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Impacts of selected dietary polyphenols on caramelization in model systems

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ABSTRACT

This study investigated the impacts of six dietary polyphenols (phloretin, naringenin, quercetin, epicatechin, chlorogenic acid and rosmarinic acid) on fructose caramelization in thermal model systems at either neutral or alkaline pH. These polyphenols were found to increase the browning intensity and antioxidant capacity of caramel. The chemical reactions in the system of sugar and polyphenol, which include formation of polyphenol-sugar adducts, were found to be partially responsible for the formation of brown pigments and heat-induced antioxidants based on instrumental analysis. In addition, rosmarinic acid was demonstrated to significantly inhibit the formation of 5-hydroxymethylfurfural (HMF). Thus this research added to the efforts of controlling caramelization by dietary polyphenols under thermal condition, and provided some evidence to propose dietary polyphenols as functional ingredients to modify the caramel colour and bioactivity as well as to lower the amount of heat-induced contaminants such as 5-hydroxymethylfurfural (HMF).

1. Introduction

Caramelization is a type of non-enzymatic browning reactions which proceed during thermal processing and storage of food products. The reaction starts with heating of carbohydrates and contributes to the formation of both volatile and non-volatile products associated with food flavour and colour. The characteristics of final caramel depend on the type and concentration of sugar as well as the preparation conditions such as heating temperature, duration and pH (Fadel & Farouk, 2002). Compared with volatile compounds whose identity has been well studied, chemical characterization of the diverse non-volatile caramelization products is poorly documented due to the lack of powerful analytical techniques in the past. Combined mass spectrometric techniques have been recently applied and taking advantage of this technology, scientists have identified a few categories of non-volatile caramel components including carbohydrate oligomers, dehydration and hydration products of sugar oligomers, disproportionation products, and coloured aromatic products (Golon & Kuhnert, 2012).

Knowledge of caramel as a class of natural pigments for food additive has a long history over a hundred years (Myers & Howell, 1992), but the colour development along with the process of caramelization is still not well understood (Quintas, Brandao, & Silva, 2007). It is generally believed that the brown colour of caramel is originated from polymeric caramelization products, with 5-hydroxymethylfurfural (HMF) and furfural as main precursors (Kroh, 1994). Previous studies attempted to correlate colour development with HMF content but the results suggested that HMF alone is not sufficient to account for colour formation (Quintas et al., 2007). In addition to contribution towards food browning, the non-volatile fraction of caramelization products was also found to possess antioxidant potential. Some studies revealed that caramel had DPPH and ABTS radical scavenging activity, reducing power as well as metal chelating activity, and could be utilized to prevent lipid oxidation (Benjakul, Visessanguan, Phongkanpai, & Tanaka, 2005; Phongkanpai, Benjakul, & Tanaka, 2006; Rhee & Kim, 1975; Tsai, Yu, Chen, Liu, & Sun, 2009; Woo et al., 2011). Other studies further pointed out that the brown pigments in caramel, instead of the colourless caramelization intermediates, likely contributed to antioxidant activity (Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001; Tsai et al., 2009).

In real foods, a variety of sources involving different chemical species and reaction pathways may contribute to the brown colour and antioxidant capacity of foods (Manzocco et al., 2001). Dietary polyphenols are a wide class of plant secondary metabolites present in food, which are structurally characterized by the presence of multiples of phenol unit. They are reputable for high antioxidant activity which contributes to health benefits associated with a lower risk of age-related diseases. During thermal treatment of certain food products, such as date juice and maple syrup which are rich in both sugar and phenolic compounds, enzymatic or chemical

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oxidation of polyphenols proceeds simultaneously with caramelization and Maillard reaction and may influence the brown appearance and antioxidants content of foods (Abou-Zaid, Nozzolillo, Tonon, Coppens, & Lombardo, 2008; Al-Abid, Al-Shoaily, Al-Amry, & Al-Rawahy, 2007). As discussed above, neither caramelization nor polyphenol oxidation solely accounts for the browning or antioxidant capacity of processed foods. However, scarce study has ever investigated the combined influence of caramelization and dietary polyphenol oxidation on the overall colour and antioxidant capacity of foods. In addition, it remains unclear whether polyphenols are reactive in caramelization reactions and their impacts on the formation of caramelization intermediates as well as the polymeric brown pigments and heat-induced antioxidants in caramel. The current study selected six important dietary polyphenols including phloretin, naringenin, quercetin, epicatechin, chlorogenic acid and rosmarinic acid, which belong to different structural subgroups and are abundant in wide dietary sources like apple, citrus, tea, coffee and herbs. The impacts of selected dietary polyphenols were examined on the colour, browning intensity, antioxidant capacity of caramel prepared by heating fructose solution at two different pH. We also tried to explore whether these phenolics could affect the formation of two caramelization intermediates, HMF and furfural. Lastly, detailed investigation was made into the chemical reactions amoung selected polyphenols, fructose and their thermal transformation products aiming to figure out how polyphenols could bring about the observed changes.

2. Materials and methods

2.1. Chemicals

Phloretin, naringenin, quercetin, chlorogenic acid, rosmarinic acid, fructose, xylose, sucrose, glucose, trolox, ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)), disodium hydrogen phosphate, sodium hydroxide, potassium peroxosulfate, 5-hydroxymethylfurfural (HMF) and furfural were all purchased from Sigma–Aldrich (St. Louis, USA). Epicatechin was purchased from Biopurify Phytochemicals Ltd. (Chengdu, PR China). All solvents and acids used were of analytical or HPLC grade and obtained from BDH Laboratory Supplies (Poole, UK).

2.2. Preparation of caramel

Fructose was dissolved in 0.1 M sodium phosphate buffer (pH = 7 or 10) at a concentration of 0.05 M. For total browning characterization resulting from sugar caramelization and polyphenol oxidation, xylose, glucose and sucrose were used at 0.05 M in pH = 7 buffer. For the analysis of furfurals, the fructose concentration was 40% (w/v). 2 ml Sugar solution was aliquoted to each 14 ml screw cap septum vials (Thermo scientific, IL, USA). Dietary polyphenols were added to vials at a concentration of 10 mM or 1 mM (quercetin, only). The solutions were then subject to heating at 120 °C in silicone oil bath for 2 h. After heating, the samples were immediately removed from oil bath and cooled in ice-water before further analysis. Polyphenol equivalent solutions were prepared by heating 10 mM or 1 mM (quercetin) polyphenols alone in 0.1 M sodium phosphate buffer (pH = 7 or 10). The caramel prepared with or without polyphenol addition is abbreviated as CP and fructose/polyphenol equivalent solution after thermal treatment is abbreviated as FE/PE in later results and discussions.

2.3. Measurement of browning intensity and colour

The sample solutions were properly diluted and the browning intensity was measured by reading absorbance at 420 nm using a spectrophotometer (UV-1206, Shimadzu, Japan). The three chromatic coordinates L^*, a^* and b^* were measured by a colourimeter (CR-400, Konica Minolta, Japan). The coordinate L^* indicates the lightness of colour ($L^* = 0$ yields black and $L^* = 100$ means diffuse white); a^* characterizes the position between red and green (negative values indicate green and positive values indicate red); b^* suggests the position between yellow and blue (negative values indicate blue and positive values indicate yellow). Chroma value was calculated as $(a^{*2} + b^{*2})^{1/2}$; E index was calculated as $(L^{*2} + a^{*2} + b^{*2})^{1/2}$; Hue angle was calculated as $\tan^{-1}(b^*/a^*)$.

2.4. Measurement of antioxidant capacity

The antioxidant capacity was quantified by Trolox equivalent antioxidant capacity (TEAC) assay based on the method of Cheng, Chen, and Wang (2007). The stock radical solution was prepared by reacting 7 mM ABTS solution with 2.45 mM potassium peroxosulfate in dark for 16 h at room temperature. Working radical solution was freshly prepared by diluting stock radical solution with MilliQ water to have an absorbance of 0.7 ± 0.05 at 734 nm. 50 μl of properly diluted sample solution or standard (trolox in mM) was mixed with 1.9 ml of working radical solution and absorbance at 734 nm was read by a spectrophotometer (UV-1206, Shimadzu, Japan) after 6-min incubation. The results were expressed as μmol trolox/ml sample solution.

2.5. Quantification of 5-hydroxymethylfurfural and furfural

The two caramelization intermediates, 5-hydroxymethylfurfural (HMF) and furfural were analyzed by an HPLC method adopted from Ferrer, Alegria, Farre, Abellan, and Romero (2005) with some modifications. The Shimadzu HPLC system is composed of a separation module (LC-20AT), an autosampler (SIL-20A), a degasser (DGU-20A3), and a photodiode array detector (SPD-M20A). Separation was conducted isocratically on a Spherisorb ODS2 column (5 μm, i.d. 4.6 × 250 mm) using a mixture of acetonitrile – 0.1% formic acid aqueous solution (5:95, *v/v*). Flow rate was set at 1 ml/min and injection volume was 10 μl. The detection wavelength for HMF and furfural was set at 280 nm. The quantitative calculation was facilitated by a calibration curve constructed by HMF and furfural standard.

2.6. HPLC and LC–MS/MS analysis of reaction products of selected polyphenols and fructose during caramelization

To characterize the reaction products of dietary polyphenols and fructose after thermal treatment, HPLC analysis was performed. The separation of reaction products was achieved on a YMC-pack Pro column (5 μm, i.d. 2.1 × 150 mm). The mobile phases were water with 0.1% formic acid (A) and acetonitrile (B) and gradient flow was as follows: 0 min, 3% B; 5 min, 3% B; 45 min, 50% B; 55 min, 80% B; 60 min, 90% B; back to 3% B in 1 min and last for 14 min. The total run time was 75 min and flow rate was 0.2 ml/min. The injection volume was 10 μl. UV detection wavelengths for phloretin and naringenin were 254 and 280 nm respectively.

The LC–MS/MS instrument was equipped with an electrospray ionization (ESI) source interfaced to a 3200 Qtrap mass spectrometer (AB Sciex, Canada). Liquid chromatography was performed on an Agilent 1290UPLC system with a binary pump (G4420A) and a thermostatted autosampler (G4226A). The separation of the reaction products was performed in the same conditions as described above. The MS parameters were as follows: negative ion mode; spray voltage, −4300 V; scan range, 100–1000 Da; temperature, 450 °C; declustering potential (DP), −65 V; collision energy (CE), −30 eV.