

The influence of pH and molar ratio on melanoidin skeleton formation

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Abstract

The Maillard reaction, a chemical mechanism involving reducing sugars and amino acids, is responsible for the distinctive flavor of browned foodstuffs. Meanwhile, melanoidin polymers are HMW molecules that range in color from brown to intense brown and are produced towards the conclusion of the Maillard reaction network. Therefore, the aim of this work was to predict the different chemical linkages such as functional groups of the melanoidin skeleton via the UV-visible method and FTIR spectra. The polymer network of numerous melanoidins synthesized from Alanine-Glucose and Alanine-Fructose in the aqueous model at 100°C for 6 hrs and diverse molar ratios under varying pH conditions after dialysis were compared in this research. Results showed that browning was more pronounced at pH 10 when UV/Vis spectra were implemented. Furthermore, Alanine-Fructose-derived melanoidins have functional groups that are extremely similar to those of Alanine-Glucose-derived melanoidins. The formation of melanoidins was further explained by employing synchronized 2D auto-correlation maps (A_{v1}/A_{v1}). The melanoidin skeleton contains a considerable amount of alanine molecules since it was kept unreacted during the cooking process at 1:1 and 1:10 ratios. In conclusion, the melanoidin structure in this analysis was alanine-enriched. In addition, the protocol of melanoidin synthesis is critical for identifying its backbone.

1. Introduction

The Maillard reaction is a sugar dehydration reaction that is aided by the presence of amines (Ismarti *et al.*, 2020; Poojary and Lund, 2021). Melanoidins are high-molecular-weight complexes composed mostly of carbonyl compounds and amino acids. Melanoidin's skeleton has been thoroughly investigated, however, it is still unspecified (Echavarría *et al.*, 2021; Mohsin *et al.*, 2022). As a result of condition reactions, melanoidins are categorized as brown to dark brown MR final products (Mohsin *et al.*, 2020). The condensation of sugar and amino acids is influenced by temperature, water, pH, time, and other parameters (Rubinsztain *et al.*, 1986; Mohsin *et al.*, 2018; Mohsin *et al.*, 2019). Several experts have looked into the impact of melanoidins on human health, however, the results are still ambiguous (Delgado-Andrade and Fogliano, 2018; Aljahdali *et al.*, 2020; Walker *et al.*, 2020; Pérez-Burillo *et al.*, 2020). Melanoidins were further isolated from bread, beef, vinegar, coffee, beer, and malt in numerous studies (Obretenov *et al.*, 1991; Obretenov *et al.*, 1993;

Bekedam *et al.*, 2008; Verzelloni *et al.*, 2010; Yang *et al.*, 2011; Mohsin *et al.*, 2019). In the synthesis of non-nitrogenous polymers, intermediate products and deoxyglucosone are critical (Yaylayan and Kaminsky, 1998). Mohsin *et al.* (2020) synthesized melanoidin from fructosylalanine, and the polymer possessed more nitrogen. The network of melanoidin polymer can be formed from heterocyclic degradation and cleavage products, according to Mohsin *et al.* (2019). Cleavage products such as glyoxal and glyoxalic acid have been discovered in the Glucose-Alanine melanoidin (Mohsin *et al.*, 2018). Investigating polymerization of 1,2-dicarbonyl intermediates (MGO or 3-DG) to interpret the synthesis of nitrogen-free substructures in melanoidin polymers was demonstrated by Wang *et al.* (2021). They showed that MGO or 3-DG can be polymerized under various pH conditions. Carbohydrates such as glucose react in the caramelization pathway without the direct participation of amino acids (Yaylayan and Kaminsky, 1998). A previous study revealed that two molecules of glucose and one molecule of alanine combine to form ARP, which is a difructosylamine (Mohsin *et al.*, 2020).

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In that case, the subsequent MR products have a lower nitrogen content than classical ARPs like fructosylalanine. Free Alanine or free Glucose could be included in polymeric MR products, resulting in different elemental compositions of the final product (glucose: alanine) (Feather and Mossine, 2005). The MR's reaction products, primarily their carbonyl compounds, are also involved in the direct oxidation of Alanine via Strecker degradation and the formation of the corresponding Strecker aldehyde, which contributes significantly to food taste and flavor (Yaylayan, 2003). To explore fundamental vibrations and rotating vibrational structure, the mid-infrared (MIR) (approximately 3900–900 cm^{-1}) can be applied. Infrared spectroscopy is becoming more accepted for determining the identification of the melanoidin skeleton (Obretenov *et al.*, 1993; Oracz and Zyzelewicz, 2019; Mohsin *et al.*, 2020). The fingerprint region is unique to each chemical molecule and is critical for identifying polymers made up of similar recurring units (Ramírez-Hernández *et al.*, 2019; Almond *et al.*, 2020). In the field of melanoidin investigation, 2DCOS has proven to be a particularly beneficial technique (Lasch and Noda, 2019). The purpose of this research is to use UV/Vis and FTIR spectra to explore the functional groups of the melanoidin skeleton, which is made up of glucose and fructose with alanine at different molar ratios and synthesized by the aqueous model following dialysis at various pH values.

2. Materials and methods

2.1 Aqueous method for making melanoidin polymers

Melanoidins were made using the protocols described by Mohsin *et al.* (2019), with minor changes. The solution was heated at 100°C for 6 hrs. The pH value was held constant at pH 4.0, pH 7.0, and pH 10, using 0.1 M NaOH and 0.1 N HCl. Melanoidin samples were prepared in three molar ratios: sugar-enriched melanoidin (10:1), the equal molar ratio (1:1), and alanine-enriched melanoidin (1:10). In dialysis tubes, 2.5 g of melanoidin was dissolved in 150 mL of distilled water. In addition, these tubes should be placed in a batch dialysis system. The distilled water was replenished after 8 hours, giving in a total dialysis period of 96 hours. The melanoidin samples were freeze-dried following dialysis.

2.2 Melanoidins analyzed utilizing FTIR spectra

A Specord 71 IR (Fa. ZEISS) was employed to measure the FTIR spectra of melanoidins in potassium bromide pellets. The samples were prepared for IR spectroscopy according to Mohsin *et al.* (2018). In a mortar, 150 mg KBr was mixed with 0.5 mg melanoidin powder for each model.

2.3 Melanoidins analyzed utilizing UV/Vis spectra

Employing a UV/Vis spectrophotometer (Specord 200 Plus, Analytik Jena, Jena, Germany), the wavelength spectra of melanoidin polymers were acquired. In 1 mL distilled water, a 0.5 mg melanoidin sample was dissolved. A blank containing exclusively distilled water was employed to measure wavelengths ranging from 400 to 800 nm.

2.4 2D correlation analysis on FTIR datasets

2D correlation comparisons were carried out on the different FTIR spectral datasets in the spectral region from 3900 cm^{-1} to 900 cm^{-1} applying the program 2Dshige vl.3. The FTIR spectral raw datasets' synchronous Φ association spectra were computed and plotted as $A(v_1)$ vs. $A(v_2)$. The amplitude of the correlation can be computed employing the equation:

$$\frac{1}{m-1} \sum_{j=1}^m A_j(v_1) A_j(v_2) = \Phi(v_1, v_2)$$

where $A_\phi(v_k)$ denotes the spectral absorbance in relation to the averaged absorbance.

3. Results and discussion

3.1 UV/VIS spectrum of melanoidins

The pH level employed has an impact on the color development of Glucose-Alanine and Fructose-Alanine melanoidins, as seen in Figure 1. Moreover, several publications have extensively described the use of UV/Vis spectra to predict melanoidin polymers (MikaMi and Murata, 2015; Kang, 2016; Yang *et al.*, 2019; Mohsin *et al.*, 2022). Browning is more intense at pH 10 than at other pH values since it is protonated and forms a cation. Besides, melanoidins with various molar ratios exhibit more brown at pH 10. Melanoidin's color is influenced by the type of sugar used. In general, Glucose-Alanine

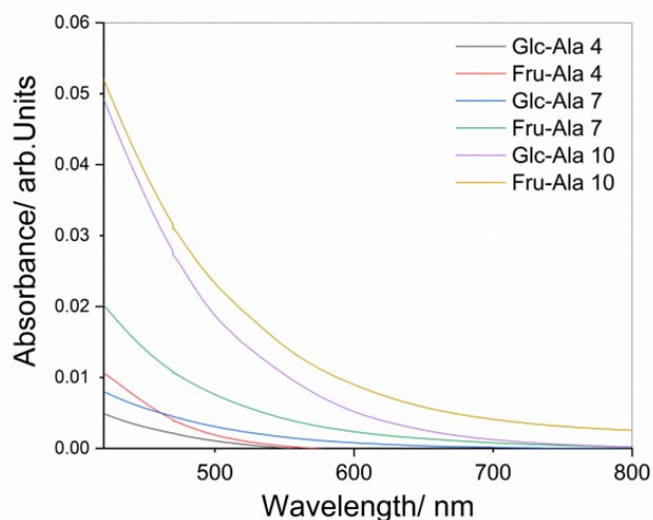


Figure 1. UV/vis spectrum of synthetic melanoidins at various pH levels and a 1:1 ratio

melanoidins have a higher absorbance than Fructose-Alanine melanoidins. Because of the color of the polymer after dialysis, the molecular weight (MW) of melanoidins at pH 10 is increased (Mohsin *et al.*, 2019).

3.2 FTIR spectra of melanoidin polymers

The structure of all bands in the Fructose-Alanine melanoidin skeleton is depicted in Figure 2. The accurate and in-depth investigation of the spectrum, together with information acquired from the publications, allows for very precise signal differentiation. In terms of the melanoidin backbone, the most exciting spectrum regions are between 1790 and 900 cm^{-1} , which is the emphasis of this work. The existence or disappearance of

distinct functional groups in a molecule is revealed by the assignments of absorption bands in the spectrum, and the spectrum as a whole serves as a "fingerprint" for detecting the sample's identification. However, all melanoidins lack a carboxyl or carbonyl group between 1790 and 1700 cm^{-1} (Mohsin *et al.*, 2019). At 1625 cm^{-1} , the amide I band could be detected (Irnawati *et al.*, 2020; Irnawati *et al.*, 2021). Furthermore, stretching vibrations of carbonyl (C=O), imines (C=N), and conjugated π bonds (C=C) are primarily responsible for this band. The C=O stretch accounts for 80% of the amide I band (Papadopoulou *et al.*, 2021). As a result of amid II, the analysis indicates sharp bands ranging from 1518 to 1300 cm^{-1} . These bands are primarily caused by N-H bending, but they can also be caused by C-N stretching vibrations. The amide II band includes 40% $\nu\text{C-N}$ and 60% δNH . However, between 1300 and 1235 cm^{-1} , amide III can be observed in the skeleton of melanoidin. The polymer's intense bands in the 1518–1300 cm^{-1} range are typical amino acid bands, implying that several alanine molecules were unreacted while the heating process (Figures 2a and c). As a result, an excess of amino acid is thought to have a catalytic effect on the formation and dehydration of MR products, but there is little evidence in the publications to support this theory (Mohsin *et al.*, 2022). Strong sugar bands were revealed in the 1112–1058 cm^{-1} range, which was caused by vibration patterns such as C-C and C-O stretching (Figure 3). Glucose-Alanine melanoidins also showed comparable bands.

3.3 Synchronous 2D correlation analysis

Synchronous 2D auto-correlation maps (A_{v1}/A_{v1}) allow for the visual decomposition between complex spectral intra-relationships and externally imposed physicochemical parameters such as T, p, or pH value.

Figure 4 displays 2D correlations maps from infrared spectra of Glucose-Alanine (a: 10:1, b: 1:1, c: 1:10) and Fructose-Alanine (d: 10:1, e: 1:1, f: 1:10) for different pH (4, 7, 10) conditions. Displayed are the corresponding spectral averages from aqueous model melanoidins.

A vast band extending from (3376–3368 cm^{-1}) is characteristic of the OH group of melanoidins and illustrated here by positive auto-peaks (red, positive correlation amplitudes Φ) located at the dashed diagonals and can be observed both for Glucose-Alanine and Fructose-Alanine melanoidins. The auto-peak intensities in these (same) spectral ranges do change simultaneously. Further, the OH band intensity, illustrated here by strong positive auto-peaks, is the strongest among all other remaining modes due to the high content of the hydroxyl moiety found in Glucose

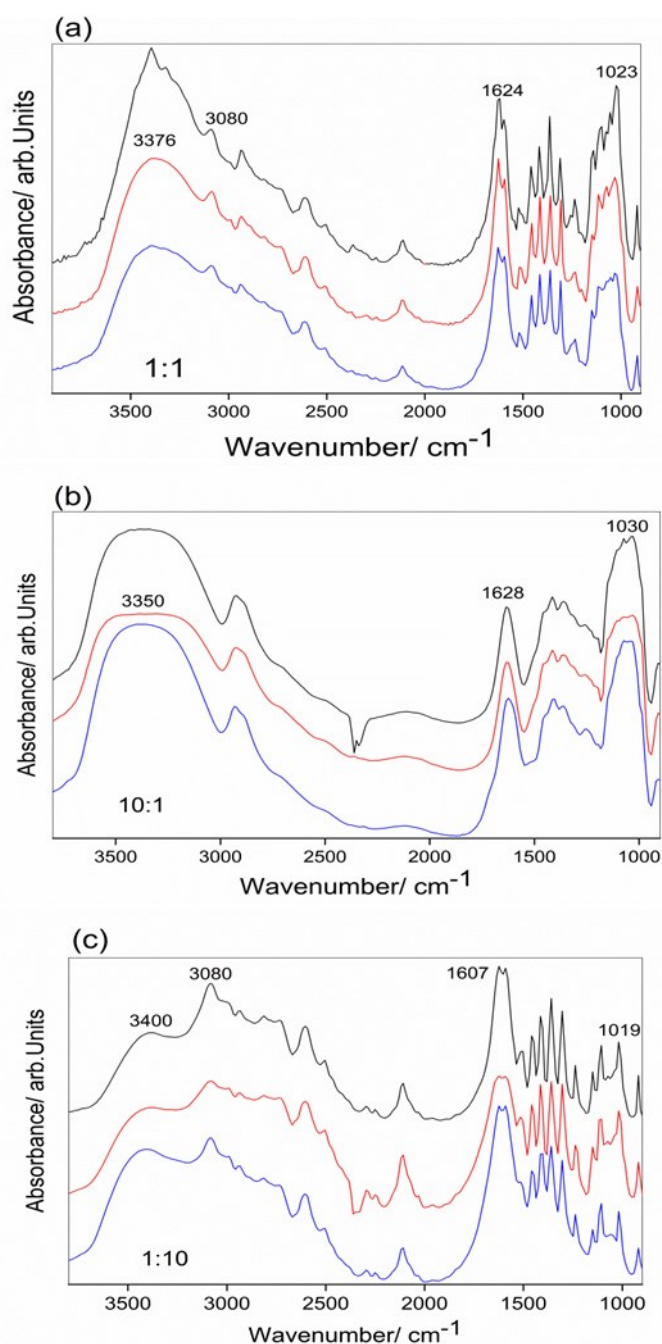


Figure 2. Infrared spectra of Fru-Ala melanoidins at different pH conditions (black line = pH4, red line = pH7, blue line = pH10). Heating treatments took place at 100°C for 6 hrs at different molar ratios (a: 1:1, b: 10:1, c:1:10).

and Fructose. Figure 4d is an exception since it shows a strong positive auto-peak relating to the water bending mode between 1800 and 2400 cm^{-1} . In Figure 4a-c, the amount of spectral contributions and auto/cross-peaks thereof, increases by the following order of molar ratios: $10:1 < 1:1 < 1:10$. Likewise, this can be observed for both types of aqueous melanoidin models. Positive (red)/negative (blue) cross-peaks occur if the band intensities considered in different spectral ranges change simultaneously/contrarily.

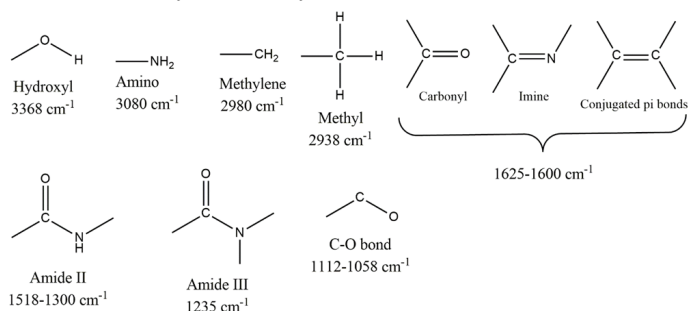


Figure 3. Main functional groups of Fru-Ala melanoidin at varied molar ratios and pH levels.

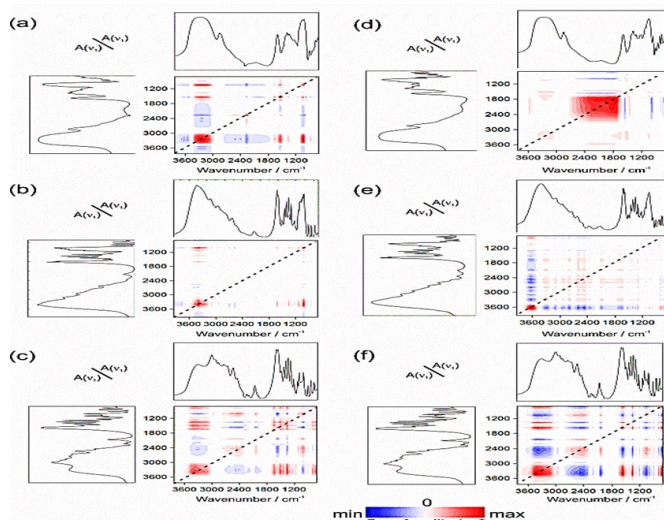


Figure 4. Synchronous 2D correlations maps from infrared spectra of Glc and Ala (a: 10:1, b: 1:1, c: 1:10) and Fru-Ala (d: 10:1, e: 1:1, f: 1:10) for different pH (4.0, 7.0, 10) conditions. Displayed are the averages of FTIR data from aqueous model melanoidins.

Out of the dashed diagonals, the lowest quantity of positive/negative cross-peaks can be seen at a molar ratio of 10:1. This observation can also be made for the auto-peaks. Assume the pH dependencies within an auto-correlation map and among the molar ratio itself were considered. In that case, auto- and cross-peaks with varying spectral intensities at various pH levels can be seen. These substantial intensity variations occur in the OH band region, depicting protonated/deprotonated states at low/high pH levels.

In comparison to (a) and (c), as well as (d) and (e), it can find lower spectral intensity variations and thus dependencies in the 1800-900 cm^{-1} spectral region for

the aqueous melanoidins synthesized at a 1:1 molar ratio (f).

This observation implies that melanoidin at a 1:1 molar ratio is a stable system. Acidic/basic pH levels in the spectral window do not have any assignable impact. A spectral contribution to the NH group at 3080 cm^{-1} , superposed with the hydroxyl group content, is detected in aqueous melanoidins at 1:1 and 1:10 ratios. This indicates that the amount of hydroxyl groups in the melanoidins decreases with an increased amino acid concentration in the preparation system.

Since the carboxyl groups of melanoidin are absent in aqueous systems, intensity strength/dependency of carboxyl or carbonyl at all pH levels cannot be observed. There are many studies related to aqueous melanoidins, in which the presence of carboxyl groups or carbonyl at different pH levels could not be proven (Mohsin *et al.*, 2019).

However, an increase in the intensity of the (C=C, C=N, and C=O) stretching modes within all ratios of melanoidins was observed. The intensity of the (C=C, C=N, and C=O) bands was high and sharp, especially for the 1:1 and 1:10 ratios (compare (a and b), (e and f), respectively). This may be due to a C=N bond than C=C, C=O with amino acid-enriched melanoidins. The role of alanine in melanoidin formation was strongly observed in melanoidins prepared by aqueous systems.

4. Conclusion

At different pH and molar ratios, the functional groups of Glucose-Alanine-derived melanoidins are quite similar to those of Fructose-Alanine-derived melanoidins. Moreover, in the field of melanoidin investigations, 2DCOS has proven to be a particularly beneficial approach. Melanoidins at 1:1 and 1:10 ratios exhibit a spectral contribution to the NH group at 3080 cm^{-1} , which is overlapped by the hydroxyl (OH) and CH groups. In melanoidins synthesized by the aqueous system, the role of alanine in melanoidin formation was seen. As a result, the structure of melanoidin is expected to be extremely complicated. However, the method of melanoidin synthesis is critical in the formation of the polymer network and its backbone. These findings suggest that a variety of parameters, including pH, molar ratio, and the method used to produce melanoidin, have a role in the conception of the melanoidin structure.

Conflict of interest

There are no conflicts of interest declared by the authors.

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