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Chemical analyses of novel colored compounds formed by the Maillard reaction

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The Maillard reaction between a carbonyl group of reducing sugars and an amino group of proteins and amino acids forms various compounds, including pigments and flavors. As almost all of foods contain these substrates, this reaction occurs during food processing and preservation, and affects qualities of various kinds of foods.

Melanoidins are known as the major brown pigments formed through the Maillard reaction, and are heterogeneous polymers formed from amino acids or proteins, and reducing sugars or their decomposed carbonyl compounds. Because of the complexities, it is extremely difficult to determine the chemical structure of melanoidins. On the other hands, several low-molecular-weight colored compounds formed by the Maillard reaction have been reported. Although the amount of each low-molecular-weight pigment is less than that of melanoidins, the intensity of color is cumulative, and low-molecular-weight pigments often form high-molecular-weight pigments or melanoidins. Therefore, to understand the process of pigmentation or browning through the Maillard reaction, it is meaningful to identify low-molecular-weight colored compounds formed by the Maillard reaction. Here I described the isolation and identification of several novel colored compounds formed in model Maillard reaction solutions.

In general, cysteine inhibits browning by the Maillard reaction; hence there were no reports on pigments formed by the Maillard reaction of cysteine. At first, I searched a pigment derived from cysteine in model Maillard reaction systems containing cysteine and glucose, lysine and glucose, or cysteine, lysine, and glucose with HPLC equipped with a diode-array detector (DAD-HPLC). Aa a result, an unidentified peak (named pyrrolothiazolate) showing two absorption maxima at 300 nm and 360 nm was detected in cysteine-glucose and cysteine-lysine-glucose reaction systems. The amount of pyrrolothiazolate increased by addition of lysine, which suggested that pyrrolothiazolate was formed by the reaction between cysteine and intermediate of the Maillard reaction. Pyrrolothiazolate was isolated from cysteine-lysine-glucose reaction systems, and identified as (3*R*,7a*S*)-6-hydroxy-5,7a-dimethyl-7-oxo-2,3-dihydropyrrolo[2,1-*b*]thiazole-3-carboxylic acid. This compound was a novel pyrrolothiazole derivative having a carboxyl group. To examine the origin of two methyl groups, pyrrolothiazolate was formed from 1-^{13}C-glucose. The 1-position of carbon of glucose was incorporated into both methyl groups. This result suggests that pyrrolothiazolate would be formed by a reaction between cysteine and derivative of 1-deoxyglucosone, an intermediate of the Maillard reaction. The formation scheme of pyrrolothiazolate was proposed from this result. Furthermore, distribution of pyrrolothiazolate in foods and beverages was examined. Pyrrolothiazolate was detected in soy sauce, miso, and beer at the concentration of 2-100 μg/ 100 mL or g. These results showed that pyrrolothiazolate was formed not only in model reaction systems, but also in foods by the Maillard reaction between amino acids and reducing sugars hydrolyzed from proteins and starch by enzymes of *koji* or yeast.

Pyrrolothiazole structure of pyrrolothiazolate was considered to be formed by the reaction between cysteine and derivatives of 1-deoxyglucosone, which suggested that a pyrrolooxazole derivative might be formed in the reaction mixture containing threonine or serine having a hydroxy group instead of cysteine having a thiol group. To search these pigments, reaction solutions containing threonine or serine and glucose were analyzed with DAD-HPLC. A peak (named pyrrolozaolates A and B, respectively) showing a similar spectrum to pyrrolothiazolate was detected in each solution. These pigments were isolated and identified as (2*R*,3*S*,7a*S*)-6-hydroxy-2,5,7a-trimethyl-7-oxo-2,3-dihydropyrrolo[2,1-*b*]oxazole-3-carboxylic acid (pyrrolooxazolate A) and as (3*S*,7a*S*)-6-hydroxy-5,7a-dimethyl-7-oxo-2,3-dihydropyrrolo[2,1-*b*]oxazole-3- carboxylic acid (pyrrolooxazolate B). These compounds were novel pyrrolooxazole derivatives having carboxyl group.

Last, a pigment formed by the Maillard reaction of protein was analyzed. Dilysyldipyrrolones formed by the Maillard reaction between lysine and xylose have a pyrrolyl-methylidene-pyrrolone ring that is composed from two amino groups of two molecules of lysine and two molecules of degradation products of two pentose. Among of these dilysyldipyrrolones, dipyrrolone B is formed by the reaction between ε-amino groups of two lysine residues and degradation products of pentoses. This suggests that dipyrrolone B is formed by a reaction between lysine residues of proteins and pentoses. Then, an acid-hydrolysate of a reaction mixture of soy protein and xylose was analyzed with DAD-HPLC. Unfortunately, dipyrrolone B was decomposed during acid-hydrolysis and not detect in the hydrolysate. Instead, a unique peak (named furpenthiazinate) showing an absorption maximum at 400 nm was detected. Furpenthiazinate was not detected in the hydrolysate of soy protein without xylose, which suggests that the compound was formed by the Maillard reaction between soy protein and xylose. Therefore, furpenthiazinate was isolated and identified. Although the NMR data showed that furpenthiazinate had partial structures of an amino acid residue, a furyl group derived from xylose, and an imine group, the structure of furpenthiazinate was not determined by only NMR and MS data. Then, furpenthiazinate was reduced by $NaBH_4$, and its reduced form was analyzed. As a result of X-ray analysis of reduced furpenthiazinate, the chemical structure of that was identified as 7-(2-furanyl)-2,3,4,4a,5,6-hexahydrocyclopenta[*b*][1,4]thiazin-4-ium-3-carboxylate, which showed the chemical structure of furpenthiazinate to be 7-(2-furanyl)-2,3,5,6-tetrahydrocyclopenta[*b*][1,4]thiazine-3-carboxylic acid.

In conclusion, four novel pigment, pyrrolothiazolate, pyrrolooxazolates A and B, and furpenthiazinate, were isolated and identified from model Maillard reaction solutions containing cysteine, threonine, serine, and protein, res Furthermore, the formation scheme of pyrrolothiazolate was proposed and distribution of the compound in foods and beverages was examined. These results showed that pyrrolothiazolate was formed not only in model Maillard reaction system but also in foods. In foods, various kinds of the Maillard reaction products are formed from various kinds of substrates. These novel colored compounds identified in this study are one of the Maillard reaction products, and cumulatively contribute to total color we recognize. This study would contribute to clarify the chemistry of pigmentation and browning through the Maillard reaction.