Review

What are melanoidins, polymers in food?

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Melanoidins are brownish heterogeneous high-molecular-weight compounds formed at the final stage of the Maillard reaction. Louis C. Maillard found that brownish polymer was formed in a reaction solution containing amino acids and reducing sugars and called those pigments melanoidins, which resulted that melanoidins have become known as the Maillard reaction end products. Melanoidins are formed during the processing and storage of various kinds of food, causing a significant influence on the quality of foods. For instance, melanoidins cause some changes in not only the color of foods but also odor and texture and show antioxidative activities and dietary fiber-like properties. The formation mechanism and the chemical structure of melanoidins are not understood fully, because of complexity and heterogeneity. However, they are being comprehended gradually through the great efforts by many scientists. Melanoidins are formed by the polymerization of a variety of reactants and intermediate products of the Maillard reaction and by cross-linking between proteins through low-molecular-weight pigments. Therefore, analyses of low-molecular-weight pigments formed by the Maillard reaction may result in the clarification of melanoidins in more detail.

1. Introduction

Melanoidins are brownish high-molecular-weight compounds formed at the final stage of the Maillard reaction (amino-carbonyl reaction). Louis C. Maillard, a French biochemist, called brownish polymers formed in a reaction solution containing amino acids and

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sugars as melanoidins in 1912 [1]. Melanoidins not only affect the preference of foods through color but also have several functions such as antioxidative activities and dietary fiber-like properties. For this reason, it is crucial to control the formation of melanoidins during food processing and storage in the food industry. There are many studies on melanoidins and some reviews for their chemical structure or biological activities [2-7]. However, the whole chemical structure, formation mechanism, or biological activities of melanoidins is still not fully understood. This review briefly introduces an overview of melanoidins from the aspect of the quality of foods, the chemical structure, and the formation mechanism. Especially we described here our studies on low-molecular-weight pigments formed by the Maillard reaction as part of structural analyses of melanoidins.

2. The outline of melanoidins and the effect on the food industry

Melanoidins are now well known as end products of the Maillard reaction. However, the term "melanoidins" was used for the first time by Schmiedeberg to define brown pigments formed by acid hydrolysis of protein and to distinguish them from melanin before the Maillard reaction was found [8-10]. After then, Louis C. Maillard demonstrated experimentally that brown polymers were formed by the reaction between amino acids and reducing sugars and called them melanoidins [1], which resulted that melanoidins have become known as the Maillard reaction end products [9].

Melanoidins have been considered to be heterogeneous high-molecular-weight compounds as well as melanin, which is commonly contained in plants and animals.

Since reactants of the Maillard reaction, amino acids or proteins and reducing sugars, are present in almost all foods, this reaction is commonly caused during the processing and storage of foods. After the discovery by Maillard, the relationship between the browning of foods and the formation of melanoidins has been made clear. Till the 1950s, it was reported that the formation of melanoidins brought browning of malt during the processing of beer [11, 12], discolorations of molasses during the processing of sugar [13, 14], and browning of processed fruits such as concentrated juice [15] and dried fruits [16]. The chemical properties of brown pigments in Japanese soy sauce were reported for the first time in 1927 [10]. Although these pigments were named soyamelanin acid or soyamelanin because of the solubility to an alkaline solution and amorphousness, nowadays the brown color of soy sauce is mainly attributed to melanoidins [17, 18].

It has been reported until now that a variety of foods such as bakery products, balsamic vinegar, cocoa, coffee, dark beer, honey, soy sauce, and wine contain melanoidins [6, 7]. Thus, melanoidins are formed during the processing and storage of many kinds of foods and cause visible changes to their color. It is well known that the Maillard reaction contributes to the flavor formation of foods. Although melanoidins are involatile, they make an effect on the flavor of foods through the adsorption of flavor

compounds [19, 20]. Protein glycation with polysaccharides through the Maillard reaction forms the structure like melanoidins, boosts emulsifying features, and improves solubilities of proteins [21, 22]. Melanoidins also affect the formation and stabilization of foam [23-25]. Thus, melanoidins have a great impact on the quality and preference of foods.

Moreover, melanoidins have several physiologically positive functions. They are antioxidant [26-28] and antimutagen [29, 30] and show function like dietary fiber [31-33]. On the other hand, the safety of melanoidins showed contradictory effects in some studies experimentally. There are both reports that melanoidins showed mutagenic or genotoxic activity [34-36] and that they did not [37-39]. It is difficult to isolate a melanoidin molecule because melanoidins are high-molecular-weight heteropolymers. These contradictory results might be due to the contamination of low-molecular-weight compounds formed through the Maillard reaction. Melanoidins are not easily digested and absorbed. Considering the total effects of melanoidins as mentioned above, melanoidins are considered to be helpful to human health, which is not estimated quantitatively. It is also necessary to examine the role of melanoidins on gut bacteria.

Melanoidins have critical influences on the quality of foods. The food industry has controlled the formation of melanoidins in desirable ways. Therefore, many scientists have tried to clarify the formation mechanism and chemical structure of melanoidins. However, their details are still not made clear, over 100 years after the term "melanoidin" appeared.

3. The formation mechanism of melanoidins

Melanoidins are end products of the Maillard reaction. Although the total formation mechanism of the Maillard reaction has not been elucidated because of its complexity, the outline summarized by Hodge [40] is well known for the chemical pathway of the reaction. This reaction is a series that begins with the nucleophilic addition of an amino group to a carbonyl group and is divided into three stages. Figure 1 shows the outline of the mechanism of the Maillard reaction, in which a reducing sugar is set as a starting material. In the initial stage, an amino group of amino acids or proteins reacts with a carbonyl group of reducing sugars, forming a Schiff base. Next, a double bond of the Schiff base migrates to lead to the Amadori rearrangement product. In the intermediate stage, the Amadori rearrangement products are decomposed into amino acids and α-dicarbonyl compounds having the skeleton derived from reducing sugars. These dicarbonyl compounds are further decomposed to other carbonyl compounds by retro-aldol reaction and/or β-carbonyl cleavage [41]. These carbonyl compounds react with amino acids to form cyclic compounds such as furan, pyran, or pyrrole through intramolecular dehydration and oxidation [42]. Melanoidins are formed in the final stage as the results of not only the complicated polymerization between carbonyl compounds formed in the intermediate stage and/or between these carbonyl compounds and amino compounds including amino acids,
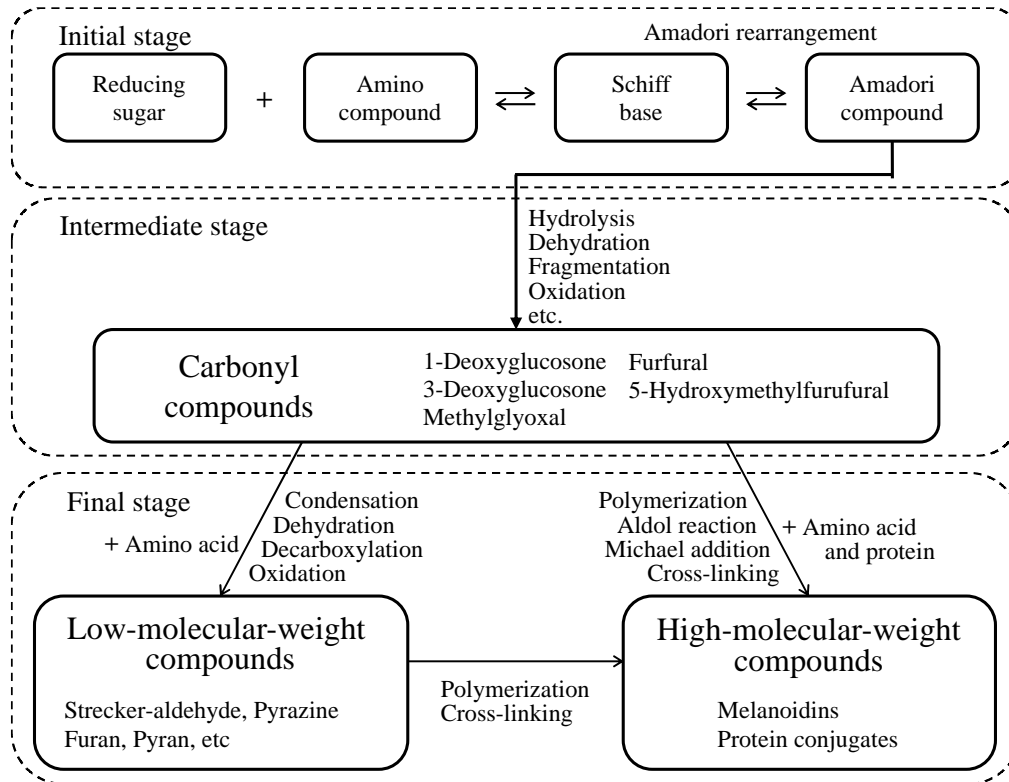
Figure 1. The outline of the Maillard reaction pathway. This outline was refined from the figure summarized by Hodge [40].

Skeleton derived from monosaccharide.

Skeleton derived from amino acid.
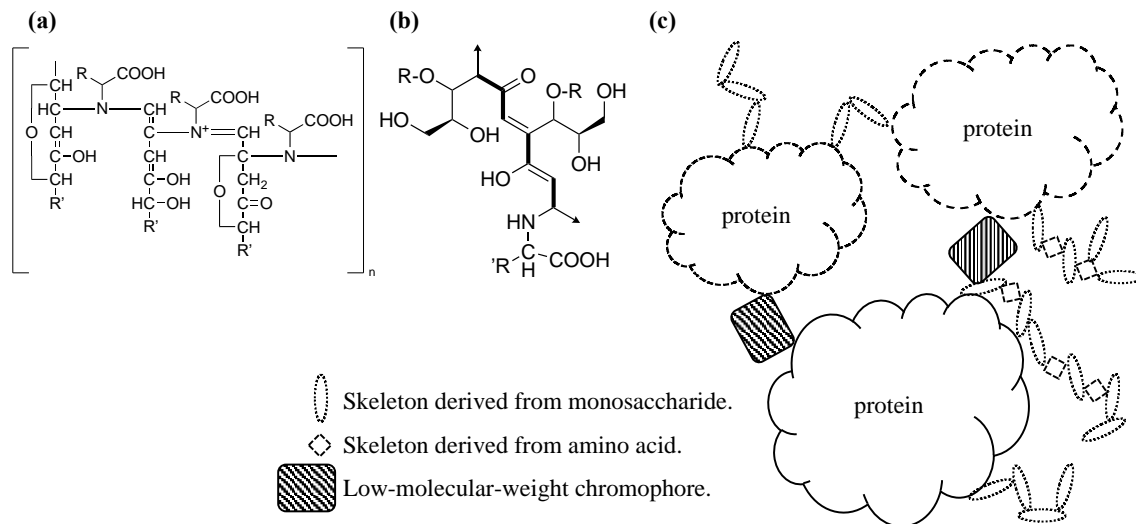
Low-molecular-weight chromophore.

Figure 2. Images of melanoidins. (a) Proposed partial structure of melanoidins by Hayase et al. [44, 52]. (b) Proposed partial structure of melanoidins by Cämmere et al. [55, 56]. (c) Image of a partial structure of melanoidins as a result of cross-linking between colorless proteins through low-molecular-weight compounds.

peptides, and proteins by aldol reaction and/or Michael addition but also cross-linking between proteins through those compounds. [43]. In the final stage, relatively stable low-molecular-weight compounds are also formed by dehydration, decarboxylation, and/or oxidation of various intermediate compounds. These reactions are affected by the kind and the concentration of reactants, temperature, time, pH, and the presence of oxygen or metal ions [44]. In addition to amino acids, proteins, and sugars, foods contain a lot of components having carbonyl or amino groups such as lipids, vitamins, and polyphenols, which may also contribute to the Maillard reaction and melanoidin formation. Hydroxycinnamates or polyphenols are involved in the formation of melanoidins in coffee during roasting [2, 4]. Thus, melanoidins in foods are formed out of a wide variety of reactants and have heterogeneous structures, which make them difficult to analyze.

4. The chemical property and structure of melanoidins

Melanoidins are heterogeneous high-molecular-weight compounds or heteropolymers formed by the polymerization of various compounds such as amino acids, peptides, proteins, sugars, etc. Their molecular weight varies considerably with the combination of reactants and the condition of the reaction. Since the 1970s, the chemical property of melanoidins has been examined with fractionation by gel filtration chromatography. Motai examined the effect of the kind of amino acids and molecular weight of melanoidins on their color formed by the reaction between several kinds of amino acids and xylose using gel filtration chromatography [45]. This study suggested that the molecular weight of melanoidins ranged from 290 to 14,200 and varied with the type of amino acids. Hofmann fractionated melanoidins prepared from glucose and amino acids or casein by ultra-centrifugation and compared the color intensity [46]. Few colored compounds having a molecular weight above 10,000 Da were formed in the reaction mixture containing amino acids and glucose, while the compounds having a molecular weight above 10,000 Da, especially above 100,000 Da, were formed from the reaction mixture containing casein and glucose and showed higher color intensity. These results show that the molecular weight of melanoidin differs from substrates and that the presence of protein in the reaction mixture causes melanoidins with high-molecular weight easily. In recent years, the Maillard reaction products are often classified by molecular weight into above 1000 as melanoidins and under 1000 as low-molecular-weight compounds expediently.

Several reviews summarized many studies and proposed partial structures of melanoidins [2-4, 47]. Figure 2 shows images of the chemical structure of melanoidins. As mentioned above, it is very difficult to analyze melanoidins in foods containing various compounds because of the complexity. Therefore, model Maillard reaction systems containing amino acids and sugars have been used to simplify the reaction and analyze melanoidins.

At the beginning of the analysis of melanoidins, its chemical structure was speculated from the chemical property. Hodge et al. proposed that melanoidins had reductones in the substructure because melanoidins obtained from Amadori rearrangement compound prepared from ethanolamine and glucose showed reducibility [48]. Reductones are stronger reducing agents having enediol or enaminol groups, and quantified by the reducibility to 2,6-dichlorophenolindophenol (Tillman's reagent) [49]. Kato et al. also reported that melanoidins formed from glycine, ammonia, or n-butylamine and glucose reduced Tillman's reagent, showing the presence of reductones in the substructure of melanoidins [49, 50]. Because of this partial structure, melanoidins show antioxidative activity and chelating abilities with metal ions [5]. Melanoidins showed anti-microbial activity for the probable reason of the chelation to metal ions such as iron [51].

In the late 20th century, the chemical structure of melanoidins has been analyzed directly using some spectroscopic measurements such as infrared spectroscopy (IR), nuclear magnetic resonance (NMR), and mass spectrometry (MS). Hayase et al. proposed a partial structure of melanoidins formed by the reaction between glycine and glucose by NMR analyses as shown in Figure 2 (a) [44, 52]. This shows that several sugars branched by linking to a nitrogen atom of amino acid. It seems that these partial structures are essential as the chromophore of melanoidins because these structures disappeared by ozonolysis with the discoloration of melanoidins. The presence of these substructures was agreed with IR analyses showing that melanoidins formed from alanine and several kinds of sugars had carboxy groups and bonds of C=C, C=O, and C=N [53, 54].

Cämmerer et al. proposed that the structure of melanoidins had the skeleton polymerized decomposed sugars by aldol reaction as shown in Figure 2 (b) because it was hardly changed the ratio of carbon to nitrogen in melanoidins formed from the mixture containing different ratios of glycine to glucose [55]. Then, melanoidins showing the same molecular weight formed by the reaction between glycine and glucose or dextrin were hydrolyzed, and the amount of free glucose in the hydrolysate was quantified. As the result, a smaller amount of glucose appeared in the hydrolysate of melanoidins derived from glucose than that derived from dextrin, suggesting that the polymerization of dicarbonyl compounds by aldol reaction contributes to the formation of the skeleton of melanoidins [56].

On the other hand, observation of the repeating structure using MS analyses suggests that cyclic compounds such as pyrrole and furan, the intermediate products of the Maillard reaction, form oligomers, resulting in the formation of melanoidins [43, 57].

Considering the presence of proteins in foods, high-molecules melanoidins or pigments are formed by cross-linking between proteins through low-molecular-weight pigments or the Maillard reaction products formed from reducing sugars and amino acids residues in proteins (Figure 2 (c)).

5. Analyses of low-molecular-weight pigments for the clarification of the structure of melanoidins

The chemical structure and chromophore of melanoidins are not identified yet. Therefore, the identification of a partial structure and/or chromophore is crucial to identifying melanoidins. Since the late 1990s, some cross-linkers between proteins and lower-molecular-weight pigments formed through the Maillard reaction have been identified, which leads to the understanding of the chemical structure of melanoidins.

Cross-linking between proteins through low-molecular-weight pigments formed by the reaction between lysine or arginine residue in proteins and reducing sugar brings the formation of the high-molecular-weight melanoidins. Hofmann identified a low-molecular-weight pigment formed by the reaction between the ε-amino group of a lysine residue and furfural, which was also observed in enzymatic degradation products of melanoidins prepared from casein and furfural (Figure 3 (a)) [58]. Moreover, he speculated on the formation mechanism and the structure of melanoidins having chromophores due to cross-linking between protein through the reaction between arginine residues of protein and reducing sugars (Figure 3 (b)) [59].

Hayase et al. determined Blue-M1 [60] and Blue-M2 [61], which are blue pigments formed by the model Maillard reaction system between glycine and D-xylose, and showed that these compounds gradually polymerized and formed melanoidins (Figure 4) [62].
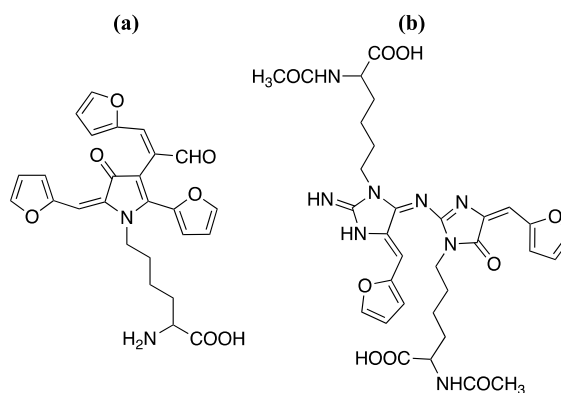

Figure 3. Chemical structures of low-molecular-weight pigments formed by the reaction between the ε-amino group of lysine and furfural. (a) [58], and by the reaction between two amino groups of arginine residues and furfural (b) [59].
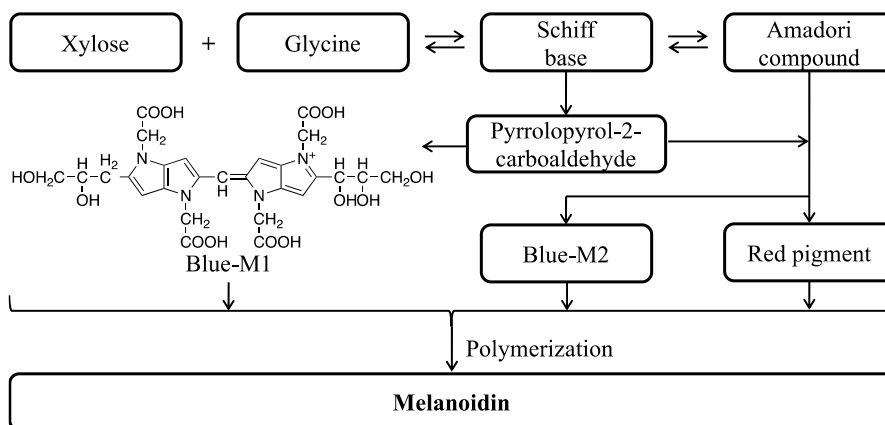

Figure 4. Proposed formation mechanism of melanoidins by the reaction between glycine and xylose via blue low-molecular-weight pigments [62].
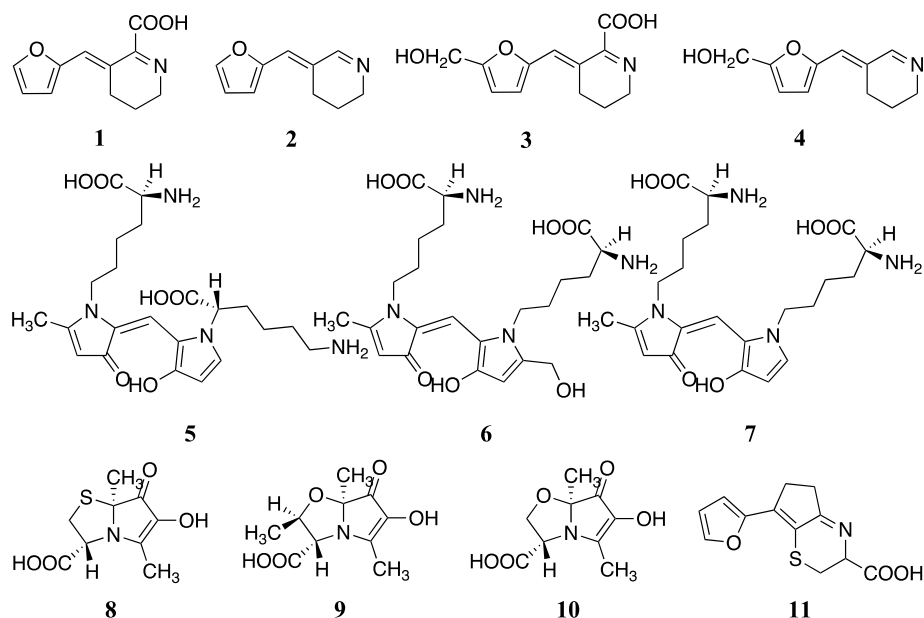
Figure 5. Chemical structures of low-molecular-weight pigments identified by the authors [47].

Because low-molecular-weight pigments are considered to be related to the formation of melanoidins, our group has conducted isolation and identification of various compounds formed in the model Maillard reaction systems as part of the determination of the mechanism of the Maillard reaction or the chemical structure of melanoidins. For example, we determined furupipates (**1-4**) [63, 64], dilysyldipyrrolones (**5-7**) [65-67], pyrrolothiazolate (**8**) [68, 69], and pyrrolooxazolates (**9**, **10**) [70], which were formed by the reaction between L-lysine and furfural or 5-hydroxymethylfurfural, L-lysine and D-xylose, L-cysteine and D-glucose, or L-threonine or L-serine and D-glucose, respectively (Figure 5). Because dilysyldipyrrolone B (DPL B; **6** in Figure 5) is formed via the reaction of the two ε-amino groups of lysine, this pigment may also be formed by the reaction between the ε-amino groups of lysine residues of protein and pentose. Therefore, the formation of DPL B in a solution of the Maillard reaction between soy protein and xylose was examined using acid hydrolysis. As a result, DPL B was not detected in the hydrolysate because DPL B was unstable under the condition. However, unexpectedly, a novel low-molecular-weight pigment, furpenthiazinate (**11** in Figure 5), was identified [71]. Although the relationship between these low-molecular-weight pigments and the formation of melanoidins is not examined yet, these compounds have a carbonyl group or a part of the structure which may cause an aldol reaction. So, it is expected that these colored compounds may polymerize or cross-link with protein resulting in the formation of melanoidins. In the future, it will be necessary to examine the relationship between low-molecular-weight pigments like these and melanoidins.

Melanoidins, formed through the Maillard reaction, were recognized over one hundred years ago. The initial and middle stages of the Maillard reaction are well understood. In the late stage, colored compounds; melanoidins and low-molecular-weight pigments, are formed. With the development of chromatographic technology and instrumental analyses, various low-molecular-weight pigments have been isolated and identified gradually. Although partial structures and formation mechanisms have been partly comprehended, present information on melanoidins is far from a unified understanding of melanoidins because of the heterogeneity. It is expected that analyses of low-molecular-weight pigments, which are understandable through current technology, may result in clarification of the structure of melanoidins.

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Communicated by Hiroe Kikuzaki