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Reduction of Nε-(carboxymethyl) lysine by (−)-epicatechin and (−)-epigallocatechin gallate: The involvement of a possible trapping mechanism by catechin quinones

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ABSTRACT

(−)-Epicatechin (EC) and (−)-epigallocatechin gallate (EGCG) (0.001–5%) were found to reduce Nε-(carboxymethyl) lysine (CML) concentrations by up to 0.80 ± 0.11 mM and 0.85 ± 0.07 mM, respectively, as quantified by HPLC-MS during heating at 80 °C and 100 °C in a glucose-lysine model system at pH 5, 7 and 8, which mimic different food processing conditions. With the addition of 1 mM EC, the free CML concentration in coconut milk was reduced to 5.7 ± 0.1 μg/mL (~2.8×10^{-2} mM), which was significantly lower than that in unheated coconut milk (6.1 ± 0.2 μg/mL (~3.0×10^{-2} mM), $p < 0.05$) indicating that the free CML may be eliminated by the addition of EC in coconut milk. Products of EC/EGCG quinones reacted with CML by a Michael addition or Schiff base addition in model systems were tentatively identified by UPLC-ESI-MS/MS.

1. Introduction

Advanced glycation end products (AGEs) are mainly generated in the late stage of Maillard reaction, and are a heterogeneous group of oxidizing compounds (Ahmed et al., 2005; Uribarri et al., 2010). Nε-(carboxymethyl) lysine (CML), which has been well-characterized and extensively studied, is a typical marker of AGEs (Ames, 2008; Assar, Moloney, Lima, Magee, & Ames, 2009; Friess et al., 2003). CML is formed by the oxidation of Amadori products rearranged from Schiff base which is generated by the condensation of free lysine (or lysine residues) and glucose (or ascorbate autoxidation products) (Dunn et al., 1990; Nguyen, Fels-Klerx, & Boekel, 2014). In addition, CML can also be generated by modification of free lysine or lysine residues with glyoxal derived from glucose degradation, polyunsaturated fatty acid peroxidation and Schiff base decomposition (Han et al., 2013; Nguyen et al., 2014). CML is widespread in foodstuffs, with 0.3 mg/kg in raw milk, 11.2 mg/kg in fried minced beef, 37.1 mg/kg in white bread crust and ca. 527 mg/L in a special kind of Japanese soy sauce (Assar et al., 2009; Li et al., 2015). Chronic ingestion of dietary CML has been found to increase the protein bound CML accumulation in kidney, heart and lung in rats (Li et al., 2015a, 2015b), and to be associated with endothelial dysfunction, arterial stiffness and aging in mice (Grossin et al., 2015).

Finding feasible strategies for controlling Maillard reactions and formation of AGEs in foods have been the aim of extensive investigation over the last decades due to the impact on not only health, but also on color, flavor, protein digestibility and protein functionality (Lund & Ray, 2017). Discovering means that reduce the CML levels in foods during processing and storage is of great important to alleviate the adverse effects of CML. It has been proven that natural antioxidants, such as polyphenols from plants, can effectively inhibit the formation of AGEs (Wu, Hsieh, Wang, & Chen, 2009; Yokozawa & Nakagawa, 2004). Green tea contains a series of polyphenols with catechol structures known as catechins; (−)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG) and (−)-epigallocatechin (EGC), which are often used as food antioxidants (Yin, Hedegaard, Skibsted, & Andersen, 2014). EC and EGCG have been found to inhibit the formation of fluorescent AGEs by ~23.0% and ~46.5%, respectively, in a model system containing bovine serum albumin (BSA) at a molar ratio of catechin to BSA of 1:15 under physiological conditions (pH 7.4, 37 °C) (Nakagawa, Yokozawa, Terasawa, Shu, & Juneja, 2002). The inhibitory effects of EC and EGC on the formation of fluorescent AGEs have been found to be more effective than that of aminoguanidine (AG, a typical positive control) (Abdallah et al., 2016) in BSA-glucose or

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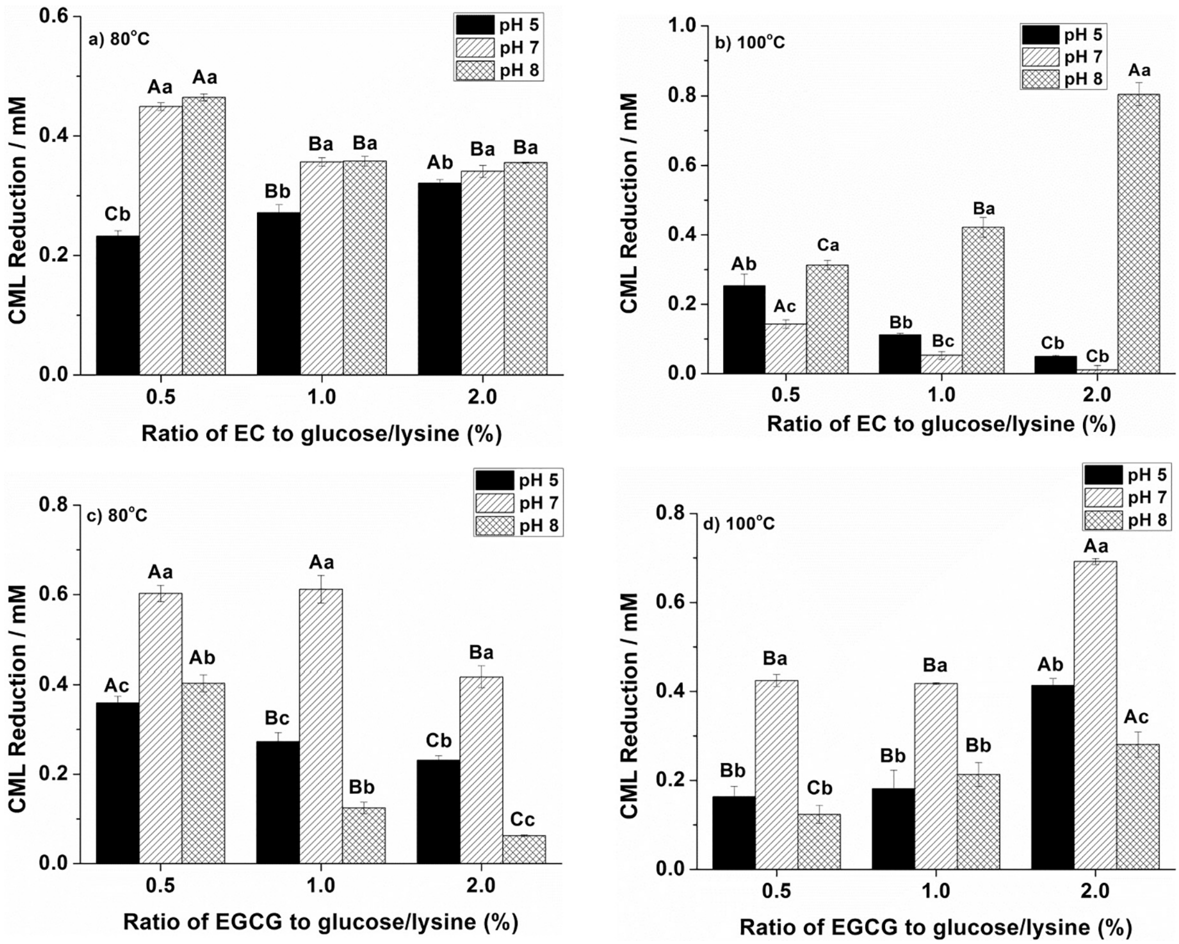

Fig. 1. Reduction of CML concentration at different EC or EGCG concentrations in model systems (0.1 M lysine and 0.1 M glucose) at pH 5, pH 7 and pH 8 incubated at 80 °C and 100 °C for 1 h. Bars followed by different lowercase letters in the same ratio of EC or EGCG to glucose/lysine are statistically different at $p < 0.05$. Different capital letters on the bars indicate the significant differences ($p < 0.05$) between different concentrations at the same pH value.

BSA-fructose systems (pH 7.4, 37 °C) (Yokozawa & Nakagawa, 2004). However, these studies have focused on the inhibition of fluorescent AGEs by catechins, and only few studies have reported effects specifically on CML formation.

The inhibitory mechanism of catechins on formation of AGEs has not been completely elucidated due to the complexity of the Maillard reactions, but the ability of catechins to trap α-dicarbonyls (such as glyoxal, a reactive Maillard intermediate) (Lo et al., 2006; Sang et al., 2007) and scavenging of free radicals formed during Maillard reactions have been widely accepted to play a role (Wu & Yen, 2005; Yin et al., 2014). There have been seldom reports on the investigation of the elimination of the existing CML in food systems by catechins. Catechol structures, such as catechins, exert their radical scavenging ability by donating the hydrogen atom of the phenolic hydroxyl group, and in effect they will be oxidized to *o*-benzoquinones via semiquinone radicals (Danilewicz, 2003; Jongberg, Gislason, Lund, Skibsted, & Waterhouse, 2011). Our previous study showed that 4-methylbenzoquinone (4MBQ, a model compound for *o*-benzoquinones) could react with CML through Michael addition with amine groups on CML (Li et al., 2018), thereby adding another type of mechanism to the action of polyphenols as inhibitors for AGEs formation. Thus, EC and EGCG quinones can also react with CML in theory, which needs to be validated.

Most studies of the inhibitory effect of catechins on the content of AGEs have mainly been performed under physiological conditions (pH 7.4, 37 °C) (Nakagawa et al., 2002; Yokozawa & Nakagawa, 2004). The stability of polyphenolic compounds is temperature- and pH-dependent, and the antioxidative and α-dicarbonyl trapping ability of catechins are altered under high temperature (Murakami, Yamaguchi, Takamura, & Atoba, 2004), alkaline conditions (Zhu, Zhang, Tsang, Huang, & Chen, 1997) or at pH ≤ 4 (Sang et al., 2007). The high temperature of food processing and wide range of pH values in food systems make it necessary to test the effects of catechins on the formation of CML under these conditions.

The aim of this study was to evaluate the effects of EC and EGCG on the formation of CML by HPLC-MS in a model system consisting of lysine and glucose and real food systems under various food processing conditions. Adducts formed via the reaction of catechin quinones with CML and lysine in model system were tentatively identified by UPLC-ESI-MS/MS.

2. Materials and methods

2.1. Chemicals and reagents

Nε-(carboxymethyl) lysine (CML, 98%) was purchased from TRC (Toronto, Canada). 4-Methylcatechol (≥95%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (HPLC grade) and acetronitrile (HPLC grade) was obtained from Oceanpak Alexative Chemical (Gothenburg, Sweden). (−)-Epigallocatechin gallate (EGCG, 98%), (−)-epicatechin (EC, ≥97%) and formic acid (HPLC grade) were purchased from Aladdin (Shanghai, China). All other reagents were of analytical grade.

Soy sauce, an amino acid functional drink, coconut milk and corn