trans-10,*cis*-12-Conjugated Linoleic Acid Isomer Exhibits Stronger Oxyradical Scavenging Capacity than *cis*-9,*trans*-11-Conjugated Linoleic Acid Isomer

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Although studies have been performed to test whether conjugated linoleic acid (CLA) acts as an antioxidant, the results were not conclusive. In addition, the CLA widely used in previous research contains 43% *cis*-9,*trans*-11 isomer, 45% *trans*-10,*cis*-12 isomer, and 10 other minor isomers. The objective of this study was to investigate the antioxidant activity of *cis*-9,*trans*-11- and *trans*-10,*cis*-12-CLA isomers using high-purity CLA isomers (>98%) by total oxyradical scavenging capacity assay (Winston, G. W.; Regoli, F.; Dugas, Jr., A. J.; Fong, J. H.; Blanchard, K. A. *Free Radical Biol. Med.* **1998**, *24*, 480−493). At all concentrations (2−200 μM), t10,c12-CLA performed as an antioxidant with a 15-min lag phase, which was more effective than c9,t11-CLA and α-tocopherol at lower concentrations (2 and 20 μM). On the other hand, c9,t11-CLA possessed weak antioxidant activity at 2 and 20 μM, whereas at 200 μM it acted as a strong pro-oxidant, which suggests that discrepancies of the results of the previous studies on the antioxidant properties of CLA may be due to the balance of the antioxidant properties of t10,c12-CLA and the pro-oxidant properties of c9,t11-CLA in different oxidation conditions.

Keywords: *Conjugated linoleic acid; antioxidant; TOSC assay*

INTRODUCTION

Conjugated linoleic acid (CLA), an anticarcinogen abundantly found in dairy products, is a mixture of positional and geometric isomers of linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid). The total content of CLA in foods varies widely, but the *cis*-9,*trans*-11-isomer was found to be the predominant form (as much as 90% of the total CLA; Chin et al., 1992). It was first found that the *cis*-9,*trans*-11-isomer was the only one incorporated into the tissue phospholipids, suggesting that the *cis*-9,*trans*-11-isomer may be the biologically active form (Ha et al., 1990; Ip et al., 1991). However, recent studies have shown that all CLA isomers, with no apparent preference for individual isomers, were incorporated in both the phospholipid and the non-phospholipid fractions of the tissues with various relative concentrations in different tissues (Belury and Kempa-Steczko, 1997; Park et al., 1995; Yu et al., 1998). The results suggest the possible importance of the individual CLA isomers in effecting response in different tissues (Park et al., 1995). However, the effects of individual isomers have not yet been substantially tested. Although several reports have shown the biological activities of the *trans*-10,*cis*-12-isomer (Park et al., 1999; Cook et al., 1999; DeVoney et al., 1999), the *cis*-9,*trans*-11-isomer was shown to activate peroxisome proliferator-activated receptor-α (PPARα), which may modulate hepatic lipid metabolism and help to elucidate the mechanism of anticarcinogenic properties of CLA (Lu and Belury, 1999). It is uncertain whether one or more of these isomers are responsible for the biological activities of CLA.

Although many studies have shown that CLA acts as an effective anticarcinogen in both animal (Ha et al., 1987; Ip et al., 1991, 1996; Belury et al., 1996) and cell culture (Shultz et al., 1992a,b; desBordes and Lea, 1995; Durgam and Fernandes, 1997) models, the mechanisms responsible for its anticarcinogenic properties have not been elucidated. One hypothesis is that CLA modulates cell oxidation (Ha et al., 1990; Shultz et al., 1992a,b). Four studies showed that CLA possessed antioxidant properties (Ha et al., 1990; Ip et al., 1991, 1996; Nicolosi et al., 1997), whereas others showed that CLA was not an antioxidant (van den Berg et al., 1995; Banni et al., 1998) and acted as a pro-oxidant (Chen et al., 1997; Cantwell et al., 1998). Moreover, the CLA used in the above research is mainly a mixture of isomers rather than a pure individual isomer. The synthetic CLA widely used in the CLA studies has 12 different isomers, in which *cis*,*trans*/*trans*,*cis*-9,11-CLA (∼43%) and *trans*,*cis*/*cis*,*trans*-10,12-CLA (∼45%) are the main isomers (Sehat et al., 1998). It is uncertain whether there are differential effects on oxidation between these isomers. The objective of this study was to investigate the total antioxidant activity of *trans*-10,*cis*-12-CLA and *cis*-9,*trans*-11-CLA using high-purity CLA isomers (>98%) by total oxyradical scavenging capacity (TOSC) assay (Winston et al., 1998). The TOSC assay is based on the reaction between peroxyl radicals [or hydroxyl or alkoxyl radicals, which are generated by thermal homolysis of 2,2′-azobis(amidinopropane) (ABAP)] and α-keto-γ-methiolbutyric acid (KMBA), which is oxidized to ethylene on reaction with various reactive oxygen species (Yang,

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1969; Winston et al., 1998):

$$CH_3SCH_2CH_2COCOOH + {}^{\bullet}OOH\ (R) \text{ or } {}^{\bullet}OH\ (R) \rightarrow$$

$$^1/_2(CH_3S)_2 + (R)\ HOO^- \text{ or } (R)\ HO^- + 2CO_2 + CH_2{=}CH_2\uparrow$$

To our knowledge, this is the first study to investigate the antioxidative role of high-purity individual CLA isomers. These data may help to answer two questions: (1) whether CLA is an antioxidant or a pro-oxidant; (2) which major CLA isomer(s) is(are) responsible for the antioxidant activity of CLA.

MATERIALS AND METHODS

Chemicals. High-purity *cis*-9,*trans*-11-CLA (c9, t11-CLA) and *trans*-10,*cis*-12-CLA (t10,c12-CLA) isomers were purchased from Matreya, Inc. (Pleasant Gap, PA). The manufacturer's analysis claimed that the purities of c9,t11-CLA and t10,c12-CLA were both >98%. Linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid, LA), α-keto-γ-methiolbutyric acid (KMBA), α-tocopherol, and L-ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO). 2,2′-Azobis(amidinopropane) (ABAP) and Tween 20 (polyoxyethylene sorbitan monolaurate) were purchased from Wako Chemicals (Richmond, VA) and Fisher Scientific Co. (Fair Lawn, NJ), respectively.

Preparation of Emulsion Samples. Stock solutions of 20 mM samples (LA, c9,t11-CLA, t10,c12-CLA, and α-tocopherol) were prepared in water with 0.0842 g of Tween 20 by vortex for 3 min. Working solutions (400 μM) were prepared from the stock solutions by dilution with water. Solutions of L-ascorbic acid (with and without Tween) were freshly made for each experiment.

TOSC Assay. The method of the TOSC assay was modified from that of Winston et al. (1998). In brief, reaction was carried out in a 4 mL Teflon/silicone septum-sealed sample vial in a final volume of 1 mL containing 0.2 mM KMBA, and 20 mM ABAP in 100 mM potassium phosphate buffer (pH 7.4) with different concentrations of CLA and LA emulsified with corresponding amounts of Tween 20 (i.e., 8.4×10^{-6}% Tween for 2 μM CLA/LA, 8.4×10^{-5}% Tween for 20 μM CLA/LA, and 8.4×10^{-4}% Tween for 200 μM CLA/LA). The generation of oxyradicals was initiated by injection of 100 μL of 200 mM ABAP in water directly through the septum into the vials, and the mixture was then incubated in a water bath at 37 °C. Ethylene production was measured by direct injection of 0.5 mL of headspace volatile into an HP5890 gas chromatograph with a flame ionization detector (Hewlett-Packard) using a heated, gastight syringe. The GC analysis was carried out isothermally at 60 °C. A 60 m × 0.53 mm i.d. fused silica capillary column (Supelcowax-10, Supelco Inc., Bellefonte, PA) with a 0.5-μm film thickness was used for elucidation of ethylene. Helium was used as the carrier gas with a flow rate of 2.4 mL/min, with a splitless injector and detector set at 180 and 200 °C, respectively.

Quantification of TOSC. The TOSC value of each concentration of different compounds was calculated as described in Winston et al. (1998). The area under the kinetic curve was calculated by integration. TOSC is then quantified according to the equation

$$\text{TOSC} = 100 - (\int \text{SA}/\int \text{CA} \times 100)$$

where ∫SA and ∫CA are the integrated areas from the curve defining the sample and control reactions, respectively. In the case of samples without Tween 20, the control was all reagents except the samples. In the case of samples with Tween 20, the control was all reagents (including the corresponding amount of Tween 20) except the samples. Samples with positive TOSC values were assigned as antioxidant, whereas those with negative TOSC values were treated as pro-oxidant.

Statistical Analysis. All values of ethylene production and TOSC values were presented as mean ± SD for at least three replications. Statistical analyses were conducted using SigmaStat version 1.0 (Jandel Corp., San Rafael, CA). Differences among treatments were determined using one-way analysis of variance (ANOVA) and the Student−Newman−Keuls pairwise test ($p < 0.05$).

RESULTS

Time Course of KMBA Oxidation with Different Concentrations of the Samples. Panels A−E of Figure 1 show the time courses for anti-/pro-oxidant activity of different concentrations of c9,t11-CLA, t10,c12-CLA, LA, α-tocopherol, and L-ascorbic acid. At lower concentrations of c9,t11-CLA (2 and 20 μM), ethylene production from KMBA was decreased compared with the control, but there are no lag phases observed (Figure 1A). At high concentration of c9,t11-CLA (200 μM), ethylene production from KMBA was elevated at all time points compared with the control (Figure 1A).

For t10,c12-CLA, ethylene production from KMBA was reduced with the increase of concentrations from 20 to 200 μM (Figure 1B). All concentrations of t10,c12-CLA (2, 20, and 200 μM) results in a 15-min lag phase in which ethylene formation was completely inhibited (Figure 1B). The ethylene production from KMBA was decreased after 15-min lag phase compared with the control.

In the presence of LA, ethylene formation from KMBA was slightly affected, with a 15-min lag time only at the concentration of 200 μM (Figure 1C).

For α-tocopherol, ethylene production was reduced with the increase of concentrations from 20 to 200 μM (Figure 1D). Higher concentrations resulted in greater inhibition effects (Figure 1D). Moreover, for concentrations of 20 and 200 μM, the presence of α-tocopherol resulted in a 15-min lag phase of KMBA oxidation (Figure 1D).

In the presence of L-ascorbic acid, a linear response was observed between TOSC value and the amount of L-ascorbic acid (2−200 μM; Figure 2). Ethylene production was quantitatively reduced beyond 30 min for 20 and 200 μM and beyond 45 min for 2 μM (Figure 1E). However, for all concentrations of L-ascorbic acid, ethylene formation was enhanced at 15 min, and higher concentrations of L-ascorbic acid possessed greater enhancing effects of ethylene formation (Figure 1E).

Effect of Tween on Ethylene Production and TOSC Value. The effect of different concentrations of Tween 20 on ethylene production and the calculated TOSC values of Tween 20 relative to control are shown in Figure 1F and Table 1, respectively. At low concentration (8.4×10^{-6}%) and high concentration (8.4×10^{-4}%) of Tween, there was weak antioxidant activity (TOSC value below 4 for 8.4×10^{-6}% and ~10 for 8.4×10^{-4}%; Table 1), with no significant difference between the TOSC values of 8.4×10^{-6}% and 8.4×10^{-4}% Tween ($p > 0.05$). However, at medium concentration (8.4×10^{-5}%), weak pro-oxidant activity was observed (TOSC value = −6.83; Table 1), and significant differences among the TOSC values of three concentrations of Tween 20 ($p < 0.01$) resulted. Figure 2 shows the comparison of the TOSC values of L-ascorbic acid in the presence and in the absence of Tween 20. The antioxidant activity of L-ascorbic acid was dose-dependent either with or without Tween. Although no significant difference of the TOSC values was obtained at a low

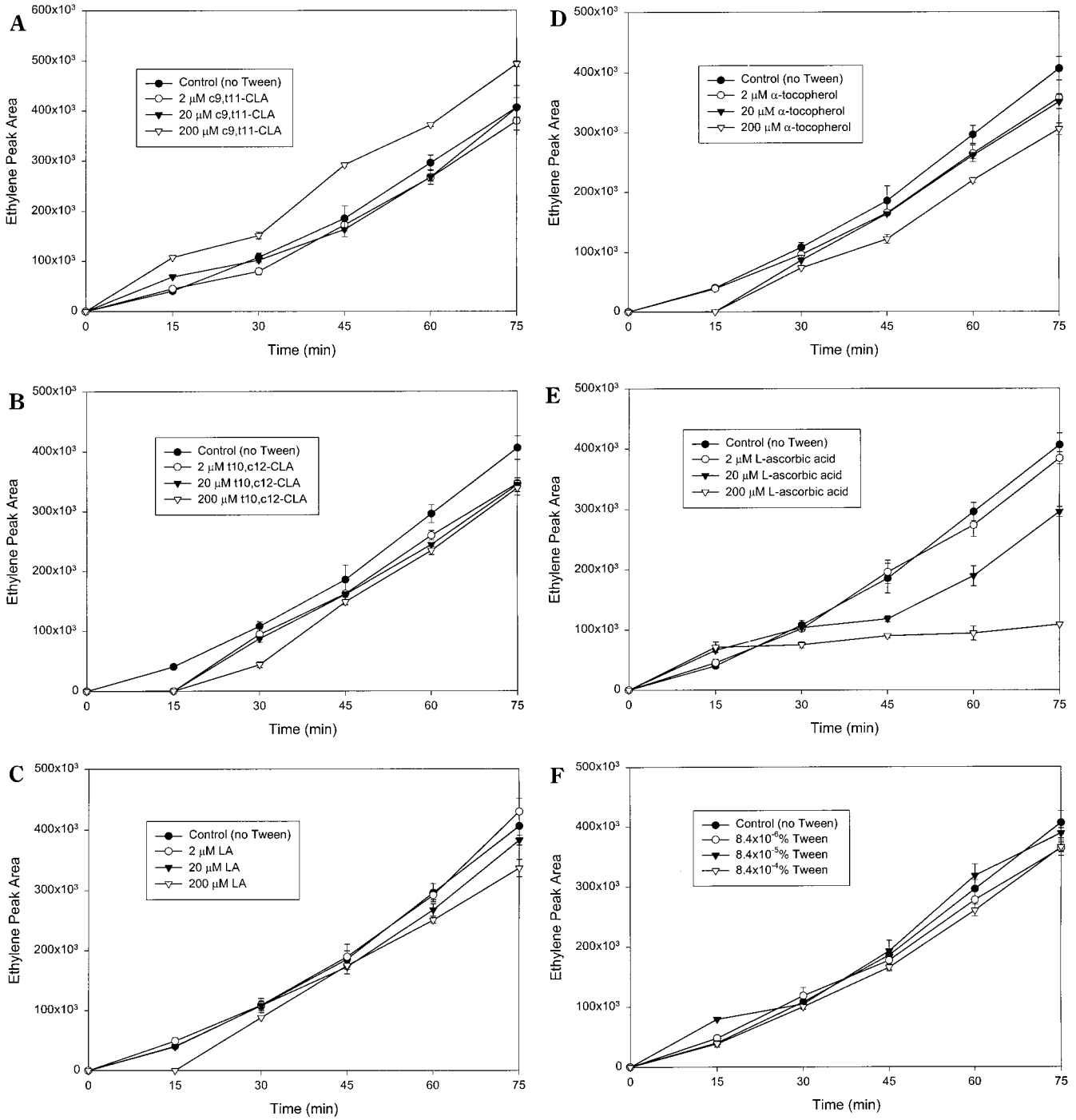


Figure 1. Time courses of KMBA oxidation by peroxyl radicals in the presence of different concentrations of (A) c9,t11-CLA, (B) t10,c12-CLA, (C) LA, (D) α-tocopherol, (E) L-ascorbic acid, and (F) Tween 20. Data are expressed as mean ± SD; $n = 3$ samples.

concentration of L-ascorbic acid (2 μM; $p > 0.05$), there was significant difference between the TOSC values of L-ascorbic acid in the presence and in the absence of Tween 20 at higher concentrations (20 and 200 μM; $p < 0.05$).

TOSC of the Samples Tested at Different Concentrations. Figure 3 compares different samples at the same concentration with the same amount of Tween 20. The resultant TOSC values of the samples at different concentrations are shown in Figure 4. At lower concentrations (2 and 200 μM), c9,t11-CLA acted as a weak antioxidant (with both TOSC values below 10; Figure 4). However, at the concentration of 200 μM, c9,-t11-CLA possessed strong pro-oxidant activity (TOSC value = −56.7). On the other hand, for all three concentrations tested, t10,c12-CLA exhibited antioxidant activity. TOSC values of t10,c12-CLA increased from 15 at 2 μM to 25 at 20 μM and then decreased to 20 at 20 μM (Figure 4). Compared at low concentration (2 μM) with α-tocopherol, the TOSC value of t10,c12-CLA was 2 times higher than that of α-tocopherol. For higher concentrations (20 and 200 μM), the TOSC values of t10,c12-CLA and α-tocopherol were similar (between 20 and 25). At low concentration (2 μM), LA possessed weak pro-oxidant activity (TOSC value = −5.84). However, at higher concentrations (20 and 200 μM), LA exhibited weak antioxidant activity (TOSC values of ∼10; Figure 4). For L-ascorbic acid, the TOSC values increased with increasing concentration, from 8.0 at 2 μM to 33.1 at 20 μM to 45.7 at 200 μM.

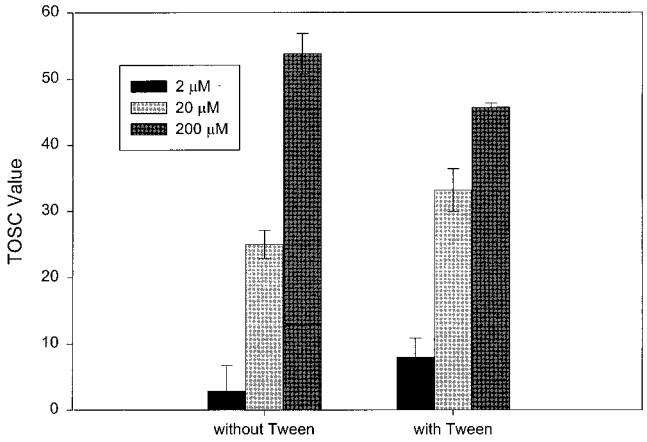


Figure 2. Effect of Tween 20 on the TOSC value of L-ascorbic acid. Data are expressed as mean ± SD; $n = 3$ samples.

Table 1. TOSC Values of Different Concentrations of Tween 20

concn of Tween 20 (%)	TOSC value (mean ± SD)
8.40×10^{-6}	3.65 ± 4.59
8.40×10^{-5}	−6.83 ± 3.50
8.40×10^{-4}	10.33 ± 2.07

DISCUSSION

The TOSC assay is a new, simple, and reliable gas chromatographic assay of total antioxidant activity developed by Winston and co-workers in 1998. There are two important pieces of information that can be generated from the TOSC assay. First, from the time courses of KMBA oxidation by peroxyl radicals in the presence of an antioxidant, the lag phase of the oxidation can be obtained when ethylene formation is largely or completely inhibited relative to the control. The lag phase, or induction period, is defined as an initial period of oxidation of an organic compound in which little oxidation occurs, which is followed by a rapid increase in the rate of autocatalysis by chain-propagating intermediates and, finally, a decrease in the rate of oxidation (Cadenas and Sies, 1998). In general, the greater the ability of an antioxidant to lengthen the lag phase, the better the antioxidant.

Second, from the calculated TOSC values of a compound at different concentrations, the antioxidant/pro-oxidant status of the compound can be determined. The greater the positive TOSC value, the higher the antioxidant activity. Although the TOSC assay was originally developed for screening the antioxidant potential of a pure solution or biological samples, it is also useful for determining the pro-oxidant status of a compound or a biological tissue. A negative value for a compound indicates that the compound acts as a pro-oxidant rather than as an antioxidant.

Effect of Emulsifier on TOSC Values of Water-Soluble and Lipid-Soluble Antioxidants. To incorporate the lipid-soluble compounds (i.e., LA, c9,t11-CLA, t10,c12-CLA, and α-tocopherol) in water-based solution, Tween 20 was used as an emulsifier in the present study. Depending on the concentration, Tween 20 acted as both an antioxidant (at 8.4×10^{-6}% and 8.4×10^{-4}%) and a pro-oxidant (at 8.4×10^{-5}%), although the effect was weak (TOSC values = ±10; Table 1). The variation of the TOSC values of Tween 20 at different concentrations can be explained by the balance between the pro-oxidant and antioxidant effects of Tween 20. Tween 20 is a nonionic surfactant produced by esterification of polyoxyethylene sorbitol with lauric acid (Benson, 1966). The sugar unit of sorbitol in Tween can possess an inhibitory effect on oxidation by scavenging radicals and hydroperoxides, which allows Tween to act as an antioxidant. Moreover, the presence of emulsifier increase the viscosity, which may reduce the mobility of the reactants and reaction products and thus lower the oxidation rate (Blaug and Hajratwala, 1974; Ponginebbi et al., 1999). Besides, KMBA dissolved in water may be protected from the peroxyl radicals and oxygen by the surfactant micelles (Blaug and Hajratwala, 1974). However, Tween can also act as a pro-oxidant because the polyoxyethylene surfactants can undergo autoxidation in the hydrophilic chain to yield hydroperoxides of the

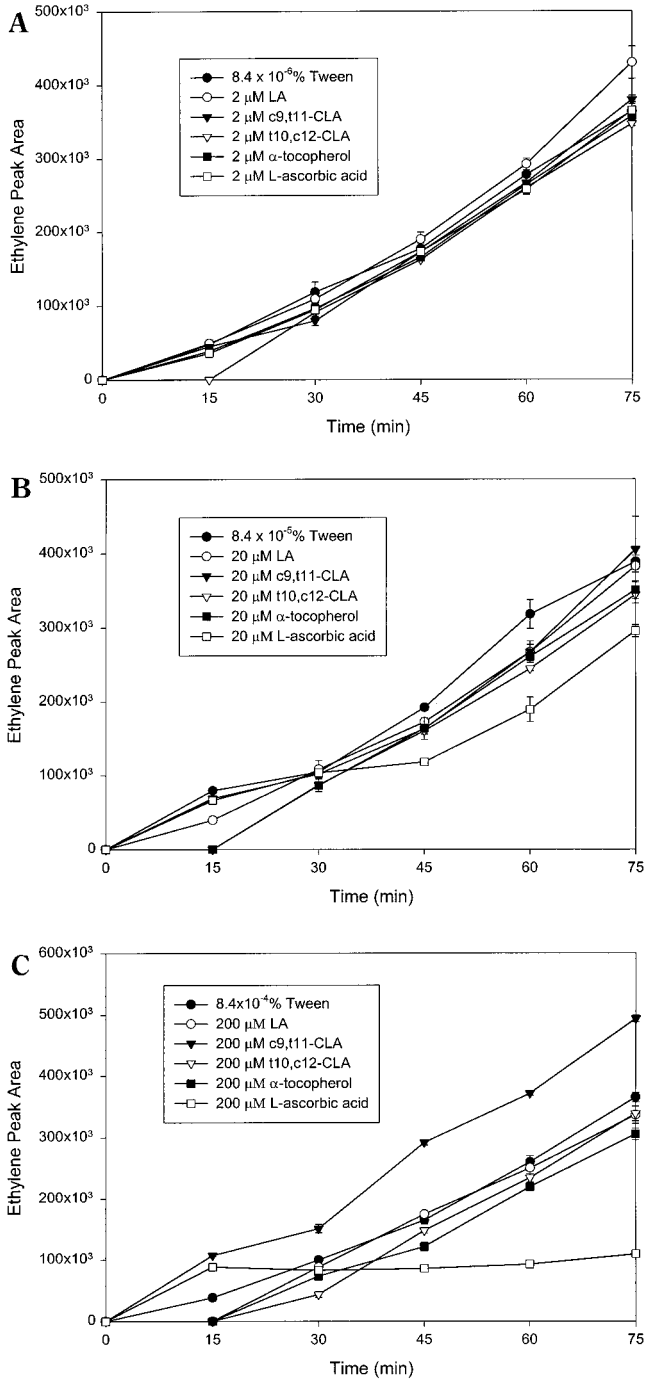


Figure 3. Time courses of KMBA oxidation by peroxyl radicals in the presence of c9,t11-CLA, t10,c12-CLA, LA, α-tocopherol, and L-ascorbic acid at (A) 2 μM, (B) 20 μM, and (C) 200 μM. Data are expressed as mean ± SD; $n = 3$ samples.

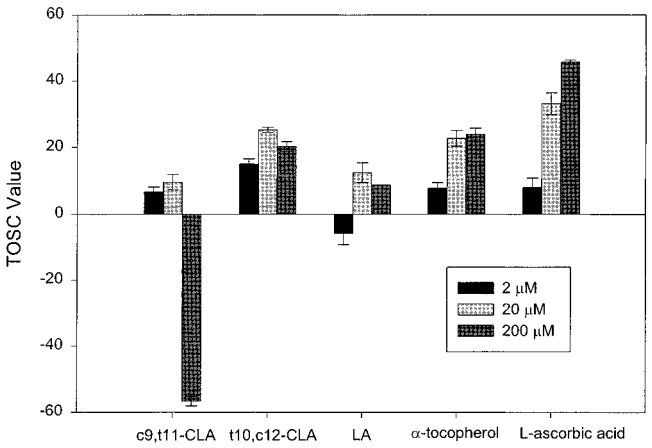


Figure 4. TOSC values of c9,t11-CLA, t10,c12-CLA, LA, α-tocopherol, and L-ascorbic acid. Data are expressed as mean ± SD; $n = 3$ samples.

oxyethylene units (Ding, 1993; Donbrow et al., 1978; Donnelly et al., 1998):

$$-(OCH_2CH_2)O- + O_2 \rightarrow -[OCH_2C(OOH)H]O-$$

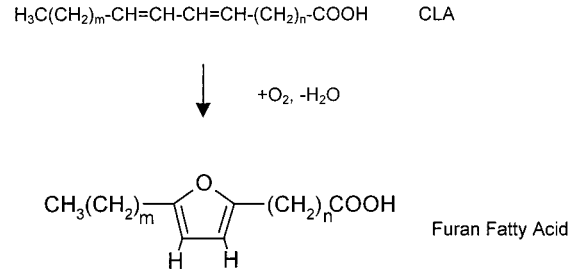
On the one hand, at the concentration of 8.4×10^{-5}%, the pro-oxidant effect of Tween 20 overrides its antioxidant effect, resulting in the negative TOSC value. On the other hand, at concentrations of 8.4×10^{-6}% and 8.4×10^{-4}%, the antioxidant effect of Tween 20 overrides its pro-oxidant effect, giving the positive TOSC values of Tween.

In a comparison of the TOSC values of L-ascorbic acid (water-soluble antioxidant) in the presence and absence of Tween 20, there were significant differences between the TOSC values of L-ascorbic acid at higher concentrations (20 and 200 μM), even after the correction by the background Tween (Figure 2). The results suggested that apart from the nonspecific antioxidant/pro-oxidant effect of Tween, other specific interactions between ascorbic acid and Tween took place that affected the results. As mentioned by Blaug and Hajratwala (1974), ascorbic acid molecules can be adsorbed on the surface of the surfactant molecules, making them more susceptible to oxidation through surface catalysis. An association complex between ascorbic acid and the surfactant molecules can be formed, which also makes ascorbic acid more susceptible to oxidation and thus increases the efficiency of peroxyl radical scavenging. On the other hand, it has been reported that hydrogen bonds can be formed between phenolic hydroxyl groups and the ether oxygen in the polyoxyethylene chain of the nonionic surfactants (Mulley and Metcalf, 1956). Ascorbic acid (which contains four hydroxyl groups) may form hydrogen bonds with Tween 20, which reduces the mobility of ascorbic acid and thus its antioxidant efficiency.

When using α-tocopherol (lipid-soluble antioxidant) as a positive control, the TOSC values of α-tocopherol at all concentrations were lower than those of L-ascorbic acid, particularly at higher concentrations (20 and 200 μM), and the TOSC values of α-tocopherol were significantly lower than those of L-ascorbic acid ($p < 0.05$ for 20 μM and $p < 0.0001$ for 200 μM). It is agreed with the previous observation that ascorbic acid performs better as an antioxidant in aqueous solution (Niki et al., 1985; Doba et al., 1985), whereas α-tocopherol is more efficient in scavenging free radicals in the inner phase of liposome (Niki et al., 1985) and emulsion (Porter et al., 1989). Moreover, the molecules of emulsifier surrounding the lipid-soluble antioxidant can form a protective layer which makes the free radicals less accessiable to the antioxidant. Although it has been shown that ABAP partitions as much as 91% into the micellar phase of sodium dodecyl sulfate (SDS) micelles due to electrostatic interactions between the positive amidino groups of ABAP and the anionic surface of the SDS micelle (Barclay et al., 1987), Tween 20 is a nonionic surfactant and the partition of ABAP into the inner phase of Tween emulsion would be expected to be much less than that of SDS micelle. The difference in physical location of water- and lipid-soluble antioxidants may be one reason studies using TOSC assay in biological samples always showed the lower TOSC value (antioxidant activity) of the proteic fraction compared with that of the soluble fraction of biological samples (Winston et al., 1998; Regoli and Winston, 1998; Regoli et al., 1998). However, even though the antioxidant activity of the lipid-soluble antioxidant was lower than that of the water-soluble antioxidant using the TOSC assay, the present assay is useful in screening the lipid-soluble antioxidant, as the antioxidant activity of α-tocopherol was generally dose-dependent. The "saturation" of the antioxidant activity of α-tocopherol at 200 μM may be due to aggregation of antioxidant-containing emulsions, which reduces the surface area of the emulsions and thus the accessibility of water-soluble oxyradicals to the lipid-soluble antioxidant.

Antioxidant/Pro-oxidant Activity of c9,t11-CLA and t10,c12-CLA. The TOSC values of c9,t11-CLA show that at lower concentrations (2 and 20 μM) c9,t11-CLA possesses weak antioxidant activity, whereas at a concentration of 200 μM it acts as a strong pro-oxidant (Figure 4). We are not sure how c9,t11-CLA at high concentration can perform as a pro-oxidant. However, as noted by Kamal-Eldin and Appelqvist (1996), the antioxidants and/or their relatively unreactive radicals often undergo other side reactions that may be classified as pro-oxidative. The degree of such reactions depends on many factors such as oxidation conditions and concentration. Indeed, even for tocopherols (which are well recognized for their effective inhibition of lipid oxidation in both food and biological systems), this "paradoxical behavior of antioxidant" (Porter, 1993) has been reported in low-density lipoproteins (Bowry et al., 1992), bulk oils, and dispersed systems (Cillard and Cillard, 1980).

An important finding of the present study is that t10,c12-CLA performed differently in the modulation of KMBA oxidation compared with c9,t11-CLA. At all concentrations tested, t10,c12-CLA performed as an antioxidant with a 15-min lag phase (Figures 1B and 4). At lower concentrations (2 and 20 μM), t10,c12-CLA was more effective in inhibiting KMBA oxidation than c9,t11-CLA and α-tocopherol (Figure 4). The results agreed with our previous finding that t10,c12-CLA was a stronger antioxidant than c9,t11-CLA (data not shown). No pro-oxidant activity was observed even at high concentration (200 μM for t10,c12-CLA). It has been suggested that the antioxidant activity of CLA is not due to CLA per se but CLA-derived furan fatty acid (Yurawecz et al., 1995). It was shown that CLA (containing 41% c9,t11/t9,c11-CLA, 44% t10,c12-CLA, and other minor isomers) was readily converted to furan fatty acids (Yurawecz et al., 1995), which were capable of scavenging free radicals (Okada et al., 1996). Accord-



CLA	m	n	Furan Fatty Acid
c9,t11	5	7	$F_{9,12}$
t10,c12	4	8	$F_{10,13}$

Figure 5. Conversion of different CLA isomers to their corresponding furan fatty acids [modified from Yurawecz et al. (1995)].

ing to the scheme by Yurawecz et al. (1995; Figure 5), it is probable that dialkyl-substituted furan fatty acids, 9,12-expoxy-9,11-octadecadienoic acid ($F_{9,12}$) and 10,13-expoxy-10,12-octadecadienoic acid ($F_{10,13}$), are produced from c9,t11-CLA and t10,c12-CLA, respectively. The only difference between $F_{9,12}$ and $F_{10,13}$ is the length of the substitution tails, *m* and n. It was shown that the length of *m* and *n* of furan fatty acids did not affect the antioxidant activity toward AAPH-induced oxidation of linoleic acid (Okada et al., 1990). Although tetra-alkyl-substituted furan fatty acids were used to investigate the effect of the length of *m* and *n* of furan fatty acids on the antioxidant activity in the study of Okada et al. (1990), such findings should be able to be extrapolated to dialkyl-substituted ones such as $F_{9,12}$ and $F_{10,13}$. Furthermore, in their study, $F_{9,12}$ revealed no significant activity in scavenging peroxyl radicals. Thus, the formation of furan fatty acid from CLA may not be a probable explanation of the antioxidant properties of CLA in our study. Moreover, it is difficult to explain the different responses of t10,c12-CLA and c9,t11-CLA by the different forms of furan acids formed (just different length of the substitution tails) from the two CLA isomers. However, we cannot eliminate the possibility that the antioxidant activity of CLA is due to the furan fatty acids-derived compounds as the oxidation products of $F_{10,13}$ (from t10,c12-CLA) may be structurally and functionally different from those of $F_{9,12}$ (from c9,t11-CLA). The pro-oxidant activity of c9,t11-CLA at high concentration may be due to conversion of the furan fatty acid formed ($F_{9,12}$) into other compounds that are highly reactive.

At higher concentrations (20 and 200 μM) LA exhibited weak antioxidant activity, although it acted as a weak pro-oxidant at low concentration (2 μM; Figure 4). It is possible that LA yielding peroxyl radicals, which are essentially water-insoluble (Ingold et al., 1993), are confined in the inner phase of Tween emulsion and fail to participate in the KMBA oxidation in water phase. In fact, the rate of lipid oxidation is much lower than that of KMBA oxidation and no hexanal could be detected for all samples including LA (data not shown).

In conclusion, using the TOSC assay, t10,c12-CLA was shown to be a stronger antioxidant than c9,t11-CLA in scavenging oxyradicals. At high concentration (200 μM) c9,t11-CLA exhibited pro-oxidant activity, whereas t10,c12-CLA acted as a strong antioxidant at all concentrations (2−200 μM). These results may help to explain the discrepancies of the results of the previous studies on the antioxidant properties of CLA. In all previous studies on the antioxidant properties of CLA, mixtures of CLA isomers (mainly 43% c9,t11/t9,11-CLA and 45% t10,c12-CLA) were used instead of individual isomers. As c9,t11-CLA can be a pro-oxidant in some situations (such as high concentration in this study), a mixed effect of the antioxidant properties of t10,c12-CLA and the pro-oxidant properties of c9,t11-CLA may occur when a mixture of CLA isomers is used. Thus, apart from the difference of oxidation conditions, the discrepancies of the results of the previous studies on the antioxidant properties of CLA may also be due to the balance of the antioxidant properties of t10,c12-CLA and the pro-oxidant properties of c9,t11-CLA. The discovery that the t10,c12-CLA isomer exhibits stronger oxy radical scavenging capacity is novel. Further research on the anticancer activity of individual CLA isomers is necessary.

ABBREVIATIONS USED

ABAP, 2,2′-azobis(amidinopropane); CLA, conjugated linoleic acid; c9,t11-CLA, *cis*-9,*trans*-11-CLA; GC, gas chromatograph; KMBA, α-keto-γ-methiolbutyric acid; LA, linoleic acid; TOSC, total oxyradical scavenging capacity; t10,c12-CLA, *trans*-10,*cis*-12-CLA.

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