JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 439−444 **439**

Total Antioxidant Capacity of Arginine-Conjugated Linoleic Acid (CLA) Complex

YOUNG JUN KIM,[†,‡] KI WON LEE,[†,§] AND HYONG JOO LEE*[,§]

Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York, 14853 and Department of Food Science and Technology, School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Republic of Korea

Conjugated linoleic acids (CLA) have shown diverse biological activities, including anti-carcinogenic, anti-atherosclerotic, anti-adipogenic, and anti-diabetogenic effects. Recent reports also showed that CLA has free radical scavenging capacity, which may give health benefits to human beings. However, the application of CLA as a bioactive ingredient has been limited due to its insolubility in water. To overcome this problem, we investigated antioxidant activities of arginine (Arg)-CLA, a water-soluble CLA salt, using both 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. CLA, Arg, and Arg−CLA all exerted radical scavenging activities in a dose-dependent manner in both assays. Arg−CLA at 20 mM scavenged 89 and 55% of ABTS and DPPH radicals in 3 h, respectively, whereas CLA alone quenched only 48 and 26% of them under the same conditions. The antioxidant activity of the Arg−CLA complex was found to be synergistic in ABTS assay and comparable to that of vitamin E in DPPH assay.

KEYWORDS: Conjugated linoleic acids; arginine; Arg−CLA; antioxidant; hydrophilic

INTRODUCTION

There is a great deal of evidence indicating that a variety of natural fatty acids has practical implications in health promotion. Among these fatty acids, conjugated linoleic acid (CLA), a collective term referring to a mixture of positional and geometric isomers of octadecadienoic acid, is currently under intensive investigation due to its health promoting potential. Two double bonds have been identified at positions 8 and 10, or 9 and 11, or 10 and 12, or 11 and 13 in CLA forming up to 14 possible isomers (*1*). CLA is primarily found in dairy foods derived from ruminants. Although the total content of CLA in nature varies widely, the *cis*-9, *trans*-11 isomer is generally the dominant form (as much as 90% of the total CLA) (*2*). Animal studies have revealed diverse biological activities of CLA including anti-carcinogenic, anti-atherosclerotic, anti-adipogenic, and anti-diabetogenic effects (*3*−7).

However, the mechanisms responsible for these physiological effects remain unclear. To demonstrate the mechanisms of CLA, some studies focused on the antioxidative roles of CLA (*3, 4, 8*−*10*). Ip et al. (*3*) showed the antioxidant activity of CLA through animal studies in the thiobarbituric-acid-reactive substances (TBARS) assay. Ha et al. (*4*) also detected the antioxidant activity of CLA by monitoring the peroxide value during oxidation. They revealed that CLA was involved in the inhibition of the Fenton-type reaction resulting from the chelation of iron by β-hydroxy acrolein derivatives of CLA. On the other hand, Cantwell et al. (*8*) observed the effect of CLA on lipid oxidation in rat hepatocytes exposed to oxysterols. They showed that the activities of both catalase and superoxide dismutase were significantly reduced in hepatocytes treated with CLA. More recent studies showed direct radical scavenging effects of CLA. Yu et al. (*9*) showed the protective effect of CLA against free radicals, whereas linoleic acid failed to show the same effect. They also reported a synergistic effect between CLA isomers existed in the reactions with 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radicals (*10*).

However, application of CLA as a bioactive food ingredient has been limited due to its lipid-soluble nature. Arginine (Arg), a water-soluble amino acid, is known to be a substrate of nitric oxide synthase and regulate vascular function and blood pressure homeostasis, and thus prevent cardiovascular disease (*11*). More recently, Lass et al. (*12*) showed that Arg has some protective roles against oxygen radical attack possibly due to its direct chemical interaction with oxygen radicals. In the present study, the antioxidative properties of the hydrophilic Arg−CLA complex were studied using two distinct assay systems, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and DPPH radical scavenging assays, in comparison to those of the free CLA and Arg.

MATERIALS AND METHODS

Chemicals. 2,2′-Azobis(2-amidino-propane)dihydrochloride (AAPH) was obtained from Wako Chemicals Inc. (Richmond, VA). Arg was purchased from Kyowa chemical (Tokyo, Japan). ABTS as diammo-

* To whom correspondence should be addressed. Tel.: 82-2-880-4853. Fax: 82-2-873-5095. E-mail: leehyjo@snu.ac.kr.
† Young Jun Kim and Ki Won Lee contributed equally to this work.
‡ Cornell University.
§ Seoul National University.

10.1021/jf030186w CCC: $27.50
Published on Web 01/06/2004

nium salt, DPPH, butylated hydroxytoluene (BHT), vitamin E, and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

CLA Preparation. CLA was prepared according to our previous method (*13*). Briefly, 1 kg of safflower oil (80% LA) was added to 250 g of sodium hydroxide (NaOH) dissolved in 100 °C polyethylene glycol (PEG) (800 g). Free fatty acids formed when the mixture was left standing for 2 h to cool to 80 °C. Phosphoric acid (2 N) was then added to the mixture to lower the pH to 3.0. The formed crude CLA was further purified by urea inclusion crystallization (*13*). The fatty acid composition of the crude CLA obtained after the first crystallization step was as follows: 78, 6, 2.5, 11, and 0.5 GC area% of CLA, palmitic, stearic, oleic, and α-linolenic acids, respectively. A 1 kg sample of crude CLA obtained from safflower oil was added into the urea-saturated methanol solution (4 L) at 70 °C in 6 portions and cooled to room temperature at 0.2 °C/min in a cooling bath (Eyela, Model NCM-3000, Tokyo, Japan) equipped with proportional-integral-derivative controller. The resulting reaction mixture was filtered through a 0.45 μm membrane filter (Millipore, Billerica, MA) to remove saturated fatty acids and most of the oleic acid in the form of urea-inclusion complex. The filtrate containing a trace amount of urea and unsaturated fatty acids, including CLA, was separated. Urea (2.5 kg) in methanol (4.7 L) was added to the residual filtrate of the fraction at 70 °C until saturated. The reaction mixture was then cooled to room temperature at a rate of 0.2 °C/min and filtered to recover highly concentrated CLA (95%) in the form of a solid urea inclusion compound, while the portion of the filtrate containing α-linolenic acid (LNA) was removed. Distilled water (2 L) was then added to the concentrated CLA to initiate the phase separation of urea from CLA. The upper layer of high-purity CLA was recovered and washed with distilled water. The residual methanol was removed using a rotary evaporator (Eyela).

Fatty Acid Analysis. Fatty acids were quantified by gas chromatography (GC) equipped with flame ionization detector (FID) (Hewlett-Packard, HP 6890, Avondale, PA). Fatty acid samples were methylated according to our previous method (*14*). Fatty acid methyl esters were separated using a Supelcowax-10 fused silica capillary column (60-m × 0.53-mm i.d., 0.5-μm film thickness; Supelco, Inc., Bellefonte, PA) and helium at a flow rate of 2.5 mL/min. Temperature was increased from 175 to 240 °C at the rate of 2.5 °C/min. Temperatures of the injector and detector were 250 and 260 °C, respectively. The CLA content was expressed as milligrams per liter of reaction mixture. Heptadecanoic acid (C17:0), as an internal reference was added before the extraction to determine the recovery of the fatty acids in the samples. The recovery of methylated CLA that was calculated in a comparison to the internal standard (C17:0) was higher than 80%.

Preparation of Arg−CLA. Arg−CLA was prepared according to our previous methods (*13*). Briefly, Arg (1 kg) was dissolved in 6 L of distilled water at 20 °C. The temperature was then slowly lowered to 0°C, during which 1.6 kg of purified CLA dissolved in 95% ethanol was added in small portions at the rate of 100 g/min, while stirring at 500−1000 rpm until the turbidity of the mixture disappeared. With equimolar ratio of Arg and CLA, the yield was about 63%. The Arg−CLA complex formation was confirmed using infrared spectroscope (FTS 3000 MX, BioRad Co., Hercules, CA). The purity of the resultant Arg−CLA salt was as high as 95%.

ABTS Radical Scavenging Assay. A method developed by van den Berg et al. (*15*) was used with slight modifications. Briefly, 1.0 mM AAPH was mixed with 2.5 mM of ABTS in phosphate buffered saline (PBS) solution (100 mM potassium phosphate buffer containing 150 mM NaCl). The mixture was heated in a 68°C water bath for 13 min. The resulting blue-green ABTS radical solution was adjusted to an absorbance of 0.3 ± 0.02 at 734 nm. Various doses of CLA and other test materials (10 μL each) were added to 190 μL of the resulting blue-green ABTS radical solution in a 96 well plate. The control consisted of 10 μL of 100% ethanol and 190 μL of ABTS radical solution. Decrease in absorbance resulting from the addition of test compounds was measured at 734 nm using an ELISA reader (Emax, Molecular Devices Co., Sunnyvale, CA). ABTS radical scavenging activities of the test compounds were expressed as % remaining ABTS radicals at each time point. All samples were analyzed in triplicate. The radical stock solution was prepared fresh daily.

DPPH Radical Scavenging Assay. A method developed by Brand-Williams et al. (*16*) was used with slight modifications. Briefly, 100 μM DPPH radical was dissolved in 100% ethanol. The mixture was shaken vigorously and allowed to stand for 30 min at 23 °C in the dark. The resulting dark blue DPPH radical solution was adjusted to an absorbance of 0.5 ± 0.05 at 517 nm. Various doses of CLA and other test materials (10 μL each) were added to 190 μL of the resulting dark-blue DPPH radical solution in a 96 well micro plate. The control consisted of 10 μL of 99% ethanol and 190 μL of DPPH radical solution. Decrease in absorbance resulted by the addition of test compounds was measured at 734 nm using an ELISA reader. The DPPH radical scavenging activities of the test compounds were expressed as % remaining DPPH radicals at each time point. All samples were analyzed in triplicate. The radical stock solution was prepared fresh daily.

Quantification of Total Antioxidant Capacity. A method developed by Winston et al. (*17*) was applied with slight modifications for quantification of antioxidant value of each test compound. The area under each kinetic curve was calculated by integration. The total antioxidant capacity (TAC) of each test compound was then quantified according to eq 1, where $\int$ SA and $\int$ CA are the integrated areas of the curve defining the sample and control reactions, respectively.

$$\mathrm{TAC} = 100 - \left(\int \mathrm{SA} / \int \mathrm{CA} \times 100\right) \quad (1)$$

Percent increase in the integrated area was measured to compare each test compound. Here, $\int$ SA is the integrated area from the sample reaction, and $\int$ CA is the integrated area from the control reaction.

Statistical Analyses and Design. All experiments were replicated at least three times, and statistical analysis was conducted using the SigmaStat program (Version 1.0; Jandel Corporation, San Rafael, CA). Treatment means were compared using Student-Newman-Keuls pairwise test, and significance of difference was defined at $p < 0.05$.

RESULTS AND DISCUSSION

CLA was prepared in a large scale by alkali-isomerization and purified up to 95% using urea inclusion crystallization. The procedure of CLA and Arg−CLA preparation was depicted in **Figure 1**. Arg−CLA was obtained by attaching to the carboxyl end of CLA (**Figure 2**). Antioxidant activities of CLA, Arg, and Arg−CLA complex were measured using ABTS radical scavenging assay. The incubation was performed at various concentrations up to 20 mM for 3 h. The antioxidant activities, expressed as remaining ABTS radicals, were decreased by the addition of CLA, Arg or Arg−CLA complex in a dose-dependent manner (**Figure 3**). The Arg−CLA complex showed the highest antioxidant capacity among the tested compounds ($p < 0.05$). At the highest concentration tested (20 mM), up to 48 and 69% of the radicals were scavenged by CLA and Arg, respectively, whereas 89% was scavenged by Arg−CLA in 3 h. Assessment of the antioxidant capacity was difficult at higher concentrations (>40 mM) due to the limitation in the solubility of CLA in the ABTS assay system. Antioxidant activities of CLA, Arg, and Arg−CLA complex were also examined using DPPH radical scavenging assay. CLA, Arg, and Arg−CLA complex exerted antioxidant activities in a dose dependent manner up to 20 mM (**Figure 4**). The Arg−CLA complex showed stronger antioxidant capacity than both CLA and Arg alone. At 20 mM, 26, 37, and 55% of the radicals were scavenged by CLA, Arg, and Arg−CLA in 3 h, respectively.

TAC of the test compounds using ABTS and DPPH radical scavenging assay are shown in **Figures 5** and **6**, respectively. The area underneath each line formed by connecting the percent of remaining radical at each time point was integrated. Each integrated value was expressed as the percent increase in the integrated area compared with control. High correlations ($r^2 > 0.95$) were found between the concentration of each compound

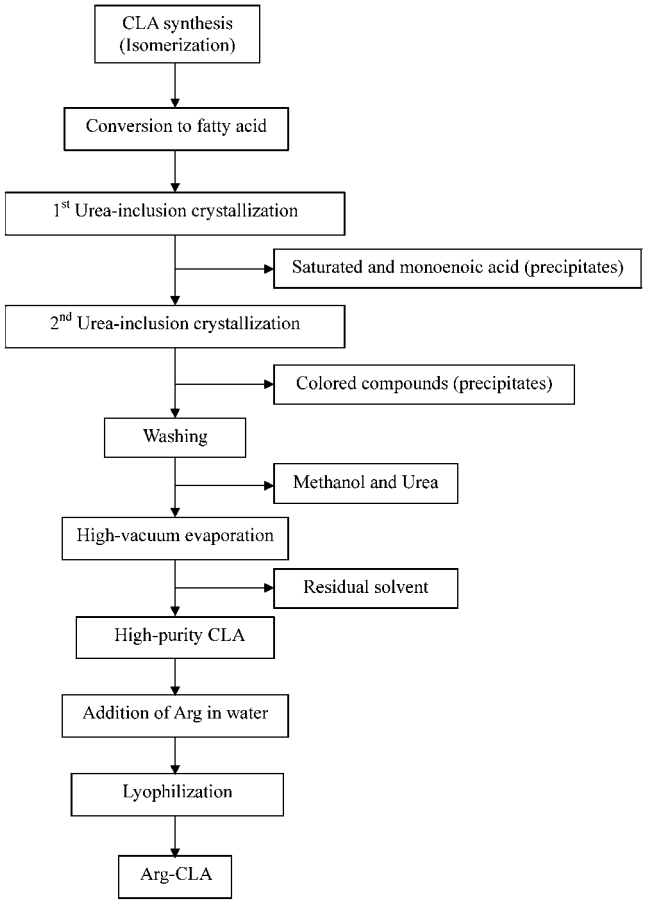

Figure 1. Flowchart for the preparation of CLA and Arg–CLA.

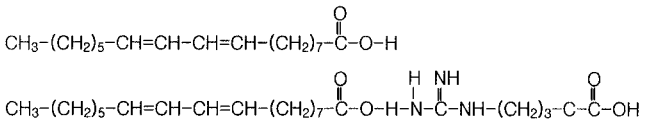

Figure 2. Proposed structures of (a) CLA and (b) Arg–CLA.

and the integrated area. Thus, the slope reflects the degree of increase in TAC. Arg−CLA showed higher TAC against both DPPH and ABTS radicals than both CLA and Arg alone ($p < 0.05$). TAC of CLA, Arg, and Arg−CLA were also compared with widely used antioxidants vitamin E and BHT. As shown in **Figure 7**, TAC of Arg−CLA was comparable to that of vitamin E, but less than BHT at 20 mM ($p < 0.05$).

CLA has some preventive roles against oxidative damages (*4*, *9*, *10*, *18*). Colorimetric assays using chromogens such as ABTS and DPPH are the most popular assays to measure antioxidant activity, because they are sensitive and ease to use. Although both compounds show good stability under certain conditions, they revealed significant differences in their responses to antioxidants (*19*). ABTS radical chromogens can be dissolved both in aqueous and organic phases, whereas DPPH radical is only dissolved in organic phase. Generally, ABTS radicals are more sensitive than DPPH radicals in the response to antioxidants (*16*). In our results, Arg−CLA, Arg, and CLA all showed higher scavenging activity for ABTS in a comparison to DPPH. The difference may be due to the solubility of the compounds, especially for Arg−CLA. The Arg−CLA complex showed higher antioxidant activity than either Arg or CLA alone against both DPPH and ABTS radicals, an indication that Arg can be stabilized by CLA attachment and vice versa in both assay systems. Our results in ABTS assay suggest that the

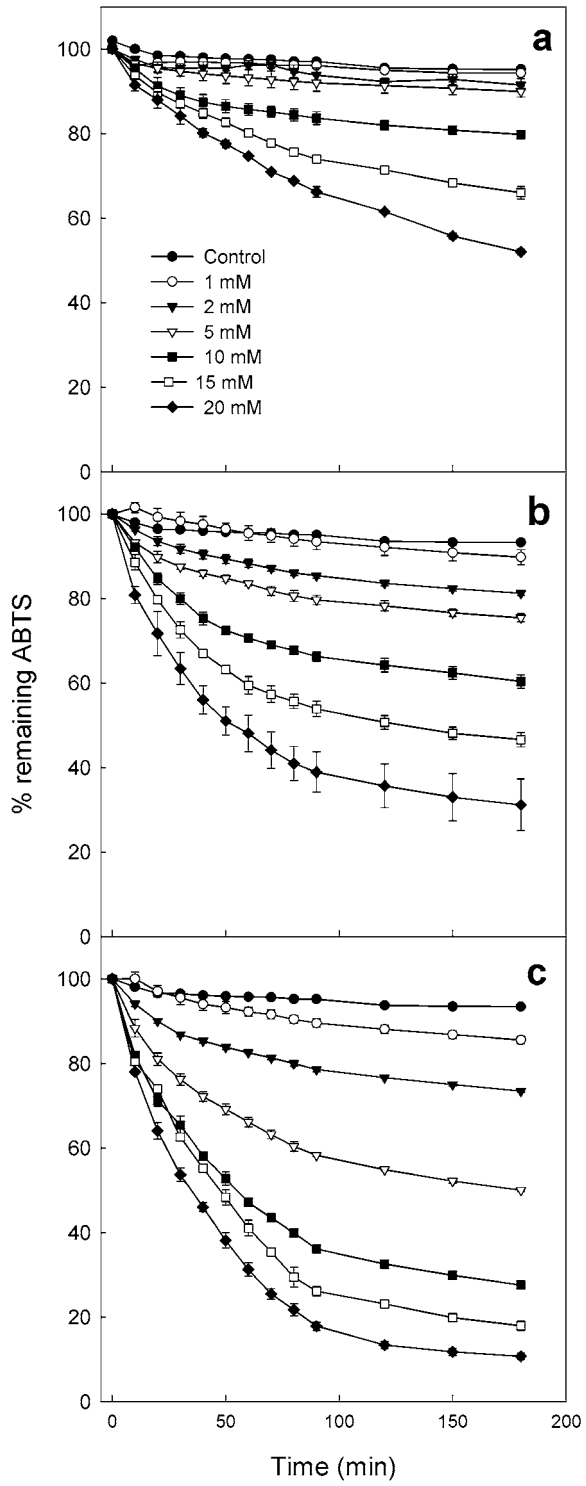

Figure 3. Kinetics of reaction of ABTS radicals with (a) CLA, (b) Arg, and (c) Arg–CLA. Each test compound at 0, 1, 2, 5, 10, 15, and 20 mM was reacted with 100 μM ABTS radicals. Error bars represent standard deviations of each data point ($n = 3$).

contributions of Arg and CLA to the TAC of Arg−CLA could be synergistic, because the activity of Arg−CLA was higher than the sum of Arg and CLA. Arg attachment may enhance the TAC of CLA in the hydrophilic assay system. This indicated that CLA play a synergistic antioxidative role in concert with Arg, with enhanced solubility in a hydrophilic system. The stability of the Arg−CLA in water may be partially attributed to the shielding of the carboxyl end by Arg from water molecule. In fact, Arg itself has also been shown to scavenge some oxygen

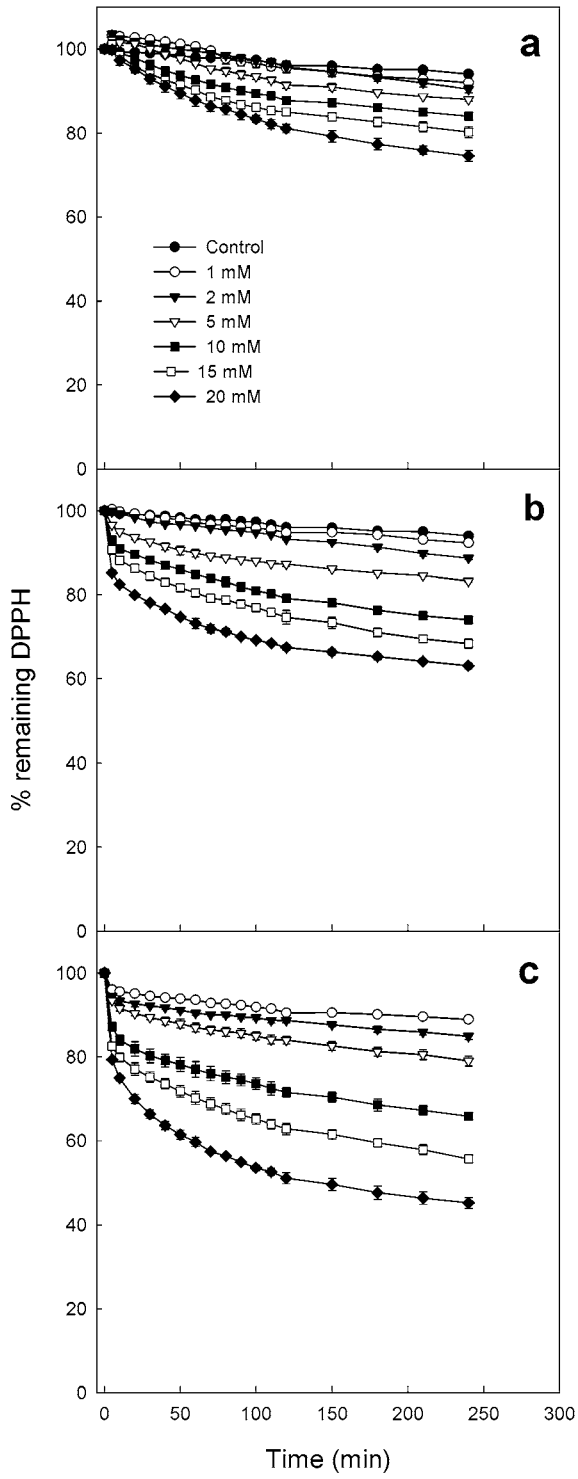

Figure 4. Kinetics of reaction of DPPH radicals with (a) CLA, (b) Arg, and (c) Arg–CLA. Each test compound at 0, 1, 2, 5, 10, 15, and 20 mM was reacted with 2.5 mM DPPH radicals. Error bars represent standard deviations of each data point ($n = 3$).

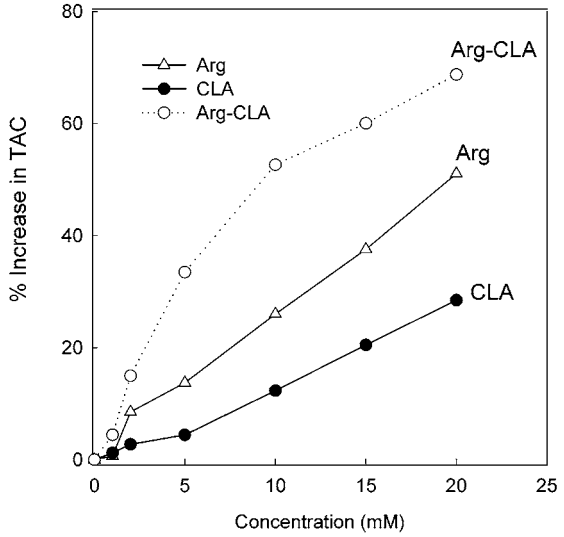

Figure 5. Comparison of total antioxidant capacities (TAC) of CLA, Arg, and Arg–CLA against ABTS radicals. Values are expressed as % increase in the integrated area (equation 1) compared to the control containing no antioxidants. Average values of three experimental data are expressed without error bars.

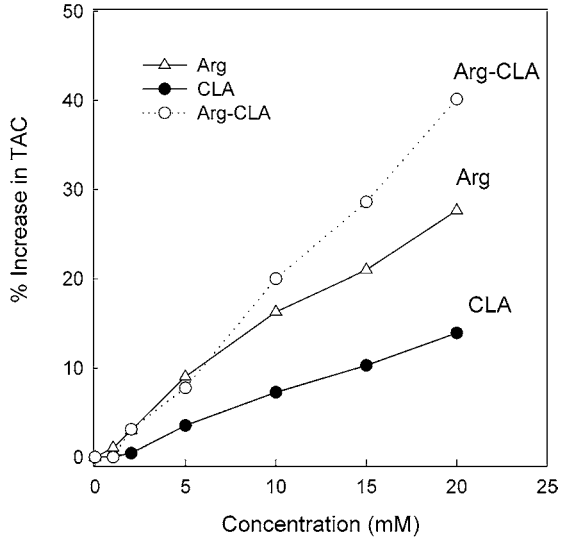

Figure 6. Comparison of TAC of CLA, Arg, and Arg–CLA against DPPH radicals. Values are expressed as % increase in the integrated area (equation 1) compared to the control containing no antioxidants. Average values of three experimental data are expressed without error bars.

radicals (*12*) and thus could also counteract oxidative stress efficiently in a complex form.

CLA could play its antioxidative roles by directly acting with free radicals to terminate the radical chain reaction or chelating transition metals to suppressing the initiation of radical formation (*10*). However, the lipid-soluble nature of CLA could be a limiting factor, not only in studying its antioxidative roles, but also in the practical application of CLA as a food ingredient. In the present study, CLA, in a complex form with Arg, was shown to quench radicals efficiently in agreements with previous reports (*9*, *10*). Thus, the antioxidant capacity of fatty acids could be enhanced through the formation of the Arg-salt. Fatty acid−amino acid complex formation could modify the miscibility and transferability of fatty acids into water phases. Therefore, the higher activity shown in ABTS assay than in DPPH assay could be ascribed to the easy access of the CLA in the complex to the ABTS radicals in the assay system. On the other hand, CLA can be efficiently protected from oxidative attacks by attaching Arg to the carboxyl end. In that the substitution of carboxyl group with arginine enhanced TAC, the carboxyl group may have little contribution to the antioxidant nature of CLA.

Yu et al. *(9, 10)* recently reported that CLA provided immediate protection against DPPH radicals in organic solvents, as revealed through electron spin resonance (ESR) spectroscopy analysis but had lower activity compared to vitamin E. Our data also showed that CLA had lower antioxidant activity than

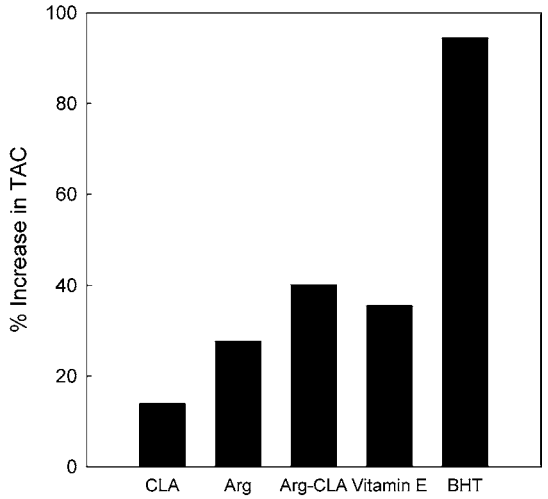

Figure 7. Comparison of TAC of CLA, Arg, Arg–CLA with selected antioxidants. All antioxidants were compared at 20 mM in 3 h by DPPH radical scavenging assay. Average values of three experimental data are represented without error bars.

vitamin E in the DPPH radical scavenging assay, in agreement with Yu's report (*9*, *10*). On the other hand, Leung and Liu (*18*) showed that CLA had antioxidant activity comparable to that of vitamin E at low concentrations (2−200 μM) based on the antioxidant activity measured by TBARS assay. Thus, the antioxidant capacity should be carefully determined in multiple model systems in vivo or in vitro, because the nature of single compound could vary depending on model system.

Despite the numerous reports on the physiological roles of CLA, the stability against high temperature and aerobic conditions has been of great concern, especially in the application of CLA as a functional ingredient in a food system (*20*). The unstable and vulnerable nature of poly unsaturated fatty acids (PUFAs) against oxidative attacks should not be overlooked, particularly when its oxidation is considered to be an important factor in the application as a food ingredient. Indeed, CLA could be an unstable ingredient, especially under aerobic or high-temperature conditions. CLA was shown to be less stable than LA against free radical attack (*20*, *21*). We also found that the stability of CLA, when attached to Arg, was enhanced to a large extent at high temperatures under aerobic conditions (unpublished observation).

Recent research has been aimed to elucidate the physiological background of CLA-inducing reduction in adipose tissue mass (*22*, *23*). However, some concerns have been aroused on the potential implication of the CLA in insulin resistance and fatty liver under certain conditions (*24*, *25*). Because Arg infusion is known to have a preventive role in the insulin resistance by decreasing the total plasma homocysteine concentration (*26*) and antioxidant capacity (*9*, *10*), the formation of Arg−CLA complex could alleviate the potential side effects, if any, resulted from the high dose of CLA. Thus, the presence of Arg in the form of complex with CLA could expand the scope of the application of CLA as a health-promoting agent.

ACKNOWLEDGMENT

We thank the R&D team of Lipozen Co. (Pyongtaek, Korea) for technical advice and valuable input. We also appreciate kind review of the manuscript by Dr. Chang Yong Lee in the Department of Food Science and Technology in Cornell University.

LITERATURE CITED

(1) Sehat, N.; Yurawecz, M. P.; Roach, J. A.; Mossoba, M. M.; Kramer, J. K.; Ku, Y. Silver-ion high-performance liquid chromatographic separation and identification of conjugated linoleic acid isomers. *Lipids* **1998**, *33*, 217−221.

(2) Chin, S. F.; Storkson, J. M.; Liu, W.; Albright, K. J.; Pariza, M. W. Conjugated linoleic acid (9, 11, and 10, 12-octadecadienoic acid) is produced in conventional but not germ-free rats fed linoleic acid. *J. Nutr.* **1994**, *124*, 694−701.

(3) Ip, C.; Chin, S. F.; Scimeca, J. A.; Pariza, M. W. Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. *Cancer Res.* **1991**, *51*, 6118−6124.

(4) Ha, Y. L.; J. Storkson; M. W. Pariza. Inhibition of benzo[*a*]-pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. *Cancer Res.* **1990**, *50*, 1097−1101.

(5) Belury, M. A. Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. *Annu. Rev. Nutr.* **2002**, *22*, 505−531.

(6) Lee, K. N.; Kritchevsky, D.; Pariza, M. W. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* **1994**, *108*, 19−25.

(7) Belury, M. A.; Kempasteczko, A. Conjugated linoleic acid modulates hepatic lipid composition in mice. *Lipids* **1997**, *32*, 199−204.

(8) Cantwell, H.; Devery, R.; Stanton, C.; Lawless, F. The effect of conjugated linoleic acid and glutathione peroxide in oxidatively challenged liver cells. *Biochem. Soc.* **1998**, *26*, 69.

(9) Yu, L. Free radical scavenging properties of conjugated linoleic acids. *J. Agric. Food Chem.* **2001**, *49*, 3452−3456.

(10) Yu, L.; Adams, D.; Gabel, M. Conjugated linoleic acid isomers differ in their free radical scavenging properties. *J. Agric. Food Chem.* **2002**, *50*, 4135−4140.

(11) Moncada, S.; Palmer, R. M. J.; Higgs, E. A. Nitric oxide physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* **1991**, *43*, 109−142.

(12) Lass, A.; Suessenbacher, A.; Wolkart, G.; Mayer, B.; Friedrich, B. Functional and analytical evidence for scavenging of oxygen radicals by L-arginine. *Mol. Pharmacol.* **2002**, *61*, 1081−1088.

(13) Kim, Y. J.; Lee, K. W.; Lee, S.; Kim, H.; Lee, H. J. The production of high-purity conjugated linoleic acid using two-step urea-inclusion crystallization and hydrophilic Arginine-CLA complex. *J. Food Sci.* **2003**, *68*, 1948−1951.

(14) Kim, Y. J.; Liu, R. H. Selective increase in conjugated linoleic acid in milk fat by crystallization. *J. Food Sci.* **1999**, *64*, 792−795.

(15) van den Berg, R.; Haenen, G. R. M. M.; van den Berg, H.; Bast, A. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem.* **1999**, *66*, 511−517.

(16) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25−30.

(17) Winston, G. W.; Regoli, F.; Dugas, A. J.; Jr., Fong, J. H.; Blanchard, K. A. A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. *Free Radic. Biol. Med.* **1998**, *24*, 480−493.

(18) Leung, Y. H.; Liu, R. H. *trans*-10, *cis*-12-conjugated linoleic acid isomer exhibits stronger oxyradical scavenging capacity than *cis*-9, *trans*-11-conjugated linoleic acid isomer. *J. Agric. Food Chem.* **2001**, *48*, 5469−5475.

(19) Arnao, M.; Cano, A.; Acosta, M. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.* **2001**, *79*, 239−244.

(20) Yang, L.; Leung, L. K.; Huang, Y.; Chen, Z. Oxidative stability of conjugated linoleic acid isomers. *J. Agric. Food Chem.* **2000**, *48*, 3072−3076.

(21) van den Berg, J. J.; Cook, N. E.; Tribble, D. L. Reinvestigation of the antioxidant properties of conjugated linoleic acid. *Lipids* **1995**, *30*, 599−605.

(22) Park, Y.; Albright, J. K.; Liu, W.; Storkson, J. M.; Cook, M. E.; Pariza, M. W. Effect of conjugated linoleic acid on body fat and energy metabolism in the mouse. *Lipids* **1997**, *32*, 853−858.

(23) West, D. B.; Delany, J. P.; Camet, P. M.; Blohm, F.; Truett, A. A.; Scimeca, J. A. Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. *Am. J. Physiol.* **1998**, *275*, R667−R672.

(24) Clement, L.; Poirier, H.; Niot, I.; Bocher, V.; Guerre-Millo, M.; Krief, S.; Staels, B.; Besnard, P. Dietary *trans*-10, *cis*-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. *J. Lipid Res.* **2002**, *43*, 1400−1409.

(25) Tsuboyama-Kasaoka, N.; Takahashi, M.; Tanemura, K.; Kim, H. J.; Tange T.; Okuyama, H.; Kasai, M.; Ikemoto, S.; Ezaki, O. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. *Diabetes* **2000**, *49*, 1534−1542.

(26) Cassone Faldetta, M.; Laurenti, O.; Desideri, G..; Bravi, M. C.; De Luca, O.; Marinucci, M. C.; De Mattia, G..; Ferri, C. L-arginine infusion decreases plasma total homocysteine concentrations through increased nitric oxide production and decreased oxidative status in Type II diabetic patients. *Diabetologia* **2002**, *45*, 1120−1127.

Received for review March 14, 2003. Revised manuscript received October 30, 2003. Accepted November 12, 2003. This work was supported by a grant from the BioGreen 21 Program, Rural Development Administration, Republic of Korea.

JF030186W