* isomers. On the contrary, no hydroperoxide isomers were detected with hydroquinone and ascorbyl palmitate, with or without α-T.

The inhibitors of the prooxidant activity of α-T could act in two different ways:

- by chelating the prooxidant metals
- by regenerating α-T, thus reducing the concentration of chromanoxy radical which would be involved in the prooxidant activity.

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Effect of TBHQ on Quality Characteristics of RBD Olein During Frying

Telingai Asap and M.A. Augustin
Faculty of Food Science and Technology, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor, Malaysia

The changes in quality characteristics of refined, bleached and deodorized palm olein (RBD olein) during heating with intermittent frying for 5 hr/day for weight consecutive days in three systems were compared. The systems were (i) RBD olein without antioxidant (system 1); (ii) RBD olein to which 200 ppm of tertiary butylhydroquinone (TBHQ) had been added prior to frying on the first day (system 2), and (iii) RBD olein which had TBHQ added to a level of 200 ppm at the start of each day. The addition of TBHQ reduced the level of polar components and polymers in the oil, decreased the rates of change in iodine value and dielectric constant and decreased the rate of oxidation of C18:2. The reduction in the rates of these undesirable changes was more pronounced when the TBHQ was added to the system on each day of frying than when there was a single addition of TBHQ prior to frying on the first day. The undesirable effect of adding TBHQ was that it darkened the oil.

The liquid fraction of palm olein is being used increasingly in frying operations. The changes in quality during frying are of importance, as frying oil is absorbed by the food being fried and forms an important constituent of the diet.

Oils undergo a complex series of changes and reactions during frying (1,2). The physical and chemical changes occurring in oils under frying conditions have been studied (3-5). However, the rate of changes occurring during frying is affected by the frying conditions and the characteristics of the fat.

This study investigated the changes in oil quality characteristics of RBD olein during frying conditions. The effect of a single addition of TBHQ to the frying oil was compared with the effect of adding TBHQ on each day.

EXPERIMENTAL PROCEDURES

Materials. RBD olein was obtained from Lam Soon Oil and Soap Mfg. Sdn. Bhd.; TBHQ (97%) was from Aldrich Chemical Co. Inc., Milwaukee, Wisconsin. Potatoes were obtained from a local supermarket. They were cleaned, then sliced with a mechanical slicer. Potato slices (2 mm) were washed with water. Excess water was drained off before the pieces were fried.

Frying experiments. The conditions used were heating with intermittent frying for 5 hr/day for eight consecutive days. The systems were (i) RBD olein without antioxidant (system 1); (ii) RBD olein to which 200 ppm TBHQ had been added prior to frying on the first day (system 2); and (iii) RBD olein which had TBHQ added up to a level of 200 ppm at the start of each day