Food Chemistry 131 (2012) 170–174

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

N^{ε}-(carboxymethyl)lysine content of foods commonly consumed in a Western style diet

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ARTICLE INFO

Article history:
Received 14 April 2011
Received in revised form 7 August 2011
Accepted 22 August 2011
Available online 27 August 2011

Keywords:
Advanced glycation end-products (AGEs)
Advanced lipoxidation end-products (ALEs)
N^{ε}-(carboxymethyl)lysine (CML)
Food processing
Western diet

ABSTRACT

The potential adverse effects on health of diet-derived advanced glycation end-products (AGEs) is of current interest, due to their proposed involvement in the disease progression of diabetic and uraemic conditions. However, accurate information about levels of AGEs in foods is lacking. The objective of this investigation was to determine the level of one particular AGE, N^{ε}-(carboxymethyl)lysine (CML), a marker of AGE formation, in a wide range of foods commonly consumed in a Western style diet. Individual foods (n = 257) were mixed, lyophilised, ground, reduced, fat-extracted, hydrolysed, and underwent solid-phase extraction. Extracts were analysed by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS). Cereal (2.6 mg/100 g food) and fruit and vegetable (0.13 mg/100 g food) categories had the highest and lowest mean level of CML, respectively, when expressed in mg/100 g food. These data can be used for estimating potential consumer intakes, and provide information that can be used to educated consumers on how to reduce their CML intake.

1. Introduction

Advanced glycation endproducts (AGEs) or advanced lipoxidation endproducts (ALEs) are formed when protein reacts with reducing sugar or lipid oxidation products (Bucala & Cerami, 1992; Singh, Barden, Mori, & Beilin, 2001) during food processing, cooking, and storage (Ahmed, 2005; Buser & Erbersdobler, 1986; Goldberg et al., 2004). Formation of AGE/ALEs involves a complex set of reactions between the carbonyl group of a reducing sugar or lipid oxidation product, with typically the epsilon amino group of lysine residues within protein (Ames, 2008). AGE/ALEs are a complex and heterogeneous group of molecules (Ahmed, 2005). (Carboxymethyl)lysine (CML) is one of the best characterised AGE/ALEs and is frequently used as a marker of AGE/ALE formation in foods (Erbersdobler & Somoza, 2007; Fu et al., 1996; Goldberg et al., 2004). CML can be formed through a number of different pathways. The condensation reaction between glucose and the ε-amino group of lysine forms fructoselysine, an Amadori rearrangement product (ARP), which is oxidised to form CML. Glyoxal (GO), which can be formed from the oxidation of glucose and lipid peroxidation, can also react directly with the ε-amino group of lysine, forming CML (Ames, 2008).

Currently there is some controversy about whether or not dietary AGEs are a risk to human health. In 2007, a debate was held to set out and discuss the opposing points of view (Ames, 2007; Šebeková & Somoza, 2007), but further research is needed to establish whether dietary AGEs are of concern and, if so, for which subject cohorts. It has been reported that dietary CML significantly increases circulatory CML levels (Uribarri et al., 2005; Vlassara & Palace, 2002), thus providing conditions that may favour the development of glycation and inflammatory associated diseases, such as renal failure and diabetes (Koschinsky et al., 1997; Uribarri et al., 2005). Therefore in order to minimise any associated disease progression, it is important to accurately determine the potential CML intake from the consumption of food.

Numerous analytical methods have been reported for the detection and quantification of CML in food (Ahmed et al., 2005; Assar, Moloney, Lima, Magee, & Ames, 2009; Chao, Hsu, & Yin, 2009; Charissou, Ait-Ameur, & Birlouez-Aragon, 2007; Delgado-Andrade, Seiquer, Navarro, & Morales, 2007; Dittrich et al., 2006; Drusch, Faist, & Erbersdobler, 1999; Fenaille et al., 2006; Goldberg et al., 2004; Hartkopf & Erbersdobler, 1994; Hegele, Buetler, & Delatour, 2008; Niquet-Léridon & Tessier, 2010; Tauer, Hasenkopf, Kislinger, Frey, & Pischetsrieder, 1999); however only limited data are available from highly specific and validated instrumental methods. These include GC–MS in powdered infant formula, liquid infant formula, cookies, toast, corn flakes, and grilled, baked or steamed, salmon and beef (Charissou et al., 2007), LC–MS/MS in raw and processed bovine milk (Hegele et al., 2008), and

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doi:10.1016/j.foodchem.2011.08.055

UPLC–MS/MS in milks processed at different temperatures, butter, cheese, infant formulae, bread, raw and cooked minced beef, and olive oil (Assar et al., 2009).

The aim of this investigation was to employ an instrumental method (UPLC–MS/MS) to determine the CML content of a wide range of foods (n = 257) typically consumed in a Western style diet, and to establish the effect of food processing on the level of CML formation in food.

2. Materials and methods

2.1. Materials

Boric acid 99.5%, sodium hydroxide 98%, sodium borohydride, trifluoroacetic acid (TFA), trichloroacetic acid (TCA), nonafluoropentanoic acid (NFPA) 97%, acetonitrile (HPLC grade), l-lysine, and C_{18} (Supelclean™ LC-18) SPE tubes were purchased from Sigma (Gillingham, UK). Other chemicals and their suppliers are as follows: methanol (HPLC grade; VWR International, UK), hydrochloric acid 37% (J.T. Baker, Deventer, The Netherlands), chloroform (Bios Europe, Skelmersdale, UK), CML, D_2-CML (NeoPMS Strasbourg, France), D_4-lysine (Cambridge Isotopes, Andover, MA).

2.2. Food selection

Foods for analysis were selected using a population-based study of young adults in Northern Ireland with detailed dietary data (1229 foods and beverages), to determine the most commonly eaten foods in a typical Western diet. A total of 257 foods were chosen based upon their high frequency of consumption amongst a Northern Irish population, where diet was assessed using the 7-day diet history methods in a representative sample of 20–25 year olds (Gallagher et al., 2002).

2.3. Source and preparation of foods

Foods were purchased from local supermarkets, and eating establishments. They were stored according to the manufacturer's instructions and used prior to their best-before date.

2.4. Preparation of cooked foods

Foods (if applicable) were prepared according to the manufacturers' instructions, or using recipes commonly used in Western-style cooking. Foods were prepared for standard cooking times with commonly used cooking methods, such as boiling, grilling, frying, and roasting.

2.5. Preparation of samples

Briefly, an amount of food equivalent to at least a representative serving was cooked and allowed to cool to room temperature (if applicable) (n = 1), and non-edible parts of the food sample were removed, such as bone. The food sample was then blended (Waring Lab Blender, Waring Laboratory, Torrington, CT), lyophilised (Modulyod Freeze dryer, Thermo-Electron Corporation, Waltham, MA) and ground (IKA A11 basic; IKA-Werke GmbH, Staufen, Germany). Samples were then extracted (in triplicate), based on the method reported by Assar et al. (2009). A quantity of food, equivalent to 6 mg protein (using food label panel data), was reduced overnight at 4 °C in sodium borate buffer (0.5 M, pH 9.2, 400 μL) and 2 M sodium borohydride (200 μL) in 0.1 M NaOH. Protein was isolated using chloroform:methanol (2:1, v:v, 3 × 1 mL), and then hydrolysed with 6 N HCl (1 mL) at 110 °C for 24 h. The acid was removed under vacuum (Genevac evaporator (EZ-2), Genevac, Ipswich, UK), and the dried hydrolysate was reconstituted in distilled water (1 mL), followed by addition of internal standard to an aliquot equivalent to 200 μg protein, which was then solid phase-extracted using a C_{18} cartridge.

2.6. Determination of equivalent protein in protein hydrolysates

The concentration of equivalent protein in the hydrolysates was determined by the rapid fluorescamine method developed by Yaylayan, Huyghues-Despointes, and Polydorides (1992). Briefly, hydrolysate solution equivalent to 40 μg protein was diluted with potassium borate buffer (0.2 M, pH 8.5, 1 mL). Diluted hydrolysate (150 μL, equivalent to 6 μg protein), fluorescamine solution (0.3 mg/mL, 50 μL) and NaOH (1 M, 25 μL) were placed into the wells of a 96-well black plate. The plate was shaken for 3 min and the fluorescence intensity was measured (λ_{ex} = 390 nm, λ_{em} = 475 nm) using a Tecan Safire micro plate reader (Vector Scientific, Moneyrea, Northern Ireland). Hydrolysate BSA (0–10 μg equivalent protein) was used to prepare a standard calibration curve.

2.7. UPLC–MS/MS analysis

Lysine and CML concentrations of hydrolysates were determined by UPLC–MS/MS. Briefly, protein hydrolysates (5 μg protein, 5 μL) were injected onto a BEH C_{18} UPLC column (2.1 × 50 mm, 1.7 μm) housed in a column oven at 50 °C and operated in gradient elution mode. Solvent **A** was aqueous nonafluoropentanoic acid (NFPA, 5 mM) and solvent **B** was acetonitrile. Gradient elution was employed starting at 10% solvent **B** which was maintained for 0.4 min, followed by a linear gradient from 10% to 80% solvent **B** in 3.8 min, with a hold at 80% solvent **B** for 1.5 min, and then returned to 10% solvent **B** for 2 min. This produced a run time of 7.5 min. The analysis was performed using a Waters Acquity UPLC (Manchester, UK) coupled to a triple quadruple MS operating in multiple reaction monitoring (MRM) mode. The flow rate was 0.3 mL/min. The MS was operated in electrospray ionisation (ESI) positive mode using MRM. Lysine and CML were quantified with the use of isotopically labelled internal standards and by reference to an external standard calibration curve. Data were reported as mean ± standard deviation of triplicates.

3. Results

Food samples were extracted in triplicate and analysed by UPLC–MS/MS. The inter-day and intra-day variability of the UPLC system was calculated by analysing (n = 3) a high, medium and low CML-containing sample, over five consecutive days (inter-day), and four times on the same day (intra-day). The reproducibility of the extraction procedure was examined by extracting a high, medium, and low CML-containing sample 10 times. The coefficients of variation obtained for the reproducibility tests described above were <8%. The LOD (0.5 mg/kg protein) and LOQ (1.6 mg/kg protein) were calculated as 3 and 10 times the standard deviation of the sample blank, respectively. The CML content of the food items are summarised in Tables 1–3, and the CML content of each individual food item can been seen in Appendices 1–11, where food items are separated into food categories. The amount of CML is expressed in mg/kg protein, mg/100 g food, mmol/mol lysine (for comparison with published data), and mg/average portion size (for estimating average consumer consumption). The average level of CML found in the foods analysed was 123.0, 1.05, 5.02 and 0.98, when expressed in mg/kg protein, mg/100 g food, and mmol/mol lysine and mg/average portion size, respectively. When expressed in mg/g protein the lowest amount of