Materials
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ARTICLE

Optimization of Extraction Conditions to Synthesize Green Carbon Nanodots from Maillard Reaction

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Carbon nanodots (CNDs) are a class of nanoparticles with unique optical properties with broad applications in various fields. However, synthesizing CNDs with high fluorescence intensity and small size using green solvent and low temperature remains challenging. In this study, we investigated using the Maillard reaction at 120°C for 12 hours for synthesizing CNDs and optimized the reaction conditions to achieve CNDs with desirable properties. The results showed glycine was the most effective amino acid for CNDs formation when combined with sucrose. A molar ratio 1:1 for glycine: sucrose resulted in the highest fluorescence intensity. The fluorescence intensity increased remarkably with 40% ethanol as the extraction solvent. Despite that, a high ethanol concentration (above 60%) had an inverse relationship with CNDs' fluorescence intensity, indicating that a high-concentration ethanol solution prevented the Schiff base formation. The purified CNDs (M-CNDs) were characterized with fluorescent spectrophotometer, UV-Vis, Raman spectroscopy, and TEM. These findings provide a purification process of sustainable CNDs from the Maillard reaction using green solvents with optimized conditions and low temperatures.

Introduction

Carbon nanodots (CNDs) - nanoparticles with sizes smaller than 10 nm have been reported for the first time since 2004.[1] CNDs were attracted because of their intense fluorescent properties, high water solubility, stability, and ease of functionalization.[1–3] Since its discovery, CNDs have garnered significant attention across various sectors due to their highly fluorescent properties and potential applications in diverse fields, including biomedical,[4] food safety,[5] and the bio-electrical industry.[6] In the food industry, CNDs have emerged as promising compounds for developing high-sensitivity probes to detect contaminants like heavy metals,[7,8] pesticides,[9,10] and food additives,[11,12] cooperating with packaging materials to detect spoilage of foods,[13–15] and even detecting foodborne pathogens.[16,17] Notably, CNDs claimed low toxic compounds[18] and biocompatible drug delivery and bioimaging applications.[19,20] Studies show that nitrogen-doped CNDs with surface modifications do not harm human lung epithelial tissue in vitro and can exhibit antioxidant and anti-cancer properties.[21,22] However, there is a research gap to prove that CNDs can be used without any health concerns. CNDs had low cytotoxicity depending on the dose, the synthesis process, and the precursors.[23–25] Therefore, choosing safe precursors for CNDs synthesis is emerging as a promising strategy to enhance their safety for food industry applications.

The Maillard reaction, a complex series of reactions between reducing sugars and amino groups, is a crucial process in the food industry, affecting food’s aesthetic, functional, and nutritional properties.[26] This reaction reported a close relationship with the formation of CNDs.[27,28] CNDs are a marker to control the Maillard reaction,[28] directly related to the formation of melanoidins, brown pigments with high aromaticity.[27]

In this study, we investigated the usage of amino acids and sucrose as a model system of the Maillard reaction for synthesizing CNDs and optimizing the reaction conditions to achieve CNDs with desirable properties. Using safe precursors (amino acids and sugars) from a fundamental food reaction is advantageous for CNDs safety. Furthermore, using lower temperatures and greener solvents, we aim to reduce the formation of toxins in the synthesis process and create safer CNDs with standardized quality. Purified CNDs were characterized using transmission electron microscopy (TEM), fluorescent, Raman, and UV-Vis spectroscopy. The obtained

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CNDs were used as a standard to measure the concentration of CNDs forming in the breadmaking process.

Materials and Methods

Materials

Amino acids (L-arginine, Glycine, L-tyrosine, L-proline, L-glutamine, and L-phenylalanine) and sucrose were obtained from Vital-Trend Ltd. (Budapest, Hungary). Hydrochloric acid (1.0 mol L^{-1}), ethanol (≥99.5%), and sodium hydroxide solution (0.01 mol L^{-1}) were used (VWR International Ltd., Pennsylvania, United States). Ultrapure water was used for all experiments. Fresh bread was bought from ALDI supermarket on the experiment day.

Characterization

The morphology and particle size of CNDs were measured using a transmission electron microscope (TEM) of JEM-2000FXII (JEOL Ltd., Tokyo, Japan). A Lambda 35 UV-Vis spectrophotometer (Norwalk, USA) was used to measure the ultraviolet-visible (UV-vis) spectra in the 200 - 600nm range. Olympus Endosonic Ultrasonicator (Olympus Co., Tokyo, Japan) was used for ultrasound steps. The Raman spectra were measured using a LabRAM HR Evolution Confocal Raman Microscope (Horiba, Ltd., Kyoto, Japan).[29] The fluorescent spectra were evaluated using a Spectrofluorometer FP-8500 produced by Jasco in Oklahoma, United States.[30]

Experimental design

The synthesis method of CNDs from the Maillard reaction was based on the published procedure with modification.[25] The optimal conditions for the Maillard reaction were investigated to achieve CNDs with high fluorescent intensity and small size following three experiments, which illustrated in **Figure 1**. (1) Initially, six amino acids—L-arginine (Arg), Glycine (Gly), L-Tyrosine (Tyr), L-Proline (Pro), L-Glutamine (Gln), and L-Phenylalanine (Phe)—were selected as precursors in combination with sucrose to form CNDs. (2) Following the selection of an amino acid formed highest quality of CNDs, the ratio between amino acid and sucrose was examined using nine combinations, including 2 mmol: 0 mmol, 1.75 mmol: 0.25 mmol, 1.5 mmol: 0.5 mmol, 1.25 mmol: 0.75 mmol, 1 mmol: 1 mmol, 0.75 mmol: 1.25 mmol, 0.5 mmol: 1.5 mmol, 0.25 mmol: 1.75 mmol, 0 mmol: 2 mmol. (3) Subsequently, various extraction solvents were assessed using nine different options: Control (DI water), DI water adjusted to pH 2.0, DI water adjusted to pH 9.0, ethanol (EtOH) at 20%, EtOH 40%, EtOH 60%, EtOH 80%, and absolute EtOH. The optimal conditions were determined based on fluorescent spectra, and each treatment was performed in triplicate.

To synthesize and purify CNDs, the solution was heated at 120°C for 12 hours and cooled naturally to room temperature. Cold ethanol was added to the product at a 1:1 (v/v) ratio to remove residual amino acids and incubated for 30 minutes at -20°C. Subsequently, the mixture was centrifuged at 10,000 rpm for 30 minutes. The supernatant obtained was passed through a cellulose column, and the resulting solution was collected and filtered through a 0.22 µm filter. After freeze-drying, the powder of the Maillard reaction-derived carbon nanodots (MR-CNDs) was collected and stored for future use. The purified CNDs were characterized using TEM, Raman, UV-Vis, and fluorescence spectroscopy.

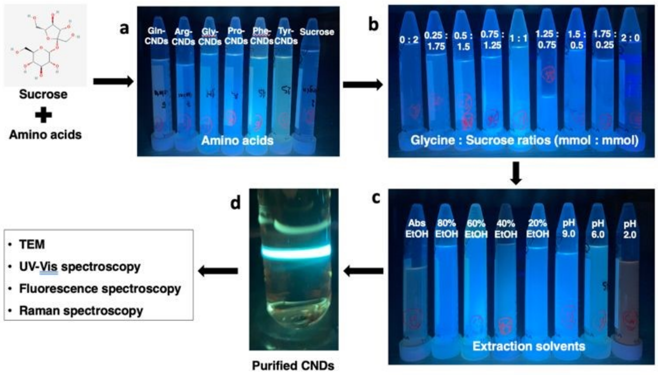

Figure 1. Schematic diagram of the experimental design listing three experiments and obtaining purified carbon nanodots (CNDs) with authentic images taken under a UV lamp. **a**, the effect of different amino acids on the fluorescence of amino acid-based carbon nanodots (AA-CNDs) including L-arginine (Arg), Glycine (Gly), L-Tyrosine (Tyr), L-Proline (Pro), L-Glutamine (Gln), and L-Phenylalanine (Phe); **b**, the impact of the ratio between glycine and sucrose (mmol/mmol) on the fluorescence of CNDs (2 mmol: 0 mmol, 1.75 mmol: 0.25 mmol, 1.5 mmol: 0.5 mmol, 1.25 mmol: 0.75 mmol, 1 mmol: 1 mmol, 0.75 mmol: 1.25 mmol, 0.5 mmol: 1.5 mmol, 0.25 mmol: 1.75 mmol, 0 mmol: 2 mmol); **c**, the effect of extraction solvents on the fluorescence of CNDs including Control (DI water), DI water adjusted to pH 2.0, DI water adjusted to pH 9.0, ethanol (EtOH) at 20%, EtOH 40%, EtOH 60%, EtOH 80%, and absolute EtOH; **d**, the purified CNDs characterized with TEM, UV-Vis, fluorescence, and Raman spectroscopy.

Statistical analysis

The statistical results were analyzed using IBM SPSS version 25.0 (New York, USA). TEM pictures and particle distribution were processed using ImageJ software version 1.54D, developed by the National Institutes of Health (New York, USA). All charts were illustrated using GraphPad Prism version 9.0 (California, USA).

Results and Discussion

Optimization of synthesis carbon nanodots from Maillard reaction.

To investigate the impact of extraction conditions on the formation of carbon nanodots (CNDs) through the Maillard reaction, all parameters, except the studied variable, were kept constant according to the following procedure. Initially, glycine (0.053 g, 1 mmol) and sucrose (0.180 g, 1 mmol) were dissolved in 20 mL of deionized (DI) water. The solution underwent heating at 120°C for 12 hours, followed by natural cooling to room temperature. Subsequently, it was mixed with cold

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ethanol in a 1:1 ratio (v/v), and the mixture was incubated in the freezer for 30 minutes. Afterward, centrifugation at 10,000 rpm for 30 minutes separated the supernatant, which was then passed through a cellulose column to eliminate impurities. The resulting liquid was filtered through a 0.22 µm filter and subjected to freeze-drying. The obtained powder represented the Maillard reaction-derived carbon nanodots (MR-CNDs) product. This method was modified with a lower temperature [31] to prevent the formation of toxic compounds in Maillard reactions like 5-HMF.[32]

The fluorescence signal was measured using a fluorescence spectrophotometer with an excitation wavelength selected after scanning the excitation and emission wavelengths of standardized CNDs, as discussed in the subsequent section.

The effect of different amino acids on the formation of CNDs.

The Maillard reaction is a complex series of reactions between reducing sugars and amino acids.[26] These reactions can lead to the formation of various new compounds, including CNDs. Furthermore, CNDs have been reported as a marker to control the Maillard reaction.[28] To understand the relationship between the Maillard reaction and the formation of CNDs, amino acids, and sucrose was chosen as a study model for the Maillard reaction. **Figure 2** illustrates the distinct fluorescent spectra of CNDs generated from sucrose and various amino acids, including tyrosine, proline, glycine, arginine, glutamine, and phenylalanine. The distinct excitation wavelengths for each amino acid are highlighted in the figure: 340 nm for tyrosine, 360 nm for proline, and 380 nm for glycine, arginine, glutamine, and phenylalanine. The fluorescence intensity of the different amino acids varies significantly (**Figure 2**). Glycine-based carbon nanodots have the highest fluorescence intensity, followed by phenylalanine, proline, glutamate, arginine, and tyrosine, as shown in **Figure 2**. It is likely due to glycine's enhanced reactivity in the Maillard reaction, which is attributed to its higher susceptibility to forming Schiff bases, intermediate products of the Maillard reaction. These Schiff bases can then react to form other fluorescent compounds, such as melanoidins and brown pigments. These melanoidins are essential precursors for forming CNDs.[27]

The different chemical structures of the amino acids can explain this phenomenon. Tyrosine and phenylalanine contain aromatic rings known as fluorescent.[33] Proline has a ring structure, but it is not aromatic. Glycine and arginine are both non-aromatic amino acids. Glutamine is an amino acid that can be converted to glutamine, a non-fluorescent amino acid.[34] Fluorescence intensity depends on the structure of amino acids; the simpler the structure, the higher the formation of CNDs, and the higher the fluorescence intensity, as depicted in **Figure 3**. **Figure 3** illustrates the fluorescence intensity of amino acids (without the synthesis process) and amino acid-based CNDs. Although phenylalanine resulted in the highest intensity (724.0 ± 4.0), the intensity of Phe-CNDs (1900.0 ± 9.1) was lower than Gly-CNDs (2024.0 ± 3.7), where glycine was the weakest in fluorescence intensity at the beginning (134.9 ± 1.9). These findings suggest glycine is a good precursor for synthesizing CNDs with high fluorescence intensity.

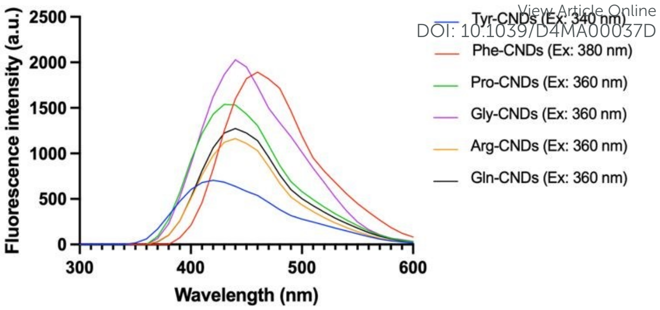

Figure 2. Fluorescent emission spectra of carbon nanodots (CNDs) formed from various amino acids and sucrose. The emission spectra plotted with fluorescence intensity (a.u.) with the excitation wavelength depending on the type of amino acid: 340 nm for Tyrosine (Tyr), 360 nm for Proline (Pro), Glycine (Gly), Arginine (Arg), Glutamine (Gln), and 380 nm for Phenylalanine (Phe).

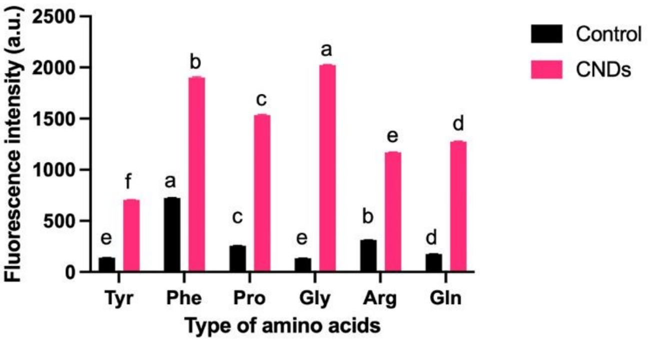

Figure 3. Fluorescence intensity (a.u.) of amino acid-based carbon nanodots (CNDs) and amino acids (Control). Each amino acid (AA) - Tyrosine (Tyr), Proline (Pro), Glycine (Gly), Arginine (Arg), Glutamine (Gln), and Phenylalanine (Phe) - was excited at specific wavelengths (340 nm for Tyr, 360 nm for Pro, Gly, Arg, Gln, and 380 nm for Phe). The error bars indicate the standard deviations of the triplicate sample. Different Ducan letters present a significant difference with a confidence interval of 95% compared with other samples at the same treatment.

Effect of the ratio between glycine and sugars (mmol/ mmol) on the formation of CNDs.

Based on glycine's selection as the precursor in the previous experiment, the excitation wavelength of 360 nm was readily adopted to synthesize CNDs. **Figure 4** shows the fluorescent emission spectra of CNDs formed from different ratios between glycine and sucrose (mmol/mmol). The fluorescence intensity of the CNDs increased with increasing glycine content until the ratio of 1 mmol glycine to 1 mmol sucrose (**Figure 4**). This is likely because glycine is more susceptible to forming Schiff bases. However, when the amount of glycine in the mixture continuously increased, the fluorescent intensity was remarkably reduced. This trend was attributed to the excess glycine interfering with the Formation of Schiff bases, which are essential for CD formation. Overall, it suggests that the fluorescence intensity of CNDs can be controlled by adjusting the glycine: sucrose ratio. The ratio between glycine and

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sucrose was maintained at 1:1 (mmol/mmol). It exhibited the highest fluorescent intensity. This could be useful for developing CNDs with specific fluorescence properties for different applications.

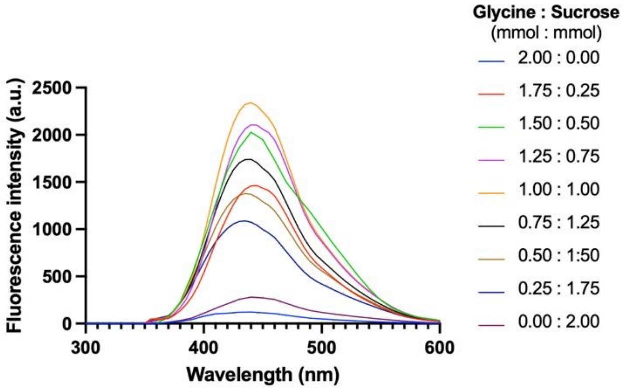

Figure 4. Fluorescent emission spectra of CNDs formed from different ratios between glycine and sucrose (mmol/mmol), measured with the excitation wavelength at 360 nm. Nine ratios was studied in this experiment, including 2 mmol: 0 mmol, 1.75 mmol: 0.25 mmol, 1.5 mmol: 0.5 mmol, 1.25 mmol: 0.75 mmol, 1 mmol: 1 mmol, 0.75 mmol: 1.25 mmol, 0.5 mmol: 1.5 mmol, 0.25 mmol: 1.75 mmol, 0 mmol: 2 mmol.

Effect of extraction solvents on the formation of CNDs

Figure 5 depicts the fluorescence intensity of carbon nanodots (CNDs) formed using various extraction solvents, with the control sample extracted using deionized (DI) water at pH 6.0. In comparison between different pH extraction solvents, the higher fluorescence intensity of CNDs is obtained when extracted with DI water at pH 9.0. In contrast, pH 2.0 seems to destroy the presence of CNDs. The structure and fluorescence properties of CNDs could be entirely converted by changing the pH of solvents in the reaction.[35] Notably, the highest fluorescence intensity of CNDs is achieved when extracted with 40% ethanol. These results were similar to the report,[36] which mentioned that ethanolic solution can significantly enhance the reaction rate of the Maillard reaction, resulting in the browning at 420 nm.

Furthermore, this phenomenon can be attributed to the favorable conditions for forming Schiff bases, crucial intermediates in the Maillard reaction. The Maillard reaction governs the complex interaction between reducing sugars and amino acids, providing strong evidence for the link between the reaction and CND formation. In contrast, an inverse relationship is observed between high ethanol concentration (60% - absolute ethanol) and CNDs fluorescence intensity, with increasing ethanol concentrations hindering Schiff base formation. These collective findings suggest a tunable control over CNDs fluorescence intensity by manipulating the pH of the extraction solvent and ethanol concentration. Specific observations highlight the maximization of fluorescence intensity at pH 9.0 and the inverse correlation with increasing ethanol concentration, reaching its lowest point with absolute ethanol extraction. These insights are valuable in tailoring CNDs for versatile applications, such as bioimaging or sensing, by optimizing fluorescence properties based on extraction conditions.

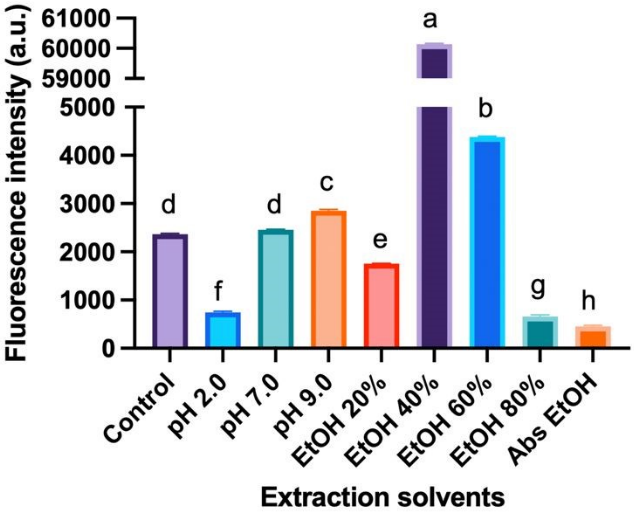

Figure 5. Formation of CNDs using various extraction solvents: Deionized (DI) Water at pH 2.0, pH 7.0, and pH 9.0; Different ethanol concentrations (20%, 40%, 60%, 80%, and absolute ethanol. The control sample was extracted with DI water measured at pH 6.0. Fluorescence intensity measurements were used to assess the formation process. The error bars indicate the standard deviations of the triplicate sample. Different Ducan letters present a significant difference with a confidence interval of 95%.

Purification and Characterization of CNDs from Maillard reaction.

The isolation procedure was performed using 1 mmol of glycine and 1 mmol of sucrose mixed in 20 mL of 40% ethanol solution at 120 °C for 12 hours, with modifications to a published procedure.[25] The resulting solution turned dark brown and exhibited intense fluorescence, from colorless without fluorescence. To extract the M-CNDs further, the mixture was sonicated for 30 minutes. The obtained solution was then mixed with cold ethanol in a 1:1 ratio (v/v) to remove impurities at -20 °C for 30 minutes. The mixture was centrifuged at 10,000 rpm for 30 minutes to obtain the purified M-CNDs. The supernatant was collected and filtered with a 0.22 µm syringe filter. The purified M-CNDs were characterized using TEM, fluorescence, Raman, and UV-Vis spectroscopy (**Figure 6**).

TEM images with 10 nm and 1 nm scales revealed the dark spherical morphology of the M-CNDs without any contaminated compounds. The size distribution of the M-CNDs ranged from 1.25 to 4 nm, with an average size of 2.5 ± 0.5 nm based on measurements of almost 300 particles in the TEM image with a scale of 10 nm (**Figure 6A**). In **Figure 6E**, the UV-Vis spectrum of M-CNDs exhibited one peak at 275 nm, indicating the presence of the π-π* electronic transition in C=C.[37] Blue solid fluorescence of M-CNDs was detected under UV light (**Figure 6B**) with a maximum emission wavelength of 438 nm and an

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excitation wavelength of 360 nm (**Figure 6D**). These findings were consistent with previous studies that used glucose and lysine or melatonin as starting materials.[25,27]

Furthermore, Raman spectroscopy analysis of the synthesized M-CNDs revealed distinct D and G bands at 1350 and 1580 cm^{-1}, respectively, as depicted in **Figure 6F**. Consistent with established literature, the low-intensity ratio of the D to G band (ID < IG) underscores the moderate graphitic structure of the M-CNDs, affirming the absence of significant carbonaceous impurities, as corroborated by similar findings in the reference study.[38] Overall, the results explain the successful isolation and characterization of M-CNDs with nanosized, intense fluorescent properties, which could be used as a safe carbon nanodot source for various applications.

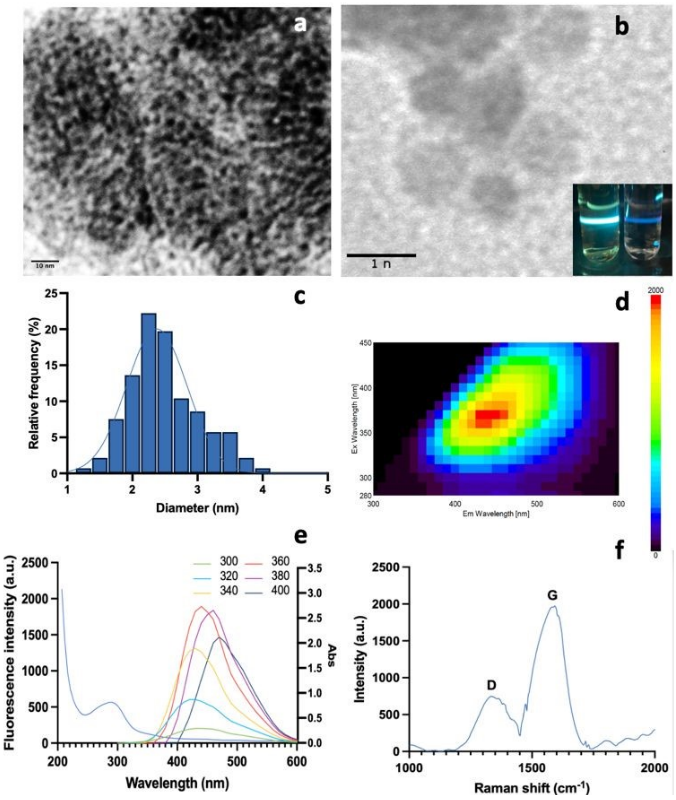

Figure 6. Characterization of isolated carbon nanodots from Maillard reaction (M-CNDs). **a**, Transmission Electron Microscopy (TEM) image of M-CNDs with a 10 nm scale. **b**, TEM image of M-CNDs with a 1 nm scale with the real image of DI water (left) and M-CNDs solution (right) on the right corner under the UV light. **c**, Particle size distribution histogram of M-CNDs with an average diameter of 2.5 ± 0.5 nm. **d**, 3-D fluorescence spectra of M-CNDs. **e**, 2D Emission-Excitation-Intensity Fluorescent Spectrum of M-CNDs (right) and UV-Vis spectrum of M-CNDs (left). **f**, Raman spectra of M-CNDs.

Use of M-CNDs as a standard for detecting CNDs in foods using a fluorescent spectrophotometer

To evaluate the purity of M-CNDs and quantitatively the carbon content of M-CNDs stock solution, the purified M-CNDs were analyzed using an elemental analyzer, resulting in a total carbon content in stock M-CNDs solution of 0.326%. Based on the carbon content, the concentration of the stock standard was 3260 mg dm-3. Standard curves were generated by measuring the intensity of the fluorescent spectrum at the excitation wavelength of 360 nm across a range of M-CNDs concentrations. The standard curve was prepared freshly on the measurement day. The resulting calibration curve for CNDs had a linear relationship for a 5-point calibration curve in the concentration range from 0 to 3200 mg dm-3 (**Figure 7**). The reproducibility of measurement was below 0.1%. The calculated detection limit of the method was 0.14 mg dm-3.

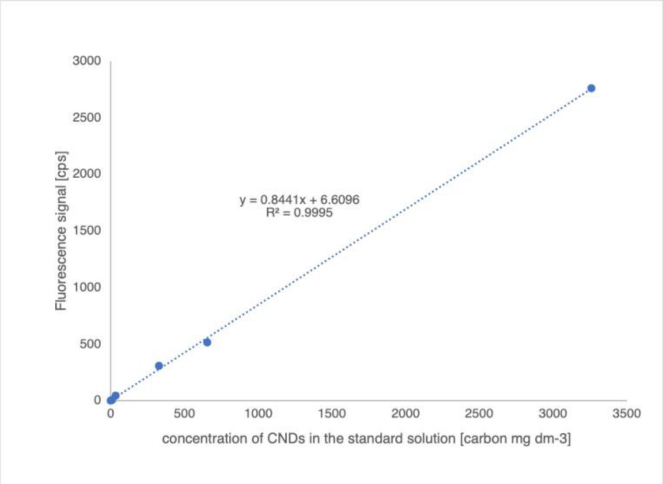

Figure 7. A calibration curve with a purified M-CNDs standard solution, measured by a Jasco FP-8500 Spectrofluorometer. The carbon content of the standard was certified by a Primacs SNC 100 Carbon Nitrogen analyzer. Each concentration was measured in triplicate.

Inter-day variation was also assessed, and a measurement was carried out with a standard addition method. The inter-day variation of the method was less than 0,1%. The calibration and the standard addition methods gave the same concentration result values.

Along with the obtained standard curve in **Figure 7**, the concentration of CNDs in breadmaking, including dough, bread crust, and bread crumb, was measured. 1g of samples were mixed with 10 mL of distilled water and then sonicated for 30 minutes. The mixture was centrifuged at 10,000 rpm for 10 mins. The supernatant was collected and mixed with cold ethanol 40% in the 1:1 (v/v) ratio at 4^{o}C in the freezer for 30 minutes and then centrifuged at 10,000 rpm for 30 minutes. The supernatant was collected and filtered with 0.22 μm, and the CNDs content was measured using fluorescent spectroscopy at the excitation wavelength of 360 nm. Our results, depicted in **Table 1**, revealed that no CNDs were detected in the dough, while the highest concentration of CNDs was detected on the bread crust with 512 ± 1 mg kg^{-1} DW. The difference in CNDs concentration in breadmaking is supposed to be related to the temperature and heat treatment time, where the bread crust directly contacted with high temperature obtained higher CNDs content than the bread crumb with 322 ± 4 mg kg^{-1} DW. The possible relationship between CNDs and heat treatment has been reported since 2012, when CNDs were found in bread and caramelized sugars for the first time.[39]

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Table 1. The concentration of CNDs in breadmaking

Food Items	CNDs concentration			
Dough	ND			
Bread crumb	322	±	4	mg kg^{-1} in DW a
Bread crust	512	±	1	mg kg^{-1} in DW b

The data are expressed as the mean ± standard deviation. Values within the same column with different letters (a-e) were significantly different ($P<0.05$).

This method's precision, repeatability, day-to-day variation, and linearity were accessed according to the validation procedure of the European Medicines Agency and AOAC International.[40,41] The standard curve was built with the regression line: $y = 0,8441x + 6.61$ with $R^2 = 0.9995$ with a limit of detection (LOD) of 0.14 mg dm^{-3} and a limit of quantification (LOQ) of 0.47 mg dm^{-3}. Considering the ten times dilution in the sample preparation, the detection limit calculated for the solid food sample is 1.4 mg kg^{-1}. With all the assessments, this method was reliable for measuring CNDs in foods.

Conclusion

In conclusion, this study has demonstrated that the Maillard reaction at low temperatures (120°C) and long time (12 hours) is a promising method for synthesizing carbon nanodots (CNDs) with high fluorescence intensity and small size (2.5 ± 0.5 nm). This study systematically explored the optimization of CNDs synthesis through the Maillard reaction, including (1) selecting an appropriate amino acid, (2) choosing the effective glycine-to-sucrose ratio, and (3) examining the influence of extraction solvents. Glycine enhanced the reactivity in the Maillard reaction, resulting in superior CNDs fluorescence intensity compared to other amino acids. Moreover, the investigation into the glycine-to-sucrose ratio revealed that a 1 mmol: 1 mmol ratio delivered the highest fluorescence intensity, offering a critical parameter for tailored CNDs synthesis. Expanding our exploration to extraction solvents, the crucial role of pH and ethanol concentration provided a valuable guide for controlling CNDs fluorescence properties, where 40% ethanol solution showed the most effective extraction to form CNDs with intense fluorescent intensity. The subsequent purification and characterization of Maillard reaction-derived carbon nanodots (M-CNDs) underscored the effectiveness of our isolation procedure. TEM imaging confirmed the nanosized, dark spherical morphology of M-CNDs, with an average size of 2.5 ± 0.5 nm. The UV-Vis spectrum and intense blue fluorescence of M-CNDs further validated their exceptional properties. Raman spectroscopy analysis supported the absence of significant carbonaceous impurities, confirming the moderate graphitic structure of M-CNDs. The obtained M-CNDs were used as a standard for quantifying the CNDs' content in the breadmaking processing, where CNDs were only found in bread crusts and crumbs after baking. It was shown that the presence of CNDs was closely related to the heat treatment process. The successful isolation and characterization of M-CNDs further elevate the potential of these nanostructures for diverse applications, emphasizing their role as a promising carbon dot source. The results of this study have the potential to advance the development of carbon nanodots for bioimaging, sensing, and other applications.

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Conflicts of interest

There are no conflicts to declare.

Author Contributions

Conceptualization, J.P. and D.H.H.N.; methodology, D.H.H.N.; doing experiments, D.H.H.N., A.M., J.P., L.N., and L.D.; analyzing data, D.H.H.N, A.M, and J.P.; writing—original draft preparation, A.M., D.H.H.N., L.N; writing-discussion and conclusion, H.E.-R., D.H.H.N., visualization, D.H.H.N., supervision, J.P., H.E.-R. All authors have read and agreed to the published version of the manuscript.

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