Food Sci. Biotechnol. 26(2): 389-391 (2017)
DOI 10.1007/s10068-017-0053-y

Food Science
and
Biotechnology

Research Note

Effect of plant polyphenols on the formation of advanced glycation end products from β-lactoglobulin

Ling Liu[1,2], Rikke V. Hedegaard[1], and Leif H. Skibsted[1,*]

[1]*Food Chemistry, Department of Food Science, University of Copenhagen, 1165 København, Denmark*
[2]*The College of Food Science, Shenyang Agricultural University, Shenyang, China*

Received February 2, 2016
Revised May 12, 2016
Accepted December 29, 2016
Published online April 30, 2017

*Corresponding Author
Tel/Fax: +45-3533-3221
E-mail: ls@food.ku.dk

pISSN 1226-7708
eISSN 2092-6456

Abstract Dietary exposure to advanced glycation end products (AGEs) formed from proteins and reducing sugars is of increasing concern to human health. AGEs may form in protein-based powders containing sugars for instant beverages during drying and storage of the product. Chlorogenic acid, a plant phenol characteristic of coffee, was found to protect against the formation of AGEs at a concentration of 50 mM during heating of β-lactoglobulin in the presence of glucose as a reducing sugar in 30% aqueous ethanol at 70°C. Epicatechin, a plant phenol characteristic of green tea, had no similar effect for the equivalent concentration of phenol on the formation of AGEs. Immunochemical detection (ELISA) using polyclonal antibodies raised against AGEs showed a dose-dependent effect of protection by chlorogenic acid on AGE formation and is recommended for routine quality control of sugar containing milk-based powders for instant beverages.

Keywords: advanced glycation end products, chlorogenic acid, epicatechin, instant milk powder, ELISA

Introduction

Advanced glycation end products (AGEs) are formed in the later stages of Maillard reactions (MR) during heat processing of food as initiated by reactions of reducing sugars with amino groups mainly present in the protein side chain of lysine or arginine. Such dietary AGEs are formed in most types of heat processed food containing proteins and reducing sugars. For dairy products, MR are important both during production and storage of milk powders as well as powders for instant beverages and protein concentrates with intermediate water activity. AGEs are also formed *in vivo* and are an increasing health concern in relation to diabetes and cardiovascular diseases. Details of the formation of AGEs, the structure of AGEs, and their effects in the body have recently been reviewed (1). For certain dairy products, addition of plant extracts containing plant phenols and carotenoids is expected to protect milk proteins against both oxidation and browning reactions. Such extracts include coffee and tea products that are mixed with sugars and milk powders for instant beverages. Although it was found that the formation of AGEs is inhibited *in vivo* by chlorogenic acid from coffee and epicatechin from tea (2,3), chlorogenic acid and other plant polyphenols may not have the same effect at the elevated temperature during food processing, where the plant polyphenols may become partly oxidized. In addition, plant polyphenols may alter the conformation of proteins, exposing reactive groups to sugar derived reactants during heat processing and thereby affecting the formation of AGEs. Control of the level of AGEs in milk powders and protein may be a future challenge for the dairy industry.

The effect of flavonoid epicatechin from green tea on the development of MR and the formation of AGEs was recently investigated in a low molecular weight model system, and epicatechin was found to have an inhibitory effect on the reaction between lysine and glucose (4). However, the effects of plant polyphenols on the formation of AGEs in heat processed proteins that are of importance in dairy products are largely unknown.

The aim of the present study was to examine two of the most relevant phenolic antioxidants as possible inhibitors in the formation of AGEs from reactions between β-lactoglobulin as a reactive milk protein and glucose as a reducing sugar when their mixture in a homogenous solution was exposed to conditions similar to heat treatment of food. To mimic the conditions of the reactions of AGEs *in vivo*, including the biological interaction with specific receptors (5), an immunochemical approach (ELISA detection) was preferred to more traditional analytical chemical methods.

Materials and Methods

Chemicals β-Lactoglobulin and (−)epicatechin were from Sigma-Aldrich (St. Louis, MO, USA). D-(+)-Glucose, gelatine, Tween 20, and

chlorogenic acid (95%) were from Sigma-Aldrich. Ethanol (99%) was from Kemetyl A/S (Køge, Denmark). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (H+at) (ABIN237503) was from Antibodies-online GmbH (Aachen, Germany), rabbit anti-AGE101 polyclonal antibody was from Biologo (Kronshagen, Germany), and TMB-one substrate was from Kem-En-Tec (Copenhagen, Denmark). Water was purified through a Millipore Q-plus purification train from Millipore Corporation (Billerica, MA, USA).

Detection of AGEs using ELISA The content of AGEs was determined by an indirect ELISA method using the polyclonal antibody against AGEs as described previously (6). Briefly, the MR mixtures were diluted 100 times by a coating buffer (0.10 M sodium bicarbonate, pH 9.6) prior to adding 100 μL/well of the samples to a 96-well plate from VWR (Albertslund, Denmark), which was left overnight at 4°C. Then the plate was washed three times with phosphate buffered saline (PBS, pH=7.4) containing 0.050% Tween 20 (PBST) and incubated for 2 h at room temperature after the addition of 300 μL/well blocking buffer (0.50% gelatine in PBST). The plate was washed three times with the PBST buffer and again incubated for 1 h after the addition of 100 μL/well of primary polyclonal antibody AGE101 (diluted 4:10,000 dissolved in the PBS buffer). The plate was washed three times with the PBST buffer and incubated at room temperature for 1 h with 100 μL/well of secondary polyclonal antibody HRP conjugated goat anti-rabbit IgG (H+L) (diluted 4:10,000 in the PBS buffer). Non-protein bound antibodies were removed by washing the plate five times with the PBST buffer, TMB-one substrate was added (100 μL/well), and the plate was left in dark conditions for 30 min. The reaction was finally stopped by adding 100 μL/well of 0.30 M H_2SO_4, and the absorbance was measured at 450 nm using a GENios plus ELISA reader from TECAN (Mannedorf, Switzerland). The development of AGEs was expressed as changes in absorbance at 450 nm. All samples were prepared in triplicate.

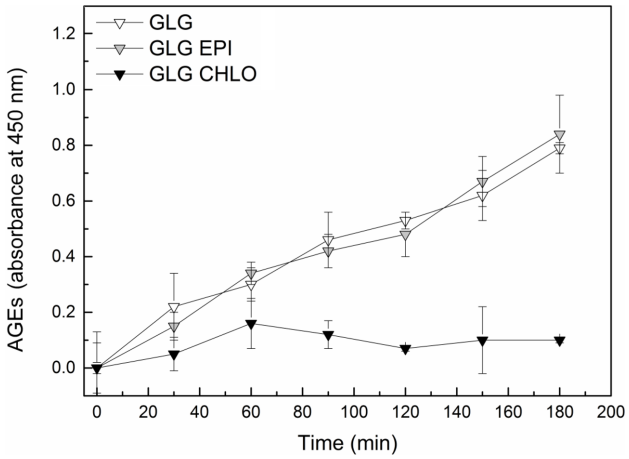

Fig. 1. Formation of advanced glycation end products (AGEs) as determined using ELISA for heating at 70°C for up to 180 min for (i) β-lactoglobulin (2.0 mM) (GLG) with glucose (2.0 M), (ii) β-lactoglobulin (2.0 mM) with glucose (2.0 M) and chlorogenic acid (50 mM) (GLG CHLO), or (iii) β-lactoglobulin (2.0 mM) with glucose (2.0 M) and epicatechin (25 mM) (GLG EPI). To ensure a homogeneous reaction, 30% (v/v) aqueous ethanol was used as the solvent.

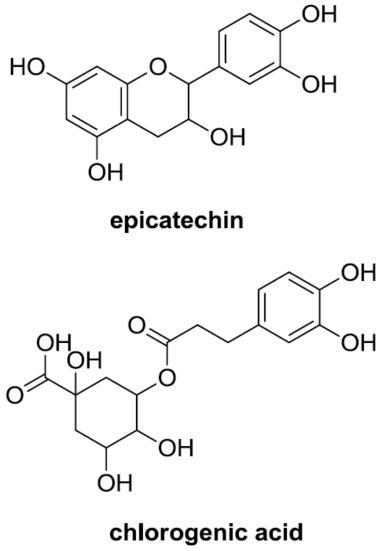

Fig. 2. Structure of the plant phenols epicatechin from green tea and chlorogenic acid from coffee

Results and Discussion

The formation of AGEs was determined in MR mixtures dissolved in 30% (v/v) aqueous ethanol at 70°C for up to 180 min in closed glass containers. The MR mixtures contained either (i) glucose (2.0 M) with β-lactoglobulin (2.0 mM), (ii) glucose (2.0 M) with β-lactoglobulin (2.0 mM) and chlorogenic acid (10 or 50 mM, investigated), or (iii) glucose (2.0 M) with β-lactoglobulin (2.0 mM) and epicatechin (5, 10, and 25 mM, investigated). A mixture of ethanol and water was preferred as the reaction solvent to ensure a homogenous solution and avoid heterogeneous catalysis. This approach is also in agreement with previous investigations of reactions of reducing sugars with whey proteins (6-8).

Inhibition of the formation of AGEs by the phenolic antioxidants (Fig. 1) was measured using ELISA as changes in absorbance at 450 nm as an indication of the relative level of AGEs. High concentration of chlorogenic acid (50 mM) was found to prevent the formation of AGEs, as shown in Fig. 1. Chlorogenic acid had no effect when tested at the lower concentration of 10 mM. Epicatechin did not inhibit the formation of AGEs at any of the investigated concentrations. The concentration used for epicatechin was adjusted for the presence of four phenol groups as compared to that of two phenolic groups in chlorogenic acid (Fig. 2) to provide conditions for direct comparison of reactivity for the two plant phenols. Chlorogenic acid is clearly seen to inhibit the formation of AGEs at a concentration of 70 mM at 70°C. Epicatechin, which is known as a good inhibitor of MR at room

temperature, has no significant effect on the inhibition of AGE formation at the high temperature used to model heat processing of food.

The mechanism behind the inhibition of the formation of AGEs by plant polyphenols is still speculative (6). Plant polyphenols may scavenge radicals formed during the browning reactions as reactive intermediates. However, a reaction mechanism in which partly oxidized forms of polyphenols react with lysine side chains of proteins is more likely to occur, thus blocking the amino group and preventing reaction with carbonyl groups of sugars or their degradation products (7). The higher reactivity of chlorogenic acid is suggested to relate to its higher acidity as compared to epicatechin, resulting in the electrostatic binding of the anion form of chlorogenic acid to the reactive lysine sites of proteins with positive charge at neutral pH (8).

Chlorogenic acid at a concentration of 70 mM inhibited the formation of AGEs from β-lactoglobulin during heating in the presence of glucose as a reducing sugar, whereas epicatechin used in the equivalent concentration had no inhibitory effect. These results should be of intermediate interest for the production of instant beverage products based on milk powder and sugars with added plant-based flavoring. Depending on the nature of the added plant extract, a milder heat treatment is recommended for some products. Further, the use of immunochemical detection using polyclonal antibiotics raised against AGEs may provide direct results related to the biological effects of AGEs and is recommended as an easy method for routine control in the food industry (9).

Disclosure The authors declare no conflict of interest.

References

1. Poulsen MW, Hedegaard RV, Andersen JM, de Courten B, Bügel S, Nielsen J, Skibsted LH, Dragsted LO. Advanced glycation endproducts in food and their effects on health. Food Chem. Toxicol. 60: 10-37 (2013)
2. Jang DS, Lee GY, Lee YM, Kim YS, Sun H, Kim DH, Kim JS. Flavan-3-ols having a gamma-lactam from the roots of actinidia arguta inhibit the formation of advanced glycation end products *in vitro*. Chem. Pharm. Bull. 57: 397-400 (2009)
3. Tsuji-Naito K, Saeki H, Hamano M. Inhibitory effects of *Chrysanthemum* species extracts on formation of advanced glycation end products. Food Chem. 116: 854-859 (2009)
4. Yin J, Hedegaard RV, Skibsted LH, Andersen ML. Epicatechin and epigallocatechin gallate inhibit formation of intermediary radicals during heating of lysine and glucose. Food Chem. 146: 48-55 (2013)
5. Reddy S, Bichler J, Wellsknecht KJ, Thorpe SR, Baynes JW. N-Epsilon-(carboxymethyl)lysine is a dominant advanced glycation end-product (AGE) antigen in tissue proteins. Biochemistry-US 34: 10872-10878 (1995)
6. Liu L, Hedegaard RV, Skibsted LH. Formation of advanced glycation end products (AGEs) are influenced by lipids in milk powders. Aust. J. Chem. 66: 1074-1079 (2013)
7. Yin J, Andersen ML, Skibsted LH. Reduction of ferrylmyoglobin by theanine and green tea catechins. Importance of specific acid catalysis. J. Agr. Food Chem. 61: 3159-3165 (2013)
8. Hedegaard RV, Liu L, Skibsted LH. Quantification of radicals formed heating β-lactoglobulin with glucose in aqueous ethanol. Food Chem. 167: 185-190 (2014)
9. Vlassara H, Stricker GE. AGE restriction in diabetes mellitus: A paradigm shift. Nat. Rev. Endocrinol. 7: 526-538 (2011)