Evaluation of Conjugated Linoleic Acid Concentrations in Cooked Beef

Nalur C. Shantha,† Andrea D. Crum,† and Eric A. Decker*,‡

Food Science Section, Department of Animal Sciences, University of Kentucky, Lexington, Kentucky 40546-0215, and 236 Chenoweth Laboratory, Department of Food Science, University of Massachusetts, Amherst, Massachusetts 01003

Conjugated linoleic acid (CLA) concentrations in raw steaks (ribeye, round, T-bone, and sirloin) ranged between 3.1 and 8.5 mg of CLA/g of fat. Freshly cooked rare (60 °C) or well done (80 °C) ground beef patties (fried, baked, broiled, or microwaved) contained between 6.6 and 8.2 mg of CLA/g of fat. When CLA concentrations were compared on a milligrams of CLA per gram of fat basis, there were no significant differences in CLA among either cooking methods or degrees of doneness. When CLA concentrations were compared on a milligrams per 100 g of cooked meat basis, the 80 °C baked patties had the highest CLA concentration (152 mg/100 g of meat), while baked patties cooked to 60 °C contained the greatest concentration of CLA on a per patty basis (113 mg of CLA/patty). During storage, cooked beef oxidized rapidly as measured by thiobarbituric reactive substances. However, oxidation did not affect CLA concentrations.

Keywords: *Conjugated linoleic acid; beef; lipids; lipid oxidation*

INTRODUCTION

Antimutagenic activity in uncooked and pan-fried ground beef was first reported by Pariza and co-workers in 1979. This beef-derived modulator of mutagenesis was later isolated from fried ground beef and identified as isomers of conjugated linoleic acid (CLA) (Ha et al., 1987). Since then, CLA has been shown to inhibit mouse skin carcinogenesis induced by 7,12-dimethylbenz[*a*]anthracene when CLA was applied on the skin surface (Ha et al., 1987). Mammary tumors in rats (Ip et al., 1991) and mouse forestomach neoplasia (Ha et al., 1990) are suppressed by dietary CLA. The 9-*cis*,-11-*trans*- (*9c, 11t*) octadecadienoic acid isomer is preferentially incorporated into the phospholipids of mice, thereby suggesting that it could be the active CLA isomer (Ha et al., 1990).

Minor changes in the fatty acid composition of foods are known to occur as a result of cooking (Kamal et al., 1989). In earlier work on cheese processing we observed an increase in CLA content of processed cheese when cheese was processed at 60 or 80 °C (Shantha et al., 1992). Ha et al. (1989) reported increased levels of CLA in grilled beef as compared to uncooked ground beef. These data suggest that cooking temperatures and methods could influence CLA concentrations in beef if CLA is formed as a result of thermal oxidation of linoleic acid. Conversely, CLA could also be destroyed in beef by high cooking temperatures and oxidative reactions that occur during subsequent storage. Since CLA is potentially a beneficial dietary component that could be obtained from meats, it is important to establish how cooking and storage affect CLA concentrations.

In the present study we report CLA concentrations in raw and cooked beef steaks (ribeye, T-bone, sirloin, and round). The effects of two cooking temperatures, different cooking methods (broiling, frying, baking, and microwaving), and refrigerated storage on CLA content in ground beef were also studied.

MATERIALS AND METHODS

Five different beef steaks were purchased over a 2-month period from separate local retailers to ensure that meat from different animals was sampled. Vacuum-packed untrimmed beef chuck was also purchased from a local retailer. *trans*,-*trans*-octadecadienoic acid methyl ester was purchased from Matreya Inc., Pleasant Gap, PA. Tetramethylguanidine was obtained from Sigma Chemical Co., St. Louis, MO. All other chemicals were of reagent grade or purer.

Ground beef was prepared by grinding deboned separable lean from beef chuck through 8-mm plates. Fat in the ground lean was measured by a modified Babcock method (Decker and Crum, 1991) and then fat was reground back into the meat to give a final fat content of 20%. One hundred grams of meat was pressed into patties (9-cm diameter), vacuum packed, and stored at −20 °C until further use. All ground beef patties were used within 1 month of purchase.

Beef patties were cooked by broiling, baking, microwaving, and frying to two internal temperatures (60 and 80 °C). Internal temperatures were determined by inserting a thermometer into the center of the patty immediately after cooking. Baked patties were cooked in a General Electric (GE) electric range at 205 °C for 4.15 min/side to attain a 60 °C internal temperature and for 11 min/side to attain an internal temperature of 80 °C. Patties were microwaved (Kenmore microwave oven) at full power for 1.15 and 2.25 min/side to obtain internal temperatures of 60 and 80 °C, respectively. Broiled patties were cooked 14 cm from the burner in a GE electric oven for 2.10 and 4.10 min/side at 288 °C to obtain internal temperatures of 60 and 80 °C, respectively. Patties were fried at medium heat on a GE electric range for 3.30 and 8.10 min/side to obtain internal temperatures of 60 and 80 °C, respectively. Cooked patties were stored at 4 °C in 500-mL Whirlpak bags and were analyzed for CLA content and thiobarbituric reactive substances after 0, 1, 2, 4, and 7 days of storage. The 0-day ground beef samples were analyzed within 3 h after cooking. Beef steaks were broiled to an internal temperature of 80 °C using the conditions listed above and were analyzed for CLA within 3 h of cooking.

* Author to whom correspondence should be addressed.
† University of Kentucky.
‡ University of Massachusetts.

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Lipid Analysis. Fat was extracted from beef using hexane and 2-propanol (3:2 v/v) (Hara and Radin, 1978) and stored at −80 °C under nitrogen until all samples could be analyzed by gas chromatography (GC). Transesterification of extracted lipids into fatty acid methyl esters was accomplished using tetramethylguanidine in methanol (TMG/MeOH) (Shantha et al., 1993). GC analysis was performed on a Perkin-Elmer auto-GC using a Supelcowax 10 fused silica capillary column (60 m × 0.75 mm i.d., phase thickness 0.1 μm; Supelco Inc., Bellefonte, PA). The GC analysis was temperature programmed from 190 to 220 °C at 20 °C/min and held at 220 °C for 30 min. Other parameters were as follows: split injection, helium (carrier gas) 11 psi; injection port temperature, 250 °C; detector temperature, 250 °C. TURBOCHROM software (PE-NELSON, Cupertino, CA) was used for data analysis. The CLA peaks were identified by comparison with the retention time of the reference standard. The areas of the CLA peaks were calculated as milligrams per gram of fat using heneicosanoic acid as an internal standard (Shantha et al., 1992). Heneicosanoic acid was added to the lipid prior to methylation. The fat content of raw and cooked beef patties was determined using the Goldfish method (AOAC, 1990). Lipid oxidation was monitored by measuring thiobarbituric acid reactive substances (TBARS) (Sinnhuber and Yu, 1977).

Statistical Analysis. Five steaks were used, each of which was sampled twice for CLA and fat content. For the comparison of cooking methods, patties were cooked in triplicate and each patty was sampled twice for TBARS and CLA concentrations and once for fat content. The coefficient of variation of GC analysis (duplicate injections) was generally less than 1%, while the coefficient of variation of fat extraction, methylation, and GC analysis of the same patty ranged from 2 to 4%. Experiments were conducted as completely randomized factorial designs which included treatment, replicate (treatment), CLA concentration, and treatment × concentration interaction. Replicate (treatment) was used as the error term for testing differences among treatments. If the F test was significant, the least significant procedure was used to determine the difference between means at the 5% level of significance (Snedecor and Cochran, 1989). Statistical calculations were conducted using GLM SAS (1988).

RESULTS AND DISCUSSION

Table 1 shows the CLA content of different beef steaks. The observed wide variations in CLA content within raw steaks is most likely due to steaks being obtained from different animals. Seasonal variations in CLA content found in milk (Riel, 1963) could be reflected in skeletal muscle of beef cattle; however, it is also possible that feed or genetic variables could influence beef CLA concentrations. Due to the wide animal-to-animal variations in CLA content observed within a steak, there were no significant differences in CLA content between steaks. Round steaks, which ranged between 5.3 and 8.1 mg of CLA/g of fat, had the highest average CLA content (6.8 mg of CLA/g of fat), while sirloin steaks, which ranged between 3.1 and 7.2 mg of CLA/g of fat, averaged the lowest concentration (5.8 mg of CLA/g of fat). In general, the CLA concentrations in the cooked steaks (broiled, 80 °C internal temperature) were higher than in raw steaks, although this was significant only between raw and cooked T-bone steaks (Table 1).

Table 2 shows the yield, fat content, and CLA concentrations of ground beef patties cooked to either 60 or 80 °C by frying, baking, broiling, or microwaving. To study the effect of cooking, experiments were conducted using a single source of meat to avoid animal-to-animal variations in CLA content as was observed in the steaks. When CLA concentrations were compared on a milligrams of CLA per gram of fat basis,

Table 1. Concentration of *9-cis, 11-trans* (*9c, 11t*) and Total Conjugated Linoleic Acid (CLA) in Raw and Cooked (Broiled, 80 °C Internal Temperature) Beef Steaks

sample		*9c, 11t* CLA[a] (mg/g of fat)	total CLA[a] (mg/g of fat)
ribeye			
raw	range	2.6−5.5	4.5−8.5
	av	3.9 (0.9)[a]	6.4 (1.2)[a]
cooked	range	3.2−6.5	4.9−8.8
	av	4.1 (1.2)[a]	6.7 (1.0)[a]
round			
raw	range	2.7−4.8	5.3−8.1
	av	3.9 (1.0)[a]	6.8 (0.8)[a]
cooked	range	4.1−5.0	7.0−8.4
	av	4.6 (0.3)[a]	7.6 (0.6)[a]
T-bone			
raw	range	2.6−5.0	4.4−6.6
	av	3.6 (0.5)[a]	6.1 (0.9)[a]
cooked	range	3.0−6.5	4.7−9.9
	av	4.5 (1.1)[b]	6.9 (1.7)[b]
sirloin			
raw	range	1.7−4.7	3.1−7.2
	av	3.4 (0.9)[a]	5.8 (1.8)[a]
cooked	range	2.3−4.8	4.0−7.5
	av	3.8 (1.0)[a]	5.9 (1.3)[a]

[a] Values represent means with SD in parentheses (n = 5). Means with different superscripts within a column and sample group (i.e., steak) are significantly different ($p < 0.05$).

Table 2. Fat Concentration, Percent Yield, and Conjugated Linoleic Acid (CLA) Concentrations in Ground Beef Patties[a] Cooked by Various Methods to Different Degrees of Doneness

cooking method	internal temp (°C)	fat content[b] (%)	yield[b] (%)	*9c, 11t* CLA[c] (mg/g of fat)	total CLA[c] (mg/g of fat)
fry	60	15.7 (0.9)	73.8 (2.5)	4.1 (0.6)[bc]	6.7 (1.1)[ab]
	80	17.1 (0.3)	57.7 (1.1)	4.4 (0.3)[ab]	7.4 (0.4)[ac]
bake	60	17.6 (0.9)	79.0 (2.0)	4.6 (0.4)[a]	8.1 (0.2)[ad]
	80	20.3 (2.0)	61.6 (0.7)	4.4 (0.5)[abc]	7.5 (1.0)[acd]
broil	60	18.2 (0.5)	83.7 (1.4)	3.9 (0.2)[bc]	6.6 (0.3)[b]
	80	18.0 (1.0)	56.3 (2.2)	4.2 (0.3)[abc]	7.3 (0.4)[ab]
microwave	60	16.3 (0.3)	74.7 (3.6)	4.2 (0.4)[abc]	7.2 (0.6)[ab]
	80	15.8 (0.5)	70.9 (2.9)	4.7 (0.6)[a]	8.2 (1.0)[d]

[a] Total and *9c, 11t* CLA concentrations were 7.4 (0.4)[a] and 4.3 (0.2)[abc] mg/g of fat, respectively, in raw beef. [b] Values represent means with SD in parentheses (n = 3). [c] Values represent means with SD in parentheses (n = 6). Means with different superscripts within a column are significantly different.

there were no large differences in CLA between either cooking method or degree of doneness, although higher internal cooking temperatures generally resulted in higher CLA concentrations within a cooking method with the exception of baking. When CLA concentrations were compared on a milligrams per 100 g of cooked meat basis, the 80 °C baked patties had the highest CLA concentration (152 mg of CLA/100 g of meat) due to a combination of high CLA (7.5 mg of CLA/g of fat) and high fat content (20.3%; Table 3). Baked patties cooked to 60 °C contained the greatest concentration of CLA on a per patty basis (113 mg of CLA/patty) due to the high yield (79%) and high CLA concentration (8.1 mg/g of fat). Although the 80 °C microwaved patties also had

Table 3. Conjugated Linoleic Acid (CLA) Concentrations in Ground Beef Patties[a] Cooked by Various Methods to Different Degrees of Doneness

cooking method	internal temp (°C)	*9c, 11t* CLA[b] (mg/100 g of meat)	total CLA[b] (mg/100 g of meat)	*9c, 11t* CLA[b] (mg/patty)	total CLA[b] (mg/patty)
fry	60	64.2 (4.4)	105.0 (7.2)	47.4 (1.7)	77.5 (2.8)
	80	75.4 (1.7)	126.8 (2.8)	43.5 (0.9)	73.1 (1.5)
bake	60	80.8 (4.9)	142.3 (8.7)	63.8 (1.8)	113.0 (3.3)
	80	89.2 (10.7)	152.0 (18.3)	54.9 (0.7)	93.5 (1.2)
broil	60	70.9 (2.4)	119.9 (4.0)	59.4 (1.0)	100.4 (1.8)
	80	75.7 (5.1)	131.6 (8.9)	42.6 (1.8)	74.1 (3.1)
microwave	60	68.5 (1.5)	117.4 (2.6)	51.2 (2.7)	87.7 (4.5)
	80	74.3 (3.3)	129.6 (5.8)	52.7 (2.4)	91.9 (4.1)

[a] Total and *9c, 11t* CLA concentrations were 7.4 (0.4) and 4.3 (0.2) mg/g of fat, respectively, in raw beef. CLA concentrations are expressed on a per patty and per 100 g of meat basis using the average fat concentrations and percent yields shown in Table 2. [b] Values represent means of CLA concentrations with SD in parentheses ($n = 6$).

a high CLA concentration on a milligrams per gram of fat basis (8.2), the loss in fat accounted for lower CLA concentrations in the cooked patty (91.9 mg of CLA/patty; Table 3).

The CLA concentration (Table 4) and oxidative deterioration (as measured by TBARS; Figure 1) of the cooked ground beef patties were followed during refrigerated storage for 7 days. In general, no significant increases in TBARS were observed over the first 2 days of storage, after which time TBARS formation became more rapid with the exception of patties broiled to 60 °C. Oxidative reactions could influence CLA concentrations by either (1) causing the formation of linoleic acid radicals, which in turn could be converted to CLA by hydrogen donors (Ha et al., 1989), or (2) causing the oxidative destruction of the conjugated double-bond system of CLA. The CLA concentrations in patties cooked by frying (60 °C), baking (80 °C), and broiling (60 °C) showed significantly higher levels of CLA on day 1 of storage as compared to day 0 (Table 4). From day 1 to day 7, there were no consistent changes in either *9c, 11t* CLA or total CLA concentration of beef cooked with by of the cooking methods to either internal temperature. The lack of changes in CLA concentrations in stored, cooked beef that is undergoing oxidative deterioration could be due to the greater stability of CLA compared to polyunsaturated fatty acids containing methylene-interrupted double bonds or a balance between CLA formation and CLA destruction. If a balance between CLA formation and destruction was occurring in stored beef, one would expect a change in the ratio of the CLA isomers. Biohydrogenation of linoleic acid in the rumen is believed to be responsible for the high proportion of the *9c, 11t* CLA (50–80%) in beef and dairy fat (Ip et al., 1991). Conversely, formation of CLA by chemical reactions leads to equal formation of both the *9c, 11t* and the *10t, 12c* isomers (Shantha et al., 1993). Therefore, if oxidative reactions are causing the formation of CLA during storage, then one would expect to see an increase in the concentration of the *10t, 12c* isomer. Neither the concentrations of the *10t, 12c* isomer nor the *9c, 11t* to total CLA ratio changed during storage, suggesting that oxidative destruction and formation of CLA were not substantial.

In conclusion, different cooking methods including frying, broiling, baking, and microwaving do not produce any major changes in the CLA content of ground beef when concentrations are compared on a milligrams of CLA per gram of fat basis. However, cooking method and degree of doneness do affect the concentration of dietary CLA from beef products since cooking methods influence both fat content and amount of edible portion (i.e., yield). In addition to cooking methods, oxidative reactions also do not alter CLA, suggesting that CLA concentrations will not change during processing and storage. Wide variations in CLA concentration (up to 4.1 mg/g of fat) were observed in steaks originating from different animals. More research is needed to deter-

Table 4. Effect of Storage on Conjugated Linoleic Acid (CLA) Concentration in Ground Beef Cooked by Different Cooking Methods and to Two Internal Temperatures

			CLA concentration[a] (mg/g of fat)				
cooking method	internal temp (°C)	CLA type	0 days of storage	1 days of storage	2 days of storage	4 days of storage	7 days of storage
fry	60	*9c, 11t*	4.1 (0.6)^{a}	4.6 (0.2)^{b}	4.5 (0.5)^{ab}	4.4 (0.2)^{ab}	4.5 (0.1)^{b}
		total	6.7 (1.1)^{a}	7.6 (0.4)^{b}	7.4 (0.8)^{ab}	7.6 (0.2)^{b}	7.6 (0.1)^{b}
	80	*9c, 11t*	4.4 (0.3)^{a}	4.6 (0.2)^{a}	4.6 (0.1)^{a}	4.4 (0.4)^{a}	4.5 (0.2)^{a}
		total	7.4 (0.4)^{a}	7.6 (0.3)^{a}	7.6 (0.2)^{a}	7.4 (0.6)^{a}	7.8 (0.4)^{a}
bake	60	*9c, 11t*	4.6 (0.4)^{a}	4.9 (0.3)^{a}	5.0 (0.4)^{a}	5.2 (0.8)^{a}	5.1 (0.6)^{a}
		total	8.1 (0.6)^{a}	8.3 (0.1)^{a}	8.1 (0.3)^{a}	8.5 (0.8)^{a}	8.5 (0.7)^{a}
	80	*9c, 11t*	4.4 (0.5)^{a}	5.0 (0.4)^{b}	4.7 (0.4)^{b}	4.6 (0.5)^{ab}	4.6 (0.3)^{ab}
		total	7.5 (1.0)^{a}	8.3 (0.5)^{b}	7.7 (0.5)^{ab}	7.6 (0.7)^{ab}	7.8 (0.4)^{ab}
microwave	60	*9c, 11t*	4.2 (0.4)^{a}	4.1 (0.2)^{a}	4.4 (0.5)^{a}	4.5 (0.5)^{a}	4.3 (0.3)^{a}
		total	7.2 (0.6)^{a}	7.3 (0.5)^{a}	7.5 (0.7)^{a}	7.6 (0.6)^{a}	7.5 (0.4)^{a}
	80	*9c, 11t*	4.7 (0.6)^{a}	4.3 (0.7)^{a}	4.1 (0.4)^{a}	4.3 (0.4)^{a}	4.2 (0.3)^{a}
		total	8.2 (1.0)^{a}	7.6 (1.1)^{ab}	7.2 (0.4)^{b}	7.6 (0.6)^{ab}	7.4 (0.3)^{ab}
broil	60	*9c, 11t*	3.9 (0.2)^{a}	4.4 (0.1)^{b}	4.3 (0.1)^{b}	4.1 (0.3)^{ab}	4.2 (0.5)^{ab}
		total	6.6 (0.3)^{a}	7.6 (0.1)^{b}	7.3 (0.1)^{b}	7.2 (0.6)^{ab}	7.3 (0.8)^{b}
	80	*9c, 11t*	4.2 (0.3)^{a}	4.3 (0.4)^{a}	4.1 (0.2)^{ab}	3.8 (0.4)^{b}	4.3 (0.1)^{a}
		total	7.3 (0.4)^{a}	7.4 (0.7)^{a}	6.9 (0.3)^{ab}	6.6 (0.6)^{b}	7.5 (0.2)^{a}

[a] Values represent means with SD in parentheses ($n = 6$). Means with different superscripts within a row and a CLA type (i.e., *9c, 11t*) are significantly different ($p < 0.05$).

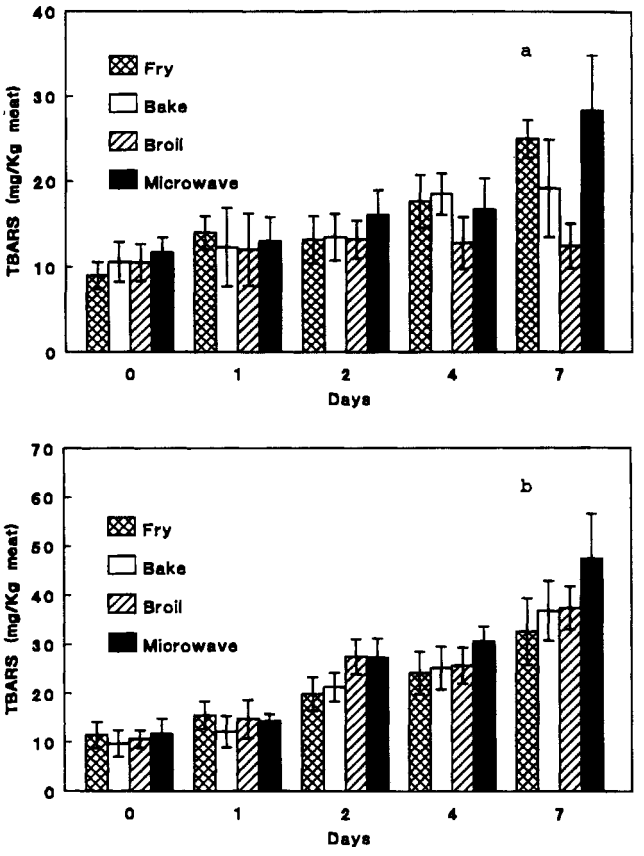

Figure 1. Thiobarbituric acid reactive substances (TBARS) in stored (4 °C) ground beef patties cooked to internal temperatures of 60 (a) and 80 °C (b) by various cooking methods.

mine if animal-to-animal variations in CLA are due to feed, seasonal, or genetic variables.

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