Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: May 2022 **2022, 19(6): 1297-1304** **E-ISSN: 2411-7986**

DOI: https://dx.doi.org/10.21123/bsj.2022.6527

Describing Polymers Synthesized from Reducing Sugars and Ammonia Employing FTIR Spectroscopy

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Received 6/9/2021, Accepted 15/12/2021, Published Online First 20/5/2022, Published 1/12/2022

Abstract:

Melanoidins can be diagnosed using the Fourier transform infrared (FTIR) technique. UV/Vis is an effective tool for both qualitative and quantitative analysis of chemical components in melanoidin polymers. The structural and vibrational features of melanoidin synthesized from D-glucose and D-fructose are identical, according to FTIR spectra, with the only difference being the intensity of bands. Using FTIR spectra, the skeleton of melanoidin is divided into seven major regions. The existence of the C=C, C=N, and C=O groups in all melanoidins formed from fructose and glucose with ammonia is confirmed by the areas ranging from 1600 to 1690 cm^{-1}, and the band is largely evident as a broad shoulder. Both melanoidins have a carboxyl or carbonyl extending around 1700 cm^{-1}. In all melanoidins, the NH^{+} group has vanished in the 3080 cm^{-1} range. However, the color intensity depends on the type of sugar employed in melanoidin synthesis. Furthermore, in comparison to Glc-ammonia, which has a higher proportion of sp^3 hybridized carbon, Fru-ammonia has a higher proportion of sp^2 hybridized carbon based on UV/Vis, FTIR and second-derivative spectra. Moreover, the data were simulated using principal component analysis. Principal component analysis (PCA) was used to interpret the data.

Keywords: Ammonia, D-Fructose, D-Glucose, FTIR spectroscopy, Melanoidin

Introduction:

Melanoidin polymer's skeleton is still unclear. Melanoidins are brown to dark brown natural condensation products of carbohydrates and proteins formed by Maillard reactions, which are non-enzymatic browning reactions[1]. Melanoidins are naturally found in a variety of foods and beverages, and they are commonly released as wastes by a range of agricultural industries, especially distilleries and breweries that use cane and beet molasses[2,3]. The network of melanoidins is uncertain, but it is thought to lack a definite structure since their elemental composition and chemical structures are highly dependent on the existence and molar content of parent reacting compounds, as well as reaction conditions such as pH, thermal processing, time, and the type of method used[1,4,5]. Melanoidins' biological and technological properties illustrate why extensive and comprehensive knowledge of their chemical structure is needed. Color, in general, is a sensory feature that is critical in determining consumer acceptance of many food items, making the analysis and control of color production during food processing important[6,7]. According to the findings of Mohsin et al.[2], the proposed basic structure of melanoidins is formed by various carbonyl compounds and alanine, but no specific molecular structure can be attributed to them in terms of their chromophore center. Each melanoidin has its own fingerprint region in the study of polymers using Fourier transform infrared (FTIR) spectroscopy[8,9]. Using FTIR spectroscopic techniques, Kim et al.[10] , investigated melanoidins produced from D-glucose and D-fructose with various amino acid enantiomers, identifying OH, NH, CH_2, CH_3, and amide I, II, and III modes in the melanoidin

polymers. Melanoidin from fructosylalanine has O–H stretching, CH symmetric stretching, (C=O, C=C, C=N), O–H deformation, C–N stretching, and C–O stretching frequencies, according to Mohsin et al.[5]. FTIR spectra analysis of the results obtained by Mohsin et al.[1], revealed the presence of OH, CH_2, CH_3, C=O, C=C, C=N, C-C, C-N, and C-O groups. Principal component analysis (PCA) is a mathematical technique that allows the representation of data by reducing data dimensionality while preserving as much detail as possible from the original data [11]. Mohsin et al.[4], employed PCA analysis to characterize different melanoidins. PCA is a useful approach for analyzing unknown polymers. The aim of this study was to use IR spectroscopy to investigate and quantify the melanoidins produced from D-glucose and D-fructose with ammonia after dialysis.

Methods and Materials:

Chemicals and Reagents

Carl Roth (Karlsruhe, Germany) provided D-(+) glucose and D-(+) fructose, Fluka (Steinheim, Germany) provided ammonia, Sigma-Aldrich (Steinheim, Germany) provided potassium bromate, and Carl Roth (Karlsruhe, Germany) provided dialysis tubing (Karlsruhe).

Preparation of Melanoidin Samples

Melanoidins were synthesized using the method described by Mohsin et al.[1]. D-fructose and D-glucose, as well as ammonia, were mixed in 1:1 molar ratios on a flat sheet and baked at 160 °C for 10 minutes. The dialysis tubing (Spectrum Por, Carl Roth, Germany) was made from cellulose with a molecular weight cut-off (MWCO) of around 12,000-14,000 Da and pore sizes ranging from 1.5 to 3.0 nm (thickness: 23 nm and width: 33mm). In batch dialysis, 5 g of melanoidin was dissolved in 300 mL of distilled water in dialysis tubes. After 10 hours, the distilled water was replaced, resulting in a total dialysis period of about 136 hours. Both samples were freeze-dried after dialysis.

Instrumentation and Data Acquisition for FTIR Spectroscopy

PTB's IR beamline 'IRMA' of the Metrology Light Source (MLS) storage ring was used to conduct FTIR measurements. Experiments were conducted with a Vertex-80v FTIR spectrometer connected to a Hyperion 3000 IR microscope (Bruker Optics GmbH, Germany) equipped with a 1282 pixel FPA (Focal Plane Array) detector (pixel size 3 m at magnification 15). For FTIR micro-spectroscopy measurements, an IR Cassegrain objective with a 15-fold magnification was used to focus a typical Globar light source onto the melanoidin sample pellets. FTIR spectra ranging from 4000 cm^{-1} to 500 cm^{-1} in transmission geometry were obtained by combining 128 scans at 4 cm^{-1} spectral resolution. Before taking sample measurements from a potassium bromide sample pellet, background images are taken and rationed against the sample spectrum.

UV/Vis Spectra

Mohsin et al.[1], described a procedure for detecting melanoidins based on the use of (Specord 200 Plus, Analytik Jena, Jena, Germany). A 0.2 mg melanoidin sample was dissolved in 1 mL of distilled water. Wavelengths ranging from 300 to 1000 nm were measured using a blank made entirely of distilled water.

PCA Analysis

Origin 9.1 was used for statistical analysis (OriginLab Corporation, Northampton, MA, USA). A PCA was first tested using normalized spectral data in the 1800–900 cm^{-1} range on the entire dataset (50 samples). The spectra were normalized with origin software 9, resulting in a simple separation of melanoidin formed from fructose and glucose.

Results and Discussion:

UV/Vis Spectra of Melanoidins

Spectroscopy in the visible ultraviolet field is a very useful tool for qualitative and quantitative research on organic molecules in melanoidin polymers[1]. As can be seen from their color variations, the spectra of both melanoidins have a similar pattern but not the same absorbance intensity (**Fig. 1**). Melanoidin derived from fructose has higher absorbance intensity in its spectra, particularly in the ultraviolet region (420 nm). In the visible region, the absorbance intensities of both spectra are nearly identical and overlap. Browning at 420 nm can be used to indicate colored Maillard reaction products, such as melanoidin polymer formation[4].

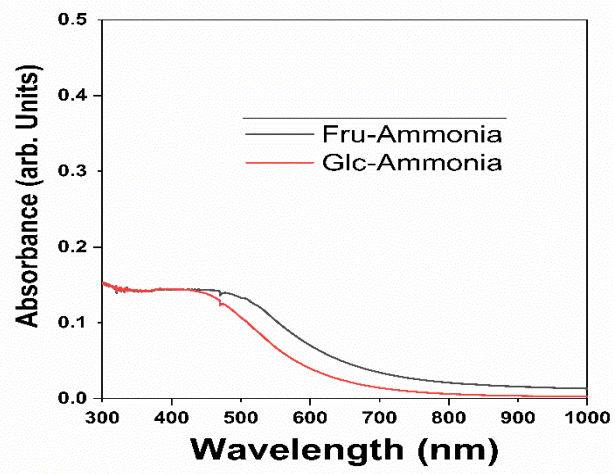

Figure 1. Ultraviolet-visible spectra of melanoidin samples.

FTIR Fingerprint of Melanoidin

The IR spectra of melanoidins formed in the Maillard reaction from glucose and fructose with ammonia are shown in **Fig. 2(a and b)**. The majority of the spectral bands observed in melanoidin IR spectra correspond to vibrational modes exhibited by sugars and proteins. In the melanoidin IR spectrum, water appears as a very distinct broad band between 3500 and 3000 cm^{-1} [12]. After heating, the FTIR spectra reveal distinct OH stretching modes that result from the relatively high content of OH groups in the sugars.

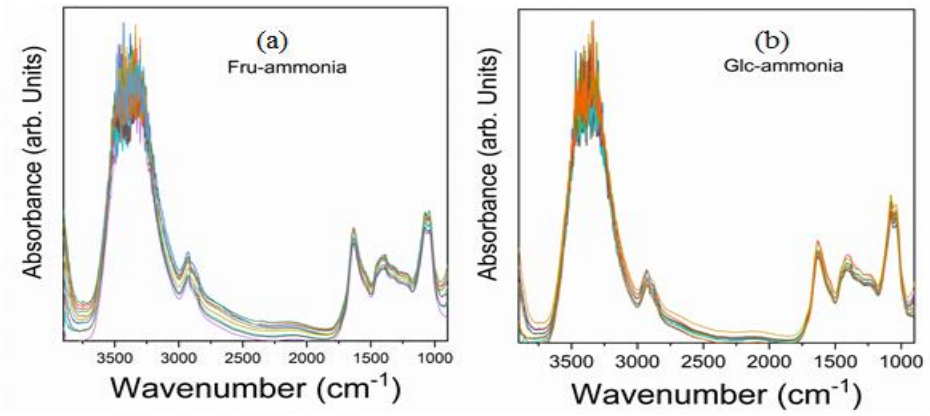

Figure 2. FTIR spectra of Fru-ammonia melanoidin (a), and Glc-ammonia melanoidin (b) between 3900 cm^{-1} and 900 cm^{-1} region

Typically, hydroxyl groups caused a wide band to appear between 3000 and 3600 cm^{-1} or 3200 and 3600 cm^{-1} [4,5]. In all melanoidins, the NH^+ group has vanished at 3080 cm^{-1} [1,5]. In addition, we can see characteristic stretching modes belonging to the CH_3 and CH_2 groups in the spectral range between 2933 cm^{-1} and 2883 cm^{-1}, respectively. There is a shoulder for COOH or C=O stretching in the 1716 cm^{-1} region[1,5] (**Fig. 3**).

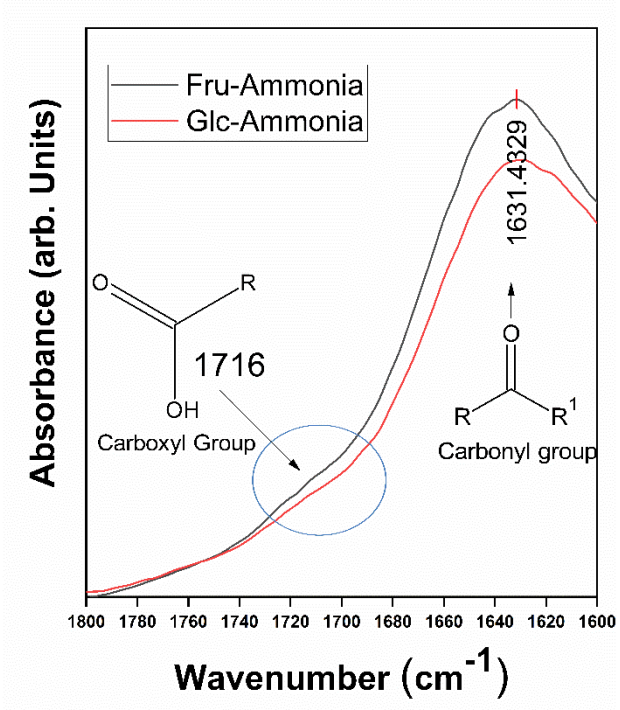

Figure 3. FTIR spectra of melanoidins samples derived from Glc and Fru with ammonia in the 1800–1600 cm^{-1} range.

The vibrational modes of carboxyl stretching and amide I band can be seen in the range between 1716 and 1600 cm^{-1}, respectively. Double bonds or aromatic compounds are formed by a large band around 1600 cm^{-1} [5]. All melanoidins showed an increase in the intensity of C=C, C=N, and C=O stretching absorption in the region of 1600 cm^{-1}. Water molecules, on the other hand, absorb a lot of light between 1640 and 1650 cm^{-1} [13]. Amadori (**Fig.4**) and Heyns compounds (C=O), Schiff bases (C=N), and intermediates (C=C) can cause a band in the 1690-1600 cm^{-1} range to become more intense. As a result, the polymers have an aromatic ring and incredibly simple functional bonding (carboxyl). This is consistent with the melanoidin chemical components studied by Mohsin et al.[1,4,5].

Baghdad Science Journal
2022, 19(6): 1297-1304
P-ISSN: 2078-8665
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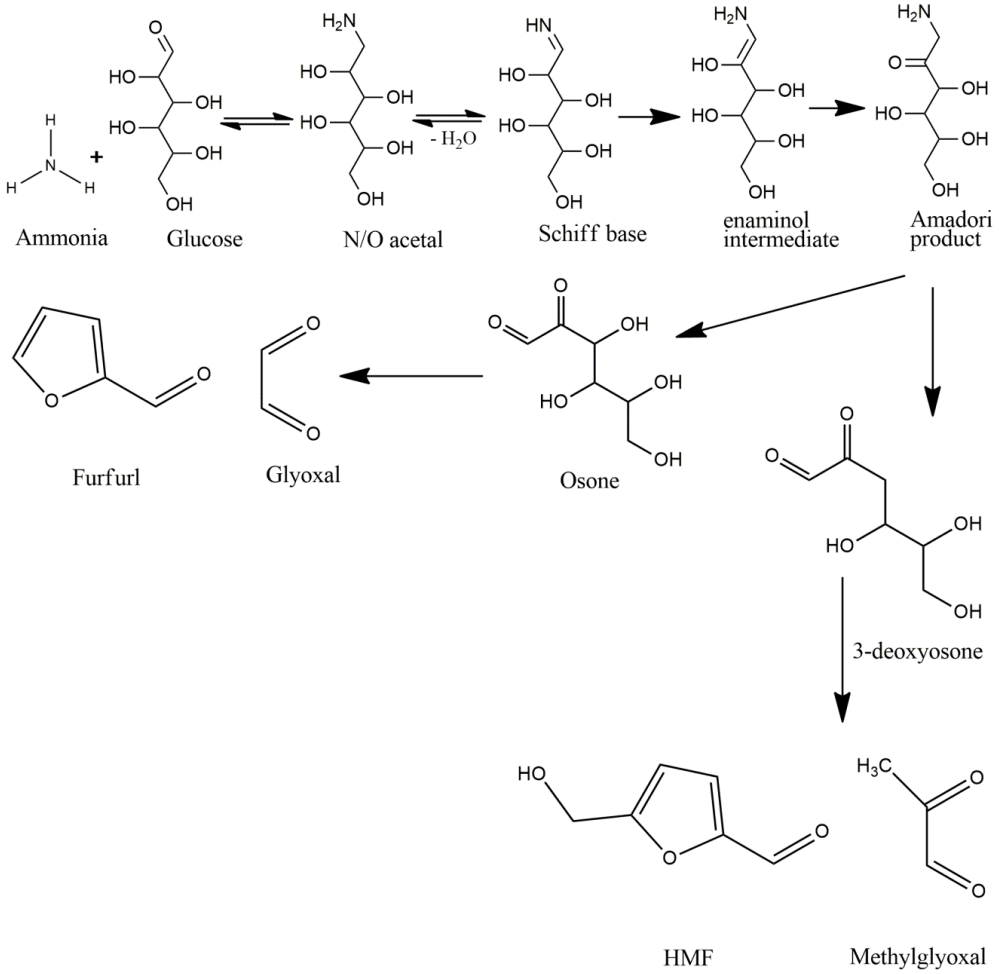

Figure 4. Formation of chromophores from Schiff bases, Amadori and intermediate compounds.

In the spectral range between 1510 and 1544 cm^{-1}, the N–H deformation and C–N stretching vibrations (amide II) of peptide's protein-based constituents occur (**Fig.5**). The spectral region between 1470 and 1280 cm^{-1} is accompanied by a variety of weak broad signals at 1459 and 1407 cm^{-1}, due to CH_2 bending and C=O asymmetric stretching (COO^-), respectively [1,4,5] and at 1337 cm^{-1}, due to C–H deformation vibration that overlaps with C–N stretching (amide III)[13]. The existence of C–O groups is responsible for the vibrations occurring between 1200 and 1300 cm^{-1} [12]. Moreover, amide III can be seen between 1200 and 1300 cm^{-1}, according to Mohsin et al.[5]. The bands discovered between 1150 and 995 cm^{-1} are referred to as the stretching and bending vibrations of C–O, C–H, and C–OH vibrations resulting from sugars[12,14].

To summarize, melanoidin's mid-IR spectrum is divided into seven unique regions:

I. Stretching of the OH occurs in a broad band between 3500 and 3000 cm^{-1}.

II. The CH_3 and CH_2 stretching is represented by weak bands between 2933 cm^{-1} and 2883 cm^{-1}, respectively.

III. In the region of 1716 cm^{-1}, a carboxyl stretching band can be diagnosed.

IV. In the 1631 cm^{-1} range, the vibrational modes of the amide I band can be detected (**Fig.5a**).

V. The amide II band can be found between 1544 and 1407 cm^{-1}.

VI. In the range of 1315-1239 cm^{-1}, an amide III band can be recognized.

VII. The C-O stretching band can be observed between 1079 and 1040 cm^{-1} (**Fig 5b**).

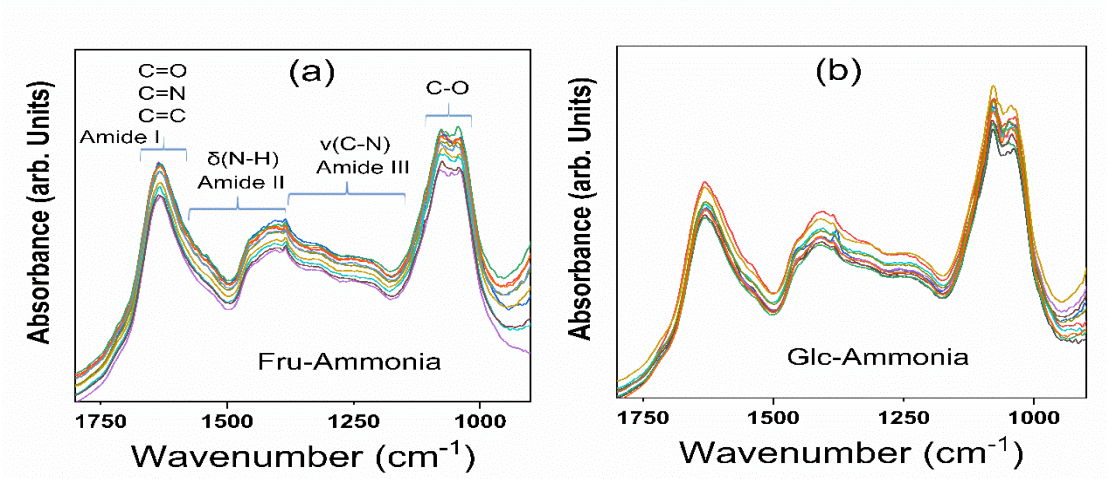

Figure 5. FTIR spectra of melanoidin samples derived from Fru-ammonia (a), and Glc-ammonia (b) in the 1800–900 cm^{-1} range.

Second-derivative Spectra of Melanoidins

Another important aspect of the current FTIR process is the second derivative treatment[15]. Melanoidins' color formation was influenced by the sugar type [4]. Fructose-derived melanoidin has a darker brown than glucose-derived melanoidin. Moreover, **Fig. 6** shows the second-derivative spectra obtained from the spectra in **Fig. 5 (a and b)**. The second-derivative procedure removes overlapped bands and corrects baseline variations. The 1710 cm^{-1} band was clearly differentiated in the second-derivative spectra in **Fig. 6**, despite the fact that the intensity decreased with melanoidin derived from glucose. The absorbance at 1710 cm^{-1} was clearly visible and strong in the spectra of melanoidin from fructose rather than glucose after second-derivative. There was also a band at 1745 cm^{-1} that was allocated to the C=O stretching vibrational mode of the ester linkage. Mohsin et al.[5], published similar findings. The C-O vibration, which is primarily found in sugars, is represented by 1079 cm^{-1} region[1,5]. Melanoidin derived from glucose has stronger C-O group bands than melanoidin derived from fructose. As a result, Fru-ammonia has a high proportion of sp^2 hybridized carbon than Glc-ammonia, which has a higher proportion of sp^3 hybridized carbon.

Table 1. FTIR spectra of absorption bands between 1631 and 950 cm^{-1} [1,4,5,16-19]

Wavelength (cm^{-1})	Assignment	Compounds
1631	Amide I, strong	protein
1456-1409	Amide II, weak	protein
1315-1239	Amide III, weak	protein
1079-1040	C-O stretching, strong	sugar
950	C-H bending, weak	sugar

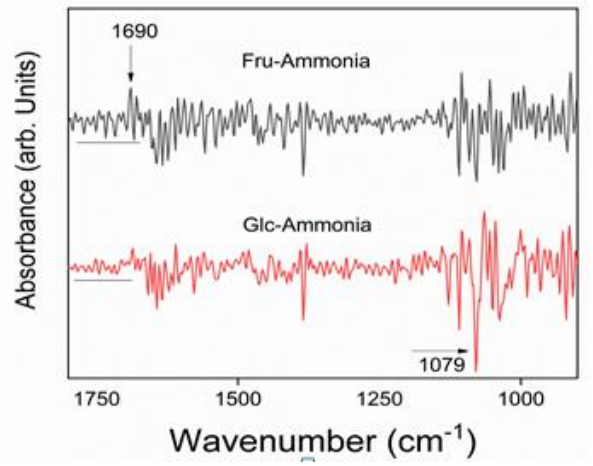

Figure 6. FTIR spectra of melanoidins derived from fructose and glucose, with Ammonia corresponding second-derivative spectra.

PCA of Melanoidins

PCA is a commonly used data mining method in the sciences that can be applied to a variety of datasets [20]. There was a clear distinction between melanoidins produced from various sugars (**Fig. 7a**). The purpose of this analysis was to determine the relationship between C=O stretching in the 1690 cm^{-1} region and C-O stretching in the 1079 cm^{-1} region. Furthermore, melanoidin derived from fructose is on the positive side, whereas melanoidin derived from glucose is on the negative. According to UV/Vis, second-derivative spectra and FTIR spectra, model melanoidin derived fructose has a larger proportion of chromophores, including C=O, C=C groups. Melanoidin derived from glucose, on the other hand, has a stronger C-O stretching intensity in the 1079 cm^{-1} range. The Fru-ammonia melanoidin has more variations (**Fig. 7b**). The color of polymers is the result of this overlap and variation. The type of sugar had an effect on the melanoidins' color development [4].

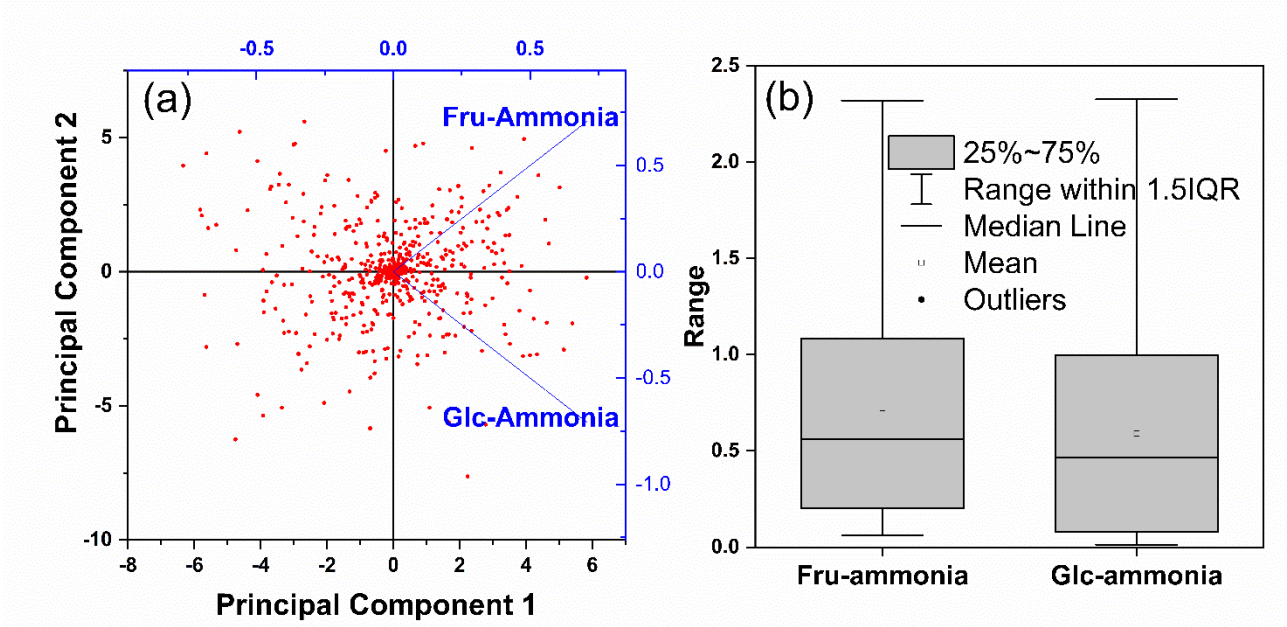

Figure 7. Principal components analysis of spectra from the melanoidins (a), and scatter interval plot for the classification of melanoidins, Box plots are used to represent mean and SEM values: mean (horizontal line), SEM (box), and SD (square) (whiskers) (b).

Conclusion:

The skeleton of melanoidin polymer is characterized using FTIR spectra. We examine the synthesis of such types of melanoidins based on sugar type using a variety of structural analytical approaches such as FTIR and UV/Vis spectra. Melanoidin derived from fructose has a higher absorbance intensity in its spectra when measured using UV/Vis, particularly in the ultraviolet region (420 nm). The structural and oscillations characteristics of polymer chains of melanoidin derived from D-glucose and D-fructose analyzed by IR spectra are extremely similar; the only difference is the intensity of bands. Both melanoidins have a small carboxyl or carbonyl extending about 1700 cm^{-1}. Melanoidins' color intensity is observed by the sugars used in their synthesis. In comparison to Glc-ammonia, which has a higher proportion of sp^3 hybridized carbon, Fru-ammonia has a higher proportion of sp^2 hybridized carbon based on UV/Vis and FTIR and second-derivative spectra. In all melanoidins, the NH^+ group has vanished in the region of 3080 cm^{-1}. The data were simulated using principal component analysis.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in Department of Vocational Education in Maysan.

Authors' contributions statement:

G. F. M. planned and carried out the experiment, and he also analyzed, interpreted, and concluded the findings. W. J. A. contributed to the FTIR measurements and the PCA simulations. A. K. A. interpreted some of the data. A. and A. supported M. in writing the manuscript.

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Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: May 2022 **2022, 19(6): 1297-1304** **E-ISSN: 2411-7986**

توصيف البوليمرات المصنعة من السكريات المختزلة والأمونيا باستخدام التحليل الطيفي FTIR

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الخلاصة:

يمكن تشخيص الميلانويد باستخدام تقنية مطيافية الأشعة تحت الحمراء (FTIR) . أن UV/Vis هي أداة فعالة للتحليل النوعي والكمي للمكونات الكيميائية في بوليمرات الميلانودين. تتطابق الميزات الهيكلية والاهتزازية للميلانويدات المُصنَّعة من D-Glucose و D-Fructose ، وفقًا لأطياف FTIR ، مع الاختلاف الوحيد في شدة القمم. باستخدام أطياف FTIR ، ينقسم الهيكل التركيبي للميلانودين إلى سبع مناطق رئيسية. تم تأكيد وجود مجموعات C = C و C = N و C = O في جميع الميلانويدات المكونة من الفركتوز والجلوكوز مع الأمونيا من خلال المناطق التي تتراوح من 1600 إلى 1690 سم $^{-1}$ ، ويتضح النطاق إلى حد كبير على أنه نطاق واسع. يحتوي كل من الميلانويدات على كربوكسيل أو كربونيل في المنطقة تقريبا 1700 سم $^{-1}$. في جميع الميلانويدينات، اختفت مجموعة NH^{+} في نطاق 3080 سم$^{-1}$. ومع ذلك ، فإن كثافة اللون تعتمد على نوع السكر المستخدم في تخليق الميلانويدين. علاوة على ذلك، بالمقارنة مع ميلانويدين المخلق من الأمونيا و الكلوكوز Glc-ammoina ، التي تحتوي على نسبة أعلى من الكربون المهجن sp^3 ، فإن الميلانويدين المخلق من الأمونيا و الفركتوز Fru-ammonia لديها نسبة أعلى من الكربون المهجن sp^2 على أساس تحليل الأشعة فوق البنفسجية / المرئية و FTIR وأطياف المشتق الثاني. علاوة على ذلك ، تمت محاكاة البيانات بأستخدام تحليل المكون الرئيسي (PCA). تم استخدام تحليل المكون الرئيسي (PCA) لتفسير البيانات.

الكلمات المفتاحية: الأمونيا، D- الفركتوز، D- الجلوكوز، التحليل الطيفي FTIR، الميلانودين.