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Formation of free and protein-bound carboxymethyllysine and carboxyethyllysine in meats during commercial sterilization

Xiaohua Sun [a], Juming Tang [b], Jing Wang [a], Barbara A. Rasco [c,*], Keqiang Lai [a], Yiqun Huang [a,c,**]

[a] College of Food Science and Technology, Shanghai Ocean University, No. 999 Hucheng Huan Road, LinGang New City, Shanghai 201306, China
[b] Department of Biological System Engineering, Washington State University, Pullman, WA 99164-6120, USA
[c] School of Food Science, Washington State University, Pullman, WA 99164-6376, USA

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ABSTRACT

The effect of commercial sterilization treatments on the levels of advanced glycation endproducts (AGEs) in meats was investigated. The amounts of both free and protein-bound N^{ε}-carboxymethyllysine (CML) and N^{ε}-carboxyethyllysine (CEL) in beef (rump, ribeye, short plate), pork (hind leg, tenderloin, belly), and chicken (chicken breasts, drumsticks) were determined using an HPLC–MS/MS method. Beef and pork had a small proportion (raw <15%; sterilized <8%) of free AGEs compared to the total AGEs, but raw chicken breasts had very high levels of free CEL (7.12 ± 9.98 mg/kg; $n = 13$) with large biological variation compared to pork (0.19 ± 0.09 mg/kg; $n = 9$) and beef (0.44 ± 0.19 mg/kg; $n = 9$). Commercial sterilization (121 °C for 10 min) did not significantly affect the amounts of free CML or CEL, but led to about 0.6- to 3.6-fold increase of protein-bound CML and CEL. The amounts of protein and fat content in beef or pork had very little effect on the formation of protein-bound AGEs during sterilization process.

1. Introduction

Meat is a rich source of advanced glycation endproducts (AGEs) compared to other foods, such as fruits and vegetables. AGEs may promote aging, oxidative stress and inflammation and have been tied to increased risks for diabetes, cardiovascular diseases and pancreatic cancer, although the role of these compounds in the diet and relation to diseases has not yet been clearly established (Ames, 2007; Jiao et al., 2015; Nguyen, 2006; Sun et al., 2015; Uribarri et al., 2010). Glycation is a non-enzymatic modification of proteins or protein derivatives by reducing sugars or sugar derivatives (Rabbani & Thornalley, 2012). AGEs in foods could be formed through the Maillard reaction and lipid oxidation (Fu et al., 1996; Rabbani & Thornalley, 2012), and the levels of AGEs in foods depend upon various factors such as the type of food, food composition, and cooking or processing method (Chen & Smith, 2015; Hull, Woodside, Ames, & Cuskelly, 2012). High protein and/or high fat foods, such as meats, generally contain relatively high levels of AGEs, and heat treatments promote AGEs formation (Goldberg et al., 2004; Sun et al., 2015; Uribarri et al., 2010).

Many different types of AGEs have been found in foods, which were mainly tied to the glycation of lysine, arginine and cysteine residues (Rabbani & Thornalley, 2012), but lysine derived AGE N^{ε}-carboxymethyllysine (CML) is the most widely studied one and usually used as a marker for AGEs in foods (Goldberg et al., 2004; Hull et al., 2012; Uribarri et al., 2010). Quantification of CML in a large variety of foods prepared with different cooking methods including boiling, frying, roasting/oven-baking, broiling has been reported. For example, Goldberg et al. (2004) used enzyme-linked immunosorbent assay (ELISA) to determine the amount CML in 250 foods typically consumed by a multiethnic urban population. Uribarri et al. (2010) quantified CML in 549 uncooked or cooked foods typically consumed by a Northeastern American urban population with ELISA. Takeuchi et al. (2015) applied ELISA to determine glucose-derived AGEs, fructose-derived AGEs, glyceraldehyde-derived AGEs and CML in 1650 beverages and foods commonly consumed in Japan. Hull et al. (2012) employed a more sophisticated instrumental method, ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS), to determine CML in 256 foods commonly consumed in a Northern Irish population, and reported their data in four commonly used forms (such as mg/100 g, and mmol/mol lysine) instead of expressing the data in units limited by the use of ELISA methods. The majority of studies on AGEs have been focused on the levels of CML in foods as affected by the culinary techniques, such as baking or broiling versus boiling, breading versus non-breading, and addition of sauces or not before the cooking (Chao, Hsu, & Yin, 2009; Chen & Smith, 2015; Hull et al., 2012).

* Corresponding author.
** Correspondence to: (Y. Huang) College of Food Science and Technology, Shanghai Ocean University, No. 999 Hucheng Huan Road, LinGang New City, Shanghai 201306, China.
E-mail addresses: rasco@wsu.edu (B.A. Rasco), yiqunh@hotmail.com (Y. Huang).

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There are very few publications on the formation of AGEs in foods during thermal treatments that simulated pasteurization or commercial sterilization conditions commonly used in the food industry (Ahmed et al., 2005; Sun et al., 2015; Zhang, Huang, Xiao, & Mitchell, 2011). In particular, there is no reported study (to the best of our knowledge) on the AGEs formation in meats during commercial sterilization, a process commonly employing heat to destroy spores of the pathogenic *Clostridium botulinum*.

The purpose of this study was to investigate the effects of commercial sterilization on the levels of CML and N^{ε}-carboxyethyllysine (CEL) in beef (rump, ribeye, short plate), pork (hind leg, tenderloin, belly), and chicken (chicken breasts, drumsticks). Since the bioavailability and physiological effects of protein-bound AGEs (protein glycation adducts) and free AGEs (glycated amino acids) are most likely different (Ahmed et al., 2005; Rabbani & Thornalley, 2012), both protein-bound and free CML and CEL in meats were quantified with a validated HPLC–MS/MS method that allows for a more accurate quantification of these AGEs compared to the commonly used immunoassay (Scheijen et al., 2016). What is more, for each type of meat cut, samples produced by three different companies were used to increase the range of variability due to different sample sources.

2. Materials and methods

2.1. Reagents

AGEs including CML (98%), CEL (98%) and d_4-CML (98%) were purchased from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada) and all other chemicals were from Sinopharm (Shanghai, China). Normal hexane, methanol, chloroform, formic acid and ammonium acetate were HPLC grade, and the others were analytical grade. Methanol–water (80:20, *v*/v) was used as solvent to prepare AGEs standard solutions including AGEs standard mixture (CML 300 μg/L, CEL 300 μg/L, and d_4-CML 400 μg/L) and the internal standard (d_4-CML 8 mg/L).

2.2. Preparation of beef, pork and chicken samples

Fresh (keep refrigerated, never frozen) meats including beef (rump, ribeye, short plate), pork (hind leg, tenderloin, belly) and chicken (breasts, drumsticks) were purchased from a Metro AG store in Shanghai, China. For each type of meat cut, meats manufactured from three different companies were collected. About 400 g of meat was cut into small pieces, and mixed in a blender (8010 s, Waring Inc., Torrington, Connecticut, USA) at low speed 4 times for about 10 s each time. About half of the minced meat was used for AGEs analyses and sterilization, and the other half was stored at −80 °C before being used for determination of water, fat and protein content (Sun et al., 2015).

To mimic commercial sterilization process, ground meat was filled in an aluminum cylindrical cell (diameter, 50 mm; height 5 mm) (Kong, Tang, Rasco, Crapo, & Smiley, 2007; Sun et al., 2015), sealed and heated at 121 °C for 10 min in an oil bath (HAAKE PC 300-S7; Thermo Fisher Scientific Inc., Waltham MA) with dimethyl silicone (Sinopharm, Shanghai, China) as the heating media. The small custom-design test cell allowed for rapid heat transfer and relatively good heating uniformity for a sample sealed inside the cell (Kong et al., 2007). The come-up time (time required for the cold spot to reach 121 °C) for the meats in the test cells were about 4–5.5 min, and the use of 10 min of heating time was to obtain F_0 of 4 min and above as required for sterilizing canned foods with a sufficient safety margin (Heinz & Hautzinger, 2007). Following the heat treatment, the cell was immediately immersed into ice-water to rapidly cool down the sample.

2.3. Sample preparation for analyses of free AGEs

A water extraction method based upon the studies of Zhang et al. (2011) and Hegele, Buetler, and Delatour (2008) with modification was used to prepare samples for free AGEs analysis. Instead of using nonafluoropentanoic acid (Hegele et al., 2008; Zhang et al., 2011), trichloroacetic acid was used in this study to precipitate proteins to avoid possible deterioration of column (Schettgen et al., 2007; Sun et al., 2015). The detailed preparation method was as follows. First, a mixture of ca. 0.5000–1.0000 g raw or sterile meat (beef, pork and chicken), pre-cooled trichloroacetic acid (2% *v*/v, 10 mL) and d_4-CML (100 μL, 8 mg/L) was homogenized (F6/10, Superfine Homogenizers, Fluko Equipment Ltd., Shanghai, China; 15,000 rpm) for about 30 s in an ice-water bath, and then centrifuged (TDL-5-A, Shanghai Anting Scientific Instrument Factory, Shanghai, China) at 5000 rpm for 20 min to precipitate protein. Next, the supernatant was mixed well with 10 mL of n-hexane, and centrifuged again at 5000 rpm for 10 min to defat and to precipitate the residual proteins. Following this, 5 mL of the recovered aqueous layer was loaded onto a pre-activated MCX column (60 mg/3 mL, Shanghai ANPEL Scientific instrument Co., Ltd., Shanghai, China). The column was washed with 3 mL water and 3 mL methanol in sequence. Finally, the target compounds were eluted with 5 mL 5% ammonia in methanol, dried in nitrogen using a nitrogen evaporator (DC12H, Shanghai ANPEL Scientific Instrument Co., Ltd., Shanghai, China) at 60 °C, reconstituted with 1–2 mL methanol–water (80:20, v:v), and filtered through a 0.22 μm filter prior to HPLC–MS/MS analysis. Since the levels of free AGEs in different meats may vary greatly, the amounts of meat sample and solvent used to extract free AGEs were modified based upon the results of our preliminary experiments so that the amounts of free CML and CEL in the final extract were within the linear range of 20–1500 μg/L for their quantification with the HPLC–MS/MS method. Triplicate experiments were conducted.

2.4. Sample preparation for analysis of protein-bound AGEs

A modified acid hydrolysis method was employed to prepare samples for protein-bound AGEs analysis (Assar, Moloney, Lima, Magee, & Ames, 2009; Niquet-Léridon & Tessier, 2011), which was described in detail in our previous study (Sun et al., 2015). In brief, a meat sample was incubated with borate buffer (0.2 M, pH 9.2) and sodium borohydride for 8 h, mixed with methanol-chloroform (1:2, v:v) and centrifuged to defat and precipitate proteins. Next, the precipitated proteins were hydrolyzed in hydrochloric acid (HCl) at 110 °C for 24 h. Following this, the diluted protein hydrolysate was spiked with d_4-CML, dried, and reconstituted in water. The sample solution was further cleaned up with an MCX cartridge and 0.22 μm filter. The dilution factor for protein hydrolysate varied depended upon the type of meat analyzed to ensure that the amounts of AGEs in the diluted hydrolysates of both the raw and sterilized meat were within the linear range of 20–1500 μg/L for HPLC–MS/MS analysis. The extraction of each sample was repeated three times.

2.5. HPLC–MS/MS analysis

The amounts of AGEs in meat extracts were determined with a Waters 2695 HPLC system (Waters Corp., Milford, MA, USA) and a Waters Quattro Micro triple–quadrupole tandem mass spectrometer (MS/MS). An Atlantis hydrophilic interaction liquid chromatography (HILIC) silica column (150 mm × 2.1 mm, 3 μm; Waters Corp.) was used in the HPLC system. The collision energy was 20 eV, and cone voltage was 20 V for CEL determination. The product ions at m/z 130 and at m/z 84 were used for quantification and confirmation of CML or CEL, respectively. All other experimental procedures (except for the concentration of AGEs standard mixture and the internal standard) were the same as our previous study (Sun et al., 2015).