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Prediction of CML contents in the Maillard reaction products for casein-monosaccharides model

Mi-Jin Oh[a], Yoonsook Kim[a], Sang Hoon Lee[a], Kwang-Won Lee[b], Ho-Young Park[a,*]

[a] Division of Functional Food Research, Korea Food Research Institute, Gyeonggi 13539, Republic of Korea
[b] Department of Food Bioscience & Technology, Korea University, Seoul 136-713, Republic of Korea

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ABSTRACT

Response surface methodology (RSM) was applied to predict the processing parameters of the casein–glucose/galactose Maillard reaction (MR) for determining the level of N^{ε}-(1-carboxymethyl)-L-lysine (CML), one of the typically harmful dietary advanced glycation end products (AGEs). The effect of industrial heating time and temperature of the MR on casein-glucose reactant (CGR) and casein-galactose reactant (CGaR) was evaluated. An increase in temperature and time was associated with an increased level of CML. A heating time of 114.8/117.9 min and a temperature of 145.1/148.8 °C maximised the formation of CML on CGR/CGaR and resulted in a CML production of 12.0/14.0 μg/mL. Evaluation of foam stability, SDS-PAGE, and energy filtering-TEM indicated that the CGR and CGaR had different characteristics. Moreover, level of intracellular reactive oxygen species was accumulated with increasing CML contents. In summary, RSM provided a basis for understanding CGR/CGaR-reactivity and for predicting the formation of CML in heat-treated milk products.

1. Introduction

The Maillard reaction (MR) is also known as the amino-carbonyl reaction or non-enzymatic browning reaction, which leads to complex changes in biological and food systems (Chuyen, 2006). Glycation during food processing leads to a reduction in the nutritional quality of food, the generation of toxic compounds such as 5-hydroxymethylfurfural (HMF) and acrylamide, and the formation of advanced glycation end products (AGEs). AGEs are heterogeneous compounds in the form of fluorescent cross-linking (e.g. pentosidine), non-fluorescent cross-linking (methylglyoxal-lysine dimers) and non-fluorescent non-cross-linking (N^{ε}-carboxymethyl-lysine (CML) and pyrraline) (Peng et al., 2008). AGEs may contribute to a decline in tissue and organ function with age and are related to chronic and degenerative disease, such as diabetes, renal failure (Šebeková & Somoza, 2007), atherosclerosis (Wang et al., 2012), and Alzheimer's and Parkinson's diseases (Li, Liu, Sun, Lu, & Zhang, 2012), as well as the formation of mutagenic compounds (Brands, Alink, van Boekel, & Jongen, 2000). The pathogenesis of this disease is due to the generation of oxidative stress such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) at the site of inflammation (Pham-Huy, He, & Pham-Huy, 2008).

Several AGEs, including N^{ε}-fructosyl-lysine (FL), pyrraline, pentosidine, CML, N^{ε}-carboxyethyl-lysine (CEL), S-carboxymethyl cysteine, glyoxal lysine dimer, methylglyoxal lysine dimer, and 3-deoxyglucosone lysine dimer, have been identified in processed foods (Henle, 2005). These markers of glycation can be formed through various pathways, such as via the condensation of glucose with the ε-amino group of lysine, where the Amadori product FL is produced as an unstable intermediate which subsequently undergoes oxidation to form CML (Poulsen et al., 2013).

Casein is the major protein in milk, accounting for almost 80% of total milk protein. It is composed of four subunits: α_{S1}-casein, α_{S2}-casein, β-casein, and κ-casein. α_{S1}-, α_{S2}-, and β-caseins are highly phosphorylated, and together with κ-casein and calcium phosphate they form colloidal protein particles called casein micelles (Yazdi, Corredig, & Dalgleish, 2014). The casein protein is used as the source of amino groups for the MR, and glucose, an aldose sugar, is the most prominently studied sugar in MR research and also the most abundant sugar in nature. According to a recent review, heat-treated dairy products showed higher concentrations of CML as compared to other food categories (0–1015 mg/kg protein) (Hull, Woodside, Ames, & Cuskelly, 2012). In addition, CML concentration increased at neutral pH compared to acidic pH, and at high temperature (Avila Ruiz et al., 2016).

Several methods have been developed to determine AGEs content in foods, including fluorescence measurement (Yanagisawa et al., 1998), and enzyme-linked immunosorbent assays (ELISAs) (Dittrich et al., 2006). To establish a predictive model of the effects of glycation on

* Corresponding author at: Division of Functional Food Research, Korea Food Research Institute, 516 Baekhyun-dong, Bundang-Gu, Gyeonggi 463-746, Republic of Korea.
E-mail address: hypark@kfri.re.kr (H.-Y. Park).

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CML level, response surface methodology (RSM), a valuable tool for simultaneous optimization of several process parameters in biological and chemical science (Ahmad, Liew, Yarmo, & Said, 2012), was employed in the present study to predict reaction parameters. Additionally, single factor tests were performed to optimize the main influential factors and ranges of parameters for RSM. Evaluation of the effects of glycation can be achieved using a smaller number of experiments (Tunga, Banerjee, & Bhattacharya, 1999) by building models based on orthogonal polynomial fitting techniques, and evaluating the effects of variable (main and interaction) model terms for the optimum response (Thys, Guzzon, Cladera-Olivera, & Brandelli, 2006).

The objective of this study was to monitor CML formation in casein-monosaccharide reactants (CMRs), using casein-glucose reactant (CGR) and casein-galactose reactant (CGaR) with RSM. The RSM method was suitable for evaluating heating temperature and time for CMRs and it was able to stimulate CML formation. We also evaluated physicochemical properties of CMRs and examined the hypothesis that CML-enriched CMRs is involved in intracellular ROS generation.

2. Materials and methods

2.1. Chemicals and materials

Casein, D-(+)-glucose, and D-(+)-galactose were purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyacrylamide gel and Coomassie brilliant blue R-250 were purchased from Bio-Rad (Hercules, USA). The CML ELISA kit was purchased from CircuLex (Nagano, Japan). 2′,7′-Dichlorofluorescin diacetate (DCF-DA) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Experimental design

RSM with a central composite design (CCD) was employed to predict CML production via industrial heating temperature (73–150 °C, X_1) and time (5–119 min, X_2). CCD with independent variables applied to a total of 13 experiments was found to be sufficient for calculating the coefficients of the model for two variables. Each variable was investigated at five levels: −1.414, −1, 0, 1, and 1.414 as shown in Supplementary Table 1. The Minitab 13.0 statistical software (Minitab Inc., State College, PA, USA) and Maple 7.0 software (Waterloo Maple Inc., Waterloo, Canada) were used to generate the experimental designs.

2.3. Preparation of CMRs

Casein mixtures that were not reacted with glucose or galactose were termed CGM and CGaM, respectively, and casein reacted with glucose and galactose reactants were termed CGR and CGaR, respectively. A model system of CMRs was modified from Gu et al. (2009). Briefly, casein (10 mM) and the monosaccharide (50 mM) were dissolved in a sodium phosphate buffer (50 mM, pH 8.5) to considering a solubility of CMRs molar ratio of casein to monosaccharide of 1:5. Then, CMRs were heated at different temperatures (73–150 °C) and for varying amounts of time (5–119 min) in a Block Heater (QBH2, Grant instrument, Cambridge, England), using a low temperature circulating bath with reflux condenser. The chosen heating temperature corresponds to sterilization temperatures usually used in the food industry. After a given heating time, CMRs were centrifuged at 2630 × *g* at 25 °C for 15 min and separated supernatants were stored at −70 °C.

2.4. Quantification of CML

CML was quantified using a CML-ELISA kit (CircuLex, Ltd, Nagano, Japan). CMRs mixed with anti-CML monoclonal antibody MK-5A10 were added to the wells of the ELISA plate on which anti-CML-BSA antibody was immobilized and allowed to react at ambient temperature for 1 h. After washing of the wells with the wash buffer provided with the kit, HRP-conjugated detection antibody was added and allowed to react at ambient temperature for 1 h. After washing of the wells again with wash buffer, tetramethylbenzidine was added and incubated for 10 min to develop colour. After the reaction was stopped, the absorbance at 450 nm was measured with a spectrophotometer. The CML concentration was calculated from the absorbance measurements using a calibration curve created with known concentrations (0.109–14.000 μg/mL) of CML-HSA.

2.5. Determination of CMRs by SDS-PAGE

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to a previously described method (Shen, Xu, & Sheng, 2017). After being heated at 100 °C for 5 min, aliquots of the sample solution (20 μL) were loaded into each sample channel on the electrophoresis gel. After the electrophoresis was completed, the gel was stained with Coomassie Blue R-250 for 10 min and destained in 3% acetic acid. SDS-PAGE was done using a 4% stacking gel and an 8% separating gel with a vertical gel electrophoresis unit (Bio-Rad, Hercules, CA, USA). Gel samples were mixed with samples buffer containing 2% SDS and 5% β-mercaptoethanol (ratio 1:2). The mixtures were then heated at 95 °C for 10 min before loading 10 μL of the mixtures to the gels. The samples were run at 200 V constant for approximately 35 min. Subsequently, the gels were soaked in a fixative solution consisting of 10% (v/v) acetic acid and 40% (v/v) methanol for 15 min. The gels were then rinsed with distilled water and stained with staining solution. After about 2 h of staining, the gels were rinsed with distilled water and destained with destaining solution of 10% acetic acid (v/v) for 24 h. The gels were photographed using an imaging system (ChemiDoc™ XRS + System with Image Lab™ Software, Bio-Rad). The protein fractions were identified using prestained protein ladder (Thermo scientific, Waltham, MA, USA).

2.6. Determination of foam stability

Foam stability was determined according to a previously described method (Augustin & Clarke, 2008) with slight modification. Five millilitres each of the CMRs and CMR-control solutions were whipped with 5 mL distilled water for 5 min at the highest speed at room temperature. The contents along with foam were immediately poured into a 10 mL measuring cylinder. Foam stability was determined by measuring the decrease in volume of foam as a function of a time at 10, 20, 30, 40, 50, and 60 min. The total volume and foam volume were recorded and expressed as a percentage of the developed foam volume in relation to the initial volume of the liquid sample according the following equation:

$$\textit{Foaming Stability}\ (\%) = \frac{\text{Foam volume after time (t)}}{\text{Initial foam volume } (t_0)} \times 100$$

t_0 = the starting time immediately after blending.
t = the time at which the foam volume in increase.

2.7. Monitoring particle size by EF-TEM

The micro-structure of CMRs and CMR-control micelles was investigated by energy filtered-transmission electron microscopy (EF-TEM) (30,000× magnification) according to a negative staining method. One drop of either CMR or CMR-control solution was deposited on to a carbon coated copper grid (200 mesh, 3 mm diameter). The excess of product was removed after 30 s using a filter paper. Subsequently, one drop of 2% uranyl acetate was placed on the grid for 2 min. After drying the grid at room temperature for 5 min, micrographs were created using a LEO 912 AB (Carl Zeiss, Germany) transmission electron microscope operating at 120 kV.