CONJUGATED LINOLEIC ACID REDUCES LIPID OXIDATION IN IRRADIATED, COOKED GROUND BEEF PATTIES

A Dissertation

by

SUNG HEE CHAE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2005

Major Subject: Food Science & Technology

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Approved by:

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ABSTRACT

Conjugated Linoleic Acid Reduces Lipid Oxidation in Irradiated, Cooked Ground Beef Patties. (December 2005)

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M.S., Texas A&M University

Chair of Advisory Committee: Dr. Stephen B. Smith

This study was conducted to examine the antioxidative effect of conjugated linoleic acid (CLA) in irradiated, cooked ground beef patties. The hypothesis was that CLA would be retained during irradiation and would reduce lipid oxidation that is caused by irradiation. The objective was to evaluate the effects of CLA alone and in combination with irradiation on lipid oxidation, fatty acid composition, cooking loss, moisture and fat content, and trained panel sensory evaluations of beef patties. CLA was added at 0, 1, 2, or 4% level during the grinding process. Addition of CLA during the grinding process increased CLA cis-9,trans-11 and CLA trans-10,cis-12 isomers in both irradiated and non-irradiated cooked ground beef patties (irradiated at 1.6 kGy) (P = 0.0001). Weight loss during cooking was greater in irradiated beef patties than in non-irradiated patties (P = 0.004). Irradiation reduced the serumy/bloody aromatic attribute and increased browned aromatic attribute, browned aftertaste, and wet dog/hairy aromatic attribute ($P < 0.05$). There was no significant main effect of irradiation on the basic tastes. The linoleic acid, CLA cis-9,trans-11, and CLA trans-10,cis-12 were decreased by irradiation ($P < 0.05$). Although irradiation decreased the CLA isomers, higher percentages of CLA isomers were retained in irradiated patties containing a 4%

free fatty acid preparation of CLA (FFA-CLA), reflecting the ability of the FFA preparation to reduce lipid oxidation that is caused by irradiation. The thiobarbituric acid reactive substances (TBARS) values were significantly higher in irradiated, cooked ground beef patties than in non-irradiated ground beef patties ($P = 0.004$). Although the FFA-CLA was effective in reducing lipid oxidation that is caused by irradiation, it increased painty aromatic attribute, bitter taste, and astringent aftertaste due to the soapy flavor of the free fatty acid (all $P < 0.05$). The FFA-CLA decreased cooked beef/brothy and serumy/bloody aromatic attribute and browned aftertaste (all $P < 0.05$). The 1% triacylglycerol (TAG) preparation of CLA reduced TBARS in irradiated, cooked patties to levels seen in control, non-irradiated patties. The 1% TAG concentration also provided good retention of CLA in the cooked ground beef.

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Thanks also to my colleagues, friends and the department faculty and staff. I would also like to extend my gratitude to the Texas Beef Council, which provided the research funds, and to the staff of the Electron Beam Center at Texas A&M University for their help.

Finally, I would like thank my husband for his patience and love and my parents for their support and nurture.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective acronym describing a mixture of octadecadienoic acids with all possible *cis* and *trans* combinations of conjugated double bonds at the 10 and 12, the 9 and 11, or the 11 and 13 carbons. Conjugated linoleic acid occurs naturally in beef and dairy products, and total CLA isomers typically sum to approximately 1% of total fatty acids in beef (Rule and others 2002) and 2% of total fatty acids in dairy products (reviewed by Baumann and others 1999). The cis -9, trans-11 (c9,t11) isomer of CLA unequivocally inhibits growth of cancer cells in animal and tissue culture systems, whereas the trans-10, cis-12 (t10,c12) CLA isomer may reduce adiposity (Pariza and others 2000).

Rising consumer demand for convenience food has generated numerous efforts to improve both the quality and stability of precooked meat products (Yu and others 2002). Irradiation of ground beef to destroy pathogens is important because of the potential for bacterial contamination during the grinding process. However, irradiation also causes oxidation of fatty acids, and thus reduces palatability of ground beef.

Early studies indicated that CLA possessed no antioxidant capacity (Van den Berg and others 1995). However, Yu (2001) demonstrated that CLA has free radical-scavenging properties, which would protect against lipid oxidation. Leung and Liu (2000)

This dissertation follows the style and format of Journal of Food Science.

previously had demonstrated that the t10, c12 isomer of CLA possessed antioxidant properties over a wide range of concentrations, whereas the c9,t11 CLA isomer was actually a pro-oxidant at high concentrations (200 μM). Thus, the t10,c12 isomer of CLA was a more effective antioxidant. Differences between isomers and the dose dependency of CLA c9,t11 may explain the disagreement across studies in the ability of CLA to serve as an antioxidant. Joo and others (2002) investigated the effects of dietary CLA on lipid oxidation in fresh pork; the thiobarbituric acid reactive susbstances (TBARS) values of loins from pigs fed CLA were significantly lower than control loins after 7 d of storage.

The hypothesis of this study was that CLA would be retained during irradiation and would reduce lipid oxidation that is caused by irradiation. The first objective of this study was to quantify the antioxidative properties of CLA by measuring lipid oxidation, chemical composition, and fatty acid composition of aerobically-packaged, irradiated raw beef patties that were subsequently cooked. The TBA test was conducted to measure the TBARS, which indicates the degree of lipid oxidation.

The second objective was to evaluate the effects of CLA alone and in combination with irradiation on lipid oxidation, fatty acid composition, cooking loss, moisture and fat content, and trained sensory panel evaluation of beef patties.

CHAPTER II

REVIEW OF LITERATURE

Conjugated Linoleic Acid

Conjugated linoleic acid is a collective acronym describing a mixture of all possible *cis* and *trans* combinations of conjugated double bonds at the 10 and 12, the 9 and 11, or the 11 and 13 carbons. The presence of fatty acids with conjugated double bonds was first demonstrated in food products derived from ruminants in 1935 (reviewed by Bauman and others 1999). Conjugated linoleic acid occurs naturally in beef and dairy products, and total CLA isomers typically constitute approximately 1% of total fatty acids in beef (Rule and others 2002) and 2% of total fatty acids in dairy products (reviewed by Baumann and others 1999).

The beneficial effects of CLA include anticarcinogenesis in mice, enhancement of immunity, alleviation of allergies and asthma, decreased blood cholesterol in hamsters, antiatherosclerotic effects in rabbits, decreased obesity in mice, and enhanced insulin sensitivity in Zucker obese rats (Mir and others 2004).

All of the known physiologic effects of CLA are induced by two isomers: the cis -9, trans-11 (c9,t11) -CLA and the trans-10, cis-12 (t10,c12) -CLA (Pariza 2004). The c9,t11 isomer of CLA unequivocally inhibits growth of cancer cells in animal and tissue culture systems, whereas the t10,c12 CLA isomer may reduce adiposity (Pariza and others 2000). The multiple physiological effects that are reported for CLA appear to be the result of multiple interactions of the biologically active CLA isomers with numerous metabolic signaling pathways (Pariza and others 2001; Pariza 2004).

Sources of CLA

Food products derived from ruminant animals are the major source of CLA in human diets (Pariza and others 2001; reviewed by Bauman and others 1999). Although ruminant species produce and deposit CLA isomers in their tissues, the concentration of total CLA isomers rarely exceeds 2% of total lipid (Smith and others 2004). The c9,t11-CLA isomer is more abundant than t10,c12-CLA isomer in both beef and milk. It represents 80 to 90% of the total CLA in milk and 60-85% in beef fat (reviewed by Bauman and others 1999; Mir and others 2004). The c9,t11-CLA is also predominant in meat from ruminants but constitutes less of the total CLA.

Kramer and others (2004) have also reported that milk fat from cows fed a normal total mixed ration consisted mainly of c9,t11-CLA and contained a small amount of t10,c12-CLA. In their study, they concluded that the t10,c12-CLA can be increased by adding fish meal to the dairy ration.

Low concentrations of CLA are present in human tissues. The average physiologic concentration of CLA in human plasma phospholipids is $1x10^{-5}$ mol/L CLA (Ohta and others 1990).

Biosynthesis of CLA

The CLA found in milk and meat fat of ruminants originates primarily from the microbial biohydrogenation of linoleic acid and linolenic acids in the rumen and secondly from the synthesis of CLA from vaccenic acid in animal tissues (reviewed by Bauman and others 1999; Pariza and others 2001).

When consumed by ruminant animals, dietary lipids undergo two

transformations in the rumen (Dawson and Kemp 1970; reviewed by Bauman and others 1999; Pariza and others 2001). The sequence begins with isomerization of linoleic acid to c9,t11-octadecadienoic and is followed by biohydrogenation of the cis-double bond of the conjugated diene to a trans-monoenoic acid. The first step, isomerization of the cis-12 double bond, is a prerequisite for the second transformation and is produced by the rumen bacteria. The enzyme, linoleate isomerase, is bound to the bacterial cell membrane and is responsible for forming conjugated double bonds from the c9,c12 double bond structure of linoleic acid as well as linolenic acid (reviewed by Bauman and others 1999).

After formation in the rumen, c9,t11-CLA may be directly absorbed or further biohydrogenated by rumen microorganisms to trans-11 octadecenoic acid (vaccenic acid). The vaccenic acid may then be converted by stearoyl-CoA desaturase (SCD) back to c9,t11-CLA in animal tissues. The conversion of vaccenic acid to CLA is a major pathway in the formation of c9,t11-CLA in cow milk (Pariza and others 2001).

Effects of CLA on Body Composition

The effects of CLA on body composition and lipid metabolism seem very conflicting across different studies and the results are species-dependent. This is due to the interaction of the two biologically active isomers (Pariza and others 2001). Most animal studies have shown that CLA decreases weight gain (Brodie and others 1999; Park and others 1999; Evans and others 2001; Pariza and others 2001; Rodriguez and others 2002; Terpstra and others 2002; Wang and Jones 2004), whereas other studies have shown no effects (Park and others 1997; Ostrowska and others 1999; Sisk

and others 2001).

It appears that the t10,c12-CLA is responsible for body composition change, which is due to a reduction in lipid uptake by adipocytes. This reduction in fatty acid uptake by adipocytes, in turn, is due to the effects of CLA on SCD and lipoprotein lipase (LPL) activities; the t10,c12-CLA reduces SCD activity and LPL activity (Pariza and others 2001). They also suggested that CLA would block body fat gain, but not necessarily reduce body fat level that had accumulated prior to CLA administration; CLA reduces the uptake of lipid by adipocytes but has little or no effect on lipolysis.

Studies have shown that the t10,c12-CLA caused triacylglycerol (TAG) reduction in cultures of preadipocytes, depending on dose, duration of treatment, and the amount of linoleic acid in the cultures, by inhibiting both proliferation and differentiation of preadipocytes in animals, whereas the c9,t11-CLA increased TAG accumulation (Brodie and others 1999; Evans and others 2001). Similarly, the study by Rodriguez and others (2002) has shown that the t10,c12-CLA treatment of brown adipocyte cell cultures from mice reduced lipid accumulation, therefore, decreasing body weight, whereas the c9,t11-CLA treatment caused increased lipid accumulation. These results are consistent with results reported by Pariza and others (2001) where the c9,t11-CLA was active in enhancing body weight gain and feed efficiency in weanling mice. Thus, to achieve optimal effects on growth, feed efficiency, and body composition in young growing animals, it seems necessary to feed a mixture containing both the c9,t11 and t10,c12-CLA.

The ability of conjugated linoleic acid to modulate human obesity remains

controversial because data from clinical trials using mixed isomers are conflicting, and the mechanism of specific isomers and their interactions in humans is unclear (Brodie and others 1999; Brown and McIntosh 2003; Pariza 2004; Wang and Jones 2004). Reductions in body weight were observed in patients with type II diabetes, whereas other clinical studies did not show any effect of CLA on body weight in healthy obese or non-obese men and women (Wang and Jones 2004). There is some evidence that CLA might slightly decrease abdominal fat, but there is no effect on body weight, body mass index, or adipose tissue fatty acid composition (Benito and others 2001; Brown and McIntosh 2003; Pariza 2004; Riserus and others 2004).

Anticarcinogenicity of CLA

It has been well established that CLA possesses anticarcinogenic properties (Ha and others 1990; Ip and others1991; Pariza and others 2001; Belury 2002; Field and Schley 2004). The c9,t11-CLA has been shown to reduce rat mammary neoplasia (Ip and others1991; Pariza and others 2001). Although the effects of isomers on carcinogenesis appear to be inconsistent, studies have shown that CLA retards promotion of mouse tumor and human breast cancer cells (Ha and others 1990; Pariza and others 2001). Conjugated linoleic acid interferes with tumor cell growth and increases tumor cell death. This is due to the alterations in membrane composition and structure, and changes in membrane-mediated functions and signals, which is enhanced by CLA (Pariza and others 2001; Field and Schley 2004).

Antioxidative Capacity of CLA

Early studies indicated that CLA possessed no antioxidant capacity (Van den

Berg and others 1995). However, Yu (2001) demonstrated that CLA has free radical-scavenging properties, which would protect against lipid oxidation (*in vitro*). Leung and Liu (2000) demonstrated that the t10,c12 isomer of CLA possesses antioxidant properties over a wide range of concentrations, whereas the c9,t11 CLA isomer is actually a pro-oxidant at high concentrations (200 μM). Thus, the t10,c12 isomer of CLA was a more effective antioxidant.

It has been hypothesized that CLA could be converted to furan fatty acid, which may contribute to the antioxidative properties of CLA (Yu 2001). Another hypothesis is that CLA modulates cell oxidation to induce anticarcinogenic properties (Leung and Liu 2000). Differences between isomers and the dose dependency of CLA c9,t11 may explain the disagreement across studies in the ability of CLA to serve as an antioxidant.

CHAPTER III

CONJUGATED LINOLEIC ACID REDUCES LIPID OXIDATION IN AEROBICALLY STORED, COOKED GROUND BEEF PATTIES

Introduction

Conjugated linoleic acid is a collective acronym describing a mixture of octadecadienoic acids that describes all possible *cis* and *trans* combinations of conjugated double bonds at the 10 and 12, the 9 and 11, or the 11 and 13 carbons. Conjugated linoleic acid occurs naturally in beef and dairy products, and total CLA isomers typically sum to approximately 1% of total fatty acids in beef (Rule and others 2002) and 2% of total fatty acids in dairy products (reviewed by Bauman and others 1999). The *cis*-9, *trans*-11 (c9,t11) isomer of CLA unequivocally inhibits growth of cancer cells in animal and tissue culture systems, whereas the *trans*-10, *cis*-12 (t10,c12) CLA isomer may reduce adiposity (Pariza and others 2000).

Numerous laboratories have fed CLA to chickens (Du and others 1999, 2001) and pigs (Demaree and others 2002; Joo and others 2002; Smith and others 2002) with the intention of increasing the concentration of CLA isomers in eggs and pork products. Beef and dairy products are primary dietary sources of CLA in the American diet (reviewed by Baumann and others 1999). However, the concentration of CLA in beef is low and is resistant to change (Madron and others 2002; Rule and others 2002). The t10,c12 CLA isomer was barely detectable in the samples of Rule and others (2002). Similar results were reported by Madron and others (2002), who fed extruded full-fat

soybeans to beef cattle with the primary goal of increasing the c9,t11 CLA isomer. Although the increase in CLA was significant, it probably was not of sufficient magnitude to alter the functional characteristics of the beef.

Scientists have demonstrated that feeding CLA to chickens and pigs retards the production of thiobarbituric acid reactive substances (TBARS) in poultry and pork (Du and others 2001; Joo and others 2002). However, it has not been possible to substantially elevate CLA in beef by dietary means. This has been especially true for the t10,c12 isomer. Because scientists cannot appreciably elevate CLA in beef by dietary means, the CLA concentration of ground beef was increased by directly adding CLA-enriched oil during the grinding process.

The proposed research had as its objective to evaluate the effects of CLA on lipid oxidation, fatty acid composition, cooking loss, moisture and fat content, and surface color in raw and cooked beef patties. The hypothesis was that CLA would be retained during cooking and would reduce lipid oxidation during cold storage.

Materials and Methods

CLA-60 was obtained from Natural of Hovdebygda, Norway. CLA-60 consists of free fatty acids, of which 60% are CLA isomers. No information was available concerning the process by which the CLA-60 was produced. By analysis, CLA-60 contained 21% c9,t11, 12% c11,t13, and 15% t10,c12 CLA isomers (Table 1; Demaree and others 2002). CLA-60 also contained nearly 16% of fatty acids that could not be identified, which probably represents additional CLA isomers. CLA-60 contained significant percentages of 16:0, 18:1, 18:1n-9, and 18:2n-6. According to the

Table 1 – Fatty acid composition of the CLA source[a]

Fatty acid	g/100 g fatty acids
16:0	5.48
16:1	0.09
18:0	3.02
18:1n-9	20.3
18:2n-6	6.74
CLA c9,t11[b]	21.1
CLA c11,t13	12.0
CLA t10,c12	15.1
18:3n-3	0.15
Other	15.9

[a]Values are based on identifiable peaks.
[b]The values for CLA c9,t11 also included trace amounts of t9,c11;
CLA c11,t13 contained trace amounts of t11,c13;
and CLA t10,c12 contained trace amounts of CLA c10,t12.

manufacturer, CLA-60 contained no antioxidants.

Preparation of Ground Beef. Lean and fat trimmings were thawed at 4 °C prior to use. For blending, each trimming source was ground separately with a Hobart grinder (model 4612, Hobart Co., Troy, Ohio, U.S.A.), fitted with a coarse (0.64 cm) grinding plate. CLA was added to the lean trimmings in the following combinations:

(a) 0% CLA + 4% fat trimmings (by weight)

(b) 2% CLA + 2% fat trimmings

(c) 4% CLA + 0% fat trimmings

The lean trimmings contained 10 to 12% extractable lipid before addition of fat trimmings/CLA, so the total fat in the ground beef patties was approximately 15% for all treatment groups. The fat trimmings contained approximately 0.4% c9,t11 CLA, and no detectable t10,c12 CLA.

Each sample was mixed by hand and then reground using a fine (0.32 cm) plate. Patties for each sample were prepared with a patty mold (Large Hamburger Press, Tupperware®, Orlando, Fla., U.S.A.), and weighed 150 g each. Ground beef containing 0, 2, or 4% CLA had pH values of 5.59, 5.58, and 5.54, respectively. One-half of the patties from each batch (n=72) was stored raw for 0, 3, or 7 d and one-half (n=72) was cooked on the same day that the patties were freshly formed, and were stored for 0, 3, or 7 d after cooking. Each patty was wrapped with clear polyethylene wrap, placed in Ziploc bags (both S. C. Johnson & Johnson, Inc., Racine, Wis., U.S.A.), and stored at 4 °C for 0, 3, or 7 d. Each patty was placed on the same tray and same shelf in the cooler with no overhead lighting. Aerobic storage was chosen to exacerbate lipid oxidation.

Cooking. Non-stick electric skillets were preset at 150 °C and preheated to 50 °C. Each patty was cooked until the center of patty reached 35 °C; it was then turned and the other side was cooked until the center of patty reached 71 °C. The internal temperature of the patties was monitored by utilizing a thermocouple probe connected to a hand-held type T thermometer (model HH501BT, Omega Engineering, Inc., Stamford, Conn., U.S.A.).

Surface Color. The surface color of each patty before and after cooking was measured with a Minolta Colorimeter (CR-200, Minolta Co., Ramsey, N.J., U.S.A.) using L* (lightness), a* (redness), and b* (yellowness) color space values. Calibration was conducted on a white tile prior to use, and the calibration values were L* 96.03, a* 0.11, and b* 1.97. Three random spots were measured on the surface of each sample and the mean value was calculated.

Moisture and Fat Content. Fat and moisture content (AOAC 1990) and cooking loss were determined for each d0 patty (cooked and raw; n = 48). To determine cooking loss, weight of each patty was measured before and after it was cooked. Cooking losses are reported as weight percentages.

Fatty Acid Composition. Fatty acid composition (Demaree and others 2002) was determined for d0 and d7 patties. Total lipid was extracted by the method of Folch and others (1957), and fatty acid methyl esters were produced as described by Morrison and Smith (1964). Fatty acids methyl esters were measured with a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler; Varian Inc., Walnut Creek, Calif., U.S.A.). Separation of the fatty acid methyl esters was on a silica capillary

colomn CP-Sil88 [100 m X 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands). Helium was the carrier gas (flow rate = 2 mL/min). After 32 min at 180 °C, oven temperature was increased at 20 °C/min to 225 °C and held for 13.75 min. Injector and flame ionization detector temperatures were 270 °C and 300 °C, respectively. Identity of fatty acid was based on retention times in comparision to standards (GlC-68D, Nuchek Prep, Inc., Elysian, Minn., U.S.A.).

TBA Test. For the measurement of TBARS, the method by Tarladgis and others (1960) and Rhee (1978) were used to include a 0.5% solution of propyl gallate (PG) and ethylenediamine tetraacetic acid (EDTA) solution. Sixty grams of meat in 90 ml of distilled water with 30 ml of 0.5% PG/EDTA was blended for 2 min. Thirty grams of the slurry was quantitatively transferred into the Kjeldahl flask, rinsing with 77.5 ml of 50 °C distilled water. Two and half milliliter of 4 N HCl was added to the flask along with boiling chips. The samples were distilled until 50 ml of distillate was collected in a graduated cylinder. Five milliliter of the distillate with 5 mL of 0.02 M TBA reagent was heated in boiling water for 35 min. For the blank, 5 ml distilled water with 5 ml TBA reagent was used. The samples were cooled in tap water for 10 min and the optical density of the sample against the blank was read in the spectrophotometer at a wavelength of 530 nm. To obtain TBARS values, the sample absorbance was multiplied by a constant (K) (where K = 7.8). TBARS are reported as mg malonaldehyde per kg of ground beef.

Statistical Analysis. The study was a 2 factor (raw and cooked) X 3 factor (0, 2, and 4% CLA) design, and three replications were performed. The data were analyzed using

SuperANOVA software (Abacus Concepts, Berkley, Calif., U.S.A.). The main effects (concentration of CLA, cooked versus raw) and their interaction were analyzed. Mean values and pooled standard errors of means (SEM) are reported. Differences among interaction and main effects means were considered statistically significant at $P < 0.05$. The effect of time of storage (0, 3, and 7 d) was analyzed as a split plot, with percentage of CLA as the whole plot and time of storage as the split plot. The error term for the whole plot was group (% CLA), where group is the main effect of cooked versus raw.

Results and Discussion

The cooking x %CLA interaction for most dependent variables was not significant, so only *P*-values for the main effects of cooking and %CLA is discussed. The whole plot was significant, and these results also are discussed.

Addition of CLA had no effect on weight loss, percentage moisture, or percentage fat after cooking (all $P > 0.85$) (Table 2), indicating that CLA was retained in the patties after cooking. There are no other reports of the retention of CLA in meat products when added during grinding. These findings indicate that, even added as an oil, CLA was retained during the cooking process.

The fresh ground beef containing added CLA appeared to be somewhat lighter, but this was not confirmed with the Minolta Colorimeter. Lightness (L*), redness (a*), and yellowness (b*) were not influenced by the addition of CLA ($P > 0.60$; Table 2). The L* color space values tended ($P = 0.068$) to be decreased by cooking, whereas a* color space values were significantly decreased by cooking ($P = 0.0001$). Hur and others (2004) reported that addition of CLA had no effect on L* or a* on d0 in uncooked

Table 2 – Characteristics of raw and cooked ground beef patties containing 0%, 2%, or 4% added CLA

Item	Added CLA (%)			Pooled SEM	*P*-values		
	0	2	4		Cooking	% CLA	Cooking x % CLA
Weight loss during cooking, g[a]	32.9	33.6	35.7	3.026	N/A[b]	0.944	N/A
Moisture, %							
Raw	66.8	66.0	65.3	1.744	0.118	0.863	0.979
Cooked	61.5	59.9	58.1				
Fat, %							
Raw	14.1	15.3	15.8	1.526	0.797	0.854	0.988
Cooked	14.3	16.4	17.4				
L*							
Raw	47.09	53.86	54.58	1.732	0.068	0.690	0.540
Cooked	45.59	45.43	44.03				
a*							
Raw	18.54	18.84	17.65	1.390	0.0001	0.909	0.990
Cooked	8.69	8.81	8.20				
b*							
Raw	8.99	11.57	11.48	0.517	0.834	0.664	0.467
Cooked	10.74	10.60	9.98				

[a]Beginning weight of patties was 150.0 g. Measured in d0 patties.

[b]N/A, not applicable.

ground beef. However, by d7 of storage in an O_2-permeable wrap, both L* and a* had increased slightly; this effect disappeared by d14 (Hur and others 2004). One of the factors affecting the color is the selective absorption of light by the meat surface (Romans and others 2001), and color is a significant factor that affects consumers' decision to purchase fresh beef. Therefore, it was important that the addition of CLA did not affect surface meat color with or without cooking, as demonstrated in the present study.

Addition of CLA to the ground beef patties before cooking decreased the extent of lipid oxidation, as measured by the production of TBARS in raw and cooked patties (Figure 1; %CLA main effect, $P = 0.0002$). For the raw patties, TBARS were reduced by 36, 43, and 9% for d0, d3, and d7, respectively ($P < 0.0001$). TBARS were reduced by 62, 69, and 72% for d0, d3, and d7, respectively ($P < 0.0001$) for the cooked patties. Thus, CLA had a more dramatic effect on the reduction of TBARS in cooked ground beef patties (%CLA x storage time x cooking interaction, $P = 0.0064$).

Early studies indicated that CLA possessed no antioxidant capacity (Van den Berg and others 1995). However, Yu (2001) demonstrated that CLA had free radical-scavenging properties, which would protect against lipid oxidation. Leung and Liu (2000) previously had demonstrated that the t10,c12 isomer of CLA possessed antioxidant properties over a wide range of concentrations, whereas the c9,t11 CLA isomer was actually a pro-oxidant at high concentrations (200 μM). Thus, the t10,c12 isomer of CLA was a more effective antioxidant. Differences between isomers and the dose dependency of CLA c9,t11 may explain the disagreement across studies in the ability of

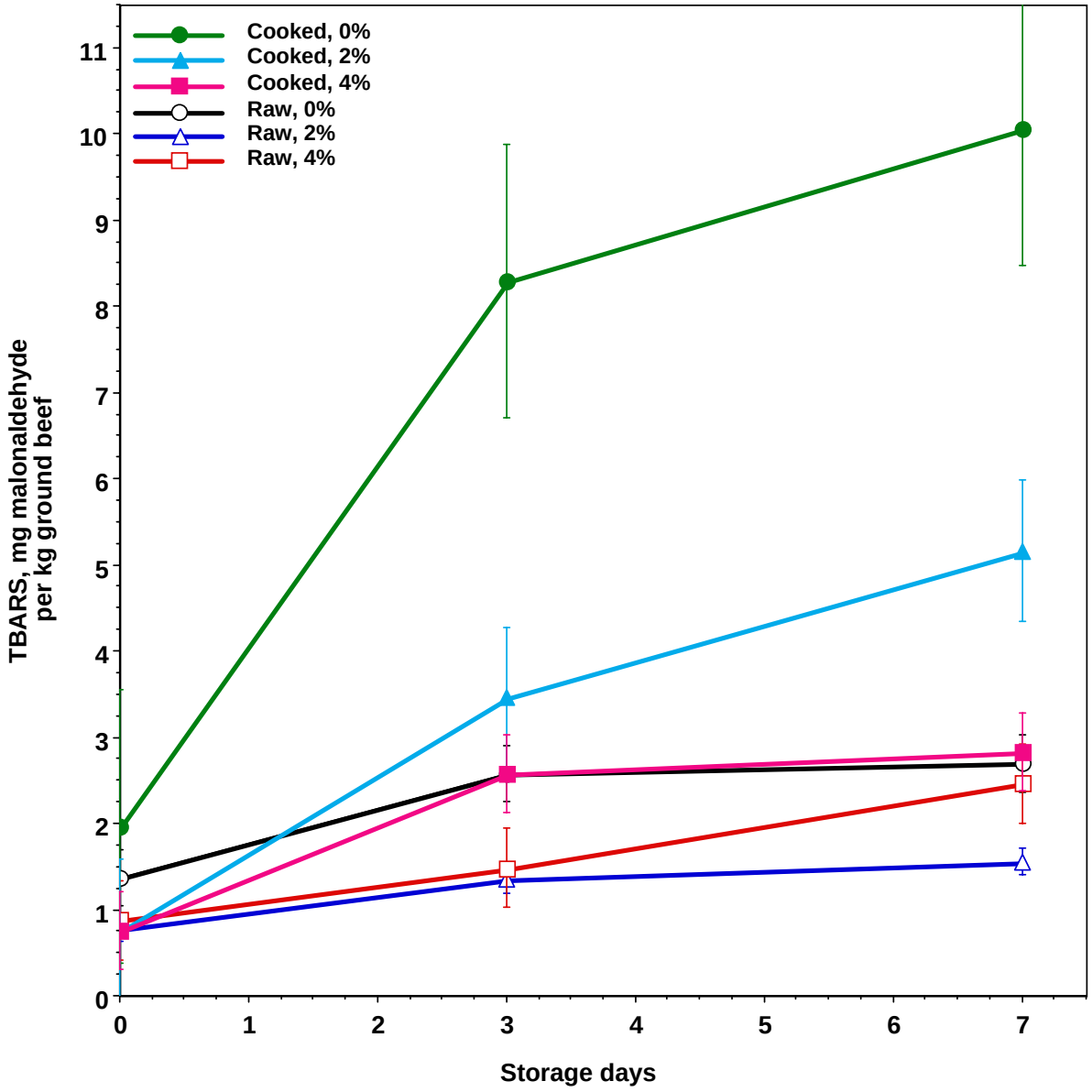

Figure 1 – TBARS values for raw and cooked beef patties. Overall SEM error bars for each treatment are attached. SEM: cooked 0%, 1.59; cooked 2%, 0.82; cooked 4%, 0.45; raw 0%, 0.33; raw 2%, 0.15; raw 4%, 0.46.

CLA to serve as an antioxidant. Joo and others (2002) investigated the effects of dietary CLA on lipid oxidation in fresh pork, measured as TBARS. In their study, TBARS were reduced from 0.7 to 0.5 mg/kg after 7 d of storage at 4 °C. Corino and others (2003) forced lipid oxidation in longissimus muscle samples of pigs fed CLA. After 300 min of forced oxidation, TBARS were reduced from approximately 2.3 nmol malondialdehyde/g in control pork muscle to 1.5 nmol malondialdehyde/g muscle in pigs fed 0.5% CLA.

Hur and others (2004) added 0.5 and 2% CLA to ground beef and stored the patties for 7 and 14 d, and observed that both concentrations of CLA reduced the production of TBARS. Hur and others (2004) did not test the effects of CLA in cooked ground beef patties.

The profound effect of CLA observed in cooked ground beef suggests that CLA would be an especially effective additive in food service institutions that use precooked ground beef.

If the added CLA was responsible for the reduction in lipid oxidation, then the CLA isomers must have been retained in the cooked patties. This was indeed the case (Table 3). Patties containing no added CLA had the expected concentration of c9,t11 CLA (approximately 0.4%) and no detectable t10,c12 CLA. Patties containing 2% added CLA had concentrations of both isomers of approximately 2%, which were similar to concentrations that was observed in the adipose tissue of pigs fed CLA as 1.5% of their diet for 35 d (Smith and others 2002). Thus, the concentrations of CLA observed in the adipose tissue of pigs only after extended feeding was achieved in

Table 3 – Main effects for fatty acid composition of raw and cooked ground beef patties containing 0%, 2%, or 4% added CLA, stored for 0, 3, or 7 d

	Main effect											
	Added CLA, %			Days of storage			Cooking		Pooled	*P*-values for main effects		
Fatty acid	0	2	4	0	3	7	Raw	Cooked	SEM	%CLA	Storage time	Cooking
	g/100 g total fatty acids											
14:0	3.48^{a}	3.18^{b}	2.81^{c}	3.14	3.11	3.23	3.13	3.18	0.071	0.01	0.16	0.29
14:1	0.80	0.62	0.67	0.74	0.60	0.76	0.66	0.73	0.044	0.22	0.31	0.40
16:0	24.2^{a}	22.5^{b}	20.8^{c}	22.3^{v}	22.4^{v}	22.8^{u}	22.5	22.5	0.344	0.01	0.04	0.82
16:1	3.31^{a}	2.99^{b}	2.74^{c}	3.01	2.97	3.06	3.05^{x}	2.97^{y}	0.059	0.01	0.23	0.03
18:0	15.6^{a}	14.5^{b}	13.2^{c}	14.3	14.4	14.6	14.3^{y}	14.6^{x}	0.244	0.01	0.23	0.04
18:1t9	7.04^{a}	6.23^{b}	5.54^{c}	6.19^{v}	6.22^{v}	6.41^{u}	6.22	6.32	0.153	0.01	0.04	0.22
18:1c9	36.9^{a}	35.5^{b}	34.4^{c}	35.6	35.5	35.7	35.8	35.4	0.278	0.01	0.72	0.08
18:2n-6	4.11^{c}	4.73^{b}	4.98^{a}	4.75^{u}	4.62^{uv}	4.44^{w}	4.70	4.51	0.100	0.01	0.05	0.06
CLAc9t11	0.38^{c}	2.72^{b}	4.42^{a}	2.67^{u}	2.65^{u}	2.20^{v}	2.44	2.57	0.411	0.01	0.03	0.49
CLAc11t13	0^{c}	0.96^{b}	1.67^{a}	0.93^{u}	0.94^{u}	0.76^{v}	0.85	0.90	0.170	0.01	0.05	0.59
CLAt10c12	0^{c}	2.20^{b}	3.86^{a}	2.14^{u}	2.17^{u}	1.73^{v}	1.97	2.07	0.393	0.01	0.05	0.61
20:4n-6	0.21	0.13	0.26	0.33	0.17	0.10	0.30^{x}	0.10^{y}	0.046	0.52	0.14	0.04

$^{abc, uvw, xy}$Means for main effects with different superscripts differ.

ground beef.

The c9,t11, t10,c12, and c11,t13 CLA isomers increased from 0.4 to 4.4%, from 0 to 3.9%, and from 0 to 1.7%, respectively, in patties containing 0, 2, and 4% added CLA ($P < 0.01$; Table 3). The CLA preparation also contained 6.7% 18:2n-6 (Table 1), and this fatty acid increased in ground beef with increasing percentage of CLA. Conversely, saturated fatty acids and 16:1n-7 and 18:1n-9 monounsaturated fatty acids decreased with added CLA ($P < 0.01$). CLA-60 is low in saturated and monounsaturated fatty acids (Table 1), so reductions in these fatty acid species is unavoidable with the addition of CLA to ground beef.

Conclusions

The concentration of CLA was increased in beef by directly adding 2% and 4% CLA mixed isomers during the grinding process. The added CLA decreased the rate of lipid oxidation in ground beef patties. This was more dramatic in cooked ground beef patties. Neither cooking nor days of storage reduced the effectiveness of CLA on the decreased rate of lipid oxidation. CLA also did not affect percentage fat and moisture, cooking loss, or meat color (L*, a*, b*).

There was insufficient benefit from 4% CLA over 2% CLA in reducing lipid oxidation to justify the higher level unless the primary focus is to increase the consumption of CLA isomers.

. CHAPTER IV

CONJUGATED LINOLEIC ACID REDUCES LIPID OXIDATION IN IRRADIATED, COOKED GROUND BEEF PATTIES

Introduction

Irradiation of Food. Irradiation is an effective means of improving microbial safety of raw and precooked meat (Greene and others 1966; Lefebvre and others 1992; Roberts and others 1998; Nam and others 2001). However, irradiation causes several off-flavors, most of which can be attributed to the oxidation of fatty acids during irradiation.

Irradiation exposes food to a source of ionizing radiation sufficient to create positive and negative charges. The amount of radiation energy absorbed is measured in units of grays (Gy). The radiation source approved for food use is gamma rays (Olson 1998). When gamma rays pass through water, ion pairs and free radicals are produced. Water molecules then yield highly reactive hydrogen and hydroxyl radicals that react with each other to produce hydrogen peroxide (Potter and others 1995).

Irradiation causes an increase in the concentration of total volatile compounds and accelerates fatty acid oxidation, causing off-flavor and rancidity (Du and others 2003; Potter and others 1995). Ozone, a strong oxidizer, is produced from oxygen during food irradiation, and may cause bleaching and discoloration of the meat by oxidizing myoglobin (Olson 1998). Du and others (2002a) suggested that the color changes induced by irradiation were due to the carbon monoxide production during irradiation.

Irradiation has also been identified as a crucial factor in initiating the oxidation of cholesterol because it causes the formation of hydroperoxides from polyunsaturated fatty acids; these indicate cholesterol oxidation (Nam and others 2001). Nam and others (2001) reported that cholesterol was oxidized in irradiated beef after 7 d of aerobic storage. These cholesterol oxidation products such as 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol in food system are directly connected to the development of atherosclerosis, coronary heart disease, and membrane impairment. Brito and others (2002) reported that irradiation increased the amount of trans fatty acids which are linked to increased incidence of coronary heart disease. Thus, a practical method to reduce lipid oxidation in irradiated meat products must be developed.

Enrichment of Meat with CLA as an Antioxidant. Numerous laboratories have fed CLA to chickens (Du and others 1999, 2001) and pigs (Demaree and others, 2002; Joo and others 2002; Smith and others 2002). This increased the concentration of CLA isomers in eggs and pork products to 3-5% of total fatty acids. Although beef is a primary dietary source of CLA in the American diet, the concentration of CLA in beef is low and is resistant to change due to ruminal modification of dietary fatty acids (Madron and others 2002; Rule and others 2002). The t10,c12 CLA isomer was barely detectable in the samples of Rule and others (2002). Similar results were reported by Madron and others (2002), who fed extruded full-fat soybeans to beef cattle with the primary goal of increasing the c9,t11 CLA isomer. Although the increase in CLA was significant (to approximately 1% of total fatty acids), it probably was not of sufficient magnitude to alter the functional characteristics of beef.

Feeding CLA to poultry or pigs reduced the TBARS value of the meat tissues (Du and others 2001; Joo and others 2002). In cooked chicken patties, irradiation (2.5 kGy) actually reduced TBARS formation in precooked chicken patties, and CLA reduced TBARS in vacuum-packaged (but not aerobically packaged) patties after 5 d of storage (Du and others 2001). Conjugated linoleic acid strongly reduced TBARS formation in non-irradiated chicken patties stored in either aerobic or vacuum packaging.

Joo and others (2002) investigated the effects of dietary CLA on lipid oxidation in fresh pork. In their study, the TBARS were reduced from 0.7 to 0.5 mg/kg after 7 d of storage at 4 °C. Thus, an important benefit of feeding CLA to livestock species and poultry may be the reduction in lipid oxidation with storage or irradiation.

Feeding CLA to monogastrics increases CLA isomers in tissues proportional to the concentration in diet (Demaree and others 2002; Smith and others 2002). Du and others (2000, 2001, 2002a, 2002b) have demonstrated that feeding CLA to chickens retarded lipid oxidation of meat tissues, but it has not been possible to substantially elevate CLA in meat by dietary means. This has been especially true for the t10,c12 isomer, which has been shown to be a more potent free radical scavenger than the c9,t11 isomer. Because scientists cannot appreciably elevate CLA in beef by dietary means, the CLA concentration was increased by directly adding CLA-enriched oil during the grinding process. The hypothesis of this study was that enrichment with CLA will protect ground beef from oxidation of fatty acids that results from irradiation.

Although effects of irradiation on the volatile production, color and sensory characteristics of raw or cooked meats are available, there is no information on the

effects of CLA in combination with low-dose irradiation (1.5 kGy) on lipid oxidation of meat. Other studies on the antioxidative effects of CLA in combination with irradiation in meat products were primarily based on dietary treatment (Du and others 2002a, 2001). Although other more common antioxidants have been tested (Kanatt and others 1998), no studies on the direct application of CLA to meat products, especially in combination with irradiation, are available. Therefore, this study represented a unique opportunity to employ the combination of a naturally occurring antioxidant with irradiation to retard off-flavor and rancid odor in ground beef.

Materials and Methods

Food-Grade CLA. Free fatty acids may cause soapy flavors when incorporated into ground beef preparations. Thus, a high quality CLA triacylglycerol (TAG) form was obtained from Loders Croklaan (Channahon, IL). The CLA free fatty acid (FFA) form was also obtained from the same manufacturer. The major CLA isomers in the CLA were CLA cis-9,trans-11 (36.6%), CLA cis-10,trans-12 (37.9%). The beef lean and fat trimmings were obtained from Rosenthal Meat Science Center at Texas A&M University.

Preparation of Ground Beef. Fresh beef lean trimmings (80:20) and fat trimmings (approximately 80% extractable lipid) were used. For blending, trimmings were ground with Hobart grinder (model 4612, Hobart Co., Troy, Ohio, U.S.A.) using a fine (0.64 cm) grinding plate. Initially, CLA was added to the lean trimmings in the following combinations to obtain a constant fat mixture with varying levels of CLA:

(a) 0% CLA + 4% fat trimmings (%, by wet weight)

(b) 1% CLA + 3% fat trimmings

(c) 2% CLA + 2% fat trimmings

(d) 4% CLA + 0% fat trimmings

This combination of treatments was intended to duplicate preliminary experiments performed with a nonesterified CLA mixture. However, it is possible that smaller amounts of CLA-TAG can be used to achieve the same level of protection from fatty acid oxidation as the nonesterified CLA because of the greater purity of the TAG mixture (> 80% CLA) as compared to the nonesterified mixture (< 50% CLA). The total fat content of each batch of ground beef was approximately 25%.

The CLA was mixed by hand with the coarsely ground beef and then ground using a fine (0.32 cm) plate. Patties (151g) for each sample were prepared with a patty mold (Large Hamburger Press, Tupperware, Inc., Orlando, FL, U.S.A.). One-half of the patties from each batch (n=112) was irradiated before cooking and one-half (n=100) served as non-irradiated controls.

Calibration Dosimetry and Irradiation. Irradiation of the patties was conducted at the Center for Food Irradiation of Texas A&M University. Each box for calibration dosimetry contained 16 patties consisting of 4 stacks of patties. The height of each stack was 7.6 cm and each patty weighed 151.3 g. Each dosimeter pallet was placed in the small plastic bag and labeled as B (bottom), 1, 2, 3, and T (top), and the pallet was placed in the mini Zip-Lock® bag. The dosimeter pallet labeled as B was placed and anchored on the bottom of each stack; those labeled as 1, 2, and 3 were placed between patties; the one labeled as T was placed and anchored on top of the patty. Each stack

was wrapped with paper towel and securely fastened with tape.

When the stack height of the samples is less than optimum 8.6 cm, the part of the depth-dose curve can be captured by using attenuation to achieve 1.2 of the maximum to minimum ratio of the dose. To narrow a range for dose distribution, half-inch-thick (1.3 cm) plywood was placed on the bottom and the top of the outside the box containing the patties. First and third boxes contained standard dosimeters 1 and 2 with sample holders; the second box contained the patties. The pallets were irradiated at speed 57 feet per min (FPM) with a dual electron beam. The dose of each dosimeter pallet was recorded. This process was repeated until desired dose, 1.5-2.0 kGy, was obtained.

Once the desired dose was established, ground beef patties used for calibration dosimetry were discarded and ground beef patties for the study were irradiated. The non-irradiated control samples were also transported to the irradiation room and put aside while the other samples were irradiated.

Cooking. Non-stick electric skillets were preset at 150 °C and preheated to 50 °C. Each patty was cooked until the center of patty reached 35 °C; it was then turned and the other side was cooked until the center of patty reached 71 °C. The internal temperature of the patties was monitored by utilizing a thermocouple probe connected to a hand-held type T thermometer (model HH501BT, Omega Engineering, Inc., Stamford, CT, U.S.A.).

Moisture and Fat Content. Fat and moisture content (AOAC 1990) and cooking loss were determined. To determine cooking loss, weight of each patty was measured before and after it was cooked. Cooking losses are reported as weight percentages.

Fatty Acid Composition. Analysis of fatty acid composition allowed for identification of the major fatty acids that are oxidized during storage. Total lipid was extracted by the method of Folch and others (1957), and fatty acid methyl esters were determined as described by Morrison and Smith (1964). Fatty acids methyl esters were measured with a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler; Varian Inc., Walnut Creek, CA, U.S.A.). Separation of the fatty acid methyl esters was on a silica capillary column CP-Sil88 [100 m X 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands). Helium was the carrier gas (flow rate = 2 mL/min). After 32 min at 180 °C, oven temperature was increased at 20 °C/min to 225 °C and held for 13.75 min. Injector and flame ionization detector temperatures were 270 and 300 °C, respectively. Identity of fatty acid was based on retention times in comparison to standards (GlC-68D, Nuchek Prep, Inc., Elysian, MN, U.S.A.).

TBA Test. For the measurement of TBARS, the method by Tarladgis and others (1960) and Rhee (1978) were used to include a 0.5% solution of propyl gallate (PG) and ethylenediamine tetraacetic acid (EDTA) solution. Sixty grams of meat in 90 ml of distilled water with 30 ml of 0.5% PG/EDTA was blended for 2 min. Thirty grams of the slurry was quantitatively transferred into the Kjeldahl flask, rinsing with 77.5 ml of 50 °C distilled water. Two and half milliliter of 4 N HCl was added to the flask along with boiling chips. The samples were distilled until 50 ml of distillate was collected in a graduated cylinder. Five milliliter of the distillate with 5 mL of 0.02 M TBA reagent was heated in boiling water for 35 min. For the blank, 5 ml distilled water with 5 ml TBA reagent was used. The samples were cooled in tap water for 10 min and the optical

density of the sample against the blank was read in the spectrophotometer at a wavelength of 530 nm. To obtain TBARS values, the sample absorbance was multiplied by a constant (K) (where K = 7.8). TBARS are reported as mg malonaldehyde per kg of ground beef.

Sensory Evaluation. Sensory analysis was performed in the Sensory Testing facility at Texas A&M University with trained panelists seated in separate booths to prevent communication between panels. An eight-member beef descriptive flavor attribute panel were selected and trained according to the Spectrum® procedures (with scale 0 = absent; 15 = extremely intense). During panel training, terminology development sessions were conducted based on a standard lexicon for beef flavors that characterize aromatic notes and chemical feeling factors. Aromatics (cooked beef/brothy, serum/bloody, cowy/grainy, cardboard, painty, fishy and liver/organy), mouth-feels (metallic and astringent) and basic tastes (sour, bitter, sweet, and salty) of each cooked patties were identified. Additionally, the panel was asked to detect aromatics that frequently are associated with irradiated ground beef (cooked fat, browned, milky, wet dog/hairy, and off-flavor) and the aftertastes of afterburn and fat mouthfeel.

Panelists were seated in separate booths with red filtered lights to mask color variation in samples (AMSA 1995). Up to 16 samples were served during two sensory sessions per day with up to 8 samples being evaluated per session. Samples were served in a randomized order using 3-digit identification codes. Distilled water and ricotta cheese were provided for cleansing of their pallets.

Statistical Analysis. There were a minimum of four batches of ground beef, with

three patties per batch per treatment group. The study was a 2 factor (irradiated and non-irradiated) x 4 factor (0, 1, 2, and 4% CLA) design, and three replications were performed. The data were analyzed using SuperANOVA software (Abacus Concepts, Berkley, CA). The main effects (irradiation and CLA) and their interaction were also analyzed. The mean values and pooled standard errors of means (SEM) are reported. Differences among interaction and main effects means were considered statistically significant at $P < 0.05$.

Results and Discussion

Weight loss, moisture, and fat content of cooked beef patties are indicated in Table 4. Weight loss during cooking was greater in irradiated beef patties than in non-irradiated patties ($P = 0.002$). The loss was primarily moisture. CLA type and level had no effect on weight loss ($P > 0.05$). Moisture content was significantly lower in irradiated beef patties than in non-irradiated beef patties ($P < 0.001$). Lower moisture content was also observed in the free fatty acid formulation of CLA (FFA-CLA) patties ($P =0.004$)

Although the beef patties were formulated to contain the same initial fat content, those patties containing CLA retained more fat than patties with no added CLA ($P = 0.005$). This result suggests that the added CLA was retained to a greater extent than the fat from the added fat trimmings. Beef patties containing the FFA-CLA retained a greater percentage of fat than patties containing the triacylglycerol formulation of CLA (TAG-CLA) ($P = 0.001$).

The TBARS values were calculated by the method of Tarladgis and others (1960)

Table 4 – Weight loss, moisture, and fat percentages of non-irradiated and irradiated ground beef patties after cooking

Treatment	Weight loss		Moisture		Fat	
	Non-Irr	Irr*	Non-Irr	Irr	Non-Irr	Irr
	Percentage					
Control	26.6^{a}	31.3^{b}	59.4^{x}	56.9^{y}	15.9^{q}	17.1^{q}
CLA-FFA %						
1	28.1^{ab}	30.6^{bc}	56.3^{xy}	54.3^{yz}	19.3^{r}	20.1^{r}
2	24.6^{ac}	27.2^{abc}	56.1^{xz}	53.4^{xyz}	19.0^{s}	21.0^{s}
4	26.6^{d}	26.5^{ad}	54.5^{w}	54.7^{wx}	21.1^{t}	21.2^{t}
CLA-TAG %						
1	25.7^{ab}	29.5^{bc}	58.8^{yx}	56.6^{zy}	17.0^{qr}	17.3^{qr}
2	25.3^{ac}	27.0^{abc}	58.2^{zx}	56.8^{zxy}	16.8^{qs}	17.4^{qs}
4	28.6^{d}	29.7^{ad}	57.9^{wxy}	55.9^{wyz}	17.7^{qt}	18.7^{qt}
Pooled SE	0.404		0.344		0.396	

CLA = conjugated linoleic acid; FFA = free fatty acid formulation of CLA; TAG = triacylglycerol formulation of CLA, 0, 1, 2, or 4%.
^{abcd} Means of subclass with different superscripts within rows differ ($p = 0.001$).
^{wxyz} Means of subclass with different superscripts within rows and columns differ ($p = 0.001$).
^{qrst}Means of subclass with different superscripts within column differ ($p = 0.001$).
*Irradiation dose was 1.6 kGy.

and Rhee (1978), and are shown in Table 5. There were no significant differences between CLA type (FFA versus TAG; $P = 0.21$) or added CLA percentage ($P = 0.36$) on TBARS (Figure 2). The TBARS values were higher in irradiated cooked ground beef patties than in non-irradiated ground beef patties ($P = 0.004$) regardless of the CLA type. These results were consistent with the results reported by Sweetie and others (1998). Du and others (2001, 2003) also reported that irradiation accelerated lipid oxidation, and the dietary CLA supplement decreased TBARS values in chicken breast rolls and turkey rolls.

Unlike the precious results (Chae and others 2004), the FFA-CLA caused an increase in TBARS at the 1% level in non-irradiated ground beef patties (Figure 2). In contrast, the TBARS declined in a linear fashion with increasing FFA-CLA concentration in irradiated ground beef patties to values observed in the control, non-irradiated patties. These data indicate that the FFA-CLA will reduce lipid oxidation that is caused by irradiation. The TAG-CLA reduced TBARS values at all concentrations in irradiated and non-irradiated ground beef patties, and was most effective at 1% TAG. The data suggest that the TAG-CLA at higher concentrations was more susceptible to lipid oxidation with or without irradiation. The TAG-CLA increased the concentration of 18:2n-6, which would have provided more substrate for lipid oxidation.

Early studies have indicated that the shelf-life of meat was improved by antioxidants as the storage time was increased (Lee and others 1999), which suggests that the FFA-CLA may be an effective antioxidant if the ground beef patties were to be stored.

Table 5 – TBARS values of non-irradiated and irradiated cooked ground beef patties after cooking

	Non-Irradiated	Irradiated[a]
Control	3.83	5.17
CLA-FFA %		
1	4.24	5.09
2	2.63	4.18
4	3.48	3.63
CLA-TAG %		
1	2.98	3.59
2	2.52	4.33
4	3.01	3.96
Pooled SE	0.178	

CLA = conjugated linoleic acid; FFA = free fatty acid formulation of CLA; TAG = triacylglycerol formulation of CLA.

[a] Main effect of irradiation, P = 0.0038. No other main effects or interactions are different.

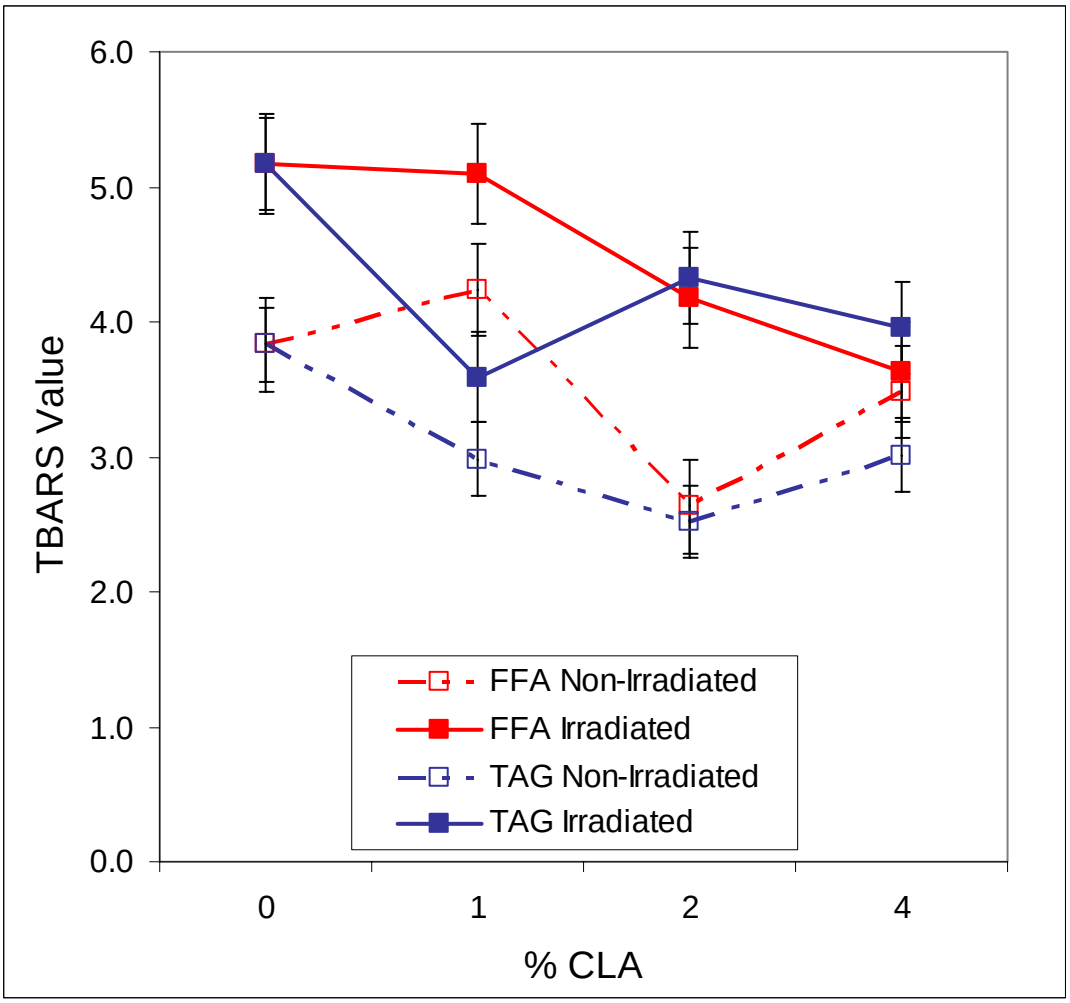

Figure 2 – TBARS values in irradiated, cooked ground beef patties containing increasing concentrations of CLA. Overall SEM error bars for each treatment are attached.

Another possible explanation for the increased TBARS value observed in the 1% FFA-CLA in non-irradiated patties is that the antioxidative effect of CLA might not be strong enough to control lipid oxidation in cooked meat. Because the progress of lipid oxidation in cooked meat under aerobic condition is very rapid, it can override the antioxidative effect of CLA (Ahn and others 1998). Therefore, the combined use of antioxidants and vacuum packaging would be a useful method to control the oxidative quality changes of irradiated raw and/or cooked meat (Ahn and others 1999; Nam and Ahn 2003).

Irradiation significantly affected several sensory attributes: serumy/bloody, soured, browned, wet dog/hairy aromatics, and browned after taste (all $P < 0.05$) (Table 6 and 7). Irradiation decreased the serumy/bloody aromatics (irradiated, 0.798; non-irradiated, 1.360) ($P = 0.001$). Soured aromatic was not detectable in irradiated ground beef patties (0.000) ($P = 0.003$), however, it was observed to be higher in non-irradiated patties (0.158) ($P = 0.003$). The wet dog/hairy aromatic was observed to be higher in irradiated ground beef patties (0.140) than non-irradiated patties (0.061) ($P = 0.04$) (Table 6). Similar results were reported by Du and others (2002a, 2003); irradiation produced off-flavor in ready-to-eat turkey breast rolls and chicken breast rolls when irradiated at 2.5 kGy. There were no significant main effects of irradiation on the basic tastes ($P > 0.38$) (Table 7). Irradiation increased browned aftertaste (irradiated, 1.044; non-irradiated, 0.807) ($P = 0.05$).

The CLA type significantly affected some aromatics, basic tastes, and aftertastes (all $P < 0.05$). The cooked beef/brothy attribute was higher in the patties with TAG-CLA

Table 6 – Aromatic attributes of irradiated and non-irradiated ground beef patties with varying concentrations of CLA

	% CLA-FFA				% CLA-TAG				Pooled	Significant
	0	1	2	4	0	1	2	4	SEM	effects*
Aromatics										
Cooked beef										
Irradiated	4.2	4.0	3.8	3.4	4.3	4.4	4.6	4.4	0.087	Type
Non-irradiated	3.6	3.9	4.4	3.4	3.7	4.2	4.3	3.8		
Serumy/bloody										
Irradiated	1.2	0.4	0.6	0.3	1.3	1.2	0.9	0.6	0.068	Type, %, Irr
Non-irradiated	1.5	1.1	1.3	0.9	1.5	1.8	2.0	1.3		
Painty										
Irradiated	0.6	0.8	1.4	1.9	0.5	0.8	0.4	0.6	0.088	Type, %
Non-irradiated	0.4	0.9	0.8	1.8	0.4	0.4	0.3	1.1		
Soured										
Irradiated	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.027	Irr
Non-irradiated	0.2	0.1	0.0	0.1	0.3	0.2	0.3	0.2		
Browned										
Irradiated	0.6	1.5	1.4	1.2	1.5	1.5	1.8	1.4	0.069	%, Irr
Non-irradiated	0.7	1.1	1.6	0.4	0.6	0.7	1.4	1.5		
Wed dog/Hairy										
Irradiated	0.2	0.0	0.3	0.2	0.2	0.2	0.4	0.1	0.024	Irr
Non-irradiated	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.1		

*Type = CLA free fatty acid form versus triacylglycerol form; % = CLA concentrations in ground beef; Irr = irradiated versus non-irradiated.

Table 7 – Basic tastes and aftertastes of irradiated and non-irradiated ground beef patties with varying concentrations of CLA

	% CLA-FFA				% CLA-TAG				Pooled	Significant
	0	1	2	4	0	1	2	4	SEM	effects[a]
Basic tastes										
Sour										
Irradiated	2.5	2.2	2.8	2.3	2.5	2.2	2.5	2.3	0.038	%
Non-irradiated	2.5	2.5	2.1	2.3	2.7	2.3	2.4	2.1		
Bitter										
Irradiated	2.7	2.7	3.3	3.4	2.5	2.4	2.6	2.7	0.048	Type, %
Non-irradiated	2.7	2.9	3.3	3.3	2.8	2.3	2.9	2.8		
Sweet										
Irradiated	0.5	0.2	0.3	0.1	0.3	0.7	0.8	0.5	0.046	Type,
Non-irradiated	0.6	0.3	0.5	0.1	0.2	0.6	0.8	0.3		Type x %
Aftertastes										
Astringent										
Irradiated	1.8	1.7	2.8	2.4	1.3	1.7	1.1	1.4	0.072	Type, %
Non-irradiated	1.5	1.7	2.4	2.6	1.2	1.7	1.0	1.6		Type x %
Bitter										
Irradiated	2.2	2.3	2.5	2.7	2.1	2.1	2.3	2.3	0.048	%
Non-irradiated	2.5	2.3	2.1	2.6	2.4	2.2	2.1	2.6		
Browned										
Irradiated	0.8	1.0	1.3	0.7	1.1	1.2	1.8	1.0	0.061	Type, %, Irr
Non-irradiated	0.6	0.8	1.3	0.4	0.8	0.6	1.3	1.2		
Sweet										
Irradiated	0.5	0.1	0.0	0.1	0.3	0.2	0.4	0.2	0.033	%
Non-irradiated	0.4	0.2	0.4	0.0	0.3	0.4	0.4	0.5		

[a]Type = CLA free fatty acid form versus triacylglycerol form; % = CLA concentrations in ground beef; Irr = irradiated versus non-irradiated.

treatment (FFA, 3.798; TAG, 4.193) ($P = 0.02$). This result indicates that the TAG-CLA contributed to the cooked beef aromatic. The serumy/bloody attribute was significantly higher in the patties prepared with TAG-CLA (FFA, 0.877; TAG, 1.281) ($P = 0.002$). The painty attribute and the bitter taste were higher in the patties with FFA-CLA preparation (FFA, 1.114; TAG, 0.596; FFA, 3.018; TAG, 2.614, respectively) ($P = 0.002$, $P = 0.001$, respectively), and the astringent aftertaste was also higher in the same patties (FFA, 2.061; TAG, 1.456) ($P = 0.001$). These results were consistent with the elevation of free fatty acids, which by themselves have these characteristics. The sweet taste and the browned aftertaste were higher in the patties with TAG-CLA treatment ($P < 0.04$).

There were several CLA % main effects for sensory characteristics. For example, the serumy/bloody aromatics and the soured taste were decreased by increasing the concentration of CLA (0%, 1.385; 4%, 0.778; 0%, 2.558; 4%, 2.250, respectively) ($P = 0.006$, 0.02, respectively), whereas the bitter taste was increased as the CLA concentration increased (0%, 2.673; 4%, 3.056) ($P = 0.0001$). Other sensory attributes such as painty aromatics and astringent and bitter aftertastes were significantly higher at 4% (0%, 0.462; 4%, 1.347; 0%, 1.462; 4%, 2.014; 0%, 2.269; 4%, 2.542, respectively) ($P = 0.0002$, 0.003, 0.04, respectively), whereas the browned aromatics and browned aftertaste were significantly higher at 2% CLA (1.531, 1.375, respectively) ($P = 0.003$, $P < 0.01$, respectively). These results were more dramatic in the patties with FFA-CLA treatment ($P < 0.05$). These results were consistent with the study by Du and others (2003) where dietary CLA treatment had negative effects on the sensory qualities at 2 and 3% levels of supplementation. Overall, the TAG-CLA contributed to the cooked

beef/brothy and serumy/bloody aromatic attributes, whereas the FFA-CLA increased painty aromatics, bitter taste, and astringent aftertaste due to the soapy flavor of the free fatty acid.

Fatty acid composition of irradiated and non-irradiated ground beef patties is shown in Table 8. Irradiation decreased the concentrations of 18:2n-6 (linoleic acid), 18:2 cis-9,trans-11 CLA, and 18:2 trans-10,cis-12 CLA isomers in cooked patties with the exception of the TAG 1 and 2% treatment (all $P = 0.04$, 0.02, 0.02, respectively). Similar results were observed in the study by Brito and others (2002) where irradiation at varying kGy decreased linoleic acid in ground beef. Their study also revealed that irradiation induced trans-fatty acid formation.

Although irradiation decreased the CLA isomers, higher percentages of CLA isomers were retained in irradiated patties containing 4% FFA-CLA ($P < 0.001$), reflecting the ability of the FFA preparation, at higher concentration, to reduce lipid oxidation that is caused by irradiation. This result was consistent with the TBARS values (Figure 2). In contrast, although the percentage of CLA isomers was not affected by irradiation in the patties containing 1 or 2% TAG, the TBARS values were decreased at 1% and increased at 2%. However, values remained below those of irradiated, cooked beef patties. These results indicate that the TAG-CLA possesses antioxidative capacity at lower concentrations and susceptibility to lipid oxidation at higher concentration in irradiated ground beef patties.

The fat content of ground beef patties containing TAG-CLA was significantly lower than that of patties containing FFA-CLA ($P = 0.02$) (Table 4), indicating less

Table 8 – Fatty acid composition of irradiated and non-irradiated, cooked ground beef patties

		% CLA-FFA			% CLA-TAG			Pooled	Significant
	Control	1	2	4	1	2	4	SEM	effects[a]
14:0 (myristic)									
Irradiated	3.03	2.76	2.66	2.51	2.69	2.56	2.46	0.058	%
Non-irradiated	3.10	2.62	2.49	2.35	2.94	2.77	2.26		
16:0 (palmitic)									
Irradiated	26.9	25.1	26.2	22.8	24.1	24.8	22.7	0.381	%
Non-irradiated	26.9	23.4	24.5	21.8	25.9	27.2	20.9		
16:1 (palmitoleic)									
Irradiated	2.95	2.75	2.45	2.46	2.78	2.60	2.53	0.053	%
Non-irradiated	3.08	2.69	2.49	2.46	2.95	2.65	2.51		
18:0 (stearic)									
Irradiated	16.3	15.2	16.4	13.5	14.5	14.7	13.6	0.265	%
Non-irradiated	15.9	14.1	15.2	12.9	15.7	16.4	12.3		
18:1cis-9 (oleic)									
Irradiated	35.7	32.9	32.8	31.8	33.7	33.0	31.7	0.288	%
Non-irradiated	35.8	33.2	32.9	32.2	35.7	34.3	31.4		
18:1cis-11 (cis vaccenic)									
Irradiated	1.36	1.30	1.46	1.31	1.31	1.24	1.29	0.021	NS
Non-irradiated	1.36	1.27	1.25	1.36	1.41	1.33	1.29		
18:1trans-11 (trans vaccenic)									
Irradiated	3.44	2.71	1.26	2.81	3.39	1.15	3.06	0.280	NS
Non-irradiated	3.45	3.24	1.18	2.75	3.45	1.20	2.60		
18:2n-6 (linoleic)									
Irradiated	0.86	0.65	0.54	1.17	1.75	1.27	1.24	0.096	Irr
Non-irradiated	1.32	1.60	1.48	1.63	1.23	0.20	2.05		

Table 8 Continued

	Control	% CLA-FFA 1	2	4	% CLA-TAG 1	2	4	Pooled SEM	Significant effects[a]
CLA cis-9,trans-11									
Irradiated	0.05	0.46	0.36	3.21	0.96	1.19	2.24	0.312	%, Irr
Non-irradiated	0.05	1.31	2.98	5.24	0.58	0.03	4.95		
CLA trans-10,cis-12									
Irradiated	0.03	0.49	0.40	3.26	0.91	1.22	2.26	0.314	%, Irr
Non-irradiated	0.04	1.21	2.93	6.24	0.54	0.07	5.11		

[a]% = CLA concentration in ground beef; Type = CLA free fatty acid form versus triacylglycerol form; NS = no significant differences ($p > 0.10$).

retention of TAG-CLA isomers with cooking. The loss of TAG-CLA during cooking, combined with the susceptibility of TAG-CLA to lipid oxidation, would have contributed to its inability to decrease the TBARS values at higher concentrations. There were no significant effects of irradiation and CLA on 18:1cis-11 (cis vaccenic) and 18:1trans-11 (trans vaccenic) in contrast to the results reported by Brito and others (2002). The different temperatures applied during irradiation may be responsible for the difference. In this study, irradiation took place at 4 °C; in the study by Brito and others (2002), irradiation was conducted at 25 °C, at which oxidation of lipid would be accelerated.

Conclusions

Irradiation caused remarkable increases in TBARS values in the patties with both FFA and TAG preparation of CLA. Although the FFA-CLA was effective in reducing lipid oxidation in irradiated, cooked ground beef patties, it increased painty aromatic attribute, bitter taste, and astringent aftertaste due to the soapy flavor of the free fatty acid. The TAG-CLA was less effective at higher concentration (4%) in preventing lipid oxidation in irradiated ground beef patties, which may have been caused by its poor retention during the cooking process and its susceptibility to lipid oxidation during irradiation. Sensory attributes were also affected by irradiation, which caused an increase in browned aromatic attribute, browned aftertaste, and wet dog/hairy aromatic attribute, and a decrease in serumy/bloody aromatic attribute. Irradiation did not affect basic tastes.

Although irradiation decreased the CLA isomers, higher percentages of both CLA isomers were retained in irradiated patties containing 4% FFA-CLA ($P < 0.001$),

reflecting the ability of the FFA preparation, at higher concentration, to reduce lipid oxidation that is caused by irradiation. The loss of TAG-CLA during cooking, combined with its susceptibility to lipid oxidation, would explain the increased TBARS values at higher concentrations.

CHAPTER V

CONCLUSIONS

The hypothesis of this study was that CLA would be retained during irradiation and would reduce lipid oxidation that is caused by irradiation. The first objective of this study was to quantify the antioxidative properties of CLA by measuring lipid oxidation of aerobically-packaged, irradiated raw beef patties that were subsequently cooked. The second objective was to evaluate the effects of CLA alone and in combination with irradiation on lipid oxidation, fatty acid composition, cooking loss, moisture and fat content, and trained sensory panel evaluation of beef patties.

Addition of CLA during the grinding process increased CLA isomers (the c9,t11 and the t10,c12) in both irradiated and non-irradiated cooked ground beef patties. Weight loss during cooking was greater in irradiated beef patties than in non-irradiated patties. The loss was primarily moisture loss. Addition of CLA and CLA type had no effect on weight loss.

The TBARS values were significantly higher in irradiated, cooked ground beef patties as in non-irradiated ground beef patties. As the concentrations of FFA-CLA increased, the TBARS values declined in irradiated ground beef patties, whereas the concentration of TAG-CLA and the TBARS values did not show linear relationship. These data indicate that the FFA-CLA will reduce lipid oxidation that is resulted from irradiation, whereas the TAG-CLA is most effective only at low concentration due to its less retention during the cooking process and its susceptibility to lipid oxidation at higher concentrations.

Irradiation significantly affected serumy/bloody and soured aromatic sensory attributes. Irradiation reduced the serumy/bloody aromatic attribute and increased browned aromatic attribute, browned aftertaste, and wet dog/hairy aromatic attribute. The soured aromatic attribute was not detectable in irradiated ground beef patties. There was no significant main effect of irradiation on the basic tastes.

The CLA type also significantly affected aromatic attributes, basic tastes, and aftertastes. The FFA-CLA increased painty aromatic attribute, bitter taste, and astringent aftertaste due to the soapy flavor of the free fatty acid. The TAG-CLA contributed to the cooked beef/brothy and serumy/bloody aromatic attributes

Irradiation had a significant effect on fatty acid composition. Linoleic acid, CLA cis-9,trans-11, and CLA trans-10,cis-12 were decreased by irradiation. Although irradiation decreased the CLA isomers, higher percentages of both CLA isomers were retained in irradiated patties containing 4% FFA-CLA, reflecting the ability of the FFA preparation, at higher concentration, to reduce lipid oxidation that is caused by irradiation.

Although the FFA-CLA was effective in reducing lipid oxidation that is caused by irradiation, it increased painty aromatic attribute, bitter taste, and astringent aftertaste and decreased cooked beef/brothy and serumy/bloody aromatic attribute and browned aftertaste. The TAG-CLA was less effective at higher concentrations in preventing lipid oxidation in irradiated ground beef patties, which may have been caused by its poor retention during cooking process and its susceptibility to lipid oxidation during irradiation. Future studies should investigate methods to stabilize the TAG-CLA in

ground beef during process to provide practical benefits for the products to be enriched with CLA isomers. These methods should also ameliorate the negative effects of irradiation.

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