Journal of Food Composition and Analysis 63 (2017) 133–138

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original research article

Formation of advanced glycation end-products in fish muscle during heating: Relationship with fish freshness

Lihong Niu[a,b], Xiaohua Sun[b], Juming Tang[c], Jing Wang[b], Barbara A Rasco[d,*], Keqiang Lai[b], Yuxia Fan[b], Yiqun Huang[b,d,*]

[a] School of Food Engineering, Ludong University, No. 186, Middle Hongqi Road, Zhifu District, Yantai, Shandong, 264025, China
[b] College of Food Science and Technology, Shanghai Ocean University, No. 999 Hucheng Huan Road, LinGang New City, Shanghai, 201306, China
[c] Department of Biological System Engineering, Washington State University, Pullman, WA 99164-6120, USA
[d] School of Food Science, Washington State University, Pullman, WA, 99164-6376, USA

ARTICLE INFO

Keywords:
Food analysis
Food composition
Advanced glycation end-products
Carboxymethyllysine
Carboxyethyllysine
Fish
Storage
Heating
Ctenopharyngodon idellus
Clarias leather

ABSTRACT

The purpose of this study was to investigate the formation of advanced glycation end-products in raw and heat-treated fish muscle as affected by fish freshness. The amounts of free and protein-bound N^{ε}-carboxymethyllysine (CML) and N^{ε}-carboxyethyllysine (CEL) in raw and heat-treated (100 °C, 5, 30 min) white muscle from cultivated grass carp (*Ctenopharyngodon idellus*) (n = 12) and catfish (*Clarias leather*) (n = 24) steaks previously stored at 0 °C for 0–21 days were quantified with an HPLC–MS/MS method. The levels of free and protein-bound CML and CEL in raw fish muscle did not change significantly during 21 days of ice storage. However, the longer the fish muscle was stored at 0 °C, the more protein-bound CML and CEL were formed upon heating. The heat-induced formation of protein-bound CML in catfish muscle previously stored at 0 °C for 21 days increased by 107% (5 min heating) or 172% (30 min heating), and CEL increased by 448% (5 min heating) or 191% (30 min heating), as compared with that in heat-treated freshly killed catfish muscle. In grass carp muscle, the increases were from 144% (5 min) to 131% (30 min) for protein-bound CML, and 135% (5 min) to 95% (30 min) for protein-bound CEL.

1. Introduction

Dietary advanced glycation end-products (AGEs) is a group of compounds generated from non-enzymatic oxidation of protein (Rabbani and Thornalley, 2012), which may increase the risk of chronic diseases like diabetes, kidney failure, and cardiovascular diseases (Chuyen, 2006; Jiao et al., 2015; Uribarri et al., 2010). Dietary AGEs are generally tied to the glycation of lysine, arginine and cysteine residues, but lysine-derived AGEs, N^{ε}-carboxymethyllysine (CML) and N^{ε}-carboxyethyllysine (CEL), are the most widely studied ones and generally used as markers for AGEs in foods (Rabbani and Thornalley, 2012; Uribarri et al., 2010). As the formation of AGEs in foods is attributed to the Maillard reaction and lipid oxidation (Fu et al., 1996), heating could promote AGEs formation and higher temperature or longer heating time would induce a greater amount of AGEs formation in foods (Chao et al., 2009; Chen and Smith, 2015; Sun et al., 2015, 2016; Zhang et al., 2011). Food storage conditions (such as storage time and temperature) also affect the levels of AGEs in foods, while the Maillard reaction and lipid oxidation that lead to AGEs formation occur continuously during storage. Reports on AGEs formation during storage are quite limited and have focused on dairy products and infant formula (Birlouez-Aragon et al., 2005; Bosch et al., 2007). There are extremely few reported studies on the changes of AGEs levels in muscle foods during storage. Typically, the study of Yu et al., (2016) showed that both CML and CEL in irradiated or non-irradiated cooked beef, mutton, pork chicken and fish were significantly increased after 6 weeks of storage at 25 °C, while the irradiated muscle foods exhibited a higher increase rate compared to non-irradiated muscle foods.

Muscle foods such as animal meat, poultry and fish are important but perishable commercial products. Raw muscle foods are usually kept at refrigerator temperature or below to slow down deterioration caused by bacterial and enzymatic activities in the muscle. However, chemical reactions, such as lipid oxidation, protein denaturation and hydrolysis, can still occur in muscle foods during cold storage, leading to quality deterioration. Although the effects of storage conditions on sensory and nutritional properties of muscle foods have been reported (Aubourg et al., 2005; Caballero et al., 2009; Hultmann and Rustad, 2004; Thiansilakul et al., 2010; Yarnpakdee et al., 2012), there is a lack of

* Corresponding authors at: School of Food Science, Washington State University, Pullman, WA, 99164-6376, USA
E-mail addresses: rasco@wsu.edu (B.A. Rasco), yhuang1@wsu.edu, yiqunh@hotmail.com (Y. Huang).

http://dx.doi.org/10.1016/j.jfca.2017.07.033
Received 14 March 2017; Received in revised form 27 June 2017; Accepted 19 July 2017
Available online 26 July 2017
0889-1575/

studies on the levels of AGEs in unprocessed raw muscle foods as affected by cold storage conditions. What is more, there is no reported study on the formation of AGEs during heating as affected by the freshness of muscle food or storage conditions. Since muscle foods are high in AGEs and are major sources of dietary AGEs (Uribarri et al., 2010), it is important to understand the levels of AGEs in muscle foods as affected by cold storage conditions and the formation rates of AGEs during heating after cold storage, to eventually reduce the amounts of AGEs in muscle foods.

The objectives of this study were to use grass carp (*Ctenopharyngodon idellus*) and catfish (*Clarias leather*) as model systems to systematically investigate the effect of cold storage (or freshness of muscle foods) on the levels of protein-bound (protein glycation adducts) and free (glycated amino acids) CML and CEL in raw and heat-treated muscle foods. This is a continuation of our previous study on the levels of free and protein-bound AGEs in raw and heat-treated fish muscle (Niu et al., 2017). To obtain fish samples varying in freshness, white muscle from live and then immediately killed cultivated grass carp and catfish and those stored at 0 °C for up to 21 days were used. We selected cultivated carp and catfish as models for muscle food animals, since these were animals that it is possible to manage in a laboratory setting. We chose these cultivated fishes also because they could be obtained alive, providing the opportunity to closely control the storage conditions upon slaughter. Grass carp are herbivores; they represent one of the most widely cultivated fishes and are a major animal protein food source across the world. *Clarias* spp., known as "walking catfish", exhibit similarities with terrestrial animals. These fish are voracious omnivores that can breathe air at the water surface and underwater *via* a suprabranchial chamber in addition to retaining gills; the role these play in gas exchange allow the fish to thrive in hypoxic conditions. Furthermore, *Clarius* spp. can move for a short distance on land, using fins adapted for terrestrial locomotion (Lefevre et al., 2014a,b).

2. Materials and methods

2.1. Regents and AGEs standards

Chemicals purchased from Sinopharm (Shanghai, China) included analytical grade trichloroacetic acid, sodium borate, boric acid, sodium borohydride, sodium hydroxide, chloroform, hydrochloric acid, as well HPLC-grade hexane and methanol. Ammonium acetate and formic acid (HPLC grade) were bought from Sigma (St. Louis, MO). Standards of d_4-CML, CML and CEL (all 98% purity) were from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada).

The mixture of AGEs standards (CML 300 ng/mL, CEL 300 ng/mL, and d_4-CML 400 ng/mL) and internal standard d_4-CML (8 μg/mL) in methanol–water solution (80:20, v:v) were prepared right before being used for analysis (Niu et al., 2017).

2.2. Fish preparation

Live grass carp (3329 ± 343 g, $n = 12$) and catfish (2858 ± 273 g, $n = 24$) were purchased from a retailer in Shanghai, China. Immediately after being killed, fish were buried in ice and transported to the lab within 30 min (Niu et al., 2017). Then, 3 steaks each of 3.5-cm thickness (210 ± 36 g) were cut off from a catfish starting from the insertion of the dorsal fin towards the posterior; but for grass carp, 4 steaks each of 2.5-cm thickness (279 ± 28 g) were cut off using the insertion of the dorsal fin as the central line. The selection of these portions was to minimize sample variance that would occur in different portions of a fish from anterior to posterior, which means that the white muscle samples from the dorsal part of each of the selected portions in an individual fish could be considered as the same (Cao et al., 2016; Niu et al., 2017). Because of the size limitation of catfish, only three steaks were sampled from each individual catfish instead of four steaks. Each fish steak was sealed in a plastic bag after being patted dry with paper towels and weighed.

To understand the effects of fish freshness on the levels of AGEs in raw fish muscle as well as the influence of fish freshness on AGEs formation during heating, 4 steaks from each of 12 grass carp were buried under ice (0 °C) and stored for 0, 7, 14 and 21 days. For catfish, two sets of experiments each including 12 fish were conducted. Three steaks cut off from each catfish were either stored under ice for 0, 7, and 14 days, or stored for 0, 10, and 21 days.

During each of the storage periods, one steak from each individual fish was patted dry with paper towels. Following this, white muscle above the lateral line of a steak was cut off, homogenized (8010 s; Waring, Inc., Torrington, CT) at low speed for 20 s and then high speed for 10 s, sealed in a plastic bag and buried under ice until further use for compositional analysis or heat treatment.

2.3. Determination of water, protein and fat concentrations in raw fish muscle

The water, protein and fat contents of white muscle from each individual fish were determined based upon AOAC methods (AOAC, 2005): an oven drying method (AOAC 950.46) for water, a Kjeldahl method (AOAC 928.08) for protein, and a solvent extraction method (AOAC 991.36) for fat determination (Niu et al., 2017).

2.4. Heat treatments

Ground fish muscle (12.1 ± 0.1 g) was sealed into a cylindrical aluminum cell (diameter: 50 mm; internal height: 5 mm) (Kong et al., 2007), and heated in boiling water (100 °C) for 5 or 30 min (Niu et al., 2017).

2.5. Extraction of CML and CEL from fish muscle

Free CML and CEL were extracted from fish muscle following the method described in our previous publications (Niu et al., 2017; Sun et al., 2016). About 1 g of fish sample, together with 10 mL 5% trichloroacetic acid and 0.2 mL d_4-CML, was homogenized to precipitate proteins, and then centrifuged at 5000 rpm for 20 min (TDL-5-A; Shanghai Anting Scientific Instrument Factory, Shanghai, China). Following this, the supernatant was mixed with hexane (10 mL) and centrifuged (5000 rpm, 10 min) again to remove fat and residual protein, and the aqueous layer (5 mL) was loaded through an MCX column (60 mg/3 mL; ANPEL Laboratory Technologies Inc., Shanghai, China). Then, the eluate was dried under nitrogen at 60 °C, and dissolved in 80% methanol-water solution. Finally, the solution was filtered through a 13 mm × 0.22 μm hydrophilic PTFE syringe filter (ANPEL Laboratory Technologies Inc., Shanghai, China), and kept at −20 °C until further analysis.

The extraction of protein-bound CML and CEL from fish muscle was based on the method described in detail in our previous publications (Niu et al., 2017; Sun et al., 2015). In brief, about 0.2 g fish muscle were reduced with 0.4 mL sodium borohydride (2 M) in 2 mL borate buffer (0.2 M, pH 9.2) overnight. Then, a mixture of 4 mL chloroform-methanol (2:1) was used to defat and precipitate proteins, and the proteins were hydrolyzed with 4 mL 6 M HCl at 110 °C for 24 h. Following this, the hydrolysate was spiked with d_4-CML and further cleaned up with an MCX column and a 0.22-μm filter, following a similar procedure to that for free CML and CEL extraction.

2.6. HPLC–MS/MS analysis

A Waters 2695 HPLC system coupled with a Waters Quattro Micro triple quadrupole tandem mass spectrometer operating in positive electrospray ionization mode (Waters Corp., Milford, MA) was used to analyze both free and protein-bound CML and CEL in the extracts of fish