

Effect of Antioxidants on Soy Oil Conjugated Linoleic Acid Production and Its Oxidative Stability

Ramesh R. Yettella, Brooke Henbest, and Andrew Proctor*

Department of Food Science, University of Arkansas, 2650 North Young Avenue, Fayetteville, Arkansas 72704, United States

ABSTRACT: Conjugated linoleic acid (CLA)-rich soy oil can be produced by photoisomerization of soy oil linoleic acid to produce a soy oil with up to 20% CLA. Recent studies indicate that mixed soy tocopherols added to refined bleached deodorized (RBD) oil produced significant increase in soy CLA yield during soy oil linoleic acid photoisomerization. However, the effect of common synthetic free radical scavenging antioxidants and specific tocopherols on CLA yield and its oxidative stability is not known. Therefore, this investigation evaluated the effects of various antioxidant systems on soy oil CLA yield and oxidative stability. Soy oil with added antioxidants consisting of combinations of mixed tocopherols (MT), ascorbyl palmitate (AP), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ) was photoisomerized to produce CLA-rich soy oil. The CLA content was determined by GC-FID analysis and oxidative stability by peroxide value (PV). The soy oil in the presence of TBHQ, MT alone and MT with 500 ppm of AP produced significantly greater CLA yields and improved oxidative stability compared to a control without added antioxidants ($p < 0.05$). However, added mixed tocopherols produced the greatest CLA yield and also reduced PV relative to the control. Tocopherols in the form of α -, γ - and δ -tocopherols were then each examined as to their relative effect on CLA yields and PV. The largest increase in CLA yield was obtained with 1800 ppm of γ -tocopherols with reduced PV. Mixed tocopherols, TBHQ and γ -tocopherols can be used to increase CLA yield and reduce PV of soy oil during linoleic acid photoisomerization.

KEYWORDS: conjugated linoleic acid (CLA), antioxidants, peroxide value, BHA, BHT, TBHQ, mixed tocopherols, ascorbyl palmitate, photoisomerization

INTRODUCTION

Conjugated linoleic acid (CLA) is a family of positional and geometric isomers of octadecadienoic acid (18:2). The double bonds in CLA are conjugated and not methylene interrupted as in linoleic acid (18:2n-6). CLA is found naturally in dairy and beef products at levels of 0.3–0.8% (w/w) of the fat. The *cis*-9, *trans*-11 isomer is the principal dietary form of CLA, but lower levels of *trans*-10, *cis*-12; *trans*-9, *trans*-11 and *trans*-10, *trans*-12 isomers are also present in ruminant milk and meat.¹ The National Academy of Sciences concluded that CLA “is the only fatty acid shown unequivocally to inhibit carcinogenesis in experimental animals.”² Previous studies indicate that CLA plays antidiabetic, antiobesity, and antiatherogenic roles.^{3,4} A daily dietary intake of 3 g of CLA has been recommended as being necessary to produce desirable physiological health effects.^{5,6} However, this intake would necessitate a simultaneous increase in dietary saturated fats, which would not be desirable.

Soy oil is the most commonly used vegetable oil in the United States. It contains about 50% linoleic acid. Jain and Proctor⁷ reported a simple way of producing high levels of CLA in soy oil on a laboratory scale by converting soy oil linoleic acid (LA) to CLA using a UV/visible lamp with 0.15% iodine. However, the photoirradiation took 144 h to produce 20% CLA. So, Jain et al.⁸ further optimized the process to produce CLA-rich soy oil on a pilot scale resulting in greater quantities of CLA in less time. Approximately 75% of total CLAs were *trans,trans*-isomers, while the remaining were *cis,trans*- and *trans,cis*-isomers. A study by Jain et al.⁸ on the effect of the degree of soy oil processing on CLA yields prior to the photoirradiation step showed that the CLA yields increased with an increasing degree of oil refining. Crude

soy oil gave the lowest CLA yield of 0.2% total CLA, while alkali RBD soy oil gave the highest yield of 16.3% total CLA. CLA processed from crude soy oil had the highest oxidative stability of 13 days induction time, whereas CLA from RBD soy oil had a low oxidative stability with 8 days of induction time. Decreasing oxidative stability with increasing degree of refining is probably due to removal of oil components with antioxidant properties.⁹ Tokle et al.¹⁰ determined the effect of concentration minor soy oil components on CLA yields and oxidative stability during photoisomerization of soy oil linoleic acid. Added peroxides, phospholipids, free fatty acids and lutein all reduced CLA yields significantly, with peroxides having the greatest affect. A PV increase from 0 to 3–4 mequiv/kg reduces CLA yield by 50%, but *Magnesol* oil adsorption prior to irradiation removed oil peroxides that significantly enhanced CLA yields. In contrast, 1400 ppm of mixed soy tocopherols produced a small, but statistically significant, increase in CLA yields, but this was not observed at other tocopherol concentrations. Therefore, a study of the effect of various commercial antioxidants and specific tocopherols on CLA yields and oxidative stability during soy oil linoleic photoisomerization was performed.

The objectives of this research were to (1) determine the effect of various antioxidants at commercially acceptable levels on CLA yields and oxidative stability, (2) identify antioxidant system that would be useful in CLA production, and (3) determine the

Received: February 22, 2011

Accepted: May 25, 2011

Revised: May 19, 2011

Published: May 25, 2011

Table 1. Combinations and Specific Concentrations of Antioxidants Used in This Study^a

combination	concentration (ppm)				
	BHA	BHT	TBHQ	AP	MT
1	100				
2	200				
3		100			
4		200			
5	50	50			
6	100	100			
7			100		
8			200		
9			50	50	
10			100	100	
11					1400
12					1600
13				250	1400
14				500	1600

^a Abbreviations: MT = mixed tocopherols; AP = ascorbyl palmitate; BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; TBHQ = *tert*-butylhydroquinone.

relationships between antioxidant, antioxidant concentrations, CLA yields and PV.

MATERIALS AND METHODS

Materials. Refined, bleached and deodorized (RBD) soy oil and mixed tocopherols (MT) were obtained from Riceland Foods (Stuttgart, AR) and used as the control. Ascorbyl palmitate (AP), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ) were obtained from Sigma-Aldrich (St. Louis, MO). α -Tocopherol was purchased from TCI America (Portland, OR). γ -Tocopherol and δ -tocopherols were obtained from Sigma-Aldrich (St. Louis, MO). Resublimed iodine crystals (EM Science, Cherry Hill, NJ) were used as catalyst. Commercial CLA methyl esters (Sigma-Aldrich, St. Louis, MO) containing a mixture of *cis*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, and *trans,trans*-CLA isomers were used as a standard, and heptadecanoic acid methyl ester (17:0; Sigma-Aldrich) was used as the internal standard. Sodium methoxide and anhydrous sodium sulfate (EMD Chemicals, Darmstadt, Germany) were used for methyl ester preparation. *Magnesol*, commercial magnesium silicate, was obtained from The Dallas Group, Inc. (Whitehouse, NJ). Helium, air and hydrogen gas was obtained from Scientific Supplies (University of Arkansas, Fayetteville, AR).

Soy Oil Adsorption Pretreatment. About 800 mL of soy oil in a 1 L beaker was mixed with 5% of *Magnesol* and stirred with a magnetic stirrer (model PC-620, Corning Inc., Lowell, MA) for 20 min on speed setting 8, adapting the method of Tokle et al.¹⁰ The oil was then vacuum filtered and deaerated with a sonicator for 30 min and placed in a 1 L beaker wrapped with aluminum foil to prevent exposure of oil to light.

Effect of Antioxidants on CLA Yields. *Oil Preparation.* Oil was heated in 1 L beaker to 70 °C with flushing with nitrogen to avoid oxidation. Then, 0.35% iodine was added to the oil, and the contents in the beaker were stirred until the iodine was completely dissolved.⁷ Twenty-six 50 g oil samples were taken from the oil and iodine solution, and antioxidants were added to obtain a range of oil samples having antioxidant concentrations described in Table 1. In this study synthetic antioxidants are tested at or below legal limits of usage in foods (200 ppm), and MT are tested at higher concentrations as their usage in foods is

Table 2. Concentrations of α -, γ -, and δ -Tocopherols Used in This Study

sample	concentration (ppm)		
	α -tocopherol	γ -tocopherol	δ -tocopherol
1	600		
2	1000		
3	1400		
4	1800		
5		600	
6		1000	
7		1400	
8		1800	
9			600
10			1000
11			1400
12			1800

unregulated. Triplicate 5 g samples taken from 50 g oil aliquots were placed in 7 mL borosilicate vials. These vials were photoirradiated using the method of Lall et al.¹¹ and Tokle et al.¹⁰ The vials were attached to the glass plate of the photoisomerization unit on the oil side at locations that provided uniform and maximum UV intensity. Irradiation was carried out for 12 h at 47 °C.¹²

GC-FID CLA Analysis. Methyl esters were prepared from RBD soy oil and photoisomerized oil by a base-catalyzed method to reduce the formation of conjugated *trans,trans* isomers during analysis.¹³ One hundred milligrams of photoisomerized soybean oil was weighed into a 25 mL centrifuge tube, and 500 μ L of 1% heptadecanoic acid methyl ester (17:0; internal standard), 2 mL of toluene, and 4 mL of 0.5 M sodium methoxide in methanol were added to the centrifuge tube and then purged with nitrogen gas. The centrifuge tube was heated to 50 °C for 10 to 12 min and then cooled for 5 min. To inhibit formation of sodium hydroxide, which could hydrolyze methyl esters to free fatty acids, 200 μ L of glacial acetic acid was added to the centrifuge tube. Five milliliters of distilled water was added to the centrifuge tube followed by 5 mL of hexane, and the tube was vortexed (model VM-3000, VWR, Thorofare, NJ) for 2 min. The hexane layer was extracted and dried over anhydrous sodium sulfate in a 7 mL glass vial. Another 5 mL of hexane was added to the centrifuge tube, the tube was vortexed for another 2 min, and the hexane layer was dried over anhydrous sodium sulfate prior to methyl ester analysis.

Methyl esters were analyzed by gas chromatography (GC) using an SP 2560 fused silica capillary column (100 m \times 0.25 mm i.d. \times 0.2 μ m film thickness; Supelco Inc., Bellefonte, PA)¹⁴ with a flame ionization detector (FID) (model 3800, Varian, Walton Creek, CA). Duplicate 2 μ L samples, prepared in hexane, were injected by an autosampler CP8400 (Varian), and gas chromatograms were collected by Galaxie Chromatography Workstation 1.9.3.2 (Varian). Commercial CLA methyl ester, methyl linoleate, and mixed methyl fatty esters (Sigma) were used as standards. Two determinations each consisting of duplicate injections were conducted for each treatment. CLA concentrations were calculated by the following equation:

$$\text{isomer concn} = \frac{[\text{internal std concn (5 mg)} \times \text{peak area} \times \text{relative response factor}]}{\text{internal std peak area}}$$

Peroxide Value. An AOCS acetic acid–chloroform method (AOCS Cd 8-53) was used to measure the oxidation in the oil samples.¹⁵ Peroxide values (PVs) of RBD soy oil and photoisomerized oil samples were measured in duplicate.

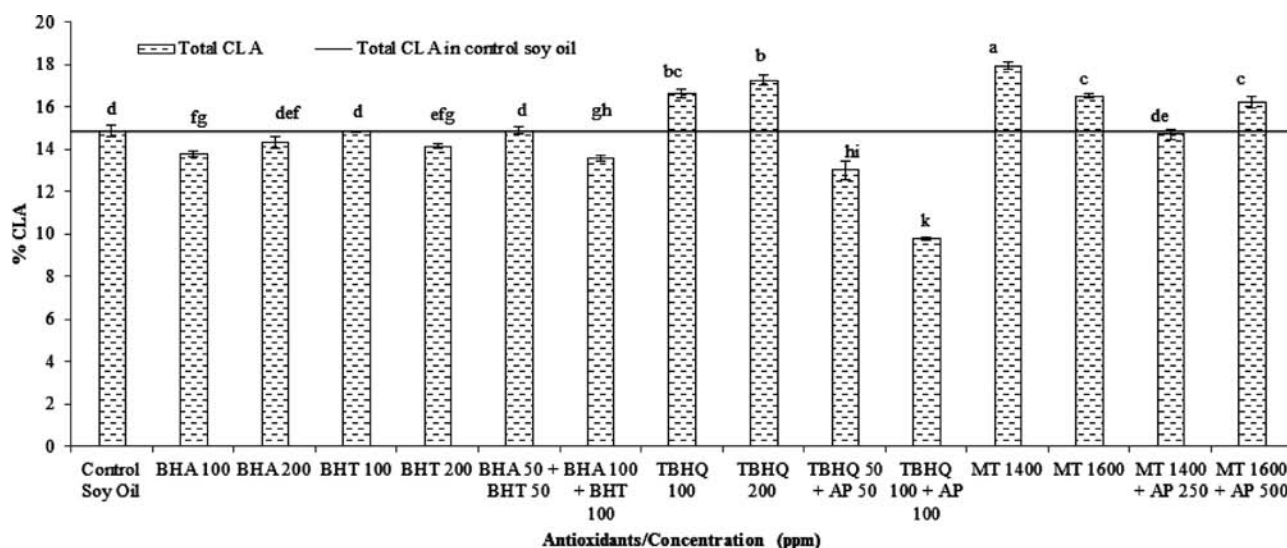


Figure 1. CLA yields obtained by photoisomerization of soy oil LA with oils of various added antioxidants (error bars represent standard error of mean ($n \geq 4$); MT = mixed tocopherols; AP = ascorbyl palmitate, BHA = butylated hydroxyanisole, BHT = butylated hydroxytoluene; TBHQ = *tert*-butylhydroquinone; bars with different letters are significantly different at $p < 0.05$).

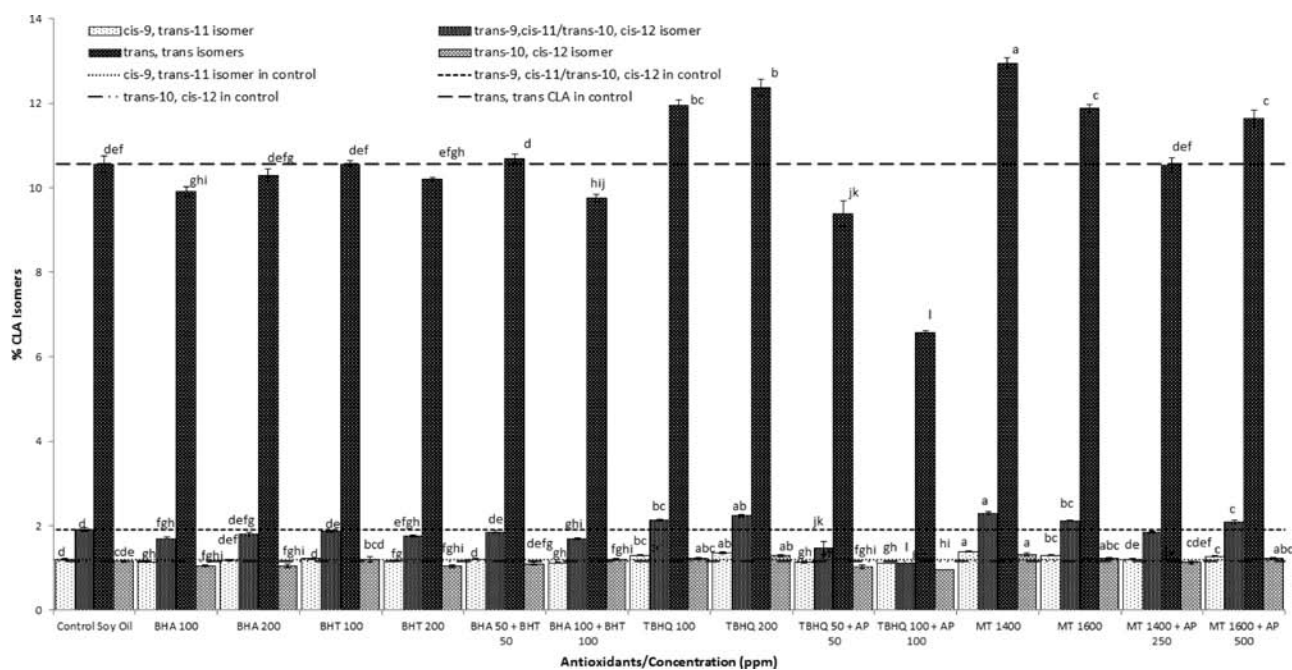


Figure 2. CLA isomer yields obtained by photoisomerization of soy oil LA with oils of various added antioxidants (error bars represent standard error of mean ($n \geq 4$); MT = mixed tocopherols; AP = ascorbyl palmitate, BHA = butylated hydroxyanisole, BHT = butylated hydroxytoluene; TBHQ = *tert*-butylhydroquinone; bars with different letters are significantly different at $p < 0.05$).

Effects of α -, γ - and δ -Tocopherols on Soy Oil CLA Yield.

Since 1400 ppm of mixed soy tocopherols produced the most CLA in the previous study, the effect of α -, γ - and δ -tocopherols on CLA yields and oxidative stability was investigated. Oil samples were prepared with 600, 1000, 1400, and 1800 ppm of α -, γ - and δ -tocopherols as presented in Table 2 and irradiated for 12 h as described earlier. The CLA content of the samples was analyzed by GC-FID, and oxidative stability was measured by PV as previously described.

Statistics. Analysis of variance (ANOVA) was conducted on all data using JMP version 5.0.1 (SAS Institute Inc., Cary, NC). A Student's *t* test

was used to differentiate mean values, with significance defined at $p < 0.05$. Standard deviations were also determined.

RESULTS AND DISCUSSION

Effect of Antioxidants on Soy Oil CLA Yield. Figure 1 shows soy oil total CLA yields with various antioxidant systems at different concentrations. The control RBD soy oil produced 14.8% total CLA after 12 h of irradiation, while BHA, BHT and

BHT/BHA combinations either did not affect CLA yield or significantly reduced it. However, TBHQ, MT, and MT with 500 ppm of AP at much higher concentration than BHA and BHT showed significantly greater CLA yields relative to control RBD soy oil ($p < 0.05$). The largest CLA yield was 18%, obtained with 1400 ppm of MT and was significantly greater than all other treatments. Tokle et al.¹⁰ also reported a small but significant

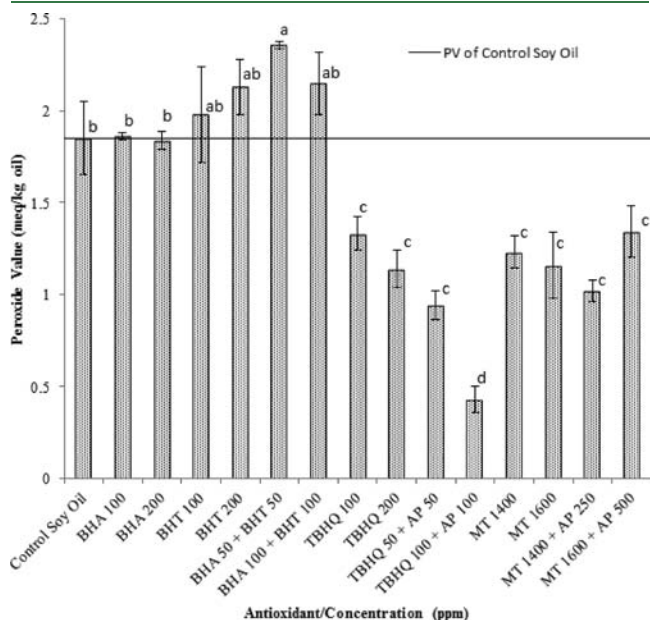


Figure 3. Peroxide values of photoisomerized soy oil with various added antioxidants (error bars represent standard error of mean ($n \geq 4$); bars with different letters are significantly different at $p < 0.05$).

increase in CLA yield on addition of tocopherols. Increasing MT to 1600 ppm and addition of AP to MT also increased CLA yields significantly in comparison to the control ($P < 0.05$). TBHQ may be more effective than BHT and BHA at similar concentrations as biphenol and BHT and BHA are monophenols.²⁴

The relative amount of each CLA isomer obtained with each antioxidant system is illustrated in Figure 2. The major CLA isomer is the *trans,trans*-isomer, whose production relative to control levels is similar to that of total CLA. Other *cis,trans* and *trans,cis* CLA isomers are at ~ 1 – 2% levels because CLA isomerizes to the more thermodynamically stable *trans,trans* CLA during processing. Processing treatments that result in reduced total CLA and *trans,trans* CLA do not proportionally reduce the minor isomers, as can be seen when reduced *trans,trans* CLA isomer contents of “TBHQ-100 + AP 100” treatment is compared with that of “MT 1400”.

Figure 3 shows the peroxide values after photoisomerization of soy oil linoleic acid with various antioxidant treatments. The PV of control soy oil after 12 h of irradiation was 1.85 mequiv/kg of oil. The PVs of soy oil with TBHQ and MT treatments either alone or in combination with AP significantly lowered PVs relative to the control ($P < 0.05$). The soy oil PV with BHA and BHT either alone or in combination did not affect PV or resulted in a slight increase. Generally, the oxidative stability was significantly high in those samples with significantly high CLA ($P < 0.05$). However, MT produced the highest CLA yields (Figure 1), although “TBHQ 100 + AP 100” treatment produced a significantly lower PV value than the other treatments possibly due to synergistic effect of TBHQ and AP.²⁵

Effects of α -, γ - and δ -Tocopherols on Soy Oil CLA Yield. Since 1400 ppm of mixed soy tocopherols produced the most CLA in the previous study, the effect of α -, γ - and δ -tocopherols on CLA yields was investigated. Figure 4 shows the total soy oil

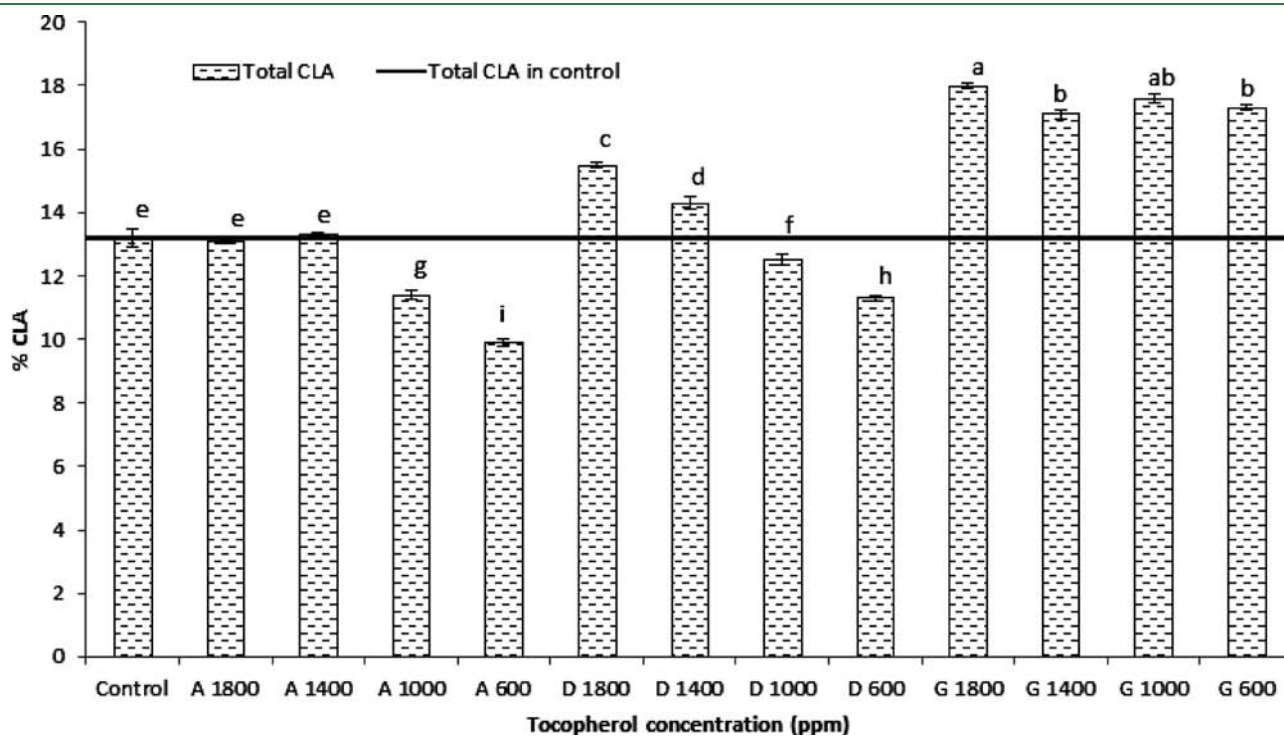


Figure 4. CLA yields obtained by photoisomerization of soy oil LA with oils of various concentrations of α -, γ - and δ -tocopherols (error bars represent standard error of mean ($n \geq 4$); bars with different letters are significantly different at $p < 0.05$; A, α -tocopherols; G, γ -tocopherols; D, δ -tocopherols).

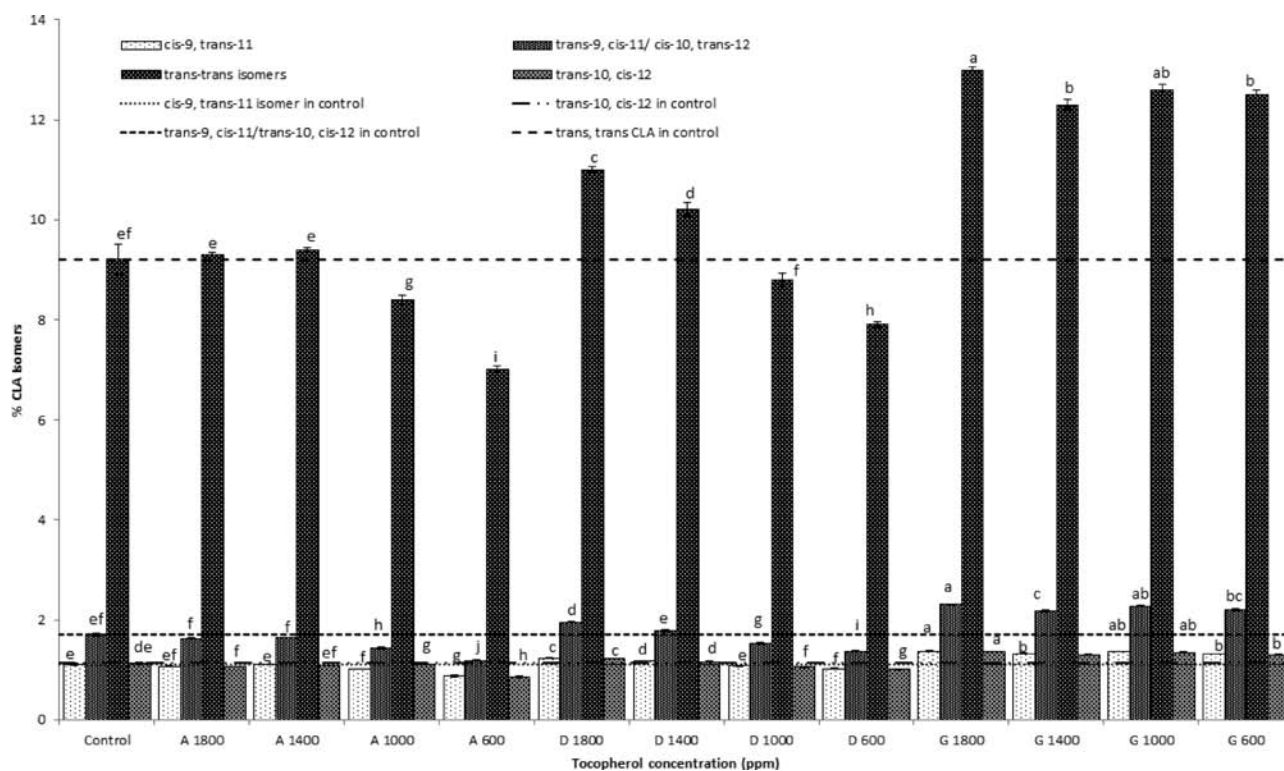


Figure 5. CLA isomer yields obtained by photoisomerization of soy oil LA with oils of various concentrations of α -, γ - and δ -tocopherols (error bars represent standard error of mean ($n \geq 4$); bars with different letters are significantly different at $p < 0.05$; A, α -tocopherols; G, γ -tocopherols; D, δ -tocopherols).

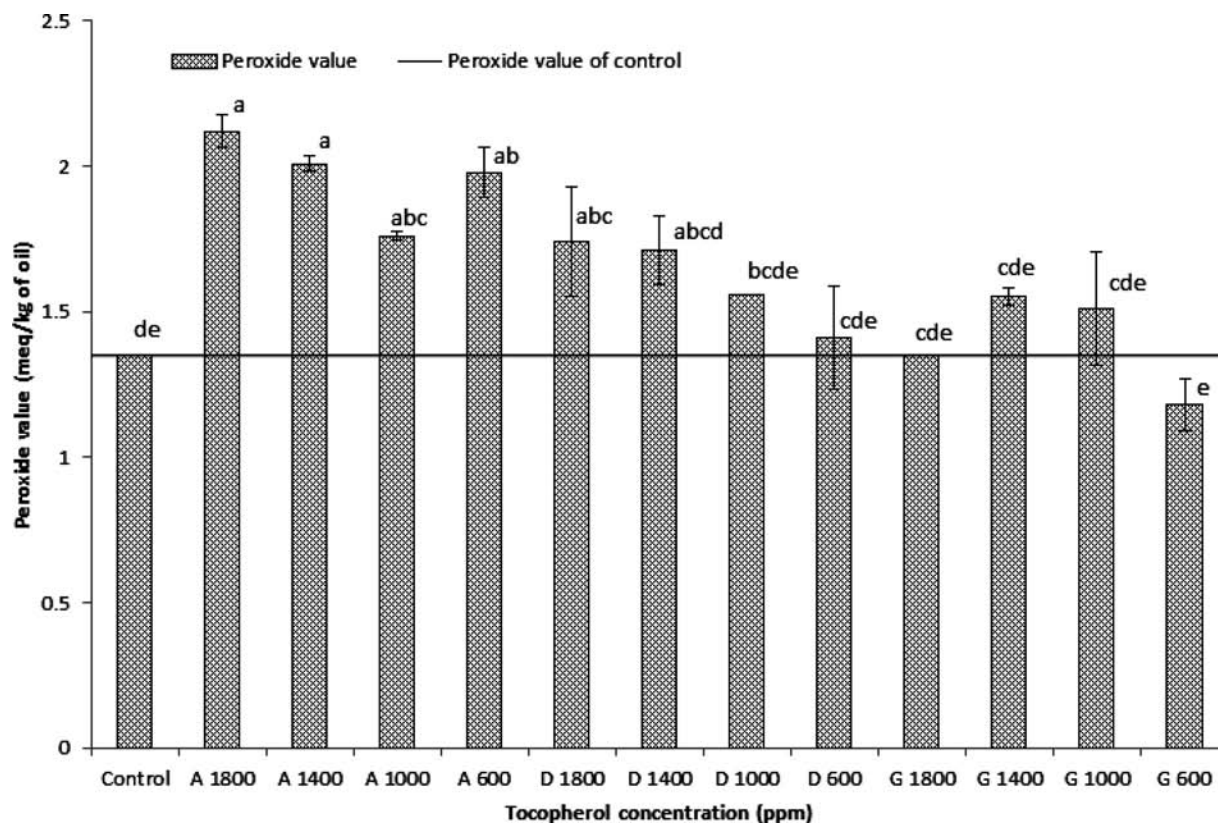


Figure 6. Peroxide values of photoisomerized soy oil with various concentrations of α -, γ - and δ -tocopherols (error bars represent standard error of means ($n \geq 4$); bars with different letters are significantly different at $p < 0.05$; A, α -tocopherols; G, γ -tocopherols; D, δ -tocopherols).

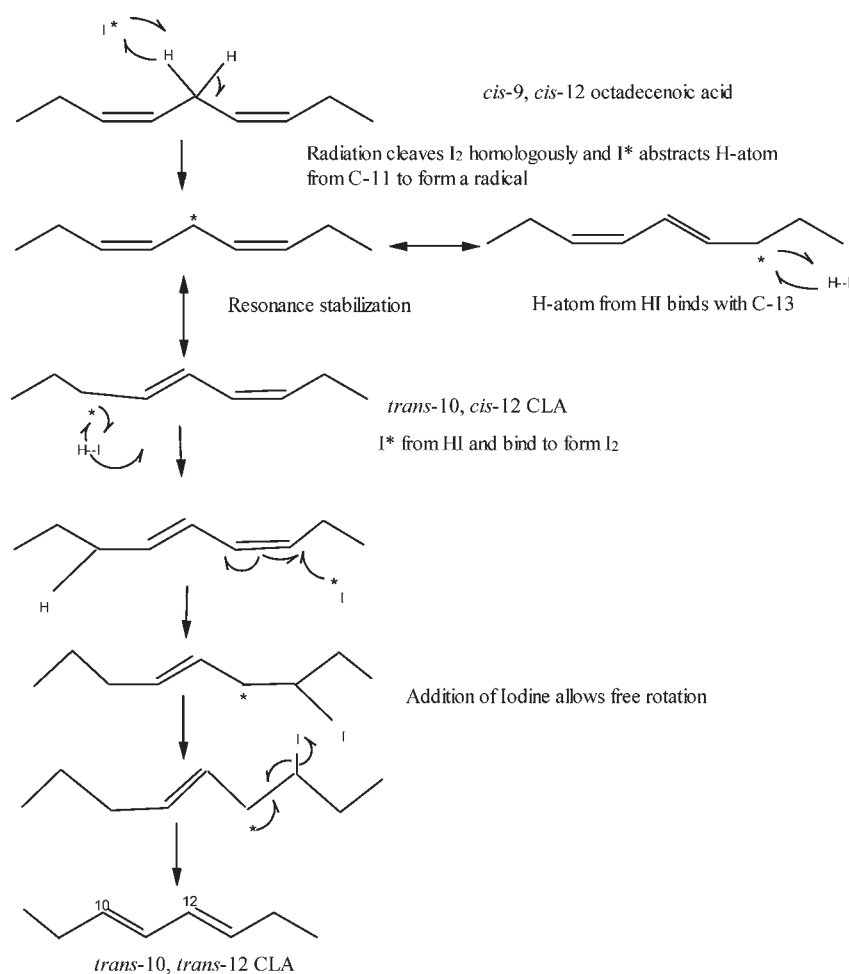


Figure 7. Free radical mechanism for the formation of CLA isomers during photoisomerization of soy oil.

CLA content obtained with various concentrations of α -, γ - and δ -tocopherols relative to a control. The γ -tocopherols at all concentrations produced significantly more CLA than the control and the other tocopherol treatments ($P < 0.05$). 1800 ppm of γ -tocopherols produced similar CLA levels as 1400 MT (Figure 4). The RBD soy oil contains ~ 1140 ppm of total tocopherols, out of which ~ 740 ppm are γ -tocopherols.⁹ So, the increase in CLA yield in the presence of mixed tocopherols is probably due to γ -tocopherols. In contrast, α -tocopherol produced similar or less CLA relative to the control.¹⁹ However, the larger δ -tocopherol concentrations at 1800 and 1400 ppm produced CLA levels greater than the control but not as high as γ -tocopherols. The lower δ -tocopherol levels reduced CLA content relative to the control.

Figure 5 illustrates the CLA isomer yields with various tocopherol treatments. The trend in *trans,trans* CLA is similar to that of total CLA, as shown in Figure 1 with the minor isomers being present ~ 1 –2% levels. There does seem to be an increase in minor isomer content with increase in *trans,trans* CLA.

The effects of various α -, γ - and δ -tocopherol concentrations on soy oil PVs is seen in Figure 6. The PV of control RBD soy oil without added tocopherols was 1.35 mequiv/kg of sample. As can be seen from Figure 3, those oils with greatest CLA levels had the lowest PVs. The α -tocopherol treatments produced significantly larger PVs than the control soy oil. The γ - and δ -tocopherol treatments produced PVs similar to the control, except

that of the 1800 ppm of δ -tocopherol treatment was significantly larger. The antioxidant activity of tocopherols as determined by PV decreased in the order γ - > δ - > α -tocopherols. This is in agreement with the results obtained by Lea et al.,¹⁶ who demonstrated that the antioxidant activity of tocopherols in methyl ester of cotton seed oil at 50 °C decreased in the order γ - > δ - > β - > α -tocopherol.

However, γ -tocopherol produced the most CLA of the tocopherols while α -tocopherol produced the lowest CLA relative to the concentrations of tocopherols investigated. This may be due to the higher stability of γ -tocopherol and faster degradation of α -tocopherol during photoisomerization. Ko et al.¹⁷ reported that α -tocopherol was destroyed faster than other tocopherols in CLA during storage at 50 °C. Isnardy et al.¹⁸ reported that α -tocopherol was destroyed faster than other tocopherols in purified rapeseed oil. Furthermore, the redox potential of α -tocopherol is low when compared to other tocopherols, which implies that it is a stronger hydrogen donor and more vulnerable to oxidation.

The main findings of this study are that CLA yields increase with increasing antioxidant capacity and antioxidant stability. Mixed soy tocopherols, probably as γ -tocopherol, produced more CLA than the synthetic antioxidant systems at the legal levels of use because they can be used at greater levels. In addition, increase in CLA levels is related to an increase in oxidative stability.

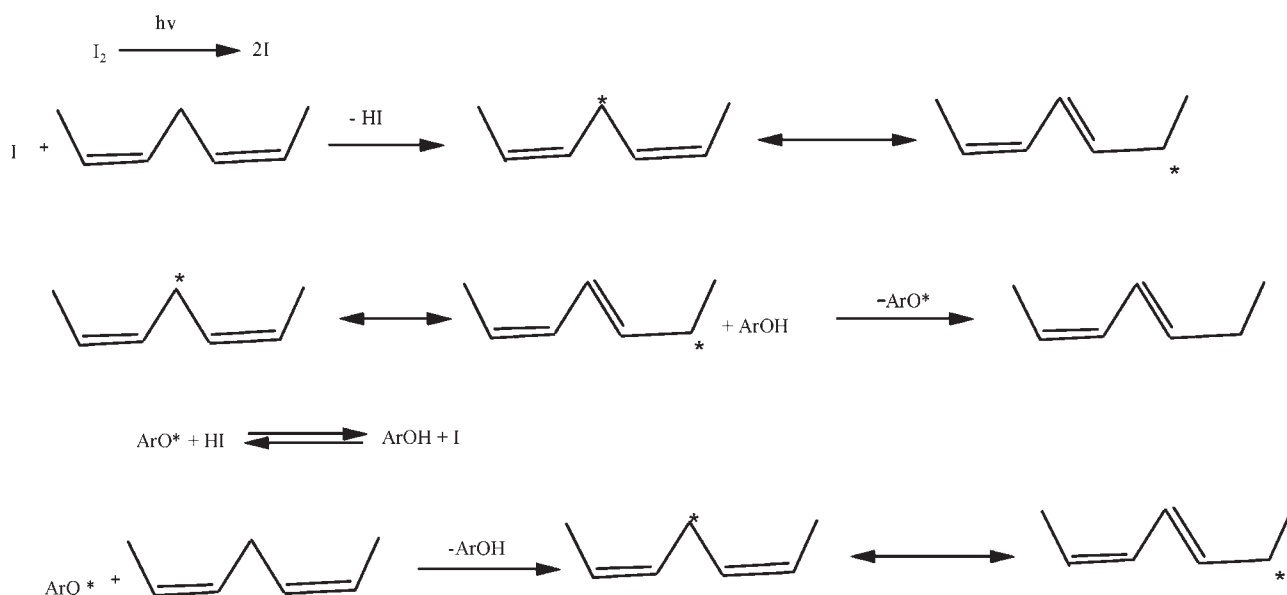


Figure 8. Proposed mechanism of the effect of antioxidants on CLA yield.

The observation of increasing antioxidant capacity being associated with promoting CLA appears counterintuitive. When the mechanism of CLA formation is considered (Figure 7), the first step in this reaction is the catalytic abstraction of a hydrogen radical from the allylic carbon 11 to form a LA radical.²⁰ Conventional understanding of radical scavenging antioxidants would predict that increasing antioxidant levels would reduce CLA formation. However, to speculate, if in this case methylene interrupted diene radicals are formed and isomerized faster than the antioxidant hydrogen radical donation, then conjugated diene radicals will be stabilized to allow CLA formation (Figure 8).

The question as to why oils with the most CLA were most oxidatively stable may be explained by the antioxidant effects of CLA. Several previous findings established the protective effect of CLA against free radicals.^{21–23} However, the mechanism by which CLA interacts with free radicals is poorly understood.

In conclusion, the present study demonstrated that TBHQ, MT and MT with 500 ppm of AP, γ - and δ -tocopherols within legal limits could be used to significantly increase soy oil CLA yields and improve oxidative stability during soy oil photoisomerization. Additional studies are necessary to elucidate the interactions between CLA, antioxidants and free radicals.

AUTHOR INFORMATION

Corresponding Author

*E-mail: aproctor@uark.edu. Phone: (479) 575-2980. Fax: (479) 575-6936.

Funding Sources

We are grateful to Arkansas Soybean Promotion Board for financial support.

ACKNOWLEDGMENT

We are grateful to Riceland Foods (AR) for providing us with the RBD soy oil and mixed tocopherol samples. We thank Dr. McIntosh (Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR) for helpful discussion and advice.

ABBREVIATIONS USED

LA, linoleic acid; CLA, conjugated linoleic acid; RBD, alkali-refined bleached deodorized oil; MT, mixed tocopherols; AP, ascorbyl palmitate; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; TBHQ, *tert*-butylhydroquinone; GC-FID, gas chromatography-flame ionization detector; PV, peroxide value; ANOVA, analysis of variance

REFERENCES

- (1) Griinari, J. M.; Bauman, D. E. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In *Advances in Conjugated Linoleic Acid Research*; Yurawecz, M. P., Mossoba, M. M., Kramer, J. K. G., Pariza, M. W., Nelson, G. J., Eds.; AOCS Press: Champaign, IL, 1999; Vol. 1, pp 180–200.
- (2) National Research Council. *Carcinogens and Anticarcinogens in the Human Diet: A Comparison of Naturally Occurring and Synthetic Substances*; National Academy Press: Washington, DC, 1996.
- (3) McGuire, M. A.; McGuire, M. K. Conjugated linoleic acid (CLA): a ruminant fatty acid with beneficial effects on human health. *Proc. Am. Soc. Anim. Sci.* **2000**, *77*, 1–8.
- (4) Khanal, R. C.; Olson, K. C. Factors affecting conjugated linoleic acid content in milk, meat and egg: a review. *Pakistan J. Nutr.* **2004**, *3* (2), 82–98.
- (5) Ip, C.; Singh, M.; Thompson, H. J.; Scimeca, J. A. Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. *Cancer Res.* **1994**, *54*, 1212–1215.
- (6) Ma, D. W. L.; Wierzbicki, A. A.; Field, C. J.; Clandinin, M. T. Conjugated linoleic acid in Canadian Dairy and beef products. *J. Agric. Food Chem.* **1999**, *47*, 1956–1960.
- (7) Jain, V. P.; Proctor, A. Photocatalytic production and processing of conjugated linoleic rich soy oil. *J. Agric. Food Chem.* **2006**, *54*, 5590–5596.
- (8) Jain, V. P.; Proctor, A.; Lall, R. K. Pilot-scale production of conjugated linoleic acid –rich soy oil by photoirradiation. *J. Food Sci.* **2008**, *73* (4), E183–E193.
- (9) Jung, M. Y.; Yoon, S. H.; Min, D. B. Effects of processing steps on the contents of minor compounds and oxidation of soybean oil. *J. Am. Oil Chem. Soc.* **1989**, *66*, 118–120.
- (10) Tokle, T.; Jain, V. P.; Proctor, A. Effect of minor constituents on soy oil conjugated linoleic acid production. *J. Agric. Food Chem.* **2009**, *57*, 8989–8997.

- (11) Lall, R. K.; Proctor, A.; Jain, V. P. A rapid micro FAME method for vegetable oil fatty acid analysis by gas chromatography. *J. Am. Oil Chem. Soc.* **2009**, *86*, 309–314.
- (12) Jain, V. P.; Proctor, A.; Lall, R. K. Pilot-scale production of conjugated linoleic acid-rich soy oil by photo-irradiation. *J. Food Sci.* **2008**, *73* (4), E183–E193.
- (13) Christie, W. W.; Sebedio, J. L.; Juaneda, P. A practical guide to the analysis of conjugated linoleic acid. *Inform* **2001**, *2*, 147–152.
- (14) Ma, D. W. L.; Wierzbicki, A. A.; Field, C. J.; Clandinin, M. T. Conjugated linoleic acid in Canadian dairy and beef products. *J. Agric. Food Chem.* **1999**, *47*, 1956–1960.
- (15) American Oil Chemists' Society (AOCS). Official Method Cd8-53. *Official Methods and Recommended Practices of the American Oil Chemists' Society*; Champaign, IL, 1997–1998.
- (16) Lea, C. H.; Ward, R. J. Relative antioxidant activities of the seven tocopherols. *J. Sci. Food Agric.* **1959**, *10*, 537–548.
- (17) Ko, S. N.; Kim, C. J.; Kim, C. T.; Kim, Y.; Kim, I. H. Effects of tocopherols and tocotrienols on the inhibition of autooxidation of conjugated linoleic acid. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 496–501.
- (18) Isnardy, B.; Wagner, K. H.; Elmadfa, I. Effects of α -, γ -, and δ -tocopherols on the autoxidation of purified rapeseed oil triacylglycerols in a system containing low oxygen. *J. Agric. Food Chem.* **2003**, *51*, 7775–7780.
- (19) Lambelet, P.; Löliger, J. The fate of antioxidant radicals during lipid autoxidation. I. The tocopheroxyl radicals. *Chem. Phys. Lipids* **1984**, *35*, 185–198.
- (20) McIntosh, M. University of Arkansas. Personal communication, 2009.
- (21) Ha, Y. L.; Storkson, J.; Pariza, M. W. Inhibition of benzo-[a]pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. *Cancer Res.* **1990**, *50*, 1097–1101.
- (22) 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.
- (23) 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.* **2000**, *48*, 5469–5475.
- (24) Buck, D. F. Antioxidants. In *Food Additives User's Handbook*; Smith, J., Ed; Blackie & Son: London, 1991; p 1.
- (25) Allam, S. S. M.; Mohammed, H. M. A. Thermal stability of some commercial natural and synthetic antioxidants and their mixtures. *J. Food Lipids* **2002**, *9*, 277–293.