THE EFFECT OF DIRECT ADDITION OF CONJUGATED LINOLEIC ACID ON THE FORMATION OF HETEROCYCLIC AROMATIC AMINES IN BEEF CHOPS

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ABSTRACT

The effect of direct addition of conjugated linoleic acid (CLA) (0.05, 0.1, 0.25 and 0.5%) to beef chops on the formation of heterocyclic aromatic amines (HCAs) was investigated. Chops were cooked at three different temperatures (150, 200, 250C). CLA addition had no significant effect ($P > 0.05$) on water content, pH, thiobarbituric acid reactive substance, L^*, a^* and b^* values and cooking loss of the samples, while cooking temperatures had a significant effect ($P < 0.01$) on these parameters except for pH value. While 4,8-DiMeIQx and MeAαC could not be detected, varying amounts of IQx (up to 0.53 ng/g), IQ (up to 0.18 ng/g), MeIQx (up to 0.09 ng/g), MeIQ (up to 0.56 ng/g), 7,8-DiMeIQx (up to 0.19 ng/g), PhIP (up to 2.61 ng/g) and AαC (up to 0.58 ng/g) were detected. Total HCA content ranged between 0.10 and 4.49 ng/g. Up to 0.1% CLA reduced the total HCA amount at all cooking temperatures.

PRACTICAL APPLICATIONS

Conjugated linoleic acid (CLA) is a bioactive compound that is found naturally in foods from ruminant. There is limited information about the direct addition of CLA to meat in the literature. In addition, to the best of our knowledge, there are no reports on the effect of CLA on some quality properties of beef chop and the formation of heterocyclic aromatic amines. Therefore, in the present study, we aim to explore the effects of CLA usage on these parameters.

INTRODUCTION

In 1979, it was firstly reported by Pariza *et al.* (1979) that anti-mutagenic substances occurred in pan-fried hamburger, and then it was found that these substances inhibited the initiation of mouse epidermal tumors (Pariza and Hargraves 1985). The fatty acid responsible for this effect was identified as conjugated linoleic acid (CLA) (Schmid *et al.* 2006). CLA is a collective term describing a mixture of octadecadienoic acids that describes all possible *cis* and *trans* combinations of conjugated double bonds in 10 and 12, the 9 and 11, or the 11 and 13 carbons (Chae *et al.* 2004). Foods from ruminants are known to have markedly higher CLA concentration than those from nonruminants. CLAs have recently received considerable attention due to positive effects on cancer, cardiovascular diseases, diabetes, body composition, immune system and bone health (Pariza *et al.* 1999; Belury 2002; Rainer and Heiss 2004). Therefore, research about CLA is still in place and will continue (Schmid *et al.* 2006).

Heterocyclic aromatic amines (HCAs) are formed in proteinaceous foods during cooking at high temperatures (Oz and Kaya 2011a). The formation of HCAs in meat and meat products is one of the most important health concerns due to the fact that most HCAs are highly mutagenic (Felton *et al.* 1984) and almost all are carcinogenic (International Agency for Research on Cancer (IARC) 1993). To date, more than 25 different HCAs have been isolated and identified in cooked foods (Sanz Alaejos *et al.* 2008; Oz *et al.* 2015); their relative formation is greatly influenced by food composition and cooking conditions such as cooking method, cooking equipment, cooking temperature and cooking duration. In addition, the formation and amounts of the HCAs are dependent on many factors such as pH, water activity,

carbohydrates, free amino acids, creatine/creatinine, heat and mass transfer, lipid level, lipid oxidation and antioxidants (Jägerstad *et al.* 1998; Pais *et al.* 1999; Oz *et al.* 2007, 2010a,b; Oz 2010, 2011; Oz and Kaya 2011a,b,c). It has been stated that human exposure to HCAs is influenced not only by the type of food and cooking method, but also by portion size and intake frequency (Skog 2004).

Several studies have so far been conducted on the effects of natural antioxidants (flavonoids, vitamins C and E), synthetic antioxidants (butylated hydroxyanisole; butylated hydroxytoluene; propyl gallate, PG; tertiary butylhydroquinone) and also some food ingredients (cherry, polyphenolic compounds from tea, spices, oligosaccharides and inulin, olive oil) on the formation of HCAs (Chen *et al.* 1992; Lee *et al.* 1992; Pearson *et al.* 1992; Weisburger *et al.* 1994; Yen and Chen 1995; Johansson and Jägerstad 1996; Britt *et al.* 1998; Murkovic *et al.* 1998; Oguri *et al.* 1998; Balogh *et al.* 2000; Tai *et al.* 2001; Shin *et al.* 2002a,b, 2003; Zöchling *et al.* 2002; Vitaglione and Fogliano 2004). However, to the best of our knowledge, the effect of CLA on the formation of HCAs in beef chop has not been investigated in the literature. The present study was therefore undertaken in an attempt to investigate the effect of direct addition of CLA on the formation of HCAs in beef chop fried at different frying temperatures (150, 200 and 250C) and some quality criteria of beef chops.

MATERIALS AND METHODS

Beef Chop and CLA

Muscles of *Longissimus dorsi* used for beef chops were obtained from a local slaughterhouse (Et ve Süt Kurumu Et Kombinası, Erzurum, Turkey). The chops were put on ice in a cooler for transportation to the laboratory. CLA was a kind gift from Mr. Mehmet GEDİK, Bio-Kim Limited Company (Gebze, Kocaeli, Turkey).

Chemicals

Chemicals and solvents were of high-performance liquid chromatography (HPLC) or analytical grade. Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA). All solutions except for HPLC grade were passed through a 0.45-μm filter (Millex, MA). IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline, CAS No. 76180-96-6), MeIQ (2-amino-3,4-dimethylimidazo [4,5-*f*]quinoline, CAS No. 77094-11-2), MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline, CAS No. 77500-04-0), 4,8-DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline, CAS No. 95896-78-9), PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, CAS No. 105650-23-5) and 4,7,8-TriMeIQx (2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline, CAS No. 132898-07-8) were purchased from Toronto Research Chemicals (Downsview, Ontario, Canada). 4,7,8-TriMeIQx was used as internal standard. The stock standard solutions were prepared according to Oz *et al.* (2015). For the solid-phase extraction, an Oasis Mixed mode Cation Exchange cartridge (3 cm^3/60 mg, 30 μm) of Waters (Milford, MA) was used.

Preparation and Cooking of Chops

Beef chops were sliced to the thickness of 1 cm and divided into five groups. One group was chosen as a control group (without CLA). Four of these groups included CLA in the ratio of 0.05, 0.1, 0.25 and 0.5% (w/w). CLA was added on the surface of chops by spreading, and then the chops were stored at 4C for 12 h.

For the cooking procedure, a hot plate was used and preheated to 150, 200 and 250C of surface temperature. Neither fat nor oil was used for frying. Samples were cooked for a total of 9 min and reversed each minute. All chops were eatable. Temperatures were measured with a digital thermocouple (part no. 0560 9260; Testo, Lenzkirch, Germany) with surface probe (0603 1992; Testo). All experiments were performed in two replications. For each replicate, one chop was used for each condition. After cooking, the samples were cooled at room temperature. The weight of the samples was measured at room temperature, before and after cooking to calculate the cooking loss. Then, the samples were homogenized by using a kitchen blender (Tefal, Istanbul, Turkey) to have a uniform sample for analyses and were stored at −18C for HCA analyses. They were thawed in a refrigerator at 4C for 12–24 h prior to use. Freezing was done so that the samples could be stored before cleanup without any risk of further reactions in the beef.

Chemical Analysis and Cooking Loss

Water content was determined by drying a 5–10 g sample at 105C to the constant weight (Gökalp *et al.* 2010). Kjeldahl method was used to determine the total nitrogen content, and crude protein content was calculated using a conversion factor of 6.25 (Gökalp *et al.* 2010). Crude fat was determined using Soxhlet extraction with diethyl ether (Gökalp *et al.* 2010). The pH of the samples was measured using the pH meter (Basic 20, E-08328 ALELLA, Barcelona, Spain) at room temperature. pH meter was calibrated with buffer solutions (pH 4.01, pH 7.00 and pH 9.21) before usage. After the cooking processes, samples were cooled at the room temperature, surface-dried with a filter paper and

reweighed. Cooking losses of the samples were calculated from differences in the raw weight and cooked samples (Oz and Kizil 2013).

Thiobarbituric Acid Reactive Substance Analysis

Thiobarbituric acid reactive substance (TBARS) value of the samples was determined according to Lemon (1975) with some modifications (Kiliç and Richards 2003). Briefly, 1 g of the sample was weighed into a 50 mL test tube and homogenized with 6 mL trichloroacetic acid (TCA) solution (7.5% TCA, 0.1% ethylenediaminetetraacetic acid (EDTA), 0.1% 1-propyl gallate (PG), 1-PG in 3 mL ethanol) using ultra Turrax for 10 s. The mixture was filtered with Whatman 1 filter paper (Sigma-Aldrich, Darmstadt, Germany). Then, 1 mL filtrate was added to 1 mL of 0.02 M thiobarbituric acid solution. This mixture was kept in a boiling water bath for 40 min, then cooled, centrifuged at 2,000× g for 5 min, and the absorbance measured at 532 nm (UV 160, Shimadzu Scientific Instruments, Columbia, MD). TBARS values were expressed as mg malondialdehyde/kg sample.

Instrumental Color Analysis

The colorimetric parameters (L^*; $L^* = 0$, black; $L^* = 100$, white [darkness – lightness]; a^*; $+a^*$ = red, $-a^*$ = green; and b^*; $+b^*$ = yellow, $-b^*$ = blue) were measured using a Minolta Chroma Meter CR-200 colorimeter (Minolta Konica, Osaka, Japan). The instrument was calibrated to a standard tile provided before the analysis. Five readings were taken for each sample at room temperature.

Extraction of HCAs

HCA content of the samples was determined by the method described by Messner and Murkovic (2004), with minor modifications (Oz *et al.* 2015). After solid-phase extraction, HCAs were identified and quantified by HPLC (Thermo Ultimate 3000, Thermo Scientific, Waltham, MA) with diode array detector (DAD-3000), auto sampler (WPS-3000), column oven (TCC-3000) and pump (LPG-3400SD). Separation was carried out on a reversed phase analytical column, Acclaim 120 C18 3 μm (4.6 × 150 mm) from Tosoh Bioscience GmbH (Stuttgart, Germany) at 35C with a mobile phase of methanol/acetonitrile/water/acetic acid (8/14/76/2, v/v/v/v) at pH 5.0 (adjusted with ammonium hydroxide 25%) as solvent A and acetonitrile as solvent B at the flow rate of 0.7 mL/min. The gradient program was: 0% B, 0–10 min; 0–23% B, 11–20 min; 23% B, 21–30 min; 0% B, 31–45 min. The injection volume was 10 μL. Standard addition method was used for the determination of recoveries, and certain concentrations of HCA mixture (10, 5, 2.5, 1 and 0.5 ng/g) were spiked to the raw samples. The recovery amounts were determined after the extraction and analysis. Quantitative determination was performed by using an external calibration curve method. Coefficients of regression line (r^2) for HCA standard curves were 0.9991 for IQx, 0.9992 for IQ, 0.9991 for MeIQx, 0.9991 for MeIQ, 0.9992 for 7,8-DiMeIQx, 0.9992 for 4,8-DiMeIQx, 0.9997 for PhIP, 0.9992 for AαC and 0.9991 for MeAαC. Recovery rates for different HCAs in the samples were determined by the standard addition method (Oz 2011).

Statistical Analysis

The data obtained in the present study were subjected to analysis of variance. The experiment was set up according to a completely random block design and employed (in two replicates). Duncan multi-comparison test was applied to data in order to determine the statistical differences between the values detected by using the Statistical Package for the Social Sciences 17.0 statistical software package (SPSS 2004).

RESULTS AND DISCUSSION

Chemical Analysis and Cooking Loss

Water, crude protein, crude fat contents and pH values of the raw *L. dorsi* muscle used as material were 75.97 ± 0.98, 17.21 ± 1.38, 4.86 ± 0.58 and 5.49 ± 0.03, respectively. TBARS value of the raw samples was 0.43 ± 0.09 mg MA/kg. On the other hand, L^*, a^* and b^* values of the raw chops were 41.98 ± 1.13, 28.93 ± 3.16 and 15.68 ± 2.72. These values are comparable with those in the related literature (Oz and Kaya 2011b,c; Oz and Zikirov 2015).

Water content of the beef chops was given in Table 1. It was observed that cooking temperature had a very significant effect ($P < 0.01$) on the water content of the samples, while the CLA concentration and the interaction between concentration and cooking temperature had no significant effect ($P > 0.05$). Indeed, Oz and Cakmak (2015) investigated the direct addition of CLA (0.05, 0.1, 0.25 and 0.5%) to meatball dough on some quality properties of meatball, and found that CLA concentration had no significant effect ($P > 0.05$) on the water content of meatballs. In addition, in their study, Chae *et al.* (2004) added different concentrations of CLA (2 and 4%) to beef meatballs, and reported that addition of CLA did not have a significant effect on the water content of meatballs.

In the present study, while the water content of the raw chops was 75.16%, cooking led to an expected decrease in water content (Table 1). Increasing the cooking temperature

TABLE 1. WATER CONTENT, pH, TBARS VALUES, COOKING LOSS AND COLOR PARAMETERS OF THE CHOPS

	Water content (%)	pH	TBARS (mg/kg)	Cooking loss (%)	*L**	*a**	*b**
Uncooked	75.16 ± 0.68	5.44 ± 0.02	0.57 ± 0.08		44.59 ± 2.28	29.12 ± 2.11	18.43 ± 3.01
T							
Control	55.24 ± 4.31	5.61 ± 0.06	1.79 ± 0.47	45.40 ± 3.97	34.06 ± 5.05	8.08 ± 1.69	12.74 ± 3.18
% 0.05 CLA	53.81 ± 1.57	5.60 ± 0.03	2.06 ± 0.50	47.24 ± 1.53	32.91 ± 2.05	7.82 ± 0.86	12.08 ± 0.74
% 0.1 CLA	52.76 ± 4.90	5.57 ± 0.06	2.04 ± 0.64	48.03 ± 4.47	31.53 ± 5.99	7.81 ± 1.90	10.69 ± 3.43
% 0.25 CLA	52.49 ± 3.82	5.59 ± 0.04	2.29 ± 0.77	48.08 ± 3.76	31.70 ± 4.54	7.73 ± 2.53	11.15 ± 4.32
% 0.5 CLA	53.62 ± 3.43	5.57 ± 0.03	2.03 ± 0.79	46.55 ± 3.70	34.70 ± 4.27	7.46 ± 1.74	11.65 ± 2.14
Significance	ns	ns	ns	ns	ns	ns	ns
CT							
150 C	56.82 ± 2.33a	5.56 ± 0.04	1.49 ± 0.27c	44.24 ± 2.11b	37.00 ± 4.15a	8.63 ± 0.79a	13.78 ± 2.35a
200 C	53.12 ± 2.02b	5.60 ± 0.04	2.00 ± 0.29b	47.34 ± 1.96a	31.08 ± 2.62b	8.40 ± 1.54a	11.62 ± 2.45ab
250 C	50.81 ± 3.58b	5.60 ± 0.05	2.64 ± 0.60a	49.59 ± 3.96a	30.85 ± 3.43b	6.32 ± 1.66b	9.58 ± 2.39b
Significance	**	ns	**	**	**	**	**
Interactions							
T × CT	ns	ns	ns	ns	ns	ns	ns

Note: Means in the same column and in the same section having the same letters (a–c) are not significantly different at $P < 0.05$.
**$P < 0.01$.
T, treatment; CT, cooking temperature; CLA, conjugated linoleic acid; ns, not significant; TBARS, thiobarbituric acid reactive substance.

caused a decrease in water content of the samples. But the water contents of the samples cooked at 200 and 250C were not statistically different from each other. Sánchez del Pulgar *et al.* (2012) have stated that three main processes cause the water losses in cooked meat. First, water can be evaporated due to increased temperature and/or reduced pressure. Second, increased temperatures during cooking cause myofibrillar proteins to shrink, a process that starts at 40C and becomes more intense with increased temperatures. Because of this shrinking process, a parallel decrease occurs in the inter-fibrillar volume, which in turn leads to a reduction in the myofibril's ability to hold water. As a consequence, part of the water retained by capillarity is lost during cooking. Finally, at temperatures between 56 and 62C, a contraction of the perimysial connective tissue seems to take place, causing a compression of the muscle fiber bundles, which in turn encourages water to be released from the meat cut.

Cooking losses of the samples were given in Table 1. As a result of cooking at different temperatures, cooking losses of the samples varied between 44.24 and 49.59%. Rodriguez-Estrada *et al.* (1997) reported that cooking caused a reduction in the weight of meat products mainly due to the water loss during cooking of meat. However, Sánchez del Pulgar *et al.* (2012) have declared that water is not the only compound lost during cooking process. According to Gerber *et al.* (2009), Laroche showed that there are various components such as myofibrillar and sarcoplasmic proteins, collagen, lipids, salt, polyphosphates and flavor compounds that are also lost together with the water. In the present study, while it was observed that cooking temperature had a significant effect ($P < 0.01$) on cooking loss, the CLA concentration as well as the interaction between concentration and cooking temperature had no significant effect ($P > 0.05$) on the parameter. Indeed, Oz and Cakmak (2015) found that CLA concentration (0.05, 0.1, 0.25 and 0.5%) had no significant effect ($P > 0.05$) on cooking loss of meatballs, while cooking temperature had a very significant effect ($P < 0.01$). In addition, Chae *et al.* (2004) observed that cooking losses of meatball including CLA at the ratios of 0, 2 and 4% were 32.9, 33.6 and 35.7%, respectively; and they determined that the addition of CLA did not have a significant effect on the cooking loss of meatballs. On the other hand, Baublits *et al.* (2007) added CLA in the form of oil or powder to beef, and determined that CLA-added meat had significantly higher cooking losses than the control group. However, Mansour and Khalil (1997) reported that the addition of wheat fibers to beef meatballs had the effect of reducing cooking loss; Anderson and Berry (2001) reported similar effects with the addition of inner pea fiber to beef meatballs, and Turhan *et al.* (2005) reported similar effects with the use of hazelnut pellicle in low-fat meat burgers.

pH values of the samples were also given in Table 1. In the present study, it was observed that CLA concentration, cooking temperature and the interaction between concentration and cooking temperature had no significant effect ($P > 0.05$) on pH values of the samples. On the other hand, Oz and Cakmak (2015) found that CLA concentration (0.05, 0.1, 0.25 and 0.5%) and cooking temperature had a very significant effect ($P < 0.01$) on pH values of meatballs, while the interactions between CLA concentration and cooking temperature had no significant effect ($P > 0.05$). However, Baublits *et al.* (2007) reported that the addition

of CLA in the form of oil and powder to beef had no significant effect on its pH value after cooking. In the present study, cooking of the beef chops led to an increase in pH values, with the pH values varying between 5.56 and 5.60 after cooking, while pH of the raw beef chops was 5.44. In the literature, numerous researchers (Rao *et al.* 1996; Serdaroglu 2006; Turhan *et al.* 2007; Modi *et al.* 2009) have described that cooking has the effect of increasing pH values of meat. Girard (1992) declared that the increase in pH values by cooking could be attributed to the cleavage of bonds involving imidazole, sulfhydryl and hydroxyl groups. Yilmaz and Daglioglu (2003) determined that the addition of oat fiber to meatballs altered their pH value and Turhan *et al.* (2007) observed a similar effect with the addition of wet okara to meatballs. The TS 10580 Meatball Standard describes that the maximum allowable pH value for raw (uncooked) meatballs is 6.3 (Anon 2002). Therefore, it was observed that the pH values of both the raw and cooked meatballs were compliant with the standard.

TBARS values of the samples were also given in Table 1. In the present study, TBARS value of raw beef chops (0.57 mg/kg) is similar to those of the literature (Houben *et al.* 2002; Ferioli *et al.* 2008). On the other hand, the TBARS value is considerably lower than TBARS values (1.8–10.4 mg/kg) that were determined in fresh beef by Formanek *et al.* (1998). While it was observed that cooking temperature had a very significant effect ($P < 0.01$) on TBARS values of the samples, CLA concentration and the interaction between concentration and cooking temperature had no significant effect ($P > 0.05$). Similarly, Oz and Cakmak (2015) found that CLA concentration (0.05, 0.1, 0.25 and 0.5%) and the interactions between CLA concentration and cooking temperature had no significant effect ($P > 0.05$) on TBARS values of meatballs, while cooking temperature had a very significant effect ($P < 0.01$). On the other hand, numerous studies in the literature demonstrate that CLA reduces lipid oxidation through its antioxidant effect (Du *et al.* 2000, 2001, 2002; Joo *et al.* 2002; Corino *et al.* 2003; Hur *et al.* 2004; Baublits *et al.* 2007). However, it is known that antioxidant materials could cause a prooxidant effect on *in vitro* and *in vivo* systems due to concentration, structure, test system and substrate (Yoshino and Murakami 1998; Maurya and Devasagayam 2010).

Cooking of the meatballs led to an increase in their TBARS values, with the TBARS values varying between 1.49 and 2.64 mg/kg, while the value was 0.57 mg/kg in raw chops. It is shown that the effect of cooking on TBARS could vary according to the cooking method. In several research, TBARS values in different animal meats increased with cooking compared to raw meat (Rao *et al.* 1996; Hernández *et al.* 1999; Serrano *et al.* 2007; Kwon *et al.* 2008; Alfaia *et al.* 2010; Peiretti *et al.* 2012; Oz 2014; Roldán *et al.* 2014), while some cooking methods had no similar effect on TBARS values (Serrano *et al.* 2007; Ferioli *et al.* 2008; Weber *et al.* 2008; Alfaia *et al.* 2010; Juárez *et al.* 2010; Peiretti *et al.* 2012). It is expected that increasing the cooking temperature and time causes an increase in lipid oxidation. On the other hand, the compounds that are analyzed by TBARS test are very reactive, so these compounds could react with protein and amino acids of meat and they could produce different compounds, thus may have an effect on the decrease of the TBARS value (Meinert *et al.* 2007; Ferioli *et al.* 2008; Sánchez del Pulgar *et al.* 2012). In the present study, TBARS values determined in the samples cooked at 150, 200 and 250C were statistically different from each other, and increasing of the cooking temperature caused an increase in TBARS value.

It has been declared that the TBARS threshold limit value was 2 mg/kg when detecting the oxidized taste–flavor of beef by panelists (Verma and Sahoo 2000; Campo *et al.* 2006; Humada *et al.* 2014). In the present study, the TBARS value of the cooked chops only at 150 and 200C was equal to the limit or lower than the threshold limit value.

Color Values

Instrumental color analysis results of the samples were also given in Table 1. The results showed that, while cooking temperature had a very significant effect ($P < 0.01$) on L^*, a^* and b^* values of the samples, CLA concentration and the interaction between concentration and cooking temperature had no significant effect ($P > 0.05$) on these color parameters. Cooking caused a reduction in L^*, a^* and b^* values. Similar results were reported by Oz (2014).

While L^* value of raw beef chop was 44.59, it ranged from 30.85 to 37.00 in cooked samples. In the literature, numerous studies have shown that L^* value of meat decreases with dry heat cooking (Oz 2014; Oz and Zikirov 2015), while sous-vide cooking and boiling of meat caused an increase in L^* values of the samples (Oz and Zikirov 2015). a^* value is often closely associated with the content of oxymyoglobin of meat and it is reported that high a^* values of cooked meat are adversely associated with the denaturation of myoglobin (Roldán *et al.* 2013). While a^* value of raw beef chop was 29.12, it ranged from 6.32 to 8.63 in cooked samples. It is known that decreasing in a^* values of meat after cooking is due to the pigment denaturation (Sánchez del Pulgar *et al.* 2012). b^* value or a yellow color intensity, which is an indicative of the yellowness value in the literature, is still unexplained. While b^* value of raw beef chop was 18.43, it was ranged from 9.58 to 13.78 in cooked samples.

The Recoveries of HCAs

The average recoveries of the HCAs varied between 28.94 and 82.15%. The values are comparable with those in the

related literature (Felton *et al.* 1994; Knize *et al.* 1994; Oz *et al.* 2007; Oz and Kaya 2011a,b). The limits of detection and limits of quantification for standard solutions were calculated with a signal-to-noise ratio of 3 (S/N = 3) and 10 (S/N = 10), respectively. The lowest detected concentrations for each compound within the sample were IQx 0.004 ng/g, IQ 0.009 ng/g, MeIQx 0.024 ng/g, MeIQ 0.014 ng/g, 7,8-DiMeIQx 0.005 ng/g, 4,8-DiMeIQx 0.008 ng/g, PhIP 0.025 ng/g, AαC 0.012 ng/g and MeAαC 0.010 ng/g. The lowest quantified concentrations for each compound in the sample were IQx 0.013 ng/g, IQ 0.029 ng/g, MeIQx 0.081 ng/g, MeIQ 0.047 ng/g, 7,8-DiMeIQx 0.018 ng/g, 4,8-DiMeIQx 0.025 ng/g, PhIP 0.085 ng/g, AαC 0.039 ng/g and MeAαC 0.035 ng/g.

HCA Content of Beef Chops

To our knowledge, this is the first study to investigate the effect of direct addition of CLA on the formation of HCAs in beef chops. Individual and total HCA amounts of the chops with and without CLA and fried at different temperatures were given as ng/g in Table 2. Table 2 also includes the calculated inhibitory or promote effect of CLA on the total HCA amount. As seen in Table 2, of the nine HCAs tested, two (4,8-DiMeIQx and MeAαC) could not be detected in the samples analyzed, while PhIP, which had the highest concentration of HCAs, was found to be up to 2.61 ng/g, followed by AαC, found to be up to 0.58 ng/g, MeIQ, found to be up to 0.56 ng/g, IQx, found to be up to 0.53 ng/g, 7,8-DiMeIQx, found to be up to 0.19 ng/g, IQ, found to be up to 0.18 ng/g, and MeIQx, found to be up to 0.09 ng/g.

In the current study, one of the most commonly observed HCAs following the cooking of CLA-containing chops at different temperatures was IQx, while IQx is an HCA on which relatively few studies have been conducted in the literature. IQx was found to range from 0.02 to 0.53 ng/g in the analyzed samples. Increasing of the cooking temperature caused an increase in the amount of IQx both in the control group chops and chops with CLA. IQx was detected as 1.5 ng/g in grilled beef (Fay *et al.* 1997), up to 0.39 ng/g in pan-fried beef, up to 0.20 ng/g in barbecued beef (Turesky *et al.* 2005), up to 0.61 ng/g in pan-fried beef chops and up to 3.65 ng/g in barbecued beef chops (Oz *et al.* 2010a). IQx was also found at the concentrations ranging from 1.59 to 3.81 ng/g in ready-to-eat meatballs and sold in restaurants in Turkey (Oz 2011). On the other hand, Pais *et al.* (1999) did not detect IQx in beef top round steak cooked for 30 min at 275C; Oz *et al.* (2010a) could not detect IQx in beef chops following cooking in a microwave oven for 6 min, in an oven at 200C for 3–12 min, and on a hot plate at 200C for 2–8 min. Similarly, Oz and Cakmak (2015) could not detect IQx in meatballs with and without CLA fried at different temperatures.

In the present study, IQ was found from nonquantifiable levels to 0.18 ng/g in the analyzed samples. Increasing of the cooking temperature caused an increase in the amount of IQ both in the control group chops and the chops with CLA. IQ was detected in the control group chops and the chops with 0.05% CLA cooked at 150C; however, the amount of IQ could not be determined. Warzecha *et al.* (2004) made a similar observation for beef cooked at 150–160C for 20 min. Similarly, Shin *et al.* (2003) were able to detect IQ in beef cooked at 225C for 20 min, although they could not quantify its amount. On the other hand, 1.1 ng/g IQ was determined in 15% fat hamburgers fried at 170C for 16 min (Britt *et al.* 1998). Felton *et al.* (1984) found 0.02 ng/g IQ in beef fried at 250C for 12 min. Up to 10.2 ng/g was determined in beef fried up to 180C for

TABLE 2. HETEROCYCLIC AROMATIC AMINE CONTENTS OF THE BEEF CHOPS (NG/G)

T (%)	CT (°C)	IQx	IQ	MeIQx	MeIQ	7,8-DiMeIQx	4,8-DiMeIOx	PhIP	AαC	MeAαC	Total HCAs	Inhibition (%)
Control	150	0.03	nq	nd	0.07	nd	nd	0.06	nq	nd	0.16	–
	200	0.06	0.05	nq	0.08	0.02	nd	0.23	0.08	nd	0.50	–
	250	0.53	0.18	0.08	0.56	0.15	nd	1.90	0.50	nd	3.90	–
0.05	150	0.05	nq	nd	0.07	nd	nd	0.12	0.03	nd	0.27	+68.75
	200	0.08	0.03	nq	0.19	0.01	nd	0.20	0.07	nd	0.58	+16.00
	250	0.40	0.17	0.09	0.52	0.19	nd	2.10	0.58	nd	4.05	+3.85
0.1	150	0.02	0.03	nd	nd	nd	nd	0.08	nq	nd	0.13	–18.75
	200	0.04	0.05	nq	0.05	nq	nd	0.18	0.06	nd	0.38	–24.00
	250	0.34	0.10	0.06	0.35	0.11	nd	1.35	0.36	nd	2.67	–31.54
0.25	150	0.02	0.02	nd	nd	nd	nd	0.06	nq	nd	0.10	–37.50
	200	0.09	0.05	nq	0.06	nq	nd	0.13	0.07	nd	0.40	–20.00
	250	0.48	0.12	0.05	0.55	0.18	nd	2.61	0.50	nd	4.49	+15.13
0.5	150	0.02	0.03	nd	nd	nd	nd	0.14	nq	nd	0.19	+18.75
	200	0.04	0.04	nd	0.03	nq	nd	0.70	0.03	nd	0.84	+68.00
	250	0.50	0.16	nq	0.35	0.17	nd	1.93	0.24	nd	3.35	–14.10

T, treatment; CT, cooking temperature; nd, not detected; nq, not quantifiable.

20 min (Murkovic *et al.* 1998). Turesky *et al.* (1988) found IQ between 0.3 and 1.9 ng/g in fried beef at 275C for 5–15 min whereas Felton *et al.* (1994) found from nd (not detected) to 1 ng/g in fried beef at 200–250C for 12 min. IQ was also found to range from 0.44 to 1.59 ng/g in meatballs consumed in Turkey (Oz 2011). Barnes *et al.* (1983) detected between 0.53 and 20.1 ng/g IQ in fried beef at 240C for 10 min. Rivera *et al.* (1996) detected up to 7 ng/g IQ in barbecued beef at 200–500C for 15 min. Abdulkarim and Smith (1998) detected up to 4.11 ng/g IQ in barbecued beef at 200–240C for 7–12 min. Klassen *et al.* (2002) detected up to 1.12 ng/g in different cooked hamburgers from four fast food outlets. Oz and Kaya (2011a) found between nd and 5.46 ng/g IQ in meatballs fried at 175–225C for 15 min. On the other hand, no IQ in cooked samples can be found in the literature (Johansson and Jägerstad 1994; Knize *et al.* 1995, 1997; Sinha *et al.* 1998; Pais *et al.* 1999; Busquets *et al.* 2004; Wong *et al.* 2005; Toribio *et al.* 2007; Oz *et al.* 2010b). Oz and Cakmak (2015) investigated the effect of direct addition of CLA at different levels to meatball dough and found that the usage of 0.1, 0.25 and 0.5% CLA in meatball production had an inhibitory effect on the formation of IQ.

In the present study, MeIQx was found from nd to 0.09 ng/g in the analyzed samples. MeIQx could not be detected in any of the chops cooked at 150C. On the other hand, it was detected in the control group chops and the chops with 0.05, 0.1 and 0.25% CLA cooked at 200C; however, the amount of MeIQx could not be determined. For the beef chops cooked at 250C, MeIQx amount could not be determined only in the samples with 0.5% CLA. Knize *et al.* (1997) found 0.89 ng/g MeIQx in hamburger. Sinha *et al.* (1998) determined MeIQx levels between nd and 1.61 ng/g in beef barbecued at 185–240C for 9–38 min; Knize *et al.* (1994) determined MeIQx at levels between nd and 7.3 ng/g in beef fried at 150–230C for 4–20 min; Polak *et al.* (2009) determined MeIQx levels between 0.19–0.36 and 0.14–0.15 ng/g in nonaged beef *L. dorsi* muscle grilled at 220C until an inner temperature of 65 and 80C, respectively; Klassen *et al.* (2002) determined MeIQx levels between 0.3 and 0.34 ng/g in beef pan-fried at 160–220C for 14 min; and Johansson and Jägerstad (1994) determined MeIQx levels between 0.03 and 2.8 ng/g in beef fried at 180–190C for 12 min. MeIQx content of the chops with and without CLA was generally lower than the values reported in the literature. There are, however, other studies in which much higher MeIQx values have been identified. MeIQx was detected between 2.7 and 12.3 ng/g in meatballs fried at 275C for 5–15 min (Turesky *et al.* 1988), between 1.1 and 1.4 ng/g in fried beef at 250C for 10 min (Gross *et al.* 1989), between nd and 5.1 ng/g in fried beef at 180–190C for 12 min (Johansson and Jägerstad 1994), between nd and 7.3 ng/g in fried beef at 150–230C for 4–20 min (Knize *et al.* 1994), up to 9 ng/g in fried beef at 250C for 22 min (Sinha *et al.* 1994), up to 16.4 ng/g in fried beef at 277C for 12 min (Thiebaud *et al.* 1994), between 0.2 and 1.6 ng/g in fried beef at 165–200C for 8 min (Johansson *et al.* 1995), between 5.5 and 5.7 ng/g in fried beef at 225C for 22 min (Shin *et al.* 2003), and 0.7 ng/g in fried beef at 175–200C for 11.2 min (Busquets *et al.* 2004). In addition, Tikkanen *et al.* (1993) did not detect MeIQx in six commercially cooked meat patty samples (cooked at 121–220C for 1.3–2.5 min). Similarly, Abdulkarim and Smith (1998) could not detect MeIQx in chops fried for 15 min at 230C. Oz and Cakmak (2015) declared that MeIQx could not be detected in the meatballs with 0.1, 0.25 and 0.5% CLA cooked at 200C, and while the usage of CLA at the levels of 0.05 and 0.1% in the production of meatballs cooked at 250C increased MeIQx amount, the usage of CLA at the levels of 0.25 and 0.5% in the production of meatballs cooked at 250C caused a decrease in the amount of MeIQx.

In the present study, MeIQ was found from nd to 0.56 ng/g in the analyzed samples. Increasing of the cooking temperature caused an increase in the amount of MeIQ in both the control group chops and the chops with CLA. MeIQ could not be detected in the beef chops with 0.1, 0.25 and 0.5% CLA cooked at 150C, while 0.7 ng/g MeIQ was detected in both control group chops and chops with 0.05% CLA cooked at 150C. There are various studies (Johansson and Jägerstad 1994; Knize *et al.* 1995, 1998; Sinha *et al.* 1998; Pais *et al.* 1999; Busquets *et al.* 2004; Oz *et al.* 2010b) in the literature in which MeIQ has not been detected in the samples. On the other hand, Abdulkarim and Smith (1998) detected MeIQ between 0.3 and 0.6 ng/g in pan-fried beef at 170C for 16 min, Britt *et al.* (1998) detected MeIQ as 0.6 ng/g in 15% fat hamburgers fried in a pan at 170C for 16 min, Balogh *et al.* (2000) detected MeIQ between 0.1 and 3.5 ng/g in pan-fried beef at 175–225C for 12–20 min, Klassen *et al.* (2002) detected MeIQ between nd and 0.1 ng/g in pan-fried beef at 160–220C for 14 min, Oz and Kaya (2011a) detected MeIQ between nd and 2.66 ng/g in pan-fried meatballs at 175–225C for 15 min, Oz and Kaya (2011b) detected MeIQ between nd and 1.34 ng/g in pan-fried beef chops at 175–225C for 15 min, Rivera *et al.* (1996) detected MeIQ up to 8 ng/g in grilled beef at 200–250C for 15 min, Oz *et al.* (2010b) detected MeIQ between nd and 0.31 ng/g in barbecued beef for 1.5–6 min. Oz and Cakmak (2015) stated that despite determining MeIQ at a level of 0.07 ng/g in control group meatballs and meatballs with 0.05% CLA cooked at 250C, meatballs with 0.25 and 0.5% CLA were also observed to contain MeIQ, but the amount of MeIQ could not be determined.

Another compound that is not commonly evaluated in the literature is 7,8-DiMeIQx. In the current study, 7,8-DiMeIQx was found from nd to 0.19 ng/g in the analyzed samples. Increasing of the cooking temperature caused an

increase in the amount of 7,8-DiMeIQx in both the control group chops and the chops with CLA. 7,8-DiMeIQx, as well as MeIQx, could not be detected in any of the chops cooked at 150C. The amount of the compound increased with the increasing cooking temperature. Turesky *et al.* (1988) determined 7,8-DiMeIQx levels between nd and 0.7 ng/g in beef fried at 275C for 5–15 min; Fay *et al.* (1997) determined 7,8-DiMeIQx at a level of 0.2 ng/g in grilled beef; Klassen *et al.* (2002) determined 7,8-DiMeIQx levels between 0.1 and 1.75 ng/g in beef fried at 160–220C for 14 min; and Busquets *et al.* (2004) determined 7,8-DiMeIQx at a level of 0.04 ng/g in beef fried at 175–200C for 14 min. Oz and Cakmak (2015) stated that the usage of CLA caused an increase in the amount of 7,8-DiMeIQx in the meatballs cooked at 150C. While 0.04 ng/g 7,8-DiMeIQx was determined in both control group meatballs and meatballs with 0.5% CLA cooked at 200C, the usage of 0.05, 0.1, and 0.25% CLA increased the amount. In the meatballs cooked at 250C, the usage of CLA (except for 0.05% CLA) caused a decrease in the amount of 7,8-DiMeIQx (Oz and Cakmak 2015).

In the literature, 4,8-DiMeIQx is one of the most commonly investigated HCAs, while the compound could not be detected in any of the samples, in the current study. Similar results can also be seen in the literature (Turesky *et al.* 1988; Gross *et al.* 1989, 1993; Knize *et al.* 1997; Sinha *et al.* 1998; Oz *et al.* 2010b). On the other hand, 4,8–DiMeIQx was detected between 0.5 and 1.2 ng/g in hamburgers cooked well at 200C (Murray *et al.* 1988), between nd and 3.9 ng/g in pan-fried beef at 275C for 5–15 min (Turesky *et al.* 1988), between 1.3 and 2 ng/g in pan-fried beef at 190C for 6–13 min (Gross 1990), up to 0.1 ng/g in grilled beef at 225C for 12 min (Murray *et al.* 1993), up to 2.7 ng/g in barbecued beef (Knize *et al.* 1995), between 0.5 and 2.7 ng/g fried at 170C for 16 min (Britt *et al.* 1998), between 0.12 and 0.43 ng/g in barbecued beef for 20 min (Turesky *et al.* 2005). In addition, by cooking at 275C for 30 min, 0.2, 0.19 and 0.4 ng/g of 4,8-DiMeIQx were determined in steak, turkey and pork, respectively (Pais *et al.* 1999). Oz and Cakmak (2015) found that 4,8-DiMeIQx could not be detected in meatballs containing 0.25 and 0.5% CLA for all cooking temperatures.

In the current study, one of the most commonly observed HCAs following the cooking of the chops at different temperatures was PhIP. In the literature, PhIP is described as one of the most common HCAs in meats subjected to heat treatment (Pais *et al.* 1999; Busquets *et al.* 2004; Warzecha *et al.* 2004; Turesky *et al.* 2005; Iwasaki *et al.* 2010; Liao *et al.* 2010; Viegas *et al.* 2012). In the present study, PhIP was found from 0.06 to 2.61 ng/g in the analyzed samples. Increasing of the cooking temperature caused an increase in the amount of PhIP in both the control group chops and the chops with CLA. Felton *et al.* (1986) stated that ground beef fried at 300C for 11 min contained PhIP at a level of 15 ng/g. Gross *et al.* (1993) stated finding 3.1 ng/g PhIP in meatballs which were grilled at 250–270C for 19 min. Gross (1990) detected PhIP between 23.5 and 48 ng/g in pan-fried samples at 190C for 6–13 min, Wakabayashi *et al.* (1993) detected PhIP up to 0.56 ng/g in pan-fried beef at 190C for 6–13 min, Felton *et al.* (1994) detected PhIP between 0.7 and 13.3 ng/g in pan-fried beef at 200–250C for 6–13 min, Murkovic *et al.* (1998) detected PhIP up to 5.28 ng/g in pan-fried beef at 180C for 20 min, Sinha *et al.* (1998) detected PhIP between nd and 2.3 ng/g in pan-fried beef at 180–191C for 6–20 min, Klassen *et al.* (2002) detected PhIP between 0.2 and 1.1 ng/g in pan-fried beef at 160–220C for 14 min. On the other hand, it was reported that no PhIP formed in some studies (Johansson and Jägerstad 1994; Knize *et al.* 1995; Sinha *et al.* 1998). In addition, Oz *et al.* (2010a) reported that they could not detect PhIP in beef cooked in oven at 200C for 3–12 min, grilled beef at 200C for 2–8 min and fried beef at 200C for 1.5–6 min. Oz and Cakmak (2015) found that increasing CLA concentration was generally associated with a decrease in the amount of PhIP in meatballs cooked at 150–200C. Control group meatballs cooked at 250C had 0.81 ng/g of PhIP, while the level of PhIP in meatballs with 0.1, 0.25 and 0.5% CLA was determined as 0.50, 0.41 and 0.40 ng/g, respectively (Oz and Cakmak 2015).

AαC is another least studied HCA in the literature. In the current study, AαC was found from nq (not quantifiable) to 0.58 ng/g in the analyzed samples. Increasing of the cooking temperature caused an increase in the amount of AαC in both the control group chops and the chops with CLA. For the chops cooked at 150C, the compound was detected in all of the chops; however, its amount was determined only in chops with 0.05% CLA. There are also studies (Felton *et al.* 1994; Knize *et al.* 1994; Fay *et al.* 1997; Pais *et al.* 1999) in the literature where AαC has not been detected. Oz *et al.* (2010a) reported that any AαC could be detected in beef cooked in oven at 200C for 3–12 min, grilled at 200C for 2–8 min and pan-fried at 200C for 1.5–6 min. On the other hand, Busquets *et al.* (2004) detected 0.04 ng/g AαC in pan-fried beef at 175–200C for 11.2 min, Turesky *et al.* (2005) detected between 0.03 and 3.32 ng/g AαC in pan-fried beef at 150–300C for 12–24 min, between 2.8 and 7.75 ng/g AαC in barbecued beef at 230–300C for 20 min, Toribio *et al.* (2007) detected up to 0.33 ng/g AαC in grilled beef at 180–210C for 4 min. Oz and Cakmak (2015) declared that 0.1, 0.25 and 0.5% CLA had an inhibitory effect that completely prevented the formation of AαC at 200C.

MeAαC is also an HCA in which relatively few studies have been researched in the literature. In the current study, MeAαC was not detected in any of the chops analyzed. MeAαC, similarly, could not be detected in some other studies in the literature (Fay *et al.* 1997; Pais *et al.* 1999; Oz

et al. 2010b). However, MeAαC was detected at the level up to 63.5 ng/g (Matsumoto *et al.* 1981). Busquets *et al.* (2004) detected up to 0.4 ng/g MeAαC in grilled beef at 180–210C for 4 min and up to 0.1 ng/g in fried beef at 175–200C for 11.2 min. On the other hand, Turesky *et al.* (2005) detected between 0.03 and 0.14 ng/g MeAαC in fried beef at 150–300C for 12–24 min, between 0.09 and 0.29 ng/g in barbecued beef at 130–300C for 20 min. Oz and Cakmak (2015) could not detect MeAαC in any of meatballs cooked at the temperatures of 150 and 200C. Among meatballs cooked at 250C, only meatballs containing 0.1% CLA were determined as containing 0.05 ng/g MeAαC (Oz and Cakmak 2015).

The total HCA content of the cooked samples varied between 0.10 and 4.49 ng/g (Table 2). For the chops cooked at 150C, while direct addition of 0.05 and 0.5% CLA to chops increased the total HCA amount compared to that of control group chops, the use of 0.1 and 0.25% CLA caused a reduction in the total HCA amount (18.75 and 37.50%, respectively). For the chops cooked at 200C as well the chops cooked at 150C, while direct addition of 0.05 and 0.5% CLA to chops increased the total HCA amount compared to that of control group chops, the use of 0.1 and 0.25% CLA caused a reduction in the total HCA amount (24.00 and 20.00%, respectively). For the chops cooked at 250C, while direct addition of 0.05 and 0.25% CLA to chops increased the total HCA amount compared to that of control group chops, the use of 0.1 and 0.5% CLA caused a reduction in the total HCA amount (31.54 and 14.10%, respectively). The results showed that while direct addition of 0.05% CLA to the beef chops increased (3.85–68.75%) the total HCA amount at all cooking temperatures, direct addition of 0.1% CLA to the beef chops decreased (18.75–31.54%) the total HCA amount at all cooking temperatures. On the other hand, the lowest and the highest total HCA amounts belonged to the chops with 0.25% CLA. In respect to cooking temperature, increasing of the cooking temperature caused an increase in the total HCA amount both in the control group chops and the chops with CLA. Oz and Cakmak (2015) investigated the effect of direct addition of CLA at different levels (0.05, 0.1, 0.25 and 0.5%) to meatball dough on the formation of HCAs and found that the addition of 0.25 and 0.5% CLA inhibited total HCA amount by 58 and 60%, respectively. Many researchers (Xu *et al.* 1996; Britt *et al.* 1998; Balogh *et al.* 2000; Santana-Rios *et al.* 2001) have described in their studies that it is possible to prevent the formation of HCAs. On the other hand, Skog (2002) describes that the acceptable daily consumption level for HCAs is between 0 and 15 μg/day. Thus, it is possible to see that consuming 100 g of the chops that had the highest total HCA content in the current study would still not have led to an intake of HCA above 0.5 μg.

It is difficult to compare the results of the present study with those in related literature when several variables such as the number of HCAs, meat type used in meatballs, preparation techniques, size of meatballs and cooking procedures are considered. However, the findings from the present study seem to be consistent with the literature data in general. On the other hand, according to Knize *et al.* (1994), who cited data by Pearson and others, the total amounts of IQ, MeIQx and DiMeIQx were 7300 ng/g in beef fried at 215C. This value is much higher than both our results and the data in the literature.

CONCLUSION

In conclusion, it was determined that the direct addition of CLA to chops did not have a significant effect on their water content, cooking loss, pH, TBARS, and L^*, a^* and b^* values. This study is the first of its kind to investigate the formation of HCA in chops prepared through the direct addition of different CLA levels. It is known that HCAs can be formed during the cooking of meat and meat products. Recent efforts have focused on ways to decrease these mutagenic and/or carcinogenic compounds. Our study indicates that the direct addition of 0.1% CLA to chops will decrease the total HCA amount.

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