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RESEARCH ARTICLE

FT-MIR Spectra of Fructose and Glutamine-Based Edible Polymer

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ABSTRACT

The Maillard mechanism frequently gives numerous varieties of food a distinctive flavor and visually appealing hue. The Maillard reaction (MR) is additionally referred to as the non-enzymatic glycosylation, and MR contain a variety of chemical compounds, including precursors for the brown hue generated in the Maillard reaction and compounds that eventually form melanoidins, which are brown that develop into dark-colored hue nitrogenous polymer molecules. Melanoidin polymer was synthesized by integrating L-glutamine and D-fructose and then scorching the resulting mixture at 130 ˚C for 20 minutes. Additionally, dialysis was conducted to eliminate low molecular weight fractions. The utilization of Fourier Transform Mid-Infrared Spectroscopy (FT-MIR) for profiling melanoidin polymers was expanding. On the other hand, the FT-MIR methodology reinforced the preliminary melanoidin structural diagnosis. This particular type of melanoidin does not exhibit discernible stretching vibrations of the NH3 band in the 3000 cm^{-1} region. There are no discernible carboxyl or carbonyl groups at the wavenumber of 1760 cm^{-1}. In the structural makeup of glutamine-fructose melanoidin, the functional groups encompass the O-H, C-H, amide I, II, and III, and C-O groups. Following being isolated from the glutamine-fructose model system, melanoidin demonstrated a potent chelating tendency for copper (Cu^{+2}) ions. A satisfactory chelating affinity for copper (II) ions could be seen in melanoidin which was derived from the glutamine-fructose model system. In the Ames test, the fructose-glutamine mixture indicated no toxicity even when tested at extremely high dosages.

Keywords: Ames, Fructose, FT-MIR spectra, Glutamine, Melanoidin

Introduction

In 1912, French chemist Louis Camille Maillard initially identified the Maillard reaction (MR), the basic process underpinning thermal flavor generation.[1] The Maillard reaction consists of a total of four stages: Initially, an interaction between reducing sugar (-C=O group) and amino acid (-NH group) takes place. In the resultant reaction, H_2O and unstable N-glycosides (N-glycosylamine) are formed. The second step, N-glycosylamine instability leads to Amadori rearrangement (AR). Further, a group of chemical constituents identified as aminoketose substances is generated via AR. In the third step, following additional rearrangement, the aminoketose substances produce taste, odor, flavors, and hues (bright hues).[1–3] Finally, high-molecular-weight chemical compounds called melanoidins, which appear at the end of the Maillard reaction, oscillate in color from dark brown to extremely black.[2] Additionally, nitrogenous moieties are included in the polymer structure of melanoidin. Black garlic, coffee, bread crust, baked products, malt, and cocoa are among the foods that are particularly high in melanoidins.[2,4–6] Due to the intricacy of the Maillard reaction system, it is challenging to characterize the molecular backbone of polymeric melanoidin. Although melanoidin possesses powerful antioxidant, metal-chelating, antibacterial, and prebiotic behavior, its skeleton is still not entirely understood.[7] By using cellulose dialysis tubing in bath dialysis, large molecules (HMW) of melanoidin can be acquired.

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Numerous authors have postulated the melanoidin form. In the findings of Tressel et al.,[8] successive pyrrole and/or furan groups can produce the melanoidin structure. Furthermore, the melanoidin network produced by the glucose-alanine system comprised two 3-deoxyglucosone molecules associated with each amino acid molecule.[9] Since it is a rapid, practical, and beneficial approach, Fourier Transform Infrared (FTIR) spectroscopy is frequently utilized in polymer diagnostics.[9,10] The FT-IR approach is increasingly being used to figure out melanoidin structures. Nevertheless, the mid-infrared spectroscopy (MIR) technique depends on the dipole bonds in groups with functional properties of molecular structures to absorb infrared radiation. Four major regions of the MIR spectra could potentially be distinguished, which is crucial for the makeup of polymers being the double bond zone spanning from 1799 to 1600 cm^{-1}.[2] However, Oracz and Zyzelewicz[2] endeavored to use the polymer extracted from cocoa beans combination for the diagnosis of its composition. They further demonstrated that despite using several kinds of techniques for analysis to promote FT-MIR, the melanoidin body structure continued to be unclear. High-molecular-weight MR products, termed melanoidin polymers, have been revealed to exhibit metal-chelating abilities. In addition, it is considered that melanoidin molecules serve as requirements for the binding of crucial metals and nutrients.[11] The present study attempts to employ the FT-MIR method in the description of the melanoidin sample extracted from the glutamine-fructose mixture. The potential of glutamine-fructose polymer to chelate metals has been studied. Academic studies have proven that many synthetic melanoidin polymers are innocuous.

Materials and methods

Chemicals

$_D$-glucose and $_L$-glutamine ($\geq$ 99%, for food chemistry experiments) were purchased from Carl Roth (Karlsruhe, Germany). Copper (II) sulfate ($CuSO_4.5H_2O$) was purchased from Merck (Darmstadt, Germany). Potassium bromide or KBr (99%, for FTIR examination) was purchased from Riedel-de Haën (Seelze, Germany). Cellulose dialysis tubes (to acquire a polymer with a high molecular weight) were purchased from ZelluTrans Carl Roth (MWCO 12.000-14.000 Da).

Melanoidin polymer preparation

After blending glutamine and reducing sugar like fructose in a molar proportion of 1:1 on a stainless-steel plate, the resultant mixture was oven-baked for 20 min at 130 °C. The polymeric raw substance was dialyzed using the procedure outlined by Mohsin et al.[12] as exhibited by Fig. 1. Moreover, eliminating low molecular weights is the main objective of dialysis. Using Cu^{+2} as a reference, the complex characteristics were examined. As a result, aqueous $CuSO_4$ typical solutions in the range of 5 to 45 mM were prepared. A combined 60 μL of distilled water was added to each Cu^{+2} solution. The procedure for preparing melanoidin polymers is depicted in Fig. 1.

Ames assay

The Ames test was carried out following Taylor et al.[13] with certain modifications. In this investigation, strains TA98 and TA100 were employed. Utilizing diverse strains is crucial as certain mutations within the particular strain increase their susceptibility to distinct mutagens. The assays were rendered in two duplicates.

Description of the FT-MIR method

OMNIC application software (Version 9.3.30) from Thermo Scientific USA was applied for collecting and examining MIR data. The method described by Mohsin et al.[12] was followed while performing FT-MIR measurements, but slight modifications were implemented. With 0.5 g of pure, freeze-dried melanoidin substances with a wavenumber range of 4000–900 cm^{-1} and a resolution of 4 cm^{-1}, the Fourier transform infrared spectra were gathered.

Fluorescence intensity measurement

Throughout the experiment, emission wavelengths from 100 nm to 900 nm at ambient temperature were captured utilizing a fluorescence spectrometer (F-7000, Hitachi, Japan) to determine the intensity of fluorescence of polymers at a wavelength of excitation of 470 nm.

Evaluation of statistics

Figs. 2 and 3 display the mean $\pm$ standard deviations of ten repetitions derived from FT-MIR and Fluorescence spectrometer measurements. Utilizing Origin 8.5 software, statistical analyses were performed.

Results and discussion

FT-MIR spectra of crystalline fructose and glutamine

Raw protein and sugar can be assessed through FTIR spectra in a range of conditions with a small

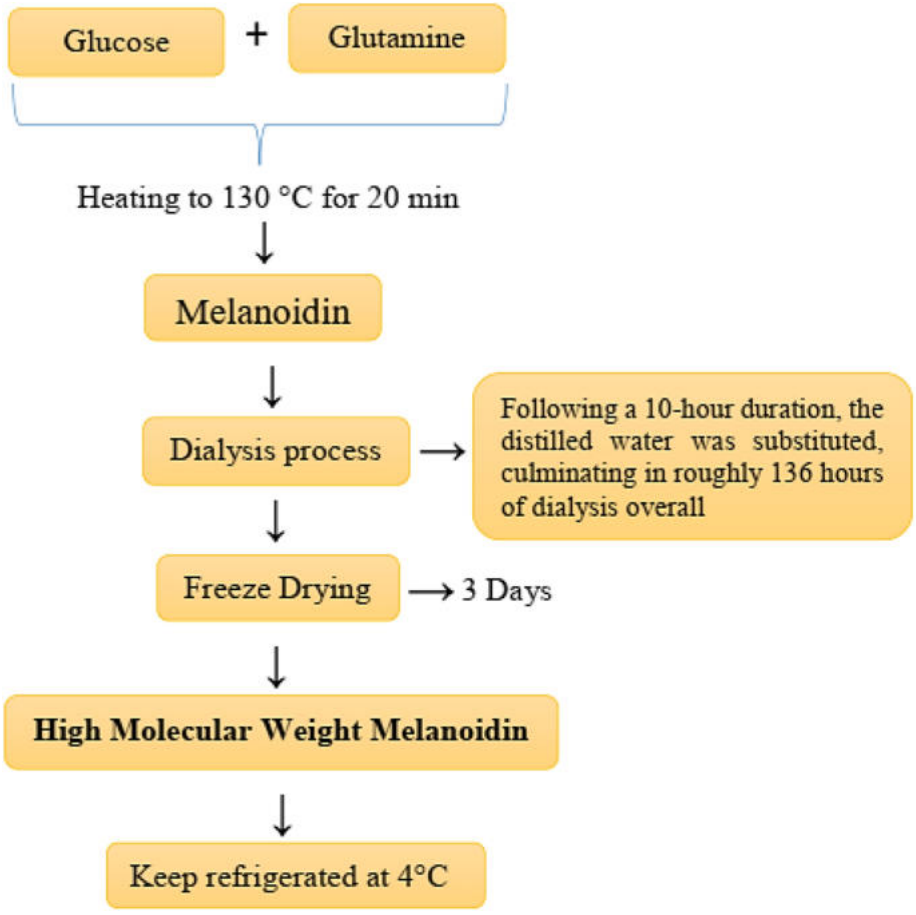

Fig. 1. Preparation of melanoidin polymer.

sample amount. The range of 3640–3070 cm^{-1} is occupied by the stretching vibrations of -OH groups in sugars (fructose)[14] as seen in Fig. 2a. Following, we detected the stretching vibrations of the C-H groups that are represented in the molecular structure of fructose in a wavelength range of 2940–2850 cm^{-1}.[15,16] The distinctive fingerprint region of pure fructose encompasses the range of 1690 to 900 cm^{-1}

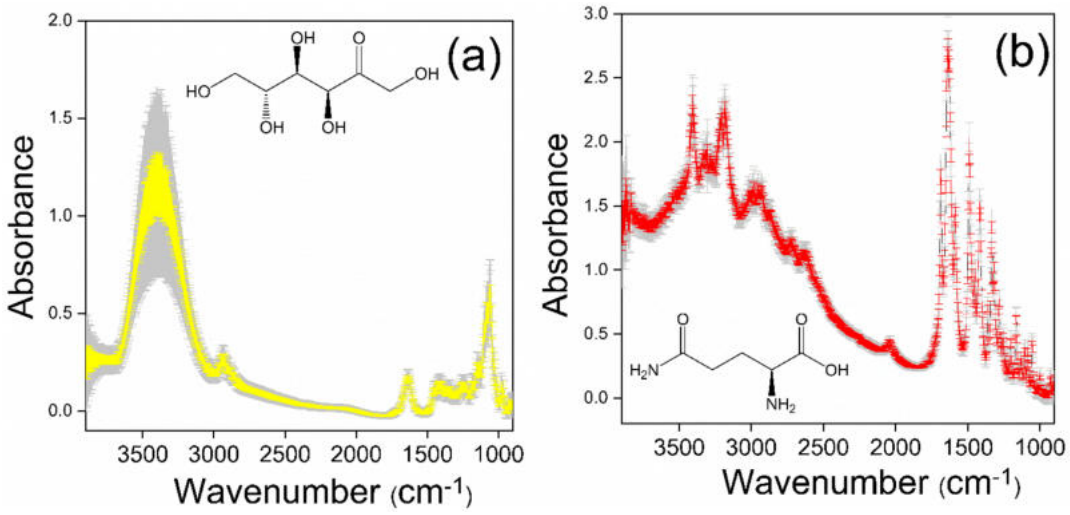

Fig. 2. FT-MIR spectra of (a) raw fructose and (b) raw glutamine.

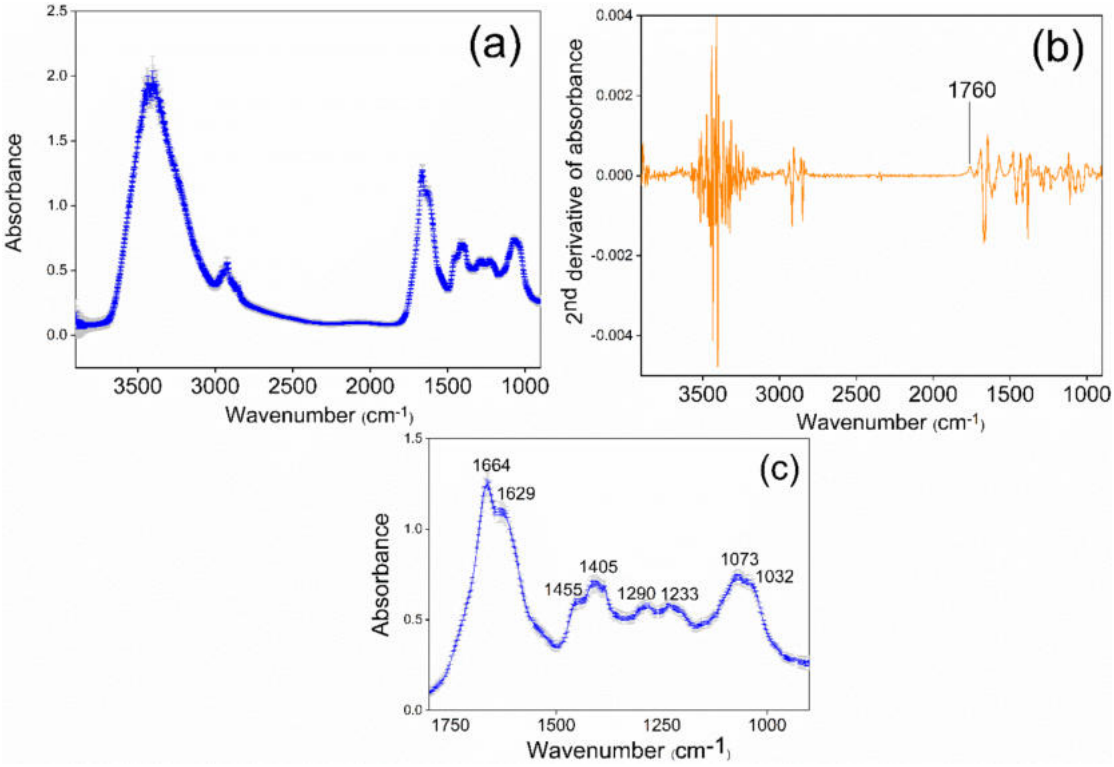

Fig. 3. (a) FT-MIR spectra of melanoidin, (b) 2nd derivative of absorbance, and (c) FT-MIR spectra of melanoidin from 1750 to 900 cm^{-1}. The standard deviation is illustrated by each confidence interval outlined in a different hue in the FT-MIR spectrum.

and consists of several bands. The C=O stretching vibrations are responsible for a very significant region that is weakly noticeable in the spectra, with a maximum of roughly 1640 cm^{-1}.[14] The region from 1066 to 965 cm^{-1} is where the band assignments of C-O in the C-OH group or C-C stretching in the sugar backbone can be noticed, as in Fig. 2a. The FT-MIR spectrum of pure glutamine is exhibited in Fig. 2b. A substantial number of bands have been assigned to NH_3 stretching from 3410 to 3180 cm^{-1}. Further, numerous bands correspond to C-H vibrations extending from 2967 to 2608 cm^{-1}. The most noticeable and perceptible vibrational bands of the glutamine structure are the amide I band from 1687 to 1600 cm^{-1}, and they are correlated to the secondary structural aspects of proteins. Owing to C=C and C=N stretching, these bands are sharp.[12,17] However, it is crucial to investigate how the amide II and III spectral regions influence the creation of the melanoidin structure. Following extensive investigation, it is determined that bands between 1490 and 1203 cm^{-1} are caused by amides II and III[18,19] as demonstrated in Fig. 2b.

FT-MIR spectra of glutamine-fructose melanoidin

Employment of FT-MIR analysis can reveal whether particular functional groups are present. It mostly examines the structural alterations of polymer utilizing the typical, recognizable bands that emerge in the mid-infrared spectrum. Fig. 3 depicts the standard FT-MIR spectra of the glutamine-fructose melanoidin employed in the present study. The spectra were gathered over a wavelength band of 4000–900 cm^{-1}. The NH_3 stretching vibrations of free glutamine in this area are not visible for this type of polymer, as illustrated in Fig. 3a. The 3900–3000 cm^{-1} range should come from the overlapped amides A and B with the O-H group rather than simply being water. A region of high intensity was perceived at 3418 cm^{-1}, corresponding to either the primary amine (NH_2) or hydroxyl (OH) groups in melanoidin's structural spectrum. No particular band is seen at the spectral wavelength of 1760 cm^{-1} attributed to the carboxyl or carbonyl group in Fig. 3a. Further, this weak band can be noticed in the 2nd derivative of absorbance at a spectral frequency of 1760 cm^{-1} as seen in Fig. 3b. The ability to differentiate overlapping bands can be improved through the application of second-derivative spectra.[12] It was found that the spectral region 1664–900 cm^{-1} that comprises the FT-MIR fingerprints of this polymer is extremely beneficial in identifying the basic structure of melanoidin. The structural modifications in proteins can be noticed applying MIR detection. The wavenumbers 1664–

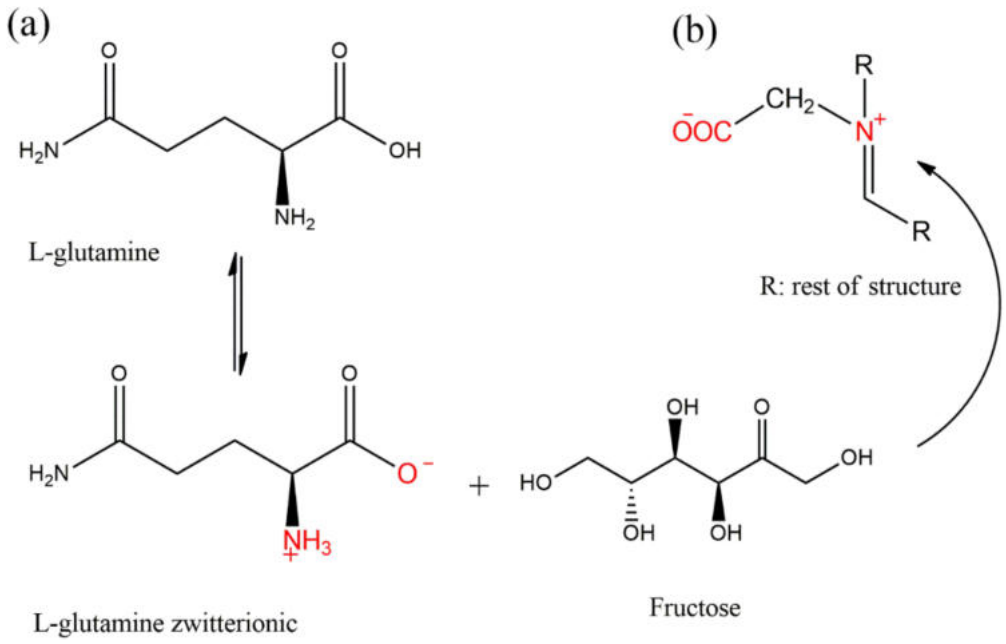

Fig. 4. (a) Structure of amino acid (glutamine) and sugar (fructose), and (b) A part of the proposed melanoidin body.

1629 cm^{-1} imply the representation of the carbonyl (C=O) and imine (C=N) groups. Additionally, the same region possesses the C=C functional group absorption bands. It has been shown that the conjugated linkages of C=C, C=O, and C=N are chromophoric molecules in the final product obtained from the glutamine-fructose model by polymerization during the reaction known as the Maillard reaction.[12] The initially generated amide band amplitude is highly responsive to the secondary structure of proteins, including α-helix, β-turn, β-sheet, and random coil sequences. Thus, it is thought that the initial amide band serves as a recognition of fingerprints for the secondary protein skeleton.[12] The range of 1455 to 1233 cm^{-1} is covered by the amide II and III bands as in Fig. 3c. The vibration of the C-O stretching bond in the skeleton of carbohydrates is represented by a wide region between 1073 and 1032 cm^{-1}.[20] Considering that the amide group linkages (C-N, N-H, and C-O) that exist in these regions are linked to the Maillard reaction products, including LMW compounds that form segments of the melanoidin body structure.[2,12] The FT-MIR method is implemented to define the overall composition of melanoidin initially. Concerning the high molecular weight melanoidin fraction, the FT-MIR analysis indicated a variety of bioactive molecules with distinct modes of function. This conclusion aligns with the findings of other investigators who demonstrated the binding of low molecular weight compounds, as subunits into the HMW melanoidin backbone.

An alpha-amino group in the protonated NH^+ form and a carboxylic acid group in the deprotonated COO^- form are both present in the $_L$-glutamine skeleton as displayed in Fig. 4. Crucial building blocks of the melanoidin body are the functional groups NH^+ at 3080 cm^{-1} and COO^- at 1664 cm^{-1}. In a study conducted by Yaylayan and Kaminsky,[21] a nitrogenous polymer derived from a glucose-glycine combination is naturally zwitterionic. Likewise, Mohsin et al.[12] displayed that the melanoidin skeleton produced from a sugar-ammonia mixture under diverse thermal processing circumstances is zwitterionic. However, zwitterionic polymer compounds are incredibly helpful for a variety of tasks, including the distribution of prescription medication and the separation of oil and water. In contrast, zwitterionic polymers are typified by several unique features, such as their temperature responsiveness, anti-polyelectrolyte behavior, and high hydration. The aforementioned characteristics are caused by their intriguing chemical compositions, which have exactly equal amounts of cationic and anionic units on the molecular chains.[22,23]

Influence of complex Cu^{+2} on glutamine-fructose melanoidin

Before dialysis, melanoidin demonstrated a significant peak due to an assortment of substances with both high and low molecular weight fractions. For the sake of allowing the low molecular weight

molecules to shift through the dialysis tubes during dialysis, distilled water is often utilized. Melanoidin thus acquires a high molecular weight (HMW). Alpha-dicarbonyl intermediates aid in building the body structure of LMW melanoidin. A tiny fraction of low-molecular-weight substances survive dialysis and serve as subunits in the melanoidin backbone.[8,12] They came to the outcome that glyoxal (Oxalaldehyde) and glyoxylic acid (2-Oxoacetic acid) are involved in the structure of melanoidin during various thermal processes. It has been observed that melanoidins, a high-molecular-weight result of the Maillard process, exhibit metal-chelating characteristics.[17,24] The capacity of melanoidin synthesized from the glutamine-fructose system to complex Cu^{+2} ions were studied as depicted in Fig. 5. Following is a synopsis of the peaks found in Fig. 5: melanoidin is represented by the red probe before dialysis, while the blue and black probes represent melanoidin after dialysis and the addition of copper ions, respectively. The generation of free radicals is a conceivable outcome of copper ions.[25] On top of that, melanoidin, which was extracted from the glutamine-fructose model system, showed a substantial chelating propensity for copper (Cu^{+2}) ions. These findings exhibit structural changes caused by an intricate or chelate formation because of the interaction of glutamine-fructose melanoidin and metal ions. When molecules present in LMW compounds that include nitrogen and oxygen are eliminated during dialysis as a result, these groups are no longer able to serve as liaisons for metal ions, and their capacity to chelate Cu^{+2} is diminished. Copper ions cause a reduction in the hue of the melanoidin polymer.

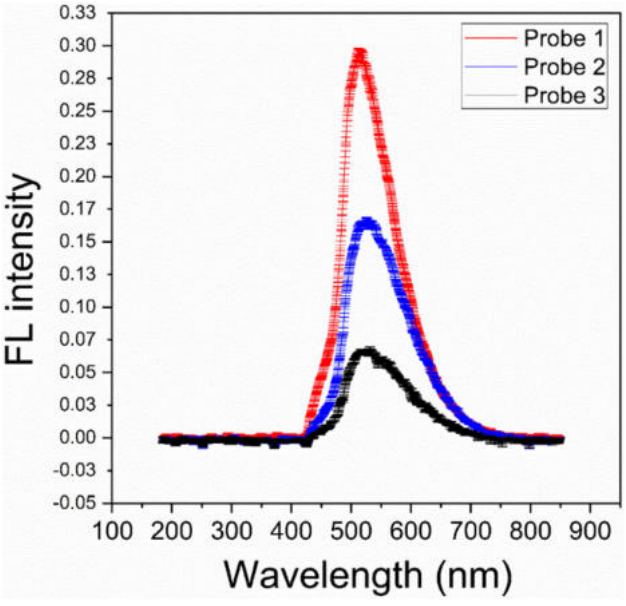

Fig. 5. Fluorescence spectrometer of various melanoidin. The standard deviation is indicated by all confidence intervals displayed in various colors in the UV/Vis spectra. It can be described as follows: glutamine-fructose melanoidin before dialysis (red line), melanoidin after dialysis (blue line), and melanoidin with Cu (II) complex (black line).

Efficacy of the glutamine-fructose model on genotoxicity

The exact process by which melanoidin forms and its composition are still obscure. Additionally, despite several research efforts, the biological consequences of melanoidin are still not entirely recognizable. Different findings from earlier investigations that have been published in the papers have been observed about the mutations of the isolated fractions of MRPs as melanoidin polymers. A great deal of research has been conducted on the mutagenicity of MRPs with the advent of rapid genotoxicity trials for example the Ames experiments. Several strains of Salmonella typhimurium are employed in the Ames examination, which is frequently used for discovering mutagenic compounds and to distinguish various kinds of gene mutations. Using the Ames assay, Taylor et al.[13] established that neither the LMW nor the HMW fractions derived from a glucose-glycine model by dialysis exhibited appreciable genotoxicity. Furthermore, Glösl et al.[26] asserted that neither the TA98 nor the TA102 strain exhibited mutagenic ability in the Ames assay when melanoidins synthesized from a glucose-glycine combination were applied. They pointed out that these melanoidins had minor but noticeable genotoxic consequences on human lymphocytes the low molecular weight melanoidins especially caused harm to cells called Caco-2. To be precise, we are the initial researchers to investigate the toxicity of glutamine-fructose-derived high-molecular-weight melanoidin. However, the Ames test was performed on the HMW fraction of fructose-glutamine melanoidin gained via dialysis. This leads us to the conclusion that the polymer can be utilized in a variety of food-related industries due to its safety. Numerous carcinogenic substances, including acrylamide and aromatic amines, are LMW and eliminated during the dialysis of melanoidin polymers. Although being evaluated at extremely high concentrations, the fructose-glutamine combination displayed no genetic toxicity in this assay. This leads us to the conclusion that the polymer can be utilized in a variety of food-related industries due to its safety. Triggering mutations are mostly dependent on the kind of sugar and amino acid implemented for the synthesis of the polymer.[27]

Conclusion

It is possible to detect whether particular functional groups exist in the polymer body through the application of a method dubbed FT-MIR. The current study illustrated the viability of characterizing glutamine-fructose melanoidin employing FT-MIR spectral fingerprinting. The amides A and B that coincide with the O-H group should contribute to the range of 3000–3900 cm^{-1} rather than simply being water. For this kind of melanoidin, the stretching vibrations of the NH_3 band in the 3000 cm^{-1} area cannot be seen. The area extending to approximately 1700 cm^{-1} is deprived of carbonyl or carboxyl groups. Through polymerization during the reaction termed Maillard, the conjugation links of C=C, C=O, and C=N have been demonstrated to be chromophoric molecules in the final product generated from the glutamine-fructose model. It emerges that functional groups consisting of OH, CH, amide I, II, and III, in addition to the C-O group, are included in the structure of glutamine-fructose melanoidin. Extracted from the glutamine-fructose model system, melanoidin exhibited a significant chelating tendency for copper (Cu^{+2}) ions. Ultimately, it can be said that melanoidin which was obtained from the glutamine-fructose model system possesses a proper chelating affinity for copper (Cu^{+2}) ions. The fructose-glutamine combination revealed no toxicity in the Ames experiments, although it was tested at highly concentrated.

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Author's declaration

- Conflicts of Interest: None.
- I hereby confirm that all the Figures in the manuscript are mine. Furthermore, any Figures and images that are not mine have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: This project was ethically cleared by the local committee at the Department of Vocational Education in Maysan.

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أطياف FT-MIR للبوليمر الصالح للأكل القائم على الفركتوز والجلوتامين

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وزارة التربية، المديرية العامة للتعليم المهني، مديرية التعليم المهني في ميسان، ميسان 62001، العراق.

الخلاصة

تمنح آلية ميلارد في كثير من الأحيان العديد من أنواع الطعام نكهة مميزة ولونًا جذابًا بصريًا. بالإضافة إلى ذلك، يُشار إلى تفاعل ميلارد (MR) بأسم الكليكوزيل غير الأنزيمي، ويحتوي MR على مجموعة متنوعة من المركبات الكيميائية، بما في ذلك سلائف اللون البني المتولد في تفاعل ميلارد والمركبات التي تشكل في نهاية المطاف الميلانويدين، وهي بنية اللون وتتطور إلى جزيئات البوليمر النيتروجينية ذات اللون الداكن. تم تصنيع بوليمر الميلانويدين عن طريق دمج L-الجلوتامين و D-الفركتوز ثم تسخين الخليط الناتج عند 130 درجة مئوية لمدة 20 دقيقة. بالإضافة إلى ذلك، تم إجراء الديلزة للتخلص من الأجزاء ذات الوزن الجزيئي المنخفض. تم التوسع في أستخدام التحليل الطيفي للأشعة تحت الحمراء المتوسطة (FT-MIR) لتحديد ملامح بوليمرات الميلانويدين. من ناحية أخرى، عززت منهجية FT-MIR التشخيص الهيكلي الأولي للميلانويدين. لا يُظهر في هذا النوع المعين من الميلانويدين اهتزازات تمدد ملحوظة لنطاق NH3 في منطقة 3000 سم-1. لا توجد مجموعات كربوكسيل أو كربونيل يمكن تمييزها عند الرقم الموجي 1760 سم-1. في التركيب الهيكلي لميلانويدين الجلوتامين-الفركتوز، تشمل المجموعات الوظيفية مجموعات OH وCH وI amide وII وIII وC-O. بعد عزله من نظام نموذج الجلوتامين-الفركتوز، أظهر الميلانويدين ميلًا قويًا لخلب أيونات النحاس (Cu+2). يمكن رؤية تقارب مخلبي مرضي لأيونات النحاس (II) في الميلانويدين المشتق من نظام نموذج الجلوتامين-الفركتوز. في اختبار أميس، لم يشير خليط الفركتوز والجلوتامين إلى أي سمية حتى عند اختباره بجرعات عالية للغاية.

الكلمات المفتاحية: أميس، الفركتوز، أطياف FT-MIR، الجلوتامين، الميلانويدين.