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Influence of home cooking conditions on Maillard reaction products in beef

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ABSTRACT

The influence of home cooking methods on the generation of Maillard reaction products (MRP) in beef was investigated. Grilling and frying hamburgers to an internal temperature below 90 °C mainly generated furosine. When the temperature reached 90 °C and 100 °C, furosine content decreased by 36% and fluorescent compounds increased by up to 98%. Baking meat at 300 °C, the most severe heat treatment studied, resulted in the formation of carboxymethyllysine. Boiling in water caused very low MRP formation. Acrylamide concentrations in grilled, fried or baked meat were extremely low. Home cooking conditions leading to low MRP generation and pleasant colours were obtained and could be used to guide diabetic and chronic renal patients on how to reduce their carboxymethyllysine intake.

1. Introduction

Adequate cooking of meat is fundamental to ensure its preservation, to eliminate pathogenic microorganisms, and to improve sensory properties. Nevertheless, thermal processing can have some unintentional and undesired consequences, such as the loss of amino acids and the synthesis of toxic compounds (heterocyclic amines, acrolein, furan, and acrylamide) and compounds exerting negative effects on flavour, texture and/or colour.

Heating foods results in the development of the Maillard reaction (MR), characterised by the formation of hundreds of different substances that are associated with the acceptance of the processed product. Maillard reactions also occur in living organisms and the products generated *in vivo*, which have biological effects, are called advanced glycation end products (AGEs) and advanced lipoxidation end products (ALEs) (Poulsen et al., 2013). The ingestion of Maillard reaction products (MRPs) from foods seems to be correlated with serum AGE levels in humans (Vlassara et al., 2002; Uribarri et al., 2005; Poulsen et al., 2013). However, it is currently not clear whether or not dietary AGEs are a risk to human health (Bastos & Gugliucci, 2015). Advanced glycation end products are associated with pathological effects *in vivo*, specifically diabetes complications, kidney damage (Zheng et al., 2002) and cardiovascular diseases (Birlouez-Aragon et al., 2010), oxidative stress (Kellow & Savige, 2013; Uribarri et al., 2005), and inflammation (Vlassara et al., 2002).

The generation of MRPs depends on food composition as well as processing temperature and time. Although there are a substantial amount of data on the MRP content of individual food products, information about the generation of MRP during home cooking and the effects of different cooking methods on recipes with the same ingredients is still lacking (Delgado-Andrade, Seiquer, Haro, Castellano, & Navarro, 2010). Dry heat cooking methods such as frying (Chao, Hsu, & Yin, 2009) and grilling (Delgado-Andrade, Seiquer, et al., 2010; Hull, Woodside, Ames, & Cuskelly, 2012) increase MRP formation by 10 times when compared to boiling.

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Compounds (markers) generated as the MR progresses are commonly used to evaluate the development of the reaction cascade. In this respect, furosine is an indirect indicator of the generation of Amadori products from lysine and is considered a marker of the early stage of the MR (Roldan et al., 2015). Furosine is produced during acid hydrolysis of Amadori products (fructosyl-lysine, lactulosyl-lysine and maltulosyl-lysine) by reaction of the ε-amino groups of lysine with glucose, lactose and maltose (Erbersdobler & Somoza, 2007). Amadori products undergo dehydration and fission as the MR progresses, generating colourless reductones and fluorescent substances (Morales, Romero, & Jimenez-Perez, 1996). Thus, the measurement of an increase or decrease in fluorescence is an effective procedure to assess the extent of the MR in foods (Delgado-Andrade, Rufian-Henares, & Morales, 2006).

Carboxymethyllysine (CML), one of the best characterised AGEs/ALEs, is an advanced Maillard product formed in both foods and biological systems and is an important marker of AGE accumulation in foods and in animal and clinical studies. Carboxymethyllysine is generated in food products by the oxidation of fructosyl-lysine (an Amadori product) and/or by the direct reaction of glyoxal, produced during lipid peroxidation, with the ε-amino group of lysine (Assar, Moloney, Lima, Magee, & Ames, 2009; Hull et al., 2012; Niquet-Leridon & Tessier, 2011).

Acrylamide is another important MRP, which is mainly generated from free asparagine and reducing sugars during high-temperature cooking (above 120 °C). Knowledge of the pathway of acrylamide generation during thermal processing is essential to reduce the acrylamide content of foods, since this substance is a potential carcinogen and mutagenic agent (Cheng, Jin, & Zhang, 2014; Heredia, Castelló, Arguelles, & Andres, 2014; Xu et al., 2014). Although carbohydrate-rich foods have been generally studied for the determination of acrylamide, protein-rich foods, such as meat cooked at high temperatures, can also contain acrylamide, reaching levels up to 300 μg/kg (Kaplan, Kaya, Ozcan, Ince, & Yaman, 2009; Delgado-Andrade, Morales, Seiquer & Navarro, 2010; Demirok & Kolsarici, 2014). The amount of dietary acrylamide intake is influenced not only by the level of acrylamide in a food, but also by how much of that food is consumed (Xu et al., 2014). Thus, the determination of acrylamide concentrations in a commonly consumed food such as meat is mandatory for the risk assessment of acrylamide consumption.

In view of the controversial debate on the relationship between dietary MRP ingestion and health and of the fact that meat is consumed worldwide after heating, it is of interest to evaluate whether the control of home cooking conditions can effectively affect MRP content in beef. In fact, the quantification of MRP in widely consumed foods could provide information for databases that can be used to estimate AGE intake in epidemiological studies (Hull et al., 2012). The present study investigated the generation of furosine, CML, acrylamide and fluorescent compounds and losses of lysine during different cooking methods of beef (grilled, breaded and fried, boiled, and baked in a gas oven).

2. Material and methods

2.1. Materials

Beef (*semimembranosus*, about 30 kg, proximate composition: 75.2% moisture, 21.7% protein, 1.6% lipids, 1.1% ash and 0.4% carbohydrates) was provided by Minerva Foods (Barretos, Sao Paulo, Brazil) and transported to the laboratory at 5 °C in ice boxes. The meat was stored at −18 °C packed under vacuum in plastic bags (Cryovac, Sealed Air, Sao Paulo, Brazil) until processing. Egg powder, white wheat flour, soy oil and commercial sodium chloride were obtained at a local market in Sao Paulo, Brazil.

Nε-(1-carboxymethyl)-L-lysine (CML) was supplied by Polypeptide Laboratories France SAS (Strasbourg, France), L-lysine dihydrochloride was obtained from Sigma–Aldrich (St. Louis, MO), Nε-(1-carboxymethyl)-L-lysine-d4 (d4-CML), and L-lysine-d4 were purchased from Alsachim (Strasbourg, France). Acetonitrile and methanol of HPLC grade were obtained from Carlo Erba (Rodano, Italy). Nonafluoropentanoic acid (97%, NFPA), sodium borohydride ($NaBH_4$), and sodium borate were purchased from Sigma–Aldrich.

The solid phase extraction cartridges [Oasis HLB, 1 cm^3, 30 mg for CML and lysine, and Sep-Pak Vac 3 cc (500 mg) for furosine] were purchased from Waters (Milford, MA). Hydrochloric acid (37%), sodium hydroxide and pronase were supplied by Merck (Darmstadt, Germany). Syringe filters (0.22 μm and 0.45 μm, PVDF) were obtained from Millipore (Bedford, MA). All aqueous solutions were prepared in water purified with a Milli-Q reagent water system (Millipore).

2.2. Meat preparation

Fifty grams of raw minced beef containing 1.0% (w/w) NaCl were shaped into a hamburger (diameter of 7 cm, thickness of 1 cm) for all cooking methods. (1) *Grilled*: Soy oil (2.5%, w/w) was added to a coated pan before grilling. The hamburger was then placed in the pan at 150 °C, cooked to an internal temperature of 30 °C, turned over every minute, and cooked to an internal temperature of 60, 70, 80, 90 and 100 °C. (2) *Boiled*: The hamburger was placed in 500 mL of water at 98 °C and cooked to an internal temperature of 30 °C. The hamburger was then turned over every minute and cooked to an internal temperature of 60, 70, and 80 °C. (3) *Breaded/fried*: Before frying, the hamburger was covered with reconstituted powdered eggs (5 g) and wheat flour (10 g). The breaded sample was fried in 150 g of soy oil at 180 °C and cooked to an internal temperature of 30 °C. The sample was then turned over every minute and cooked to an internal temperature of 60, 70, 80, 90 and 100 °C. (4) *Baked*: Two hamburgers were baked at 180, 240 and 300 °C for 30 min in a domestic gas convection oven. A thermocouple (type J; G Controls Sistemas Ltda., Sao Paulo, Brazil) was placed inside the oven. Images of the hamburgers are shown in Table 1.

The meat was grilled, boiled or fried in a coated pan (without lid) using a domestic gas stove. A thermocouple (type J, G Controls Sistemas Ltda., Sao Paulo, Brazil) was inserted in the geometric centre of each hamburger and connected to a digital display (GC 2000I; G Controls Sistemas Ltda., Sao Paulo, Brazil) to permit monitoring of the temperature profile. All heat treatments were performed in sextuplicate.

Cooking yield was calculated according to the USDA (2012):

$$\text{yield } (\%) = 100 \times (\text{sample hot cooked weight}/\text{sample raw weight}).$$

After cooking, the hamburgers were weighed, freeze-dried, packed in plastic bags (Cryovac; Sealed Air, Sao Paulo, Brazil), and stored at −18 °C until the time of analysis.

2.3. Analysis of furosine

Furosine was determined as described by Pompei and Spagnolello (1997), with some modifications. Briefly, 8 mL of 8 N HCl were added to a quantity of freeze-dried sample, corresponding to 40 mg protein, in 50-mL screw-cap Pyrex tubes. After nitrogen was bubbled for 2 min, the tubes were sealed and kept at 110 °C for 23 h. The hydrolysates were cooled at room temperature and subsequently filtered through a 0.45-μm PVDF membrane. An aliquot (0.5 mL) of the filtrate was applied to a Sep-Pak C_{18} cartridge pre-wetted with 5 mL methanol, followed by 5 mL water.