Oxidative Instability of CLA Concentrate and Its Avoidance with Antioxidants

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ABSTRACT: This study evaluated the effectiveness of antioxidants, such as BHA, BHT, TBHQ, propyl gallate (PG), α-tocopherol (α-T), green tea extract (GTE), and rosemary extract (RE) on oxidative stability of CLA concentrate. Stability of CLA concentrate stored in air at 45°C up to 44 d was assessed by PV. During the storage period, the PV of the control CLA concentrate sample increased from 0.20 (fresh oil) to 1654 meq/kg (oxidized oil). On the other hand, the PV for CLA concentrates treated with 200 ppm of the single synthetic antioxidants, BHA, BHT, TBHQ, and PG, increased from 0.20 to 81, 107, 78, and 101 meq/kg, respectively. Also, the PV of CLA concentrate with the addition of 200 ppm single natural antioxidants α-T, GTE, and RE lowered the final PV to 122, 140, and 110 meq/kg, respectively. Under our experimental conditions, the protective effect of 200 ppm antioxidant was in the order of TBHQ > BHA > PG > BHT > RE > α-T > GTE. These results suggest that the appropriate use of antioxidants prolongs the oxidative stability of CLA concentrate.

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KEY WORDS: BHA, BHT, CLA, green tea extract, propyl gallate, rosemary extract, TBHQ, α-tocopherol.

CLA is a mixture of geometric (*cis,cis*; *cis,trans*; *trans,cis*; and *trans,trans*) and positional (C_7,C_9; C_8,C_{10}; C_9,C_{11}; C_{10},C_{12}; C_{11}, C_{13}; and C_{12},C_{14}) isomers with two double bonds that are conjugated and that are not separated by a methylene group (–CH_2–), unlike linoleic acid (LA, 18:2n-6) (1). Among the isomers, *cis*-9,*trans*-11, *trans*-10,*cis*-12, *trans*-9,*trans*-11, and *trans*-10,*trans*-12 CLA account for more than 89% of total CLA in dairy products, especially cheeses (2).

The report that CLA reduces skin carcinogenesis in mice induced with 7,12-dimethyl-benz(a)anthracene (3) led to numerous studies on the anticarcinogenic activity of CLA. CLA inhibited (i) benzo(a)pyrene-induced forestomach neoplasia in mice (4), dimethylbenz(a)anthracene-induced mammary tumor in rats (5), and (iii) growth of human lung adenocarcinoma, malignant melanoma, colorectal, (6,7), and breast cancer cells (8,9). In addition to anticarcinogenic activity, CLA has potent fat-to-lean repartitioning (10) and improves feed efficiency (11), stimulates the immune system (12), protects against arteriosclerosis (13), and reduces cardiovascular risk factors (14) in animal models. A recent publication indicated that the percentage of body fat and fat mass in CLA-supplemented human subjects was significantly reduced (15).

The current human intake of CLA by dietary sources, which is estimated at 1 g CLA/d in the United States (2), seems insufficient to exert the aforementioned potential beneficial effects. Ip *et al.* (16) estimated, on the basis of a rat model, that a 70-kg human should consume 3 g CLA/d to obtain its health-promoting capabilities. These needs could be met by designing vegetable oils that are rich in CLA as a dietary source of CLA isomers. These would be produced under alkaline isomerization of vegetable oils (i.e., safflower oil) containing a high concentration of LA (17). Presently, CLA supplements containing approximately 70% of CLA content are being marketed as nutraceuticals.

Although the pro-oxidative property of CLA remains controversial, CLA in the FFA form is reportedly extremely unstable, like DHA, (18), and is more vulnerable to autoxidation than LA, linolenic acid, and arachidonic acid (19,20). Therefore, the oxidative stability of CLA products should be considered before they are consumed. A few investigations have considered how to protect CLA against oxidative instability. The addition of 200 ppm green tea extract (GTE) or BHT to CLA decreased the O_2 uptake as compared with the control CLA sample during incubation for 4 h at 90°C (20). However, to the best of our knowledge, there has been no study on preserving the oxidative stability of CLA at longer storage times and lower temperatures. We assess here the oxidative stability of CLA concentrate with four synthetic and three natural antioxidants under such conditions.

EXPERIMENTAL PROCEDURES

Antioxidants and reagents. α-Tocopherol (α-T), BHA, BHT, TBHQ, and propyl gallate (PG) were purchased from Sigma Chemical Co. (St. Louis, MO). GTE and rosemary extract (RE) were donated by the Nongsim Company (Seoul, Korea). Safflower oil was purchased from a retail store in Korea. All FA standards including CLA isomers were purchased from Matreya (State College, PA). Other chemicals used in this study were purchased from Sigma (Chemical Co.), and were analytical grade unless otherwise mentioned.

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Preparation of CLA concentrate by alkaline isomerization. CLA concentrate was prepared from safflower oil by alkaline isomerization according to the slightly modified method of Chin *et al*. (21). Briefly, 150 g of safflower oil was slowly added to 150 g of diethylene glycol and 22.5 g of sodium hydroxide, and the reaction mixture was refluxed for 2 h as a stream of nitrogen was bubbled slowly through it. The isomerized FA were isolated by dissolving the reaction mixture in two parts of water, acidifying with 10 N sulfuric acid, and extracting with *n*-hexane. The hexane extract was washed four times with distilled water for neutralization, filtered through anhydrous sodium sulfate, evaporated at 30°C, and used as the CLA concentrate in this study. The recovered CLA concentrate was stored at −20°C under nitrogen prior to use.

FA composition. The CLA concentrate was esterified according to a modification of our previous method (22). FAME were extracted with hexane. Then 1-μL aliquots of the extracts were injected into a gas chromatograph (Varian 3800) equipped with a SUPELCOWAX 10 fused-silica capillary column (30 m × 0.32 mm i.d.; Supelco, Bellefonte, PA), and an FID was used. The column was held at 190°C for 2 min, and programmed to increase to 220°C at the rate of 2°C/min. The carrier gas was helium, and the total gas flow rate was 20 mL/min. The injector and detector temperatures were 240 and 260°C, respectively. FAME were identified by comparison with the retention times of standards.

Preparation of samples and oxidation. Samples of CLA concentrate (100 g) were accurately weighed into a 500-mL beaker, and each antioxidant, dissolved in ethanol, was added at a 200 ppm level based on the CLA concentrate amount. After addition of the antioxidants, the oils were mixed for 10 min using a stirring bar. Then samples, 10 g each, were weighed into 50-mL beakers for the oxidative stability test. Before the oil was weighed, ethanol was removed under vacuum with a rotary evaporator to remove any possible effects of ethanol on the oxidative stability. For GTE, total phenolic content was determined according to the Folin–Ciocalteu colorimetric method (23). The amount of GTE added was based on total phenolic compounds. Oxidation was carried out for 44 d in the dark at 45°C, and the oxidative stability was evaluated by PV (24).

Statistical analysis. All measurements were replicated three times. The results obtained for PV were statistically analyzed by ANOVA and Duncan's multiple range test. Statistical significance was accepted at a level of $P < 0.05$.

RESULTS AND DISCUSSION

The CLA concentrate prepared by alkaline isomerization in this study had a CLA content of 74.6% (Table 1). The ratio of CLA to total linoleic acid in this concentrate was 98.7%, and the contents of the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA were 32.7 and 34.9%, respectively. To assess the oxidative stability of CLA concentrate in the presence of seven commonly used synthetic and natural antioxidants, each at a concentration of 200 ppm, the reaction mixtures were exposed to air at 45°C for up to 44 d. The addition of 200 ppm of an antioxidant is generally allowed in fats and oils for human consumption in most countries (25). The stability of each mixture was expressed in PV. Over 44 d, the PV of the control CLA concentrate sample increased from 0.20 (fresh oil) to 1654 meq/kg (oxidized oil). The PV of CLA concentrate samples containing antioxidants are presented in Figures 1 and 2. PV for CLA concentrates treated with 200 ppm BHA, BHT, TBHQ, and PG increased from 0.20 to 81, 107, 78, and 101 meq/kg, respectively (Fig. 1). Although BHA, BHT, TBHQ, and PG are widely used as antioxidants in food lipids, possible safety concerns with these phenolic synthetic antioxidants have been expressed for many years (26). The effect of BHA on biotransformation of ingested material into toxic substances is related to increased activities of microsomal enzymes (27). BHT reportedly BHT causes forestomach cancer in rats (28). In addition, TBHQ has not been authorized for use in food in Europe and Canada. Furthermore, Miyauchi *et al.* (29) reported that PG is carcinogenic in rats. As a result, natural antioxidants have gained popularity in recent years. Figure 2 shows the PV of CLA

TABLE 1
FA Composition[a] (wt%) of Safflower Oil and CLA Concentrate

FA composition	Safflower oil	CLA concentrate[b]
14:0	0.1	0.1
16:0	6.4	7.4
16:1	0.1	0.1
18:0	2.6	2.6
18:1	14.4	14.2
18:2	76.4	1.0
*c*9,*t*11 CLA	ND[c]	32.7
*t*10,*c*12 CLA	ND	34.9
*c*9,*c*11; *t*11,*t*13 CLA	ND	2.4
*t*8,*t*10; *t*9,*t*11; *t*10,*t*12 CLA	ND	4.6
Total CLA	—	74.6

[a]Values are the means of three determinations.
[b]CLA concentrate was prepared from safflower oil by alkaline isomerization.
[c]ND, not detected.

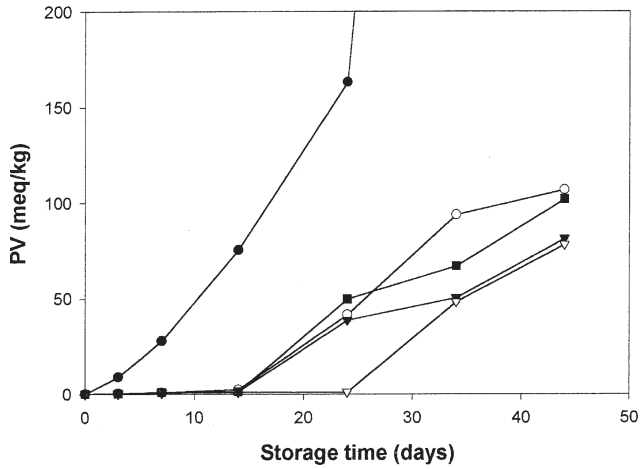

FIG. 1. Effect of 200 ppm synthetic antioxidants on oxidative stability of CLA concentrate during 44 d at 45°C: hydroperoxide formation. (SD for each point was within ±1% of the mean value; $n = 3$). (●) Control, (○) BHT, (▼) BHA, (▽) TBHQ, and (■) propylgallate.

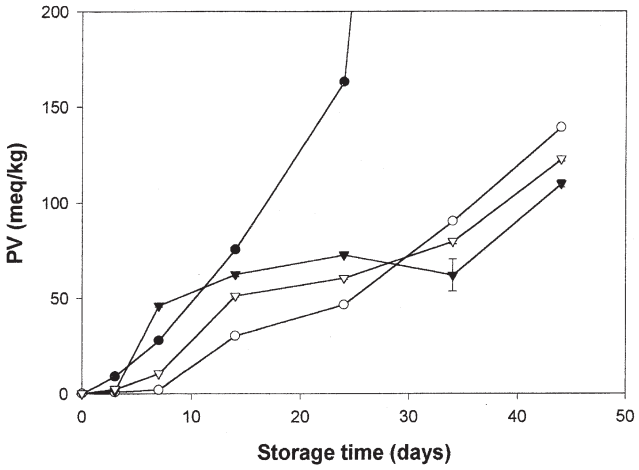

FIG. 2. Effect of 200 ppm natural antioxidants on oxidative stability of CLA concentrate during 44 d at 45°C: hydroperoxide formation. (SD for each point was within ±1% of the mean value; $n = 3$). (●) Control, (○) green tea extract, (▼) rosemary extract, (▽) α-Tocopherol.

concentrates with added single natural antioxidants. Added at levels of 200 ppm, α-T, GTE, and RE lowered the final PV after 44 d from 1654 meq/kg (control sample) to 122, 140, and 110 meq/kg, respectively.

Our results showed that the addition of 200 ppm BHT to CLA concentrate was more effective than 200 ppm GTE after the reaction mixtures were held at 45°C up to 44 d of storage. On the other hand, Yang *et al.* (19) reported that headspace-oxygen depletion by CLA with 200 ppm green tea catechins (GTC) or 200 ppm BHT in airtight glass tubes, which were reacted with oxygen at 90°C for 4 h, was significantly decreased compared with CLA alone, and GTC addition was more effective than 200 ppm BHT in protecting CLA from oxidation. This discrepancy may be due to the different GTE preparations, substrate concentrations, O_2 partial pressures, reaction times, and temperatures. On the other hand, RE and α-T were quite effective at 200 ppm in protecting CLA concentrate from oxidation, and gave a slightly higher PV than RE-treated CLA concentrate. However, α-T has either weak antioxidant activity or pro-oxidant activity, depending on its concentration and the physical state of the oils being reacted (30). In the experiment of Huang *et al.*, on the basis of PV, 200 ppm α-T in corn oil had maximal antioxidant activity, and at levels >250 ppm α-T showed slight, initial pro-oxidant activity. For α-T (200 ppm) in our system using CLA concentrate, an antioxidant effect was observed that was comparable to that of 200 ppm RE. However, the present results do not take into consideration the differences existing in the molecular ratio of the antioxidants employed.

Under the conditions of the test, after 44 d of storage at 45°C, the protective effect of 100 ppm antioxidants was in the order of TBHQ > BHA > PG > BHT > RE > α-T > GTE. CLA concentrate was not stable under our experimental conditions. The oxidative instability of CLA should not be overlooked when it is prepared as a concentrate for use as a dietary supplement, stored, and transported for general consumption. Therefore, the appropriate use of antioxidants will prolong the biological effects of CLA.

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