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Chemical properties and reactive oxygen and nitrogen species quenching activities of dry sugar–amino acid maillard reaction mixtures exposed to baking temperatures

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ABSTRACT

Maillard reaction products (MRPs) derived from 10 different, dry sugar–amino acid reaction model systems were examined for changes in color index (E), sugar loss, and formation of α-dicarbonyl compounds; the changes were correlated with relative activities to quench both reactive oxygen (ROS) and reactive nitrogen (RNS) species. Reducing sugars, xylose, ribose, fructose, glucose, and non-reducing sucrose were reacted with glycine (Xyl–Gly, Rib–Gly, Fru–Gly, Glc–Gly, and Suc–Gly), or lysine (Xyl–Lys, Rib–Lys, Fru–Lys, Glc–Lys, and Suc–Lys), respectively, at temperatures of 150 °C and 180 °C for time periods ranging from 5 to 60 min. ROS quenching capacity was negatively correlated with color index (E) ($r = -0.604$, $P < 0.001$), and positively correlated with sugar loss ($r = 0.567$, $P < 0.001$). MRPs also exhibited activity to quench RNS as assessed by nitric oxide (NO) inhibition in differentiated Caco-2 cells that were induced with interferon-γ (IFN-γ) and phorbol ester (PMA) cocktail. We also showed a correlation between RNS and color index, sugar loss, and ROS quenching activities for MR mixtures that were heated for a short time (e.g. 10 min) at 150 °C. MRP quenching of ROS was largely influenced by sugar type, whereas, RNS quenching was dependent more so on the interaction between reactants and reaction conditions used to generate MRPs.

1. Introduction

A typical Western diet comprising of roasted meat, bakery products, and coffee beverages contains Maillard reaction products (MRPs) (Fogliano and Morales, 2011). These products are generated when reducing sugars react with amino acids, peptides or proteins during heat processing. Recent studies have shown that the presence of MRPs in many thermally processed MR mixtures provides bioactive properties that quench both reactive oxygen (ROS) and reactive nitrogen (RNS) species (Chen & Kitts, 2011b; Chen, Chen, Chen, Zhang, & Kitts, 2015; Chen & Kitts, 2015). These findings accompany the more established positive and negative effects of MRPs reported on food sensory and nutritional quality, respectively (Waller & Feather, 1983). Some bioactive properties for MRPs include both pro- and anti-oxidant activities (Cai et al., 2002; Chen & Kitts, 2008a; Chen & Kitts, 2012; Kitts, Chen, & Jing, 2012), carcinogenesis (Skog, Johansson, & Jägerstad, 1998) or anti-cancer activity (Marko et al., 2003), genotoxicity and mutagenicity (Glösl et al., 2004; Kitts, Wu, Stich, & Powrie, 1993), and pro- and anti-inflammatory activities (Chen & Kitts, 2011b; Chen & Kitts, 2015; Chen et al., 2015; Muscat et al., 2007).

The capacity of MRPs to quench ROS is influenced by various factors such as the concentration and type of reactants, starting pH, duration of reaction, temperature, water activity, and the presence of buffering agents, metal ions or salts (Jing & Kitts, 2002; Jing & Kitts, 2002; Matmaroh, Benjakul, & Tanaka, 2006; Wijewickreme & Kitts, 1997). Our previous study using MRPs derived from aqueous reaction systems reported that the type of sugar was the most important variable in evoking MR browning, the generation of α-dicarbonyl compounds, and capacity to quench ROS (Chen & Kitts, 2011c). Inhibition of nitric oxide by MRPs derived from heated aqueous glucose–lysine (Glu–Lys), in differentiated Caco-2 cells (Chen & Kitts, 2011b; Chen & Kitts, 2012; Chen & Kitts, 2015; Chen et al., 2015) has also been reported, albeit the major factors that influence NO inhibition in dry heat processing, typical of baking conditions, has not been clearly defined.

Herein, we examine the relationship between the bioactive properties of MRPs to quench both ROS and RNS, with pigment development and formation of α-dicarbonyl compounds, when generated from dry reactants processed at two moderate temperatures used in baking.

Abbreviations: ACN, acetonitrile; 3-DG, 3-deoxyglucosone; 3-DP, 3-deoxypentosone; DPPH, 2,2-diphenyl-1- picrylhydrazyl radical; 3-MCP, 3-methyl-1,2-cyclopentanedione; DAN, 2,3-diaminonaphthalene; Fru, fructose; Glc, glucose; Gly, glycine; GO, glyoxal; Lys, lysine; MEM, minimum essential medium; MGO, methylglyoxal; MR, Maillard reaction; MRPs, Maillard reaction products; NO, nitric oxide; PMA, phorbol ester; Rib, ribose; RNS, reactive nitrogen species; ROS, reactive oxygen species; Suc, sucrose; Xyl, xylose.

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The contribution of reactants and reaction conditions on the changes in multiple physicochemical and functional properties such as ROS and RNS quenching activity of MRPs and their correlations was evaluated to predict the potential antioxidant and anti-inflammatory activities.

2. Materials and methods

2.1. Preparation of MRPs

Four reducing sugars, including xylose (Xyl), ribose (Rib), fructose (Fru), glucose (Glc), and a non-reducing sugar, sucrose (Suc), were reacted with glycine (Gly) and L-lysine (Lys) (Sigma, St Louis, MO, USA), respectively, at a sugar to amino acid ratio of 0.01 mol: 0.01 mol. The dry mixtures were mixed evenly in a coffee grinder and heated in the 100 mL glass beaker without cover at 150 °C and 180 °C, respectively, for 5, 10, 20, 40, and 60 min time periods in a convection oven (Blue M Electric Company, Blue Island, Illinois, USA). After baking, samples were rapidly chilled on ice and ground to a powder, before being stored at −20 °C for further analysis. All experiments were performed in triplicate.

2.2. Color measurement

Colorimetric measurements were performed with a HunterLab Labscan 600 spectrocolorimeter, using a 5-cm-diameter aperture and Labscan II software (version 3.0) (Hunter Associates Laboratory Inc., VA, USA) according to the method described by Jing, Yap, Wong, & Kitts (2011). The Hunter scale parameters were expressed as L = Lightness (0 = black, 100 = white), a = red or green (+a = red; −a = green) and b = yellow or blue (+b = yellow; −b = blue). The color index (E) was calculated according to Eq. (1) (Morales & Van Boekel, 1998).

$$E = \left(L^2 + a^2 + b^2\right)^{1/2} \quad (1)$$

2.3. Determination of sugar losses

The recovery of pentose, hexose sugars and sucrose from heated reaction mixtures over time was measured using HPLC (Agilent 1100 LC series, Agilent, USA) equipped with a refractory index detector. Samples were dissolved in 50% acetonitrile (ACN) aqueous solution at a concentration of 20 mg/mL and separated by a Zobax carbohydrate analysis column (4.6 × 150 mm, 5 μm, Agilent, USA). MR samples and standards (5 μL) were eluted by 75% ACN aqueous solution for 8 min with a flow rate of 1.2 mL/min. The percentage (%) of sugar loss in the heated mixture was calculated.

2.4. Determination of α-dicarbonyl compounds

α-Dicarbonyl compounds were derivatized and quantified according to Chen and Kitts (2011c) with minor modification. Briefly, 1 mL of 10 mg/mL samples, or varying concentrations of standards (glyoxal (GO) and methylglyoxal (MGO) (Sigma, St Louis, MO, USA); glucosone and 3-deoxyglucosone (3-DG) (Toronto Research Chemicals Inc., Toronto, Canada)) in 10 mmol/L phosphate buffer (pH 7.4) were incubated with 2,3-diaminonaphthalene (DAN) (100 μL; 5 mg/mL) (Sigma, St Louis, MO, USA) overnight at room temperature, followed by the addition of 400 μL of ACN. Benzoquinoxaline derivatives were quantified using HPLC (Agilent 1100 LC series, Agilent, USA) equipped with a Sphereclone ODS2 column (4.6 × 150 mm, 3.5 μm, 80 Å; Phenomenex, Torrance, CA, USA). Samples (10 μL) were eluted using a step-wise gradient of ACN and 0.2% formic acid as follows: 0–13 min, 28–45% ACN; 13–20 min, 45–85% ACN; and 20–23 min, 85% ACN at a flow rate of 1 mL/min with a temperature control at 30 °C. Compounds were detected using a fluorescence detector with excitation at 267 nm and emission at 503 nm. Pentosone and 3-deoxypentosone (3-DP) were quantified based on standard curves generated from glucosone and 3-DG, respectively. The amount of α-dicarbonyl compounds is expressed as μg/g dry matter.

2.5. Measurement of oxygen radical absorbance capacity

Chemical-based antioxidant activity was determined using the oxygen radical absorbance capacity (ORAC) method described by Chen and Kitts (2008a). ORAC was expressed as μmol Trolox/g dry matter (d.m.).

2.6. Measurement of NO in Caco-2 cells

NO production was measured in IFN-γ + PMA induced Caco-2 cells as described by Chen and Kitts (2008b). Caco-2 cells were seeded in 96-well plates at a density of 10^5 cells/cm^2, cultured in minimum essential medium (MEM). After three weeks, cells were incubated with or without MRPs for 24 h. MRPs or culture medium was removed and cells were then stimulated with 8000 U/mL IFN-γ + 0.1 μg/mL PMA for 48 h. NO levels were determined in the culture medium using the Griess reagent after reducing nitrate to nitrite with nitrate reductase (Sigma, St. Louis, MO, USA). The % inhibition was calculated using Eq. (2):

$$(NO_{st} - NO_{sample}) / (NO_{st} - NO_{blank}) \times 100\% \quad (2)$$

where NO_{st} is the NO concentration of the cell supernatant incubated with IFN-γ + PMA for 24 h; NO_{blank} is the NO concentration of the cell supernatant without sample and inducers; NO_{sample} is the NO concentration of cells incubated with MRPs for 24 h and then stimulated with IFN-γ + PMA for 48 h. The cell viability was tested using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma, St. Louis, MO, USA) assay described by Chen and Kitts (2011b). Experiments were conducted with cell viabilities that were greater than 95%.

2.7. Statistics

A 4-way analysis of variance (ANOVA) was performed using the general linear model (GLM) of MINITAB software (Version 14, Minitab Inc., State College, PA., U.S.A.) to investigate the effects of various factors (type of sugar and amino acid, reaction time and reaction temperature) and the interactions among them. Significant differences among treatments were compared using Tukey's test. Data are expressed as means ± SD of triplicate experiments with $P < 0.05$ representing a statistically significant difference. The correlations between the chemical properties and ROS and RNS scavenging activities were also determined using MINITAB.

3. Results and discussion

3.1. Color index (E)

Color was assessed using the *E* index; a decrease in *E* is related to the shift from lightness to darkness (Morales & Van Boekel, 1998). The formation of color in foods during heat processing is contributed to non-enzymatic browning, generated from both MR and caramelization. Caramelization occurs more so at high heating temperature (>120 °C) along with extreme pH (<3 or >9) (Reyes, Poocharoen, & Wrolstad, 1982). Dramatic decreases in *E* index occurred within 5 min of heating at both 150 °C and 180 °C temperatures in pentose–amino acid models (Xyl–Gly, Xly–Lys, Rib–Gly, and Rib–Lys) (Fig. 1A and B). The *E* index decreased faster in ribose–amino acid compared to xylose–amino acid MR models and at a lower baking temperature, indicating a relatively faster MR rate for the ribose. In hexose–amino acid models, the *E* index decreased faster in MR models that contained fructose compared