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Determination of advanced glycation endproducts in cooked meat products

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ABSTRACT

Advanced glycation endproducts (AGEs), a pathogenic factor implicated in diabetes and other chronic diseases, are produced in cooked meat products. The objective of this study was to determine the AGE content, as measured by Nε-carboxymethyllysine (CML) levels, in cooked chicken, pork, beef and fish (salmon and tilapia) prepared by three common cooking methods used by U.S. consumers: frying, baking, and broiling. The CML was detected in all the cooked samples, but the levels were dependent on types of meat, cooking conditions, and the final internal temperature. Broiling and frying at higher cooking temperature produced higher levels of CML, and broiled beef contained the highest CML content (21.8 μg/g). Baked salmon (8.6 μg/g) and baked tilapia (9.7 μg/g) contained less CML as compared to the other muscle food samples.

1. Introduction

Advanced glycation endproducts (AGEs) are a group of complex and heterogeneous compounds that are formed through the Maillard reaction, a nonenzymatic reaction between reducing sugars and free amino groups (Ahmed, 2005). Although the mechanism of the Maillard reaction is still not fully known, the AGEs exist in the body as well as in food. The common AGEs found in food are Nε-carboxymethyl lysine (CML), methylglyoxal-lysine dimers (MOLD), pentosidine and pyrraline (Wu, Huang, Lin, & Yen, 2011). Current research suggests excessive consumption of these compounds may contribute to metabolic chronic diseases including diabetes, renal disorders, and Alzheimer's disease (Brownlee, 1994; Kim, Reddy, Rahbar, Lanting, & Natarajan, 2002; Koschinsk et al., 1997). Some epidemiological studies have shown that consumption of certain dietary AGEs are indicators of oxidative stress and inflammation such as 8-isoprostanes, which may play an important role in disease pathologies (Uribarri et al., 2007). Moreover, reductions of inflammatory mediators were also found in diabetic subjects by restricting their dietary AGEs (Vlassara et al., 2002). Based on some animal studies, AGE-rich diets fed to mice was associated with kidney disease and accumulation of AGEs in tissue (Hofmann et al., 2002; Vitek et al., 1994). Thus, information on the levels of dietary AGEs and the prevalence of these compounds in food items is desirable.

It is established that the diet is a significant source of exogenous AGEs. In addition, long-term storage and cooking procedures can increase AGEs content in foods (Forster & Henle, 2003). The concentrations and types of AGEs in cooked meat depend on several factors including cooking method, cooking temperature and time, and the presence of protein and fat (Goldberg et al., 2004). Traditional cooking methods may play a key role in AGEs consumption and exposure. Compared to some Asian countries, it is estimated that broiling or grilling was used more to cook steak (34%), and pan frying was used more to cook chicken (56%) and fish (54%) in the U.S. (Keating & Bogen, 2004). All of these cooking methods have been reported to induce AGE formation (Ames, 2008; Delgado-Andrade, Seiquer, Pilar, & Morales, 2007). For example, Goldberg et al. (2004) found that higher levels of CML in meats cooked by broiling and frying with higher temperatures.

Although some previous studies have investigated AGEs levels in food (Dittrich et al., 2006; Drusch, Faist, & Erbersdobler, 1999; Goldberg et al., 2004; Hull, Woodside, Ames, & Cuskelly, 2012), direct comparison of results is difficult because of the various preparation methods, meat types, and cooking conditions. For instance, meat samples have been cooked to different internal temperatures in past studies, which yielded inconsistent results. Therefore information on AGEs levels should include some standard parameters such as the internal temperature of the cooked samples.

CML has been studied extensively as an oxidation product, and is reported to be formed by numerous pathways in food systems (Ahmed, Thorpe, & Baynes, 1986). In the process of cooking meat

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products, CML may form though the oxidation of fructose lysine or the direct reaction of glyoxal and lysine. Many of the AGEs are not as stable to acids as CML is, so it is often used as an indicator in foods (Tauer, Hasenkof, Kislinger, Frey, & Pischetsrieder, 1999).

This study was performed to evaluate the AGEs content, as measured by CML levels, in meat and fish samples cooked to the internal temperatures recommended by the U.S. Department of Agriculture, Food Safety and Inspection Service (1998). The results can be used as a guideline for evaluating the risk associated with AGE consumption and give some reasonable advice about dietary habits for consumers.

2. Materials and methods

2.1. Materials

The Nε-carboxymethyl lysine (CML) standard was purchased from NeoMPS (Strasbourg, France), boric acid, sodium hydroxide, hydrochloric acid, 2-mercaptoethanol, sodium borohydride, and Na tetraborate decahydrate, were purchased from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade), chloroform (HPLC grade), methanol (HPLC grade), and ortho-phthalaldehyde (OPA) reagent were obtained from Fisher Scientific (Fairlawn, NJ, USA).

2.2. Chemical analyses

Crude protein for uncooked meat samples was measured with a Leco FP-2000 protein analyser (Leco Corp., St. Joseph, MI, USA) according to AOAC Int method 992.15 (Kingbrink & Sebranek, 1993). Fat and moisture content were determined with a CEM Smart Trac system (CEM Corp., Matthews, NC, USA) using AOAC Int method 2008.06 (Leefler et al., 2008).

The pH of each sample was measured according to the method of Jang et al. (2008). Five grams of fine ground tissue was added to 45 mL of distilled water, and mixed for 30 s at medium speed in a Waring blender (Waring Laboratory, Torrington, CT, USA) followed by measurement with an Accumet AP115 pH metre (Fisher, Pittsburgh, PA, USA). The raw meat chemical analyses of the samples are reported in Table 1.

Table 1
Chemical analyses of raw meat samples.

Meat type	Moisture (%)	Fat (%)	Protein (%)	pH
Beef	69.25 ± 1.06	7.18 ± 0.94	21.53 ± 0.57	5.56 ± 0.11
Pork	74.49 ± 1.41	6.16 ± 1.52	19.01 ± 2.38	6.08 ± 0.17
Chicken	73.92 ± 2.99	4.25 ± 1.06	21.88 ± 0.59	6.16 ± 0.10
Salmon	75.03 ± 2.96	1.94 ± 1.27	18.59 ± 1.70	6.85 ± 0.07
Tilapia	77.21 ± 1.78	1.68 ± 1.41	17.98 ± 2.42	7.95 ± 0.09

Each value is represented as mean ± standard deviation ($n = 3$).

2.3. Preparation of meat samples

Fresh meat samples were purchased from regional supermarkets: beef rib round steak, pork top loin, skinless chicken breast, and fish fillet (tilapia and salmon). Fresh meat products were tempered to room temperature prior to cooking. A thermocouple temperature probe was inserted in the middle of each sample, and temperature was recorded with a data logger (USB-TC model, Measurement Computing, Norton, MA, USA).

The description of the cooking methods for the samples are presented in Table 2. The cooking methods preferred by U.S. meat consumers were used in the experiments. Meat samples were prepared by pan frying at different desired surface temperatures, oven broiling at 232 °C (450 °F), and oven baking at 177 °C (350 °F). To eliminate foodborne illness, the internal cooking temperature was used according to recommendation of USDA-FSIS (1998): 63 °C (145 °F) for fish, 71 °C (160 °F) for pork, 74 °C (165 °F) for chicken, and 71 °C (160 °F, well done) for beef. To compare the AGEs levels in cooked meat with different degrees of doneness, the pork samples were fried to 63 °C (145 °F, medium), and the beef steak samples were also fried to 54 °C (130 °F, very rare), 63 °C (145 °F, medium), 71 °C (160 °F, well done) and 77 °C (170 °F, over done). In order to compare the AGE contents in cooked meat by different frying methods, the beef samples were cooked to the same internal temperature of 71 °C (160 °F) by turning once (after 5 min) or multiple times (interval of 1 min). No salt, spice, and oil were used in the cooking procedures. Approximately 2 mm of the surface or 2 mm of the middle part of the meat was excised from the cooled samples with a motorised meat slicer

Table 2
Cooking conditions of the meat and fish samples.

Meat type[a]	Cooking method[b]	Internal temperature (°C)	Cooking temperature (°C)	Cooking time (min)	Cooking loss[c] (%)
Beef	Frying	71	204	20	25.23 ± 1.80
	Broiling	71	232	16	30.81 ± 4.10
	Baking	71	177	45	21.59 ± 2.69
Pork	Frying	71	204	16	24.83 ± 1.79
	Broiling	71	232	14	31.25 ± 3.93
	Baking	71	177	35	22.50 ± 3.03
Chicken	Frying	74	204	18	29.66 ± 2.08
	Broiling	74	232	16	35.90 ± 2.31
	Baking	74	177	35	27.24 ± 1.45
Salmon	Frying	63	204	12	19.64 ± 2.42
	Broiling	63	232	10	25.98 ± 2.64
	Baking	63	177	14	16.71 ± 1.24
Tilapia	Frying	63	204	12	18.12 ± 1.89
	Broiling	63	232	8	25.26 ± 2.54
	Baking	63	177	12	19.84 ± 2.82

[a] Beef: rib round steak, 270–310 g, 2.5 thickness; Pork: top loin: 220–250 g, 2.3 cm thickness; Chicken: breast without skin, 230–250 g, 2.3 thickness; Salmon: 180–200 g, 1.8 cm thickness; Tilapia:140–160 g, 1.5 thickness.

[b] Frying: meat was fried in a Teflon-coated frying pan; Broiling: the meat was prepared on a broiler pan to be out of the drippings and cooked in an oven; Baking: the meat was prepared on a baking pan and baked in an oven.

[c] % Cooking loss = (before cook weight − after cook weight)/before cook weight * 100%.