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Formation of advanced glycation endproducts in ground beef under pasteurisation conditions

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ABSTRACT

Advanced glycation endproducts (AGEs) in food products may pose health risks, and thermal processing of foods accelerates the formation of AGEs. The effects of heat treatments (65–100 °C, 0–60 min) on the formation of AGEs including N^{ε}-carboxymethyllysine (CML) and N^{ε}-carboxyethyllysine (CEL) in ground beef were investigated. The levels of CML and CEL in ground beef steadily increased with heating time and heating temperature. A strong linear relationship ($r^2 = 0.920$) between the amounts of CML (2.76–19.96 mg/kg) and CEL (2.32–11.89 mg/kg) in raw and thermally treated beef was found. The formations of both CML and CEL in ground beef during heat treatments basically fitted zero-order reactions (CML: $r^2 = 0.851$–0.995, rate constant = 0.031–0.224 mg kg^{-1} min^{-1}; CEL: $r^2 = 0.907$–0.971, rate constant = 0.044–0.118 mg kg^{-1} min^{-1}) with an activation energy of 61.01 kJ/mol for CML and 29.21 kJ/mol for CEL.

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1. Introduction

The Maillard reaction involves a series of complicated reactions starting from the reaction between the carbonyl group of reducing sugars and amino group of amino acids or free amino groups of proteins to form a Schiff base, then undergoing Amadori rearrangement. From the Amadori products, various reactive intermediate products are produced following different pathways before more stable products are formed, such as aroma and browning compounds, and advanced glycation endproducts (AGEs). AGEs were initially believed to be formed at later or advanced stages of the Maillard reaction as a result of the attachment of saccharides (most often monosaccharides) or their derivatives to a protein or its derivatives, from which the term "advanced glycation endproducts" came (Rabbani & Thornalley, 2012). Recent studies indicate that AGEs in foods can be formed in the early stages of the Maillard reaction through potent glycating agents such as α-dicarbonyl compounds formed directly from degradation of glucose or Schiff base, or from lipid oxidation (Fu et al., 1996; Poulsen et al., 2013; Rabbani & Thornalley, 2012). AGEs can be formed *in vivo* and in foods during thermal processing and storage. In the human body, AGEs may promote aging and oxidative stress and increase risks for various diseases such as diabetes, obesity, and cardiovascular diseases, though the risk effects of dietary AGEs on human health are still controversial (Ames, 2007; Nguyen, 2006; Poulsen et al., 2013; Uribarri et al., 2010).

Assessments of various foods such as infant formulas (Birlouez-Aragon et al., 2004), milk and beverages (Ahmed et al., 2005), almonds (Zhang, Huang, Xiao, & Mitchell, 2011), sauces and sauce treated meat and fish (Chao, Hsu, & Yin, 2009), as well as several hundred commonly consumed foods (Goldberg et al., 2004; Hull, Woodside, Ames, & Cuskelly, 2012; Uribarri et al., 2010) indicate that heat treatments significantly affect the levels of AGEs in foods; and high protein/high fat foods (such as meat, pork, chicken) have higher levels of AGEs compared to low fat or carbohydrate-rich foods (such as fruits, vegetables, grains, legumes, and nonfat milk). Although the effects of heating on the levels of AGEs in foods have been acknowledged (Chao et al., 2009; Wellner, Huettl, & Henle, 2011; Zhang et al., 2011), very few studies have been published on how thermal processes (such as heating temperature and heating time) affect the amounts of AGEs in foods. What is more, among these limited number of reported studies on AGEs, simple model systems (such as monosaccharide-lysine mixtures) instead of real food systems were often used (Fu, Li, & Li, 2012; Morales & Van Boeckel, 1996). Since foods in general contain numerous components that may participate in or interfere with the formation

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of AGEs of varying molecular structures, the use of simple model systems cannot reveal what actually happens regarding AGEs formation in real food systems.

The goal of this study was to determine the overall amounts of two commonly occurring AGEs, N^{ε}-carboxymethyllysine (CML) and N^{ε}-carboxyethyllysine (CEL) formed in ground beef, one of the most widely consumed foods, under pasteurisation conditions covering a wide range of heating temperatures (65–100 °C) and heating times (0–60 min). In addition to exploring the kinetics of formation for both CML and CEL, the relationship between the levels of CML and CEL formed in thermal processed ground beef was investigated.

2. Materials and methods

2.1. Reagents

Analytical grade hydrochloric acid 37%, sodium hydroxide, sodium borohydride, and boric acid were purchased from Sinopharm (Shanghai, China). HPLC-grade methanol, chloroform, formic acid, and ammonium acetate were purchased from Sigma (St Louis, MO). CML (98%), CEL (98%) and d_4-CML (98%) were purchased from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada). Stock standard solutions for CML (390 mg/L), CEL (310 mg/L) and the internal standard d_4-CML (200 mg/L) were prepared in methanol–water (80:20, v/v) and stored at −20 °C. Working standard solutions including AGEs standard mixture (CML 195 μg/L, CEL 155 μg/L, and d_4-CML 100 μg/L) and the internal standard (d_4-CML 4 mg/L) were prepared by diluting the stock solutions in methanol–water (80:20, v/v) solution.

2.2. Sample information

Ground beef brisket packaged in modified atmosphere was purchased from a local retailer in Shanghai, China. Ground beef was mixed with a homogeniser (8010S; Waring, Inc., Torrington, CT) at low speed for 20 s, packed in plastic bags with about 120 g of beef per bag, and stored at −50 °C. The beef sample was thawed overnight at 4 °C before further testing.

The moisture content of ground beef was determined based upon an oven drying method (Lawrence J., 2005) in which *ca.* 4.0000 g of sample was heated at 105 °C until reaching a constant weight, taking about 24 h. Fat content was determined using a solvent extraction method (Lawrence, 2005) with an automatic Soxhlet solvent extractor (SZF-06; Shanghai Jiading Food and Oil Instrument Ltd, Shanghai, China). Protein content was measured using the Kjeldahl Method (Lawrence, 2005) with an automatic Kjeldahl apparatus (UDK 159; VELP Scientific, Inc., Italy). A conversion factor of 6.25 was used for the calculation of the protein content from nitrogen content. The moisture, fat and protein contents for the ground beef were 70.92% (±0.07%), 5.92% (±0.42%), and 20.54% (±0.16%) on a wet weight basis, respectively.

2.3. Thermal treatments

Ground beef (13.0 ± 0.1 g) was sealed into cylindrical aluminium cells (Kong, Tang, Rasco, Crapo, & Smiley, 2007) with inner diameter of 50 mm and inner height of 5 mm and heated at 65, 70, 75, 80, 85, 90, or 100 °C for 2.5, 5, 7.5, 10, 15, 20, 40, or 60 min in a water bath (Isotemp 5150 H7; Fisher Scientific Inc., Pittsburgh PA) or in boiling water directly (for 100 °C treatment only). The selected treatment of temperatures and times covered a range used in cooking and in-package pasteurisation protocols. Following heating, test cells containing the beef samples were immediately immersed in ice-water mixture. After cooling, both the meat and exuded juice were mixed well in a mortar before AGEs analysis. Each thermal treatment was repeated twice on different days, and duplicate analysis for AGEs was conducted for each sample. The come-up time, defined as the time required for the sample central temperature to reach 0.5 °C below the targeted temperature, was determined based on the methods of Kong et al. (2007).

2.4. Sample preparation for AGEs analyses

Sample preparation for AGEs analyses was based upon an acid hydrolysis method, in which the beef sample was reduced with sodium borohydride before the acid hydrolysis step and the protein hydrolysate was cleaned up with solid-phase extraction (Assar, Moloney, Lima, Magee, & Ames, 2009; Niquet-Léridon & Tessier, 2011). First, *ca.* 0.2000 g raw or cooked beef was incubated with 2 mL borate buffer (0.2 M, pH 9.2) and 0.4 mL sodium borohydride (2 M in 0.1 N NaOH) at 4 °C for 8 h. The reduction step was to prevent the formation of CML or CEL from Amadori products during the acid hydrolysis which would lead to overestimation of CML or CEL (Niquet-Léridon & Tessier, 2011). The sample was then vigorously mixed with 4 mL of methanol-chloroform (1:2, v:v) and centrifuged (TDL-5-A; Shanghai Anting Scientific Instrument Factory, Shanghai, China) at 5000 rpm for 10 min to defat and precipitate protein. The precipitated protein was hydrolysed with 4 mL of 6 M hydrochloric acid (HCl) at 110 °C for 24 h. The protein hydrolysate was diluted with water to 20 mL, from which 4 mL were withdrawn and spiked with 100 μL d_4-CML (4 mg/L, internal standard) and dried in a vacuum oven (DZF-6050; Shanghai Jinghong Laboratory instrument Co., Ltd, Shanghai, China) at 50 °C. The dried protein hydrolysate was reconstituted in 4 mL water (the final concentration of d_4-CML was 100 μg/L) and 2 mL of sample solution was passed through an MCX cartridge (60 mg/3 mL; Shanghai ANPEL Scientific instrument Co., Ltd, Shanghai, China) pre-activated with 3 mL methanol and 3 mL water in sequence. The cartridge was then washed with 3 mL water and 3 mL methanol, respectively. Finally, the target compounds were eluted with 5 mL methanol (containing 5% aqueous ammonia), dried in nitrogen with a nitrogen evaporator (DC12H; Shanghai ANPEL Scientific Instrument Co., Ltd, Shanghai, China), reconstituted with 2 mL methanol–water (80:20, v:v), and filtered through a 0.22-μm filter before chromatographic analysis. The MCX cartridge was chosen in our study since our preliminary results indicated that a higher recovery rate could be achieved with the use of MCX than the most commonly used C_{18} cartridge due to the strong polarity of CML and CEL.

Recovery experiments were conducted by spiking the protein hydrolysate with CML or CEL at three different levels (CML: 19.5, 97.5, 195 μg/L; CEL: 15.5, 77.5, 155 μg/L for CEL) and the internal standard d_4-CML (100 μg/L). In addition, protein hydrolysate with only d_4-CML added was used as a blank. Since raw ground beef contained CML and CEL, the amount of CML or CEL in the blank was deducted from that of the spiked samples to calculate the recovery of CML or CEL. Recovery experiments were repeated six times.

2.5. HPLC–MS/MS analysis

Liquid chromatography analysis was performed with a Waters 2695 HPLC system (Waters Inc., Milford, MA). An Atlantis hydrophilic interaction liquid chromatography (HILIC) silica column (150 mm × 2.1 mm, 3 μm; Waters Inc.) was used. Since CML and CEL are highly polar, the use of common reversed-phase columns (such as C_{18}) would not be able to retain both chemicals well, unless an ion-pairing reagent such as nonafluoropentanoic acid is used (Assar et al., 2009; Zhang et al., 2011). However, the strong acidity of ion-pairing reagents may result in rapid deterioration

of the column (Schettgen et al., 2007; Zhang et al., 2011). HILIC is a combination of hydrophilic stationary phase and an aqueous mobile phase containing a high proportion of organic solvent, which successfully separates polar compounds such as CML, as demonstrated by Schettgen et al. (2007) and confirmed in our preliminary study. The sample injection volume was 10 μL, and the column temperature was set at 35 °C. The binary mobile phase used was composed of (A) methanol containing 2 mM ammonium acetate and 0.1% formic acid, and (B) water containing 2 mM ammonium acetate and 0.1% formic acid. The flow rate was 0.2 mL/min with the percentage of mobile phase changed as follows: 0–3 min, 80–50% A; 3–6 min, 50% A; 6–6.1 min, 50–80% A; 6.1–12 min, 80% A.

Mass spectrometric analysis was performed using a Waters Quattro Micro triple-quadrupole tandem mass spectrometer (MS/MS) (Waters Inc., Milford, MA) operated in positive electrospray ionisation (ESI) mode. The source and the desolvation temperature were 120 °C and 350 °C, respectively. The extractor voltage was 3 V, and the capillary voltage was 3 kV. Nitrogen was used as desolvation gas at a flow rate of 500 L/h, and as cone gas at 50 L/h. Argon was used as collision gas with collision pressure set as 3×10^{-3} mbar. Multiple reaction monitoring (MRM) mode was applied to detect CML and CEL with 200 ms as dwell time for each transition; the transition ions, collision energy, as well as cone voltage are specified in Table 1. The product ion at m/z 130 was used for quantification of both CML and CEL, while the ions at m/z 84 and m/z 173 were used for confirmation of CML and CEL, respectively (Table 1). Response factors (spectral peak area to the amount of substance) of CML, CEL and d_4-CML were calculated based on the LC–MS/MS analysis results of AGEs standards mixture (CML 195 μg/L, CEL 155 μg/L, and d_4-CML 100 μg/L), and the LC–MS/MS analysis of the standards mixture was conducted each time before analysing ground beef samples. The ratio of response factor of CML or CEL to that of the internal standard was used for calculation of CML or CEL in beef samples.

2.6. Data analysis

To evaluate how fast the levels of CML and CEL changed in ground beef during heating, the amounts of CML and CEL during different heating times at a specific temperature were used to fit the kinetic function,

$$dC/dt = kC^n \quad (1)$$

where k is the rate constant, C is the concentration of CML or CEL at time t, n is the order of reaction and $n = 0$, 0.5, 1, 2 were tested in this study. Plots of C ($n = 0$), $\sqrt{C}$ ($n = 0.5$), ln$C$ ($n = 1$), and 1/C ($n = 2$) against t were obtained, and the r^2 values of their linear regression trendlines were compared. The best fitted reaction order resulted in the highest r^2 value.

The effect of temperature on reaction rate was modelled with the Arrhenius equation,

$$k = Ae^{\frac{-Ea}{RT}} \quad (2)$$

where A is a pre-exponential factor (unit the same as k), E_a is the activation energy (J/mol), R is the universal gas constant (8.314 J mol^{-1} K^{-1}), and T is absolute temperature (K). Data analyses were conducted using Microsoft Excel 2007 (Redmond, WA).

Table 1
Mass spectrometric parameters for multiple reactions monitoring AGEs.

Compounds	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)	Cone voltage (V)
d_4-CML	209	88	15	20
CML	205	130*	10	20
		84	20	20
CEL	219	130*	12	25
		173	12	25

* Ion used for quantification.

3. Results and discussion

3.1. LC–MS/MS chromatogram of AGEs, sensitivity and recovery

Fig. 1 shows the LC–MS/MS chromatogram of mixture of AGEs standards including d_4-CML, CML and CEL obtained through MRM in positive-ion mode. Mass transitions of m/z 209 → m/z 88 for d_4-CML, m/z 205 → m/z 130 for CML, m/z 219 → m/z 130 for CEL were the most prominent ions detected. The retention time for both d_4-CML and CML was 3.88 min, and for CEL was 3.67 min. The collision induced dissociation of these three AGEs was due to the loss of fragments of –NHCH$_2$COOH and/or –COOH, which was consistent with other reports (Teerlink, Barto, Brink, & Schalkwijk, 2004; Zhang et al., 2011), although the most prominent ions identified by separate research groups may be different due to the use of different parameters (such as collision energy) in the tests.

The LC–MS/MS method had high sensitivity for CML and CEL, leading to a limit of detection (*LOD*) of 4 and 5 μg/kg, respectively, while the limit of quantification (*LOQ*) was 12 and 15 μg/kg for CML and CEL, respectively. Zhang et al. (2011) reported similar *LOD* and *LOQ* for analysis of CML (*LOD*, 3 μg/kg; *LOQ*, 9 μg/kg) and CEL (*LOD*, 4 μg/kg; *LOQ*, 12 μg/kg) with LC–MS/MS for standard solutions. The quantification linearity range of the LC–MS/MS was 20–1000 μg/kg, and the levels of CML and CEL in all tested beef extracts (from 0.2 g beef to 20 mL solution during sample preparation for LC–MS/MS analysis, diluted 100 times) were within the linear range. The recovery of CML ranged from 78% (±10.7%) to 98% (±5.9%) for protein hydrolysate spiked with CML of 19.5 μg/kg to 195 μg/kg, and for CEL (spiked with 15.5 μg/kg to 155 μg/kg) ranged from 81% (±11.1%) to 108% (±5.4%). A lower recovery of CML and CEL (78–81%) was found for hydrolysates spiked with less than 20 μg/kg, which is very commonly seen at these low concentration levels; the recovery of CML and CEL from this study were comparable with other similar studies (Teerlink et al., 2004; Zhang et al., 2011).

3.2. CML and CEL in raw and heat treated ground beef

The raw ground beef contained 2.76–4.32 mg/kg CML, and the heat-treated ground beef contained 3.12–19.96 mg/kg CML. As shown in Fig. 2a, the CML levels in ground beef samples were greatly affected by heat treatments. The amounts of CML in ground beef steadily increased with heating time at all seven tested temperatures. For the same heating time, the higher the temperature, the more CML produced. For example, 60 min of heating at 65 °C led to 50% increase of CML in ground beef. The same heating time resulted in 154% increase of CML at 75 °C, 263% at 85 °C and 361% at 100 °C, respectively.

The amounts of CML in raw or cooked beef varied greatly in a limited number of reported studies. Chao et al. (2009) reported that the raw beef and processed beef strips (average 50 g per strip, boiled at 100 °C, fried at 180 °C, and baked at 230 °C for 15 min) contained about 0.13 mg/kg to 0.6 mg/kg CML (data estimated from published figures). The boiled strips had 230% increase in CML (from 0.13 to 0.43 mg/kg), which was similar to the 190% CML increase in our 100 °C/15 min treatment for ground beef. Assar et al. (2009) reported that the amount of CML in raw minced beef, minced beef boiled for 3 min (50 g beef in 250 g water), and minced beef pan-fried for 3 min (50 g, detailed unknown) were

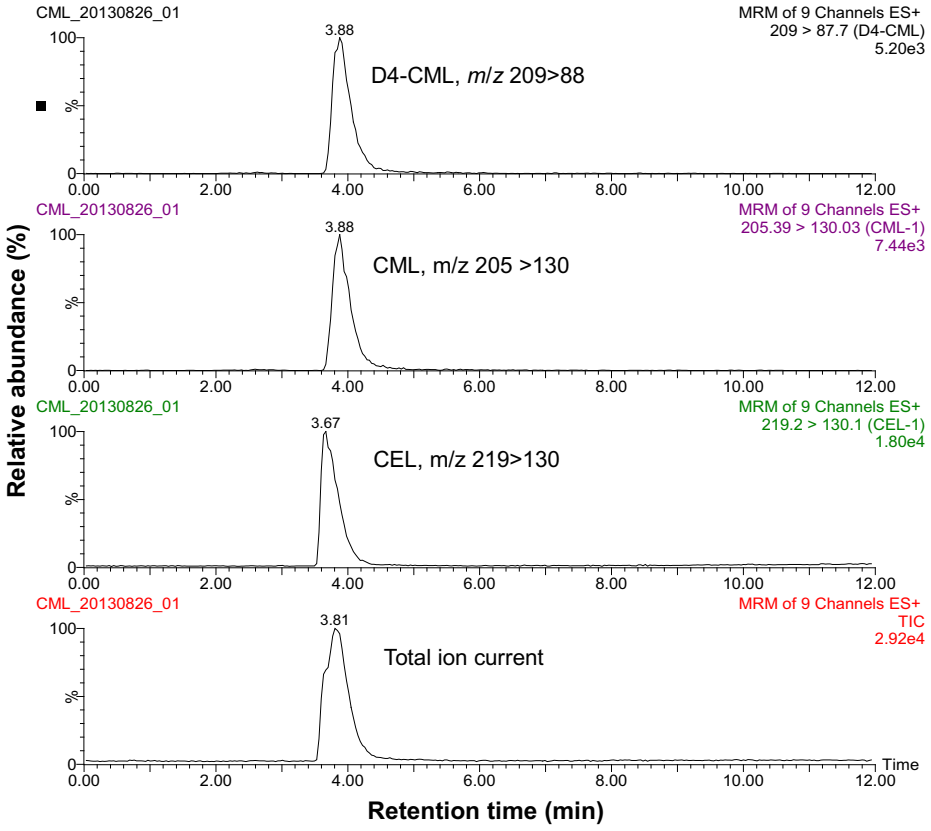

Fig. 1. LC–MS/MS chromatogram of a mixture of AGEs standards obtained through multiple reaction monitoring in positive-ion mode. The concentrations of AGEs were: CML 195 μg/kg, CEL 155 μg/kg, and d_4-CML 100 μg/kg.

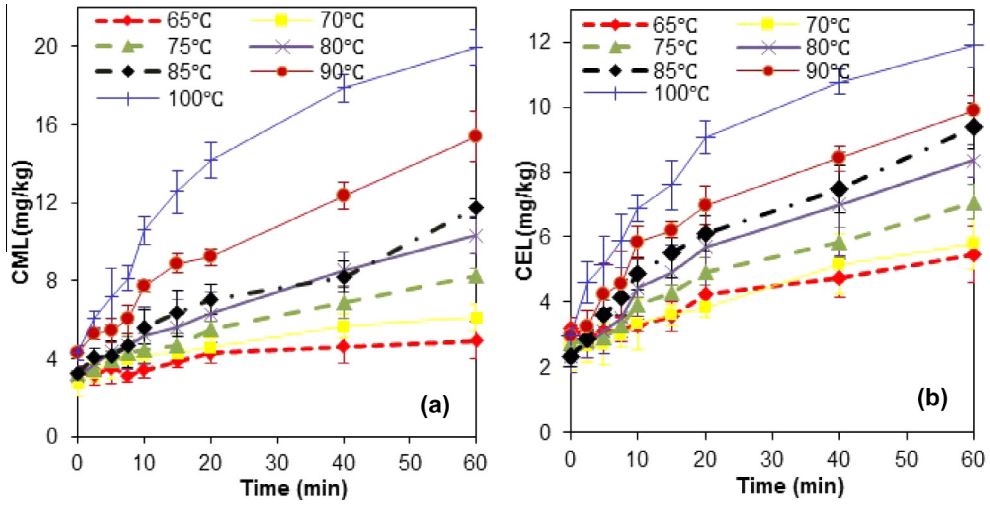

Fig. 2. Change of (a) CML and (b) CEL concentrations in ground beef heated at 65–100 °C for 0–60 min. Data are presented as mean ± standard deviation of four measurements.

0.72, 5.02, 11.2 mg/kg, respectively. The amount of CML in their boiled minced beef increased six times in only 3 min of heating. Hull et al. (2012) reported that CML in roasted beef ranged from 1.5 mg/kg for raw, 2.5 mg/kg for medium, to 4.2 mg/kg for well-done beef. Obviously, the source, freshness and process methods of beef samples used in different studies varied greatly, leading to great differences in these reported data. It is very likely that ground beef is more susceptible to lipid oxidation, which forms glycating agents such as α-dicarbonyl compounds during storage, accelerating the formation of AGEs and leading to relatively high levels of CML as reported in our study.

The levels of CEL in raw ground beef were 2.32–3.18 mg/kg, and in heat-treated ground beef ranged from 2.65 to 11.89 mg/kg. Similar to that for CML, the amount of CEL increased with heating time and temperature. After 60 min of heat treatments, the levels of CEL increased 72% to 301% (Fig. 2b). In addition, we observed a strong linear relationship ($r^2 = 0.920$) between CEL and CML contents in raw and heated treated beef samples (Fig. 3), indicating that CEL and CML were affected in a similar pattern by heat treatments and may be formed under similar pathways. A linear relationship between CEL and CML ($r^2 = 0.87$) for almonds roasted at 129 °C for 32 to 70 min was found based upon the data reported by

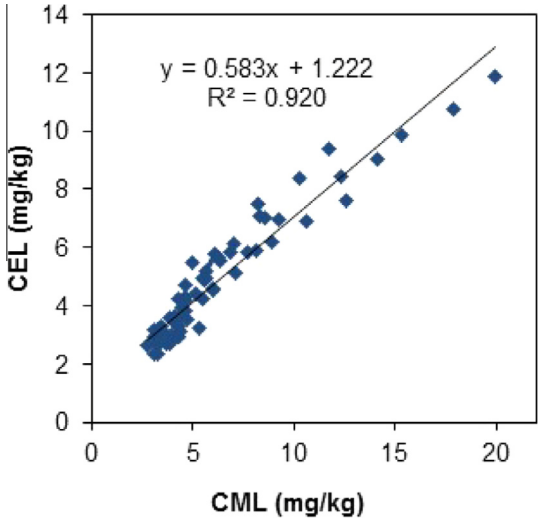

Fig. 3. Plot of CEL *versus* CML content in raw and heat-treated ground beef.

Zhang et al. (2011), although there was no linear relationship between CEL and CML in almonds roasted at 138 °C to 182 °C.

3.3. Kinetics of formation of CML and CEL in ground beef during thermal treatments

Since the come-up time for the central temperature of ground beef reaching the targeted temperature was about 3.5–4 min, the CML and CEL data before 5 min of heating time were not used for determining reaction order. As shown in Table 2, the higher the reaction order used, the less satisfactory were the results for fitting the kinetic models; a zero-order reaction resulted in the highest r^2 value for both CML and CEL at all seven different temperatures (CML: r^2 = 0.851–0.995; CEL: r^2 = 0.907–0.971) (Table 2). However, at high temperature conditions including 90 and 100 °C, although zero-order reactions fitted well for both CML and CEL at heating time up to 20 min (CML, r^2 = 0.903 at 90 °C, r^2 = 0.965 at 100 °C; CEL, r^2 = 0.918 at 90 °C, r^2 = 0.981 at 100 °C), the rate of AGEs formation seemed to decrease at longer heating time; yet zero-order reaction still fitted very well for the data from 20 to 60 min of heating (CML: r^2 = 1 at 90 °C, r^2 = 0.976 at 100 °C; CEL, r^2 = 1 at 90 °C, r^2 = 0.986 at 100 °C) (Fig. 4a and b). Because of limited data for 20 min or longer heat treatments, no specific two-step zero-order models were developed for the formation of AGEs at 90 and 100 °C and further studies are needed. A zero-order reaction

Table 2
Correlation of determination (r^2) for kinetic models (n = 0, 0.5, 1, or 2) of CML and CEL formation during heating.

	CML				CEL			
	0	0.5	1	2	0	0.5	1	2
65 °C	0.851	0.830	0.808	0.761	0.959	0.946	0.932	0.898
70 °C	0.978	0.973	0.967	0.951	0.971	0.955	0.932	0.871
75 °C	0.990	0.981	0.968	0.929	0.951	0.921	0.882	0.788
80 °C	0.995	0.984	0.967	0.915	0.943	0.906	0.859	0.748
85 °C	0.960	0.944	0.914	0.816	0.965	0.938	0.899	0.797
90 °C	0.967	0.940	0.892	0.780	0.933	0.898	0.856	0.758
100 °C	0.902	0.856	0.804	0.687	0.907	0.875	0.837	0.750

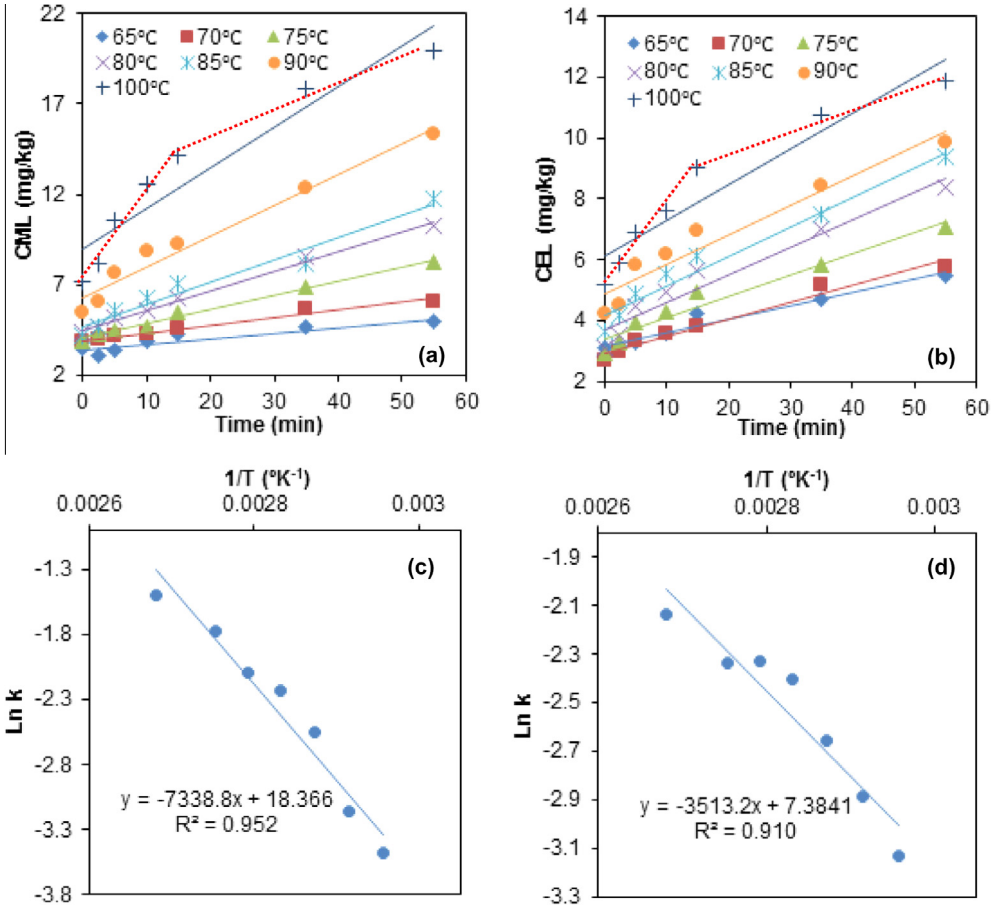

Fig. 4. (a and b) Plot of CML and CEL *versus* time at different temperatures, respectively. Each solid line is the linear regression trendline at a specific temperature. The red dashed lines show linear regression trendlines for two different heating time periods at 100 °C. (c and d) Plot of ln k for CML and CEL *versus* 1/T, respectively. The unit for k is mg kg^{-1} min^{-1}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

indicated that the amount of CML or CEL in ground beef did not affect the reaction rate. Zero-order reactions are very common for food systems when the product formed is much less than the precursors present in foods (Van Boekel, 2008), and such a finding is typically tied to products from the Maillard reaction (Boonchiangma, Chanthai, Srijaranai, & Srijaranai, 2011; Güneser, Toklucu, & Karagül-Yüceer, 2013; Morales & Van Boeckel, 1996; Van Boekel, 2001).

The reaction rate constant calculated at each temperature based upon a zero-order reaction for CML increased from 0.031 to 0.224 mg kg^{-1} min^{-1}, and for CEL from 0.044 to 0.118 mg kg^{-1} min^{-1} as reaction temperature increased from 65 to 100 °C. The rate constants for both CML and CEL in ground beef during heating basically fitted the Arrhenius equation (Fig. 4c and d; CML, $r^2 = 0.952$; CEL, $r^2 = 0.910$) with activation energy of 61.01 kJ/mol (±2.40 kJ/mol) for CML, and 29.21 kJ/mol (±2.19 kJ/mol) for CEL.

The study of Morales and Van Boeckel (1996) for sugar-casein model systems (110–150 °C for 0–30 min) showed that the formation of L-lysyl pyrraline (an AGE) was zero-order with an activation energy of 109.1–138.1 kJ/mol. Several other studies indicated that the formation of another typical Maillard reaction product, hydroxymethylfurfural, also followed zero-order reaction with an activation energy of 96.31 kJ/mol in goat milk heated at 75–95 °C for 0–540 min (Güneser et al., 2013) and 135.4–139.6 kJ/mol in honey (25–85 °C for 0–14 days) (Boonchiangma et al., 2011). In our study, the activation energies for CML and CEL were relatively lower than the other reported Maillard reaction products, such as hydroxymethylfurfural and L-lysyl pyrraline. This may indicate that the formation of CML and CEL is less temperature dependent than other reported Maillard products (Van Boekel, 2008), but further study is needed since test conditions were not the same across different studies.

4. Conclusions

The levels of CML and CEL in ground beef increased with heating time and heating temperature, and the amounts of these two AGEs in thermally-treated ground beef were highly correlated. The formation of both CML and CEL in ground beef could be considered to be zero-order reactions. A zero-order reaction implies that the amounts of CML and CEL in the samples did not affect the production rate of CML and CEL, which was expected, since the single- or two-digit ppm-levels of CML and CEL in the ground beef samples were much less than precursors such as lysine.

The formation of CML and CEL results from at least four identified pathways involving numerous intermediate products in complex food systems. The dominant pathway(s) leading to the formation of AGEs may change at different temperatures. Meanwhile, some of the intermediate products may follow different pathways to form other end products in addition to AGEs. This makes it very difficult, if not impossible to apply kinetic models to reveal the mechanisms of AGEs formation in complex food systems. In this study, an order of reaction was determined to help understand the quantitative changes of AGEs in ground beef under pasteurisation conditions. Although the study could not reveal the underlying mechanism for the formation of AGEs, a systematic study like this helps advance our knowledge on the overall formation of AGEs under pasteurisation conditions in complex food systems.

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