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ABSTRACT

Dietary conjugated linoleic acid (CLA) is known to be effective in avoiding many obesity related diseases. Conjugated linoleic acid is a product of ruminant fermentation and 3.4 g/day are needed to obtain the clinical benefits. However, it is difficult to obtain sufficient CLA to realize these benefits from a healthy diet containing dairy and beef products, without increasing levels of dietary cholesterol and saturated fat. A 20% CLA soy oil with low saturated fat and no cholesterol has been produced by photoisomerization of linoleic acid in the triacylglyceride oil. Further increasing the CLA yields has been possible by addition of tocopherol antioxidants. The objectives of this research were to determine the effects of other natural phenolic antioxidants on CLA yield and oxidative stability during photoisomerization. Rosemary extract (RME), rosmarinic acid (RA), gallic acid (GA), caffeic acid (CA), and chlorogenic acid (CHA) were each added to refined bleached deodorized soy oil at levels they were reported to serve best as an antioxidant. The oil was then photoisomerized to produce CLA-rich oil. The CLA levels in soy oil were determined by gas chromatography - flame ionization detector (GC-FID) as fatty acid methyl esters (FAMES). The oxidative stability was determined by peroxide value (PV). The order of effectiveness as a CLA promoter was CHA>RME>RA>CA>GA. Chlorogenic acid at 11 ppm showed the greatest increase in CLA yield and a much lower PV than the control. Rosemary extract was less effective than CHA while the CA, GA and RA were ineffective. A balance of polarity/non-polarity and antioxidant concentration seem to be the most important factors in determining CLA yields, oil solubility, and antioxidant performance.

* Camille Schaffner is a 2012 graduate with a major in Food Science.
† Andy Proctor is a faculty mentor and a professor in the Department of Food Science.

MEET THE STUDENT-AUTHOR

Camille Schaffner

I was born in Dallas, Texas. My dad is French, and at the age of 3 we moved to Lyon, France. Moving back to the Dallas area at the age of 10, I went on to graduate high school in 2008 from Berkner High School. Starting my college career in the fall of 2008, I was a part of the Razorback Marching Band, Sigma Alpha Iota Fraternity for Women, Alpha Epsilon Delta, and the Food Science Club. I graduated from the University of Arkansas in May of 2012 with Honors, a B.S. in Food Science, and a minor in French. I plan on starting medical school in the fall of 2013.

With the expertise and encouragement of my honors mentor, Andy Proctor and post-doc, Ramesh Yettella, I was able to successfully complete an honors research project studying the effects of natural antioxidants on conjugated linoleic acid yields during the photoisomerization of soy oil linoleic acid. The research and communication skills I acquired during this process will be invaluable to my future endeavors.

INTRODUCTION

Conjugated linoleic acid (CLA) isomers and dietary 9,12 linoleic acid (LA) are both octodienoic acids, but in CLA the diene structure is conjugated and can be either *cis-9*, *trans-11* or *trans-10*, *cis-12* whereas linoleic acid has *cis-9*, *cis-12* methylene-interrupted double bonds producing very different isomers. Conjugated linoleic acid was discovered as a product of ruminant fermentation and found in beef and dairy products in the 1930s, but it was not until the 1980s were its potential health benefits discovered in *in vitro* and animal studies. These CLA health benefits include anti-carcinogen (Ip et al., 1994), anti-obesity (West et al., 1998), and anti-diabetic activity effects (Houseknecht et al., 1998).

Unfortunately, the average daily CLA consumption is not sufficient to obtain the recommended 3.4 g of CLA necessary to realize the associated health benefits (Ip et al., 1994). This is because fat in beef and dairy products contains only low levels of CLA at 0.3-0.8%. If an increased CLA intake was achieved through increased dietary bovine and dairy products, there would be a corresponding increase in the consumption of saturated fats and cholesterol. This would be undesirable, as saturated fats and cholesterol increase the risk of cardiovascular disease and of cancer. Therefore, alternative ways to obtain high levels of dietary CLA from a low saturated fat, low cholesterol food source would be helpful.

Conjugated linoleic acid may be produced directly from linoleic acid through fermentation (Martin and Jenkins 2002; Vahvaselka et al., 2004; Lin et al., 2005) and organic synthesis (Yang and Liu, 2004). However, these methods are time consuming, produce low CLA yields, are expensive, tedious, and are not commercially viable. Soy oil is an ideal candidate as a source of CLA as it contains 50% LA, is low in saturated fats (<10%), naturally contains no cholesterol and is the most common, inexpensive vegetable oil in the U.S. Jain et al. (2008a) developed a simple method to photoisomerize soy oil linoleic acid to CLA to produce 20% CLA-rich oil. This was done in a pilot plant setting requiring only elemental iodine and 12 hours UV/vis light. The iodine was then removed by either adsorption or distillation. Jain et al. (2008b) showed that the higher the degree of oil processing in this process, i.e. the more minor crude oil components were removed, the greater the CLA yields. Tokle et al. (2009) investigated the effect of each minor crude oil component on soy oil CLA yields and found that free fatty acids (FFA), peroxide oxidation products, and phospholipids all decreased CLA yields with peroxide oxidation products having the greatest effect. Lutein and free fatty acids had very little effect on CLA yield whereas tocopherols, a soy oil antioxidant, increased CLA yields. In a subsequent study, Yetella et al. (2011) showed that adding 1400 ppm of mixed tocopherols significantly increased CLA yields while also decreasing peroxide values, which indicates greater oil oxidative stability.

The objectives of this study were to (1) determine the effect of chlorogenic acid (CHA), rosemary extract (RME), rosmarinic acid (RA), caffeic acid (CA) and gallic acid (GA) on CLA yield during the photoisomerization of soy oil

linoleic acid and (2) determine the effect of CHA, RME, RA, CA and GA on oxidative stability of CLA-rich soy oil during photoisomerization.

MATERIALS AND METHODS

Refined, bleached, and deodorized (RBD) soy oil was obtained from Riceland Foods (Stuttgart, Ark.) and used as the control. Resublimed iodine crystals (EM Science, Cherry Hill, N.J.) were used as a 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) was used for methyl ester preparation. Magnesol®, commercial magnesium silicate was obtained from The Dallas Group of America, Inc. (Whitehouse, N.J.). Helium, air and hydrogen gas were obtained from Scientific Supplies (University of Arkansas, Fayetteville, Ark.). Chlorogenic acid, caffeic acid, rosmarinic acid, and gallic acid were obtained from Sigma-Aldrich (St.Louis, Mo.) and rosemary extract was obtained from Danisco (Copenhagen, Denmark).

Oil Processing

Pretreatment of Soy Oil. Five percent Magnesol® magnesium silicate adsorbent, was added to 800 g of refined bleached deodorized (RBD) soy oil and mixed for 15 min using a magnetic stirrer to remove oxidation products that would reduce CLA yields. The oil was then vacuum filtered, 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.

Iodine and Antioxidant Addition to the Oil. Oil was heated to 70 °C while flushing with nitrogen to avoid oxidation and 0.35% iodine was added to the oil. The oil was then stirred until the iodine was completely dissolved and allowed to cool to room temperature (Jain and Proctor, 2006). One hundred gram aliquots of a range of concentrations of chlorogenic acid, caffeic acid, gallic acid, rosmarinic acid, and rosemary extract were prepared as shown in Table 1. The selected concentration range of each antioxidant was based on the concentration range they were found to be most effective as an antioxidant (Chen and Ho, 1997; Sasaki et al., 2010; Frankel et al., 1996; Frankel and Huang, 1997). Duplicate 5-mL samples were pipetted into separate 7-mL borosilicate vials for photoirradiation. Duplicate control samples of oil without added antioxidant were included with each treatment.

Photoisomerization. These vials were placed on a photoirradiation unit in areas to facilitate maximum, uniform UV light exposure and irradiated for 12 hours as described by Jain et al. (2008b) and Lall et al. (2009).

Oil Analysis

Each duplicate oil sample was subjected to fatty acid analysis as fatty acid methyl esters (FAMES) by gas chromatography-flame ionization detection (GC-FID) to determine the CLA content. Peroxide value (PV) analysis was also conducted to determine oil oxidative stability after processing. Each duplicate sample was subjected to duplicate analysis for each method.

Fatty Acid Methyl Ester (FAMES) Formation for GC-FID Analysis. One hundred milligrams of photoisomerized soybean oil was weighed into a 25-mL centrifuge tube, and 500 µL 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 min and then cooled for 5 min. After the tube had cooled, 200 µL of glacial acetic acid was added to the centrifuge tube to prevent the formation of sodium hydroxide. Five milliliters of distilled water was added to the centrifuge tube followed by 5 mL of hexane, and the tube was vortexed for 2 min. The hexane layer was extracted and dried over anhydrous sodium sulfate in a 7-mL glass vial (Christie et al., 2001). The extracted layer was then taken from the glass vial and placed in a gas chromatograph vial. Methyl esters were analyzed by gas chromatography (GC) using an SP 2560 fused silica capillary column (100 m × 0.25 mm i.d. × 0.2 µm film thickness; Supelco Inc., Bellefonte, Pa.) (Ma et al., 1999) with a flame ionization detector (FID) (model 3800, Varian, Walton Creek, Calif.). Duplicate 2-µL 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). Two determinations each consisting of duplicate injections were conducted for each treatment. Conjugated linoleic acid concentrations were calculated by the following equation:

$$\text{Isomer conc.} = \frac{[\text{ISC (5 mg)} \times \text{peak area} \times \text{RRF}]}{\text{ISPA}}$$

where ISC stands for internal standard concentration; RRF, relative response factor; and ISPA, internal standard peak area.

Peroxide Value Analysis. Peroxide values (PV) of the photoisomerized samples were measured in duplicate according to an AOCS acetic acid-choloroform method (White and Crowe, 2001).

Statistical Analysis. All samples were prepared in duplicate and duplicate analysis of each sample was done. Analysis of variance (ANOVA) was conducted on all data using JMP version 5.0.1 (SAS Institute Inc., Cary, N.C.). 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

Chlorogenic Acid (CHA). Figure 1a shows the effect that various chlorogenic acid (CHA) concentrations had on soy oil total CLA yields, relative to the control. Chlorogenic acid levels of 11, 14 and 71 ppm produced CLA levels of 25.1%, 24%, and 22.5% respectively, which were significantly greater than the level of 20.5% found in the control. Figure 1b shows the effect of CHA on the peroxide value. All oil CHA concentrations had statistically significant lower PV relative to the control. Treatments producing the greatest antioxidant effect appear to also produce the greatest CLA levels. Thus, CHA would be a viable additive to increase the CLA content of CLA-rich oil.

Rosemary Extract (RME). Figure 2a shows the effect that various RME concentrations had on total CLA in soy oil. The RME levels of 300, 500, and 600 ppm produced a small but significant increase in CLA of 18.7%, 18.1%, and 19.4%, respectively, relative to the control value of 17.8%. The 400 ppm RME level significantly reduced total CLA yield producing only 13.2% CLA. Only RME at 300 ppm had a statistically significant effect in reducing the PV during processing, relative to the control (Fig. 2b). Oil PV of oil with 400 ppm and 500 ppm RME were not significantly different from that of the control, while 600 ppm produced a greater PV.

Rosmarinic Acid (RA). The effect that various concentrations of RA had on total CLA in soy oil is seen in Fig. 3a. None of the treatments produced an increase in CLA relative to the control level of 21.8%. The 50 ppm level produced less CLA than the control and other treatments. Rosmarinic acid is polar and hydrophilic making it water soluble (Frankel et al., 1996). Its hydrophilicity made solubilization in the soy oil difficult, which may explain the results. The PV data in Fig. 3b show that at lower RA levels there was no significant difference in PV relative to the control. However, there was an elevated PV value at the higher RA concentrations of 60 ppm and 100 ppm. The greater PV at higher levels may be due to the greater mixing needed to achieve dissolved RA and thus the greater probability of incorporating oxygen into the oil.

Caffeic Acid (CA). None of the CA concentrations produced a statistically significant increase CLA yield relative to the control (Fig. 4a). A significant decrease in CLA yield was seen at 9 ppm (25.16% CLA) and 36 ppm (24.94% CLA). Figure 4b represents the PV at various concentrations of CA. All concentrations of CA produced a significantly higher PV relative to the control, which had a PV of 1.23 mequiv/kg. Thus, the presence of CA seemed not to affect or reduce CLA yields while reducing oxidative stability at the concentrations used.

Gallic Acid (GA). Figure 5a shows the effect of GA on total CLA production. Increasing GA concentration at lower levels had no effect on CLA production, but inhibited production at higher levels relative to the control. Figure 5b shows the PV data for various concentrations of GA. The low GA levels did not protect against oxidation, relative to the control. A GA concentration of 25.5 ppm resulted in a significant decrease in PV relative to the control.

The effectiveness of the phenolic antioxidants were chlorogenic acid (11-106 ppm) > rosemary extract (300-600 ppm) > rosmarinic acid (40-100 ppm) > caffeic acid (9-36 ppm) > gallic acid (8.5-34 ppm), with only chlorogenic acid and rosemary extract increasing CLA yields. Therefore, the nature of the antioxidant should be considered. It has been suggested that the antioxidant concentration in oil is critical to its performance (Shahidi and Zhong, 2011). However, CHA at 11 and 14 ppm were the most effective treatments throughout this study. The literature values of optimum antioxidant concentrations that were used in this study did not pertain to the UV irradiation processing conditions.

In order for the antioxidant to be effective in CLA production it should be sufficiently non-polar to dissolve in oil; but in order have antioxidant activity in oil it has to be polar enough to migrate to air-oil interface of microscopic air bubbles to serve as a radical scavenger. Chlorogenic acid seems to have both of these characteristics to perform as a CLA promoter and antioxidant at the interface. Carnosic acid and carnosol in RME (Frankel et al., 1996) would seem to have these characteristics, but to a much lesser degree. In contrast RA, CA, and GA are more polar, therefore requiring more time to dissolve and increasing the possibility of mixing oxygen in the oil while stirring, even under a nitrogen blanket. Thus, this would result in higher PV levels at higher RA, CA and GA levels.

CONCLUSION

Chlorogenic acid at 11 and 14 ppm was the most effective of the selected phenolic compounds of those selected in ranges used in promoting CLA formation and serving as an effective antioxidant. The most ineffective compounds, GA and CA, were the most polar and used at lowest concentrations. A balance of polar and non-polar characteristics at a critical concentration seems important to dissolve in the oil (non-polar characteristics) and serve as an antioxidant (polar characteristics). Further studies of the selected compounds under a common equimolar and ppm range could be conducted to better understand the interaction of concentration and molecular structure on both CLA yields and PV.

ACKNOWLEDGEMENTS

I would like to thank Andy Proctor, Ramesh Yettella, and Brooke Henbest for their continued support, guidance, and motivation throughout this process. Their knowledge inside and outside of the lab made my research possible. I would also like the thank Riceland Foods for providing the soy oil used in this study.

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Table 1. Antioxidants and their concentrations used in this study.

Antioxidant	Concentrations (ppm)	Citation
Caffeic Acid (CA)	9, 18, 27, 36	(Chen and Ho, 1997)
Chlorogenic Acid (CHA)	11, 14, 18, 71, 106	(Sasaki et al., 2010)
Gallic Acid (GA)	8.5, 17, 25.5, 34	(Frankel and Huang, 1997)
Rosmarinic Acid (RA)	40, 50, 60, 100	(Frankel et al., 1996)
Rosemary Extract (RME)	300, 400, 500, 600	(Frankel et al., 1996)

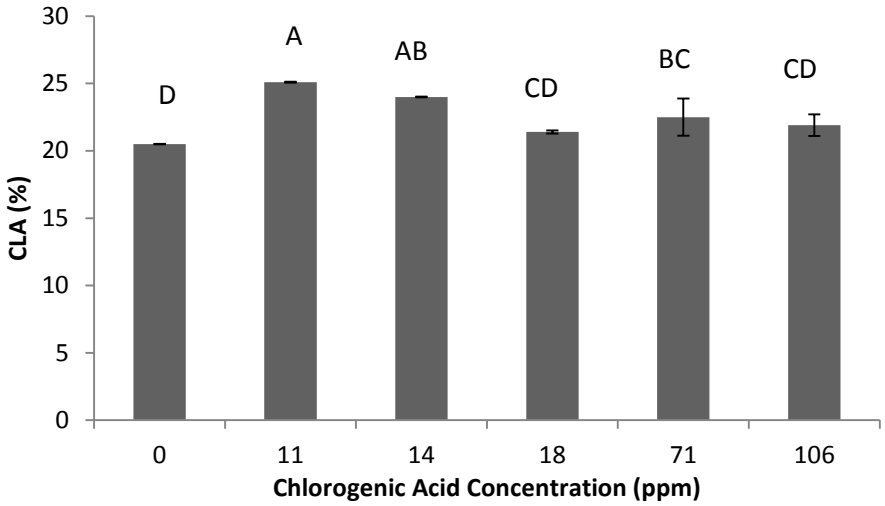

Fig. 1a. Effect of chlorogenic acid concentration on total conjugated linoleic acid yield in refined, bleached, deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours. Data points with letters in common are not statistically significantly different.

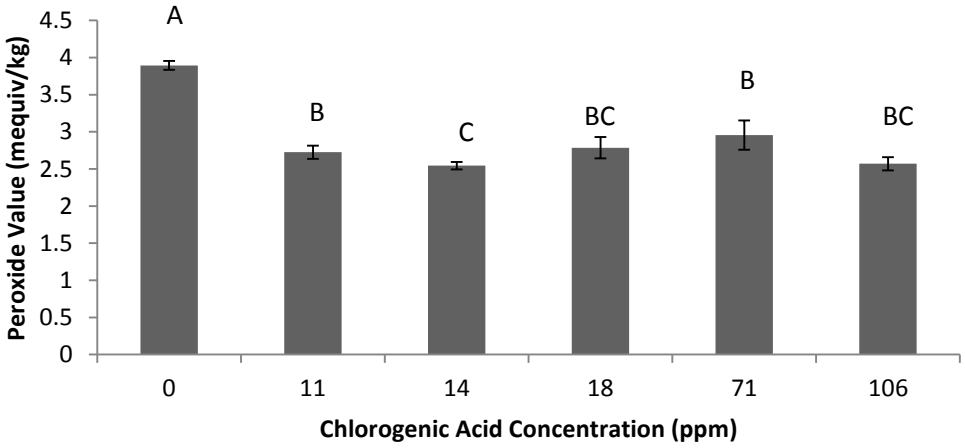

Fig. 1b. Effect of chlorogenic acid concentration on peroxide value in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours. Data points with letters in common are not statistically significantly different.

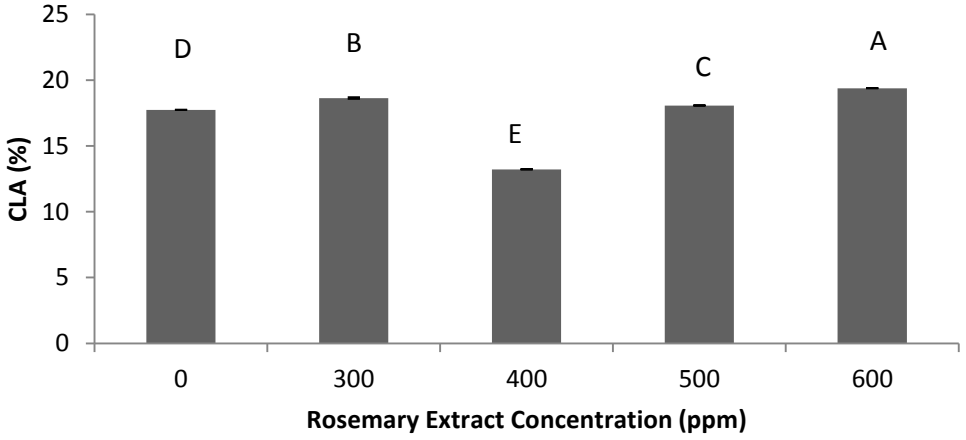

Fig. 2a. Effect of rosemary extract concentration on total conjugated linoleic acid yield in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours. Data points with letters in common are not statistically significantly different.

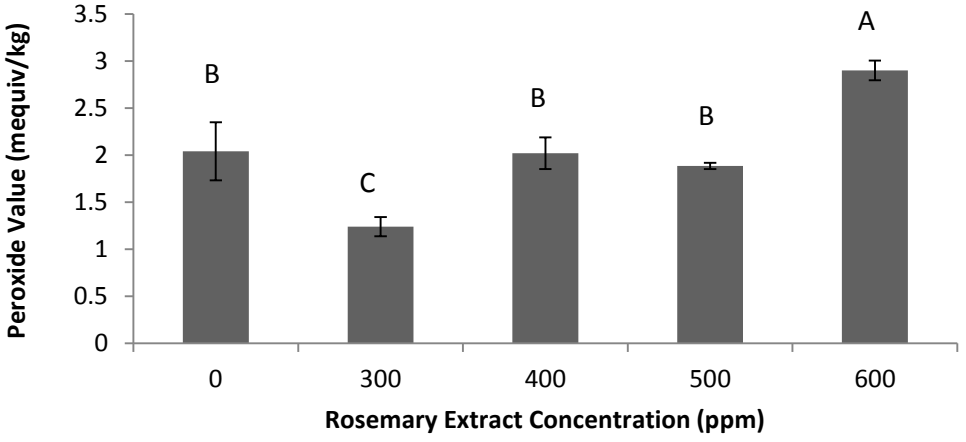

Fig. 2b. Effect of rosemary extract concentration on peroxide value in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours. Data points with letters in common are not statistically significantly different.

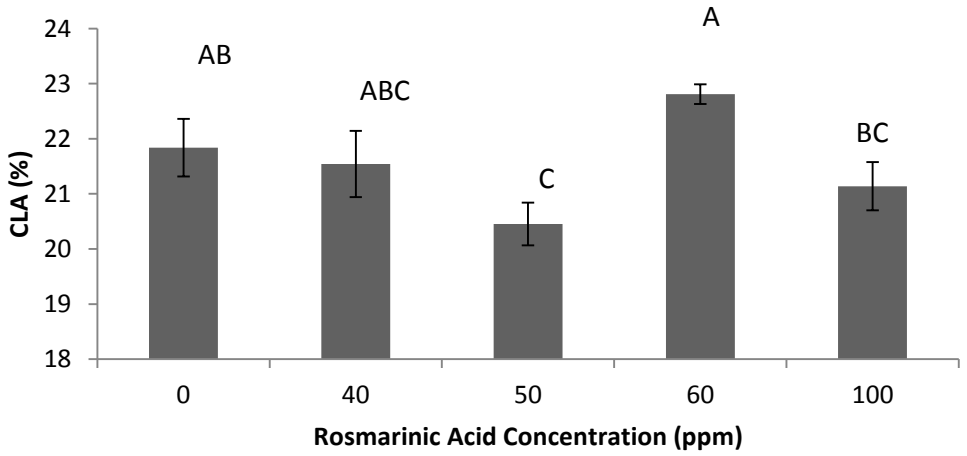

Fig. 3a. Effect of rosmarinic acid concentration on total conjugated linoleic acid yield in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours. Data points with letters in common are not statistically significantly different.

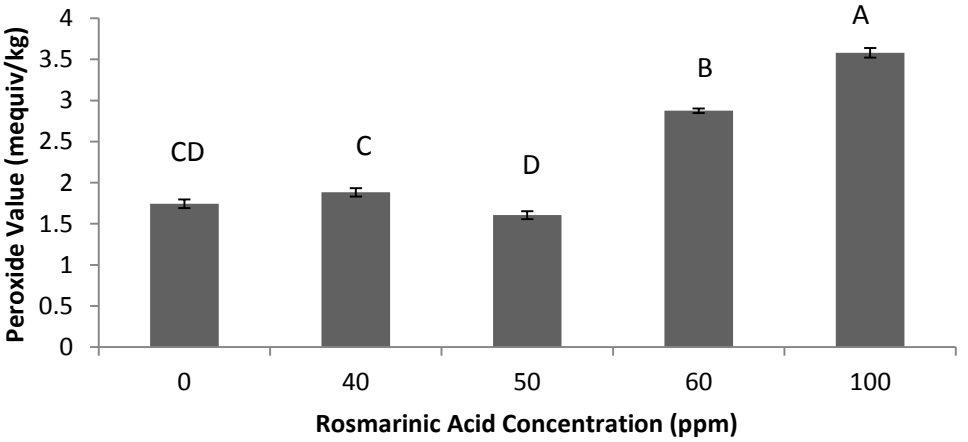

Fig. 3b. Effect of rosmarinic acid concentration on peroxide value in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours. Data points with letters in common are not statistically significantly different.

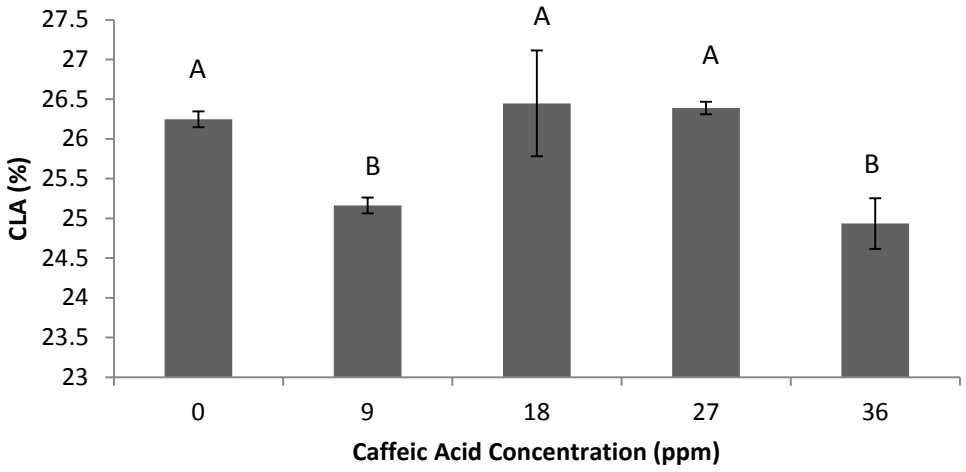

Fig. 4a. Effect of caffeic acid concentration on total conjugated linoleic acid yield in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours. Data points with letters in common are not statistically significantly different.

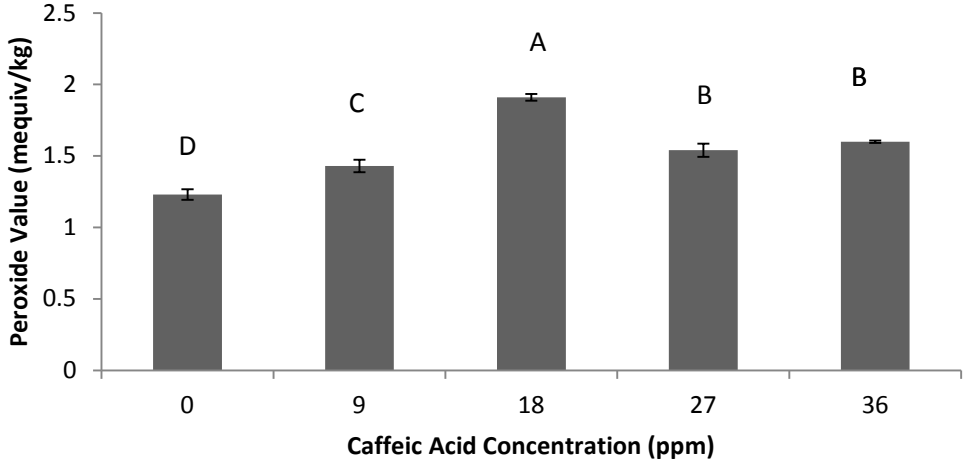

Fig. 4b. Effect of caffeic acid concentration on peroxide value in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours. Data points with letters in common are not statistically significantly different.

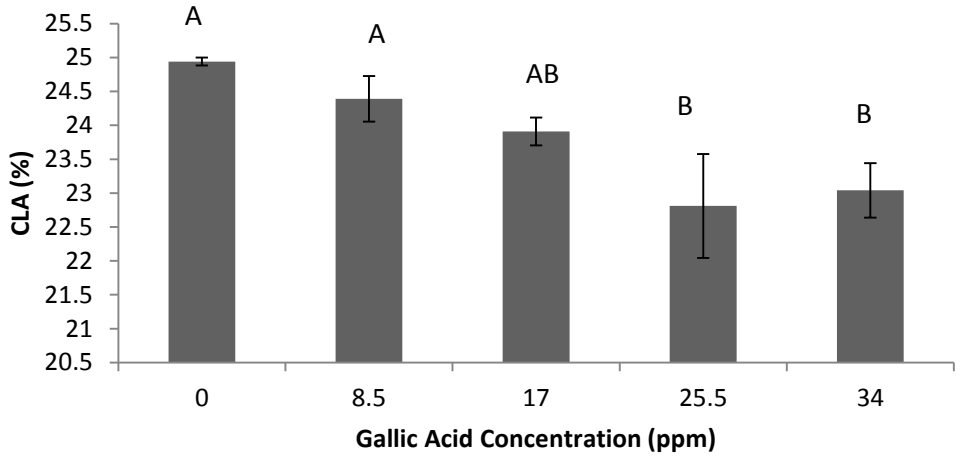

Fig. 5a. Effect of gallic acid concentration on total conjugated linoleic acid yield in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours. Data points with letters in common are not statistically significantly different.

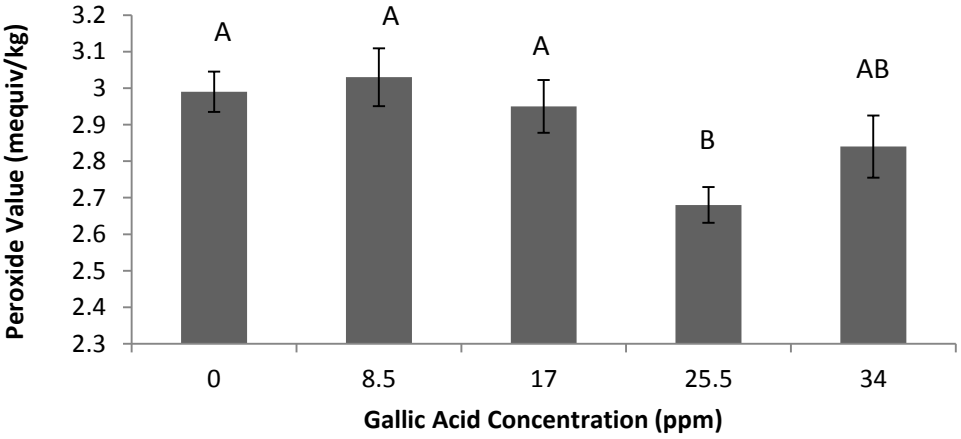

Fig. 5b. Effect of gallic acid concentration on peroxide value in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours. Data points with letters in common are not statistically significantly different.