# Oxidative Stability of Conjugated Linolenic Acids

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Interest in conjugated linolenic acid (CLnA) and conjugated linoleic acid (CLA) as functional lipids is growing. The present study was (i) to study the oxidative stability of individual CLnA isomers and (ii) to compare the oxidative stabilities of CLnA and CLA with their corresponding nonconjugated counterparts, α-linolenic acid (LN) and linoleic acid (LA). The oxidation was carried out in air at 50 °C and monitored by the gas−liquid chromatography (GC) and the oxygen consumption test. First, it was found that CLnA was most unstable followed by CLA, LN, and LA in decreasing order. Second, analyses of silver ion high-performance liquid chromatography (Ag$^+$-HPLC) demonstrated that t,t,t-CLnA isomers had greater stability than c,t,t-CLnA and c,t,c-CLnA isomers. Finally, both green tea catechins (GTCs) and butylated hydroxytoluene (BHT) were capable of preventing the CLnA oxidation, with the former being more effective than the latter.

## INTRODUCTION

The human diet contains saturated, monounsaturated, and omega-3 and omega-6 polyunsaturated fatty acids. In addition, two types of structurally related conjugated octadecaenoic acids, namely, conjugated linoleic acids (CLA) and conjugated linolenic acids (CLnA), are also present in the human diet, although their quantity is small. CLA refers to a group of positional and geometrical isomers of octadecadienoic acids that have two conjugated double bonds, whereas CLnA is a group of octadecatrienoic acids that have three conjugated double bonds (*1*, *2*). CLA and CLnA are present in various foods, with CLA being present mainly in dairy foods and ruminant-derived meats (*3*), whereas CLnA is rich in several kinds of seeds from bitter melon, pomegranate, tung, and catalpa (*4*). Both CLA and CLnA can be also formed during the processing of vegetable oils as a result of the isomerization or dehydration of secondary oxidation products of linoleic acid (LA) and α-linolenic acid (LN) (*5*, *6*).

CLA has been the subject of extensive investigations for its various biological activities. It has been reported that CLA is antiatherosclerotic (*7*), anticarcinogenic (*8*), hypotensive (*9*), antilipogenic (*10*), immunoenhancing (*11*), and antioxidative (*12*). Similarly, CLnA has been shown to be a potent suppressor of the growth of various human tumor cells (*13−15*). In addition, CLnA is more effective than CLA in reducing body fat mass in rodents (*16*, *17*). In this regard, genetically modified rapeseed oil containing CLnA has been developed (*16*). Most interestingly, CLnA is able to be converted to CLA in the liver, kidney, mammary gland, and intestine via endogenous Δ13 saturation system in vivo (*18*, *19*).

Although the mechanism of CLA and CLnA bioactivities is considered to be related to oxidation, many controversial results are reported. For CLA, Ha et al. (*20*) and Ip et al. (*21*) showed that CLA had antioxidative activity and proposed this as a possible explanation for the anticarcinogenic and antiatherosclerotic effects. However, other investigations have found that CLA was a prooxidant (*22*). We have examined the stability of CLA as its chemical form of free fatty acids, methyl esters, and triacylglycerols in various conditions. It was found that CLA as the free fatty acid form was extremely unstable, similar to docosahexanenoic acid (*23*), and had an oxidation rate considerably greater than that of LA, linolenic acid, and arachidonic acid (*24*). We also found that the stabilities of 12 CLA isomers were also different. The four c,c-CLA isomers were most susceptible to oxidative degradation, whereas the four t,t-CLA isomers were most stable in air under the same conditions. Their stability at least partially was determined by the *cis-* or *trans*-configuration (*25*). Miyazawa et al. found that the oxidation rate of CLA and CLnA was slowed by triacylglycerol esterification and α-tocopherol (*26*). Yurawecz et al. (*27*) reported that CLA was rapidly decomposed to form furan fatty acids when it was oxidized in air.

For CLnA, some thought the mechanism of antitumor affects of α-CLnA was the apoptosis induction via lipoperoxidation (*28−30*). In vivo, CLnA increased the level of plasma hydroperoxide and live lipid oxidation significantly (*31*). In contrast, Dhar et al. reported the antioxidant effects of α-CLnA on plasma lipid peroxidation (*32*). In fact, it had already been found that different CLnA isomers expressed different bioactivities (*33*). Such difference perhaps was attributed to the different oxidation stability of each isomer. However, stability comparison of each isomer is very difficult because extremely small differences in positional and

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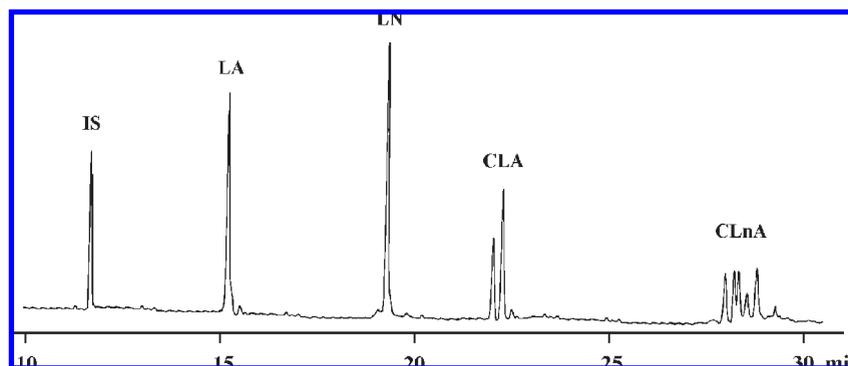
**Figure 1.** Typical GC chromatogram of conjugated linolenic acid (CLnA), conjugated linoleic acid (CLA), α-linolenic acid (LN), linoleic acid (LA), and internal standard heptadecanoic acid (17:0).

geometric configuration of individual isomers make their separation and analysis very difficult. Neither GC nor reversed-phase HPLC method could separate all CLnA isomers completely (*2*).

It is essential to protect CLA and CLnA from autoxidation before they are consumed. In this regard, the oxidative stability of CLA as a whole as well as that of 12 CLA isomers has been studied, demonstrating that both synthetic and natural antioxidants are able to partially inhibit the oxidation of CLA in oils (*25*). However, no studies to date have investigated the oxidative stability of CLnA. The objectives of the present study were therefore (i) to compare the relative oxidative stability of CLnA with those of CLA, LA, and LN; (ii) to investigate the relative degradation rate of 10 CLnA isomers by silver ion high-performance liquid chromatography (Ag$^+$-HPLC); and (iii) to ascertain if synthetic antioxidant butylated hydroxytoluene (BHT) and natural antioxidant green tea catechins (GTCs) were able to inhibit the CLnA oxidation.

## MATERIALS AND METHODS

**Materials.** Air-dried seeds of pomegranate were obtained from San He Tian Co. (Chengdu, Sichuan Province, China). A CLA mixture (9c,11t, 45.3%; and 10t,12c, 44.6%) was purchased from Larodan Fine Chemicals AB (Malmö, Sweden). LN, LA, and BHT were purchased from Sigma Chemical Co. (St. Louis, MO). Jasmine tea was purchased from Cheong Hing Tea Co. Ltd. (Hong Kong). Hexane and acetonitrile were of HPLC grade. All other solvents and chemicals were of analytical grade.

**Preparation of CLnA.** A CLnA mixture was prepared from pomegranate seeds as we previously described (*34*). In brief, air-dried seeds of pomegranate were ground and extracted in hexane. Pomegranate seed oil (2 g) was methylated to form CLnA methyl esters (CLnAME) by the addition of 200 mL of 14% BF$_3$/methanol at 80 °C for 30 min under a gentle stream of nitrogen gas. In this way, punicic acid in pomegranate seed oil was isomerized to produce a mixture of 10 isomers. After the mixture had been saponified in 150 mL of 0.3 M KOH in 90% ethanol at 50 °C for 2 h, CLnAMe was acidified by the addition of 30 mL of 1.0 M H$_2$SO$_4$. The resultant free fatty acid form of CLnA was extracted in hexane (3 × 10 mL) and recrystallized twice in hexane at −20 °C. GC analyses showed that the purity of total CLnA reached 98%.

**Preparation of GTCs.** Jasmine green tea was extracted to isolate GTCs according to the method given in our previous study (*35*). In brief, 10 g of dry tea leaves was soaked three times in 150 mL of hot distilled water (80 °C). The infusion was cooled at room temperature, filtered, and then extracted with an equal volume of chloroform to remove caffeine and pigment. GTCs in the remaining aqueous layer were then extracted twice with an equal volume of ethyl acetate. After ethyl acetate had been removed in a vacuum rotary evaporator, the resulting crude GTCs were dissolved in 10 mL of distilled water and freeze-dried overnight. The individual catechin derivatives in jasmine tea extract were quantified using a HP-1100 HPLC equipped with a ternary pump (*36*). The yield in this study reached 7.5 g of GTCs/100 g of jasmine green tea leaves with a

purity of 95%, in which (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epicatechin (EC) accounted for 62.3, 19.2, 8.3, and 4.6%, respectively.

**Oxidation of CLnA, CLA, LN, and LA.** To compare the oxidative stabilities of CLnA and CLA with those of their nonconjugated counterparts, a mixture containing four types of fatty acids was oxidized. In brief, CLnA, CLA, LN, and LA each (0.5 g each) was mixed thoroughly in a 5 mL beaker with 0.2 g of heptadecanoic acid (17:0) being added as an internal standard. The mixture was oxidized in air under continuous stirring at 50 °C. Five samples (20 mg each) were periodically taken at 0, 12, 14, 16, 18, 20, 22, 28, 35, 44, and 48 h, respectively. The experiments were performed in triplicate. After being methylated as described below, all of the samples were subjected to GC analyses. The amounts of unoxidized fatty acids were quantified by GC according to the amount of heptadecanoic acid added.

**Oxidation of Individual CLnA Isomers.** To study the oxidative stability of individual CLnA isomers, 0.5 g of CLnA mixture with the addition of 60 mg of heptadecanoic acid was oxidized at conditions described above. Similarly, the experiments were performed in triplicates, and five samples (20 mg each) were periodically taken at 0, 2, 4, 6, 14, and 16 h. The CLnA isomers were then methylated and subjected to GC and Ag$^+$-HPLC analyses to quantify each CLnA isomer on the basis of heptadecanoic acid added.

**Effect of BHT and GTCs on Stability of CLnA.** To investigate if GTCs were more effective than BHT in preventing the oxidation of CLnA, 200 ppm BHT and 200 ppm GTCs were added into CLnA. The rationale for choosing this concentration is that the maximum 200 ppm of antioxidant is generally permitted in fats and oils in most countries. CLnA with or without addition of 200 ppm BHT or 200 ppm GTCs was similarly oxidized at the conditions described above. The experiments were conducted in triplicates, and the mixture was sampled five times at each time interval. All of the samples were methylated and subjected to GC analyses.

**Methylation.** A gentle acid-catalyzed methylation condition (1.5 mol/L H$_2$SO$_4$/methanol, at 40 °C for 10 min) was used in the present study to minimize the isomerization of CLnA and CLA obtained above (*34*). In brief, fatty acid samples (10−20 mg) were transferred into a test tube followed by the addition of 2 mL of 1.5 mol/L H$_2$SO$_4$/methanol reagent. The tube was then flushed with nitrogen gas and sealed tightly. The methylation tube was placed in a heat block at 40 °C for 10 min and then cooled at room temperature. Hexane (4 mL) and distilled water (3 mL) were then added and mixed thoroughly. After centrifugation, the top hexane layer containing fatty acid methyl esters (FAME) was saved and subjected to GC and Ag$^+$-HPLC analyses.

**GC Analysis.** FAMEs were analyzed on a flexible silica capillary column (SP 2560, 100 m × 0.25 mm i.d., Supelco, Inc., Bellefonte, PA) in an HP 5980 series II gas−liquid chromatograph equipped with a flame ionization detector and an automated injector (Palo Alto, CA). The column temperature was programmed from 170 to 210 °C at a rate of 2 °C/min and then held for 5 min. The injector and detector temperatures were set at 250 and 300 °C, respectively. Nitrogen gas was used as the carrier at a head pressure of 35 psi. A typical GC chromatogram is shown in **Figure 1**.

**Ag⁺-HPLC Analysis.** The individual CLnAMEs were separated using an HP-1100 HPLC equipped with a ternary pump delivery system. In brief, 5 μL of the CLnAMEs (5 μg/mL) in hexane were injected onto a silver ion impregnated column (250 × 4.6 mm i.d., 5 μm, Chrompack, Bridgewater, NJ) via a Rheodyne valve injector. Hexane, containing 0.1% acetonitrile, was chosen as a mobile phase at a flow rate of 1.0 mL/min. The separated individual CLnAMEs were monitored at 284 nm. Each CLnAME was identified and quantified by Ag⁺-HPLC as we previously reported (*2*).

**Oxygen Consumption Test.** Headspace oxygen analysis was used to compare the oxidative stability of each fatty acid by monitoring oxygen consumption (*25*). In brief, 100 mg each of CLnA, CLA, LN, LA, or CLnA with the addition of 200 ppm BHT or GTCs was transferred into glass tubes (150 × 16 mm, o.d.). After flushing with air, these reaction tubes were sealed tightly with a rubber stopper obtained from an evacuated blood collection tube (100 × 16 mm o.d., Becton-Disckinson, Rutherford, NJ), which usually maintains a vacuum for 2−3 years. The sealed tube was leak free and verified by filling the tube with nitrogen gas and monitoring the headspace oxygen concentration by gas chromatography. Oxidation was conducted at 50 ± 2 °C. The headspace oxygen was sampled periodically with a gastight syringe and analyzed in a Shimadzu GC-2010 gas−solid chromatograph (Tokyo, Japan) fitted with a ¹/₈ in. × 6 in. stainless steel column packed with molecular sieves 5A (60/80 mesh) and a thermal conductivity detector. The percent oxygen in the headspace was calculated from the ratio of the oxygen to nitrogen. Each experiment was performed in triplicate.

## RESULTS

**Relative Oxidative Stabilities of CLnA, CLA, LN, and LA.** The oxidation of CLnA, CLA, LN, and LA was conducted in air at 50 °C. The oxidative stability of four types of fatty acids was expressed as a percentage of its original amount. All four fatty acids were stable at 50 °C for 13 h; thereafter, they demonstrated varying oxidative stability (**Figure 2**). To be specific, CLnA and CLA were unstable, with 95.3% CLnA and 58.0% CLA being lost after 22 h in air at 50 °C. In contrast, LN and LA were relatively stable, with 35.8% LN and 32.6% LA being thermally degraded under the same conditions. In general, CLnA and CLA degraded thermally more rapidly than their corresponding nonconjugated counterparts, LN and LA, whereas CLnA with three conjugated double bonds demonstrated greater instability compared with CLA with two conjugated double bonds.

To further confirm the results described above, an oxygen consumption test was also performed. The stability of each fatty acid was expressed as the percentage of headspace oxygen at each time interval. The faster the headspace oxygen drops, the more susceptible to oxidation the fatty acid is. When the oxidation reaction lasted for 4 h, the headspace oxygen concentrations for CLnA, CLA, LN, and LA were 7.3, 17.6, 20.2, and 20.1%, respectively (**Figure 3**). The headspace oxygen analyses clearly showed that CLnA was most unstable followed by CLA. In contrast, LA and LN were relatively stable compared with their conjugated counterparts.

**Relative Oxidative Stability of 10 CLnA Isomers.** To assess the stability of each CLnA isomer, Ag⁺-HPLC was developed, showing the CLnA mixture consisted of 10 isomers, namely, peaks 1−10 (**Figure 4**). As we previously identified (*2*), eight isomers was identified as t10,t12,t14-18:3 (peak 2), t9,t11,t13-18:3 (peak 3), t8,t10,t12-18:3 (peak 4), c11,t13,t15-18:3 (peak 6), c10,t12,t14-18:3 (peak 7), c9,t11,t13-18:3 (peak 8), c8,t10,t12-18:3 (peak 9), and c9,t11,c13-18:3 (peak 10), respectively, by Ag⁺-HPLC.

Silver-ion impregnated column of Ag⁺-HPLC can separate conjugated fatty acids into geometrical configuration groups, and different positional isomers within the same geometrical group can also be separated completely. The separation
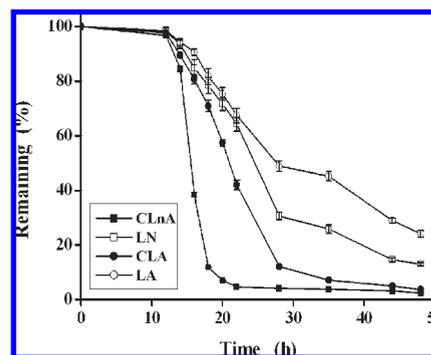


**Figure 2.** Time course of the remaining conjugated linolenic acid (CLnA, solid squares), α-linolenic acid (LN, open squares), conjugated linoleic acid (CLA, solid circles), and linoleic acid (LA, open circles) heated at 50 °C in air. Data are expressed as mean ± SD for *n* = 5 samples.
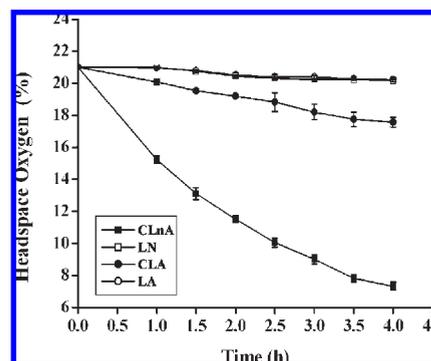


**Figure 3.** Headspace oxygen consumption profile of conjugated linolenic acid (CLnA; solid squares), α-linolenic acid (LN, open squares), conjugated linoleic acid (CLA, solid circles), and linoleic acid (LA, open circles) heated at 50 °C. Data are expressed as mean ± SD for *n* = 5 samples.
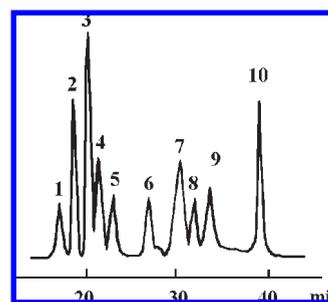


**Figure 4.** Ag⁺-HPLC chromatogram of 10 isomers of conjugated linolenic acid (CLnA). Peaks 1−10 were t11,t13,t15-18:3; t10,t12,t14-18:3; t9,t11,t13-18:3; t8,t10,t12-18:3; t7,t9,t11-18:3; c11,t13,t15-18:3; c10,t12,t14-18:3; c9,t11,t13-18:3; c8,t10,t12-18:3 and c9,t11,c13-18:3, respectively.

principle is that CLnA geometrical groups elute as the order of t,t,t < c,t,t < c,t,c. For positional isomers, the farther the double bonds are from −COOCH₃, the earlier the isomer elutes. That is, the eluting order should be 12,14,16-; 11,13,15-; 10,12,14-; 9,11,13-; 8,10,12-; 7,9,11-; 6,8,10-, respectively. According to such principle, peak 1 was identified as t11,t13,t15-18:3 because it eluted before and adjacent to t10,t12,t14-18:3 (peak 2). Similarly, peak 5 was t7,t9,t11-18:3 because it eluted after and adjacent to t8,t10,t12-18:3 (peak 4).

These 10 CLnA isomers could further be classified into three groups, namely, t,t,t-CLnA isomers (peaks 1−5), c,t,t-CLnA isomers (peaks 6−9), and a c,t,c-CLnA isomer (peak 10), on the basis of their positional and geometric configurations. In general, t,t,t-CLnA isomers as a whole were most stable, whereas c,
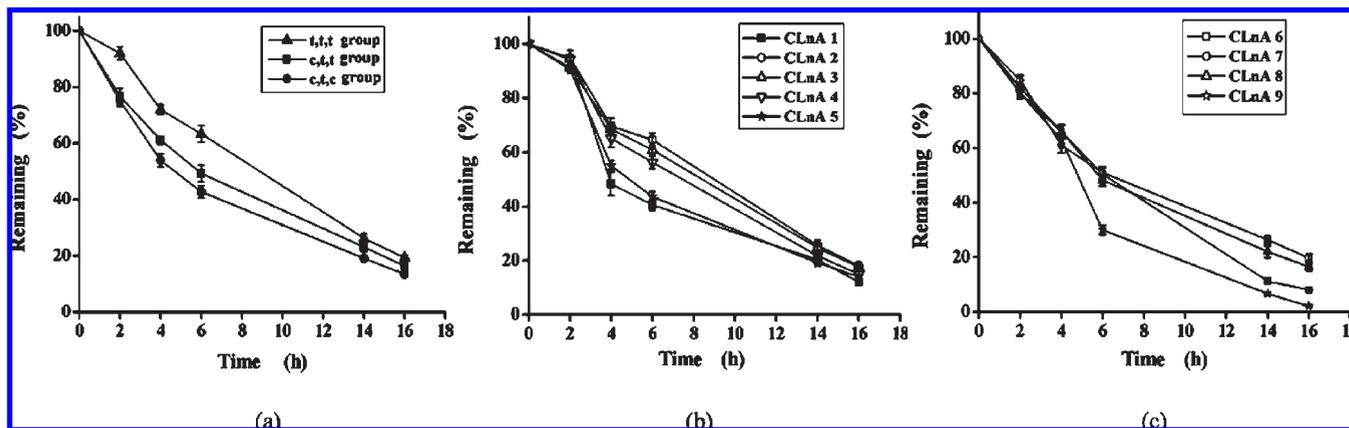
**Figure 5.** Time course of the remaining individual isomers of conjugated linolenic acid (CLnA) at 50 °C. CLnA1, CLnA2, CLnA3, CLnA4, CLnA5, CLnA6, CLnA7, CLnA8, and CLnA9 refer to t11,t13,t15-18:3; t10,t12,t14-18:3; t9,t11,t13-18:3; t8,t10,t12-18:3; t7,t9,t11-18:3; c11,t13,t15-18:3; c10,t12,t14-18:3; c9, t11,t13-18:3; c8,t10,t12-18:3; c9,t11,c13-18:3, respectively. Data are expressed as mean $\pm$ SD for $n$ = 5 samples.

t,c-CLnA isomers as a whole were most unstable (**Figure 5a**), suggesting that conjugated fatty acids with *trans*-configuration are more stable than those with *cis*-configuration. In this regard, total t,t,t-CLnA remained 26.2%, whereas total c,t,c-CLnA isomers retained was only 19.0% when incubation time was 14 h. As expected, total c,t,t-CLnA isomers had an intermediate stability compared with t,t,t-CLnA and c,t,c-CLnA isomers (**Figure 5a**).

CLnA isomers among each group had various oxidative stabilities (**Figure 5b**). It was found that t11,t13,t15-18:3 (peak 1) and t7,t9,t11-18:3 (peak 5) were less stable than t10,t12, t14-18:3 (peak 2), t9,t11,t13-18:3 (peak 3), and t8,t10,t12-18:3 (peak 4). For instance, the amounts of t10,t12,t14-18:3 (peak 2), t9,t11,t13-18:3 (peak 3), and t8,t10,t12-18:3 (peak 4) remained 25.5, 24.7, and 21.6%, respectively, whereas those of t11,t13,t15-18:3 (peak 1) and t7,t9,t11-18:3 (peak 5) remained at only 20.2 and 19.2% after 14 h of oxidation, suggesting a CLnA isomer could become unstable if the three conjugated double bonds were closer to either the methyl or carboxyl terminal (**Figure 5b**). Among the c,t,t-CLnA isomers, c8,t10,t12-18:3 (peak 9) and c11,t13,t15-18:3 (peak 6) were less stable than c10,t12,t14-18:3 (peak 7) and c9,t11,t13-18:3 (peak 8) (**Figure 5c**).

**Antioxidant Activity of BHT and GTCs in Oxidation of CLnA.** Both BHT and GTCs were able to prevent partially the oxidation of CLnA. The fatty acid analyses clearly demonstrated that addition of 200 ppm BHT or GTCs significantly increased the stability of CLnA. As shown in **Figure 6**, 95.3% of CLnA in the control was lost when the oxidization reaction lasted 22 h, whereas only 69.3 and 53.0% of CLnA were lost in the samples with the addition of 200 ppm BHT and GTCs, respectively. It appeared that GTCs were more effective than BHT in preventing the oxidation of CLnA under the same experimental conditions.

The result from the headspace oxygen consumption test was consistent with that from the fatty acid analyses. When the reaction lasted 4 h, the headspace oxygen concentration in the CLnA control decreased to 7.3%, whereas that in the samples with the addition of BHT and GTCs decreased only to 9.2 and 13.0%, respectively (**Figure 7**). This reaffirmed that GTCs had greater protection than BHT in the oxidation of CLnA.

## DISCUSSION

The present study was the first of its kind to compare the oxidative stabilities of CLnA and CLA with their nonconjugated counterparts, LN and LA. First, both the fatty acid analysis and
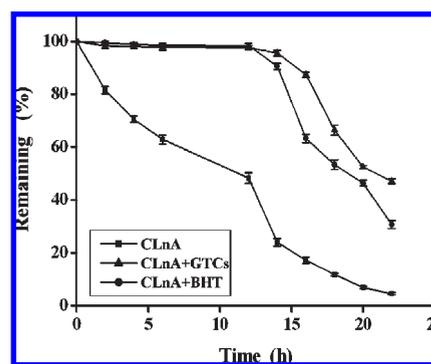


**Figure 6.** Time course of the remaining conjugated linolenic acid (CLnA, solid squares), CLnA with addition of 200 ppm green tea catechins (GTC, solid triangles) or 200 ppm butylated hydroxytoluene (BHT, solid circles) at 50 °C in air. Data are expressed as mean $\pm$ SD for $n$ = 5 samples.
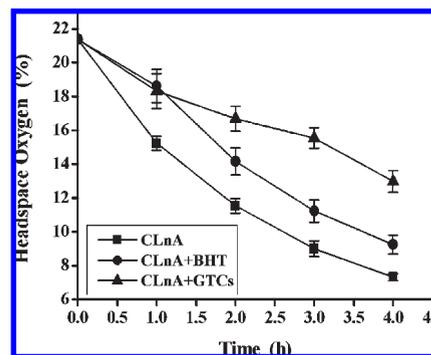


**Figure 7.** Headspace oxygen consumption profile of conjugated linolenic acid (CLnA, solid squares), CLnA with addition of 200 ppm green tea catechins (GTCs, solid triangles) or 200 ppm butylated hydroxytoluene (BHT, solid circles) at 50 °C. Data are expressed as mean $\pm$ SD for $n$ = 5 samples.

the oxygen consumption test demonstrated clearly that CLnA and CLA were less stable compared with LN and LA, suggesting that conjugated fatty acids are more susceptible to autoxidation than their corresponding nonconjugated ones. Second, CLnA oxidized much more quickly than CLA, implying that the oxidative stability of conjugated fatty acids decreases with increasing number of conjugated double bonds. The result was in agreement with that of Tsuzuki et al. (*26*), who showed that α-eleostearic acid (9c,11t,13t-18:3) degraded more quickly than LA and CLA, and the oxidative stability of α-eleostearic acid in

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which the carboxylic acid group was esterified with triacylglycerol was greater than that of the free α-eleostearic acid. The mechanism by which conjugated fatty acids are more vulnerable to oxidation than nonconjugated fatty acids is attributable to their low free energy level because both CLnA and CLA can readily donate an electron or a proton to form a free radical intermediate due to their resonance delocalization (*25*). By the same deduction, CLnA should have greater susceptibility to oxidative degradation than CLA because conjugated fatty acids with a greater number of double bonds would resonance better and have lower free energy than those with lesser numbers of conjugated double bonds. In this regard, CLA free radicals have been shown to be rapidly decomposed to furan fatty acids (*27*). However, no study to date has examined the decomposition of CLnA free radicals.

The present study was also the first to compare the relative stabilities of 10 CLnA isomers. The results indicated their stability was a function of geometric configuration of conjugated double bonds. The results showed that t,t,t-CLnA isomers were most stable followed by c,t,t-CLnA isomers and then c,t,c-CLnA isomers, suggesting a *cis*-double bond is chemically more unstable than a *trans*-double bond because the former has a higher free energy level and is more vulnerable to autoxidation. We had previously studied the oxidative stability of 12 CLA isomers and had made the similar observation, showing that the oxidative stability of CLA isomers was t,t-CLA > c,t-CLA > c,c-CLA (*25*). As t,t,t-CLnA isomers had three *trans* double bonds, they exhibited greater stability compared with c,t,t-CLnA and c,t,c-CLnA isomers. Similarly, c,t,t-CLnA isomers as a whole were more stable than c,t,c-CLnA isomers because the former had two *trans* conjugated double bonds, whereas the latter only had one.

The oxidation stability of CLnA isomers in each geometrical group was also a function of the location of the three conjugated double bonds. This was reflected by the fact that among five t,t,t-CLnA isomers, t11,t13,t15-18:3 and t7,t9,t11-18:3 were less stable than t10,t12,t14-18:3, t9,t11,t13-18:3, and t8,t10,t12-18:3, whereas among the c,t,t-CLnA isomers, c8,t10,t12-18:3 and c11,t13,t15-18:3 degraded more rapidly than c10,t12,t14-18:3 and c9,t11,t13-18:3 under the same experimental conditions. It was concluded that a CLnA isomer could become less stable if the three conjugated double bonds were closer to either the methyl or carboxyl terminal along the fatty acid chain. It is expected that a CLnA is more likely to donate an electron or a proton to produce a free radical intermediate due to its resonance delocalization when the methyl and carboxyl groups are closer to the three conjugated double bonds.

It seems necessary to prevent the oxidation of CLnA in foods. In this study, GTCs were chosen as an antioxidant to prevent the oxidation of CLnA compared with BHT, a most commonly used synthetic antioxidant used in frying oil and fat. Both the GC analysis and the oxygen consumption test revealed that the addition of GTCs at 200 ppm significantly inhibited the oxidation of CLnA, with the protective effect of GTCs being stronger than that of BHT under the same conditions. Similarly, GTCs have been previously proven to be more effective than BHT against the oxidation of CLA in canola oil (*25*). In this regard, GTCs are indeed capable of preventing lipid oxidation in various food systems (*37, 38*).

The reason GTCs can inhibit polyunsaturated fatty acids from oxidation is that they have a specific polyphenolic chemical structure and three rings, which are able to scavenge effectively the free radicals. Comparatively, BHT has only one ring and should have a lower free radical scavenging activity than GTCs.

In conclusion, CLnA and CLA had greater oxidative instability than their corresponding nonconjugated counterparts, LN

and LA, under the present experimental conditions. Compared with CLA, CLnA was more susceptible to oxidative degradation. Among the three groups of CLnA isomers, t,t,t-CLnA isomers had greater stability followed by c,t,t-CLnA and c,t,c-CLnA isomers in decreasing order. Both GTCs and BHT were able to partially prevent CLnA oxidation. However, GTCs appeared to be more potent than BHT under the same experimental conditions.

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