

Theses of Doctoral (PhD) Dissertation

CARBON NANODOTS IN FOOD: FROM FORMATION TO APPLICATION

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UNIVERSITY OF DEBRECEN
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DEBRECEN, 2025

1. THE ANTECEDENTS AND THE OBJECTIVES OF THE DOCTORAL DISSERTATION

Carbon-based nanomaterials, particularly Carbon Nanodots (CNDs), have garnered significant scientific attention due to their unique properties, such as strong luminescence, small size, and high biocompatibility. The 2023 Nobel Prize in Chemistry was awarded for the discovery and development of quantum dots – a unique nanoscale luminescent carbon-based material (Nobel-Prize 2023). CNDs, as part of the quantum dots family, share characteristics like size-dependent fluorescence and offer superior water dispersibility, making them applicable in fields such as food safety, smart packaging, and nutrient delivery (Gao et al., 2024; D. Nguyen, El-Ramady, et al., 2024; D. H. H. Nguyen, Muthu, et al., 2025; Rossini et al., 2019).

CNDs are small, quasi-spherical fluorescent particles typically less than 10 nm in size, known for their photoluminescence, aqueous solubility, low toxicity, and biocompatibility (Kang et al. 2020)(Shi et al. 2019). The discovery of CNDs dates back to Xu et al. (2004, p. 2), who first reported fluorescent carbon nanoparticles produced during the purification of single-walled carbon nanotubes. These particles introduced a new category of photoluminescent carbon-based nanomaterials.

Interestingly, CNDs are not only synthetically produced in controlled laboratory conditions but are also formed naturally during food processing, such as roasting, baking, grilling, and caramelization (Y. Li et al., 2017; D. Nguyen, Muthu, El-Ramady, Béni, et al., 2024; Semsey et al., 2024; Sk et al., 2012). These thermally driven reactions, particularly the Maillard reaction between amino acids and reducing sugars, result in the formation of fluorescent nanoparticles within food matrices (D. Li et al., 2019; D. Nguyen, Muthu, El-Ramady, Daróczi, et al., 2024; D. H. H. Nguyen, El-Ramady, Törös, et al., 2025). Foods like coffee, roasted meat, bread crusts, and fried spices have been shown to contain measurable amounts of CND-like structures (D. Nguyen, Muthu, El-Ramady, Béni, et al., 2024; Semsey et al., 2025; Sk et al., 2012). Additionally, the green synthesis of CNDs from food waste offers sustainability advantages (Pajewska-Szmyt et al., 2020; Wu et al., 2024). However, batch variability and matrix-dependent behaviors complicate their integration into food industry workflows (D. Nguyen, El-Ramady, et al., 2024).

The presence of CNDs in food products spans three critical contexts: (1) unintentional formation during thermal food processing, (2) intentional green synthesis from food-derived precursors, and (3) incorporated applications in food and agricultural systems, such as contaminant detection and intelligent packaging. These contexts give rise to three major

challenges: (1) a limited understanding of the physicochemical mechanisms governing CND formation in real food systems, (2) unclear toxicological implications and regulatory oversight, and (3) unresolved questions regarding the application of food-derived CNDs in real-world conditions (Bhatlawande et al., 2024; D. H. H. Nguyen, Muthu, et al., 2025). While lab-synthesized CNDs have shown low toxicity and biocompatibility (Liao et al., 2021; Yang et al., 2009), cytotoxicity may depend on factors like particle size, surface functional groups, dosage, and light-induced degradation (Havrdová et al., 2022; Liu et al., 2021; D. Nguyen, El-Ramady, et al., 2024).

This PhD research aims to bridge these knowledge gaps by investigating how CNDs form in real food systems, developing analytical methods for detection, and exploring the risks and potential applications of CNDs in food production, consumption, and regulation. The overarching aim is to study the formation, detection, and application of CNDs in processed food systems, with a focus on green synthesis, analytical detection, sustainability, and food safety considerations.

Specific Objectives:

- **Objective 1:** To optimize the synthesis of CNDs using Maillard-reaction model systems under various reaction conditions, and to characterize their properties via spectroscopic, microscopic, and chromatographic methods, aligning with sustainability and circular-economy principles.
- **Objective 2:** To establish and validate analytical methods for detecting CNDs in real food matrices ($\text{LOD} \leq 0.05 \text{ mg/L}$, $R^2 \geq 0.995$, recovery 80–120%, inter-day RSD $\leq 5\%$).
- **Objective 3:** To determine the occurrence of CNDs in six categories of food products (bakery, roasted cereals, beverages, chocolate products, spices, and black foods) and to elucidate their formation mechanisms through case studies.
- **Objective 4:** To develop applications of synthesized CNDs for the food industry, including (i) a rapid sensing method using CNDs, and (ii) valorization techniques for producing CNDs from food wastes.

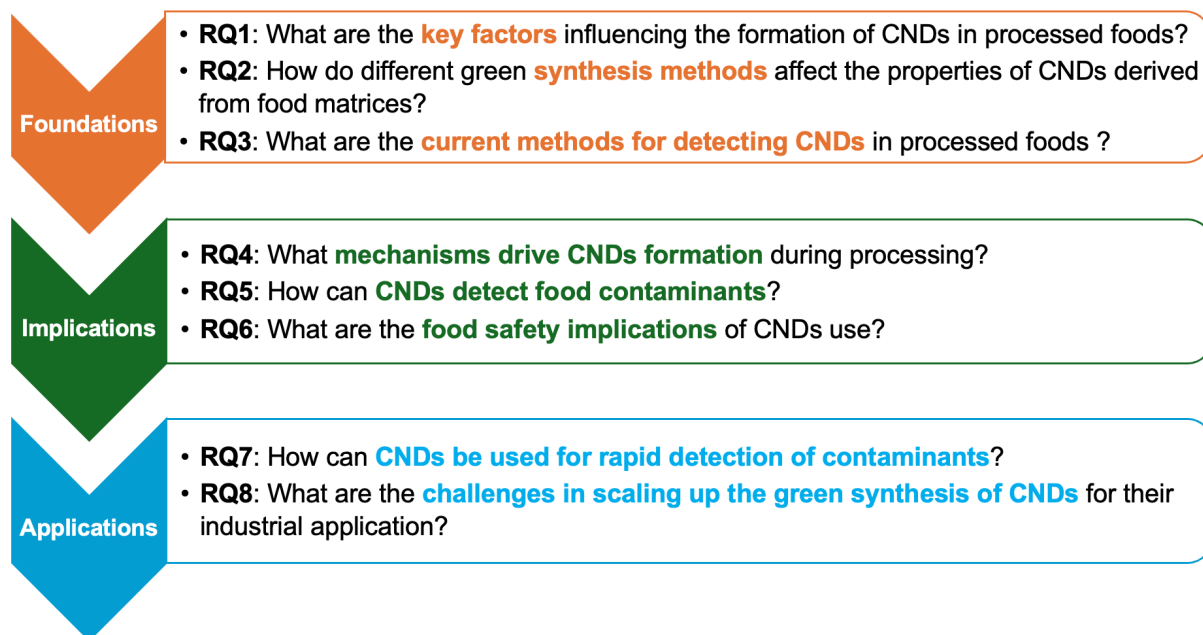


Figure 1. Logical structure of research questions

2. MATERIALS AND METHODS

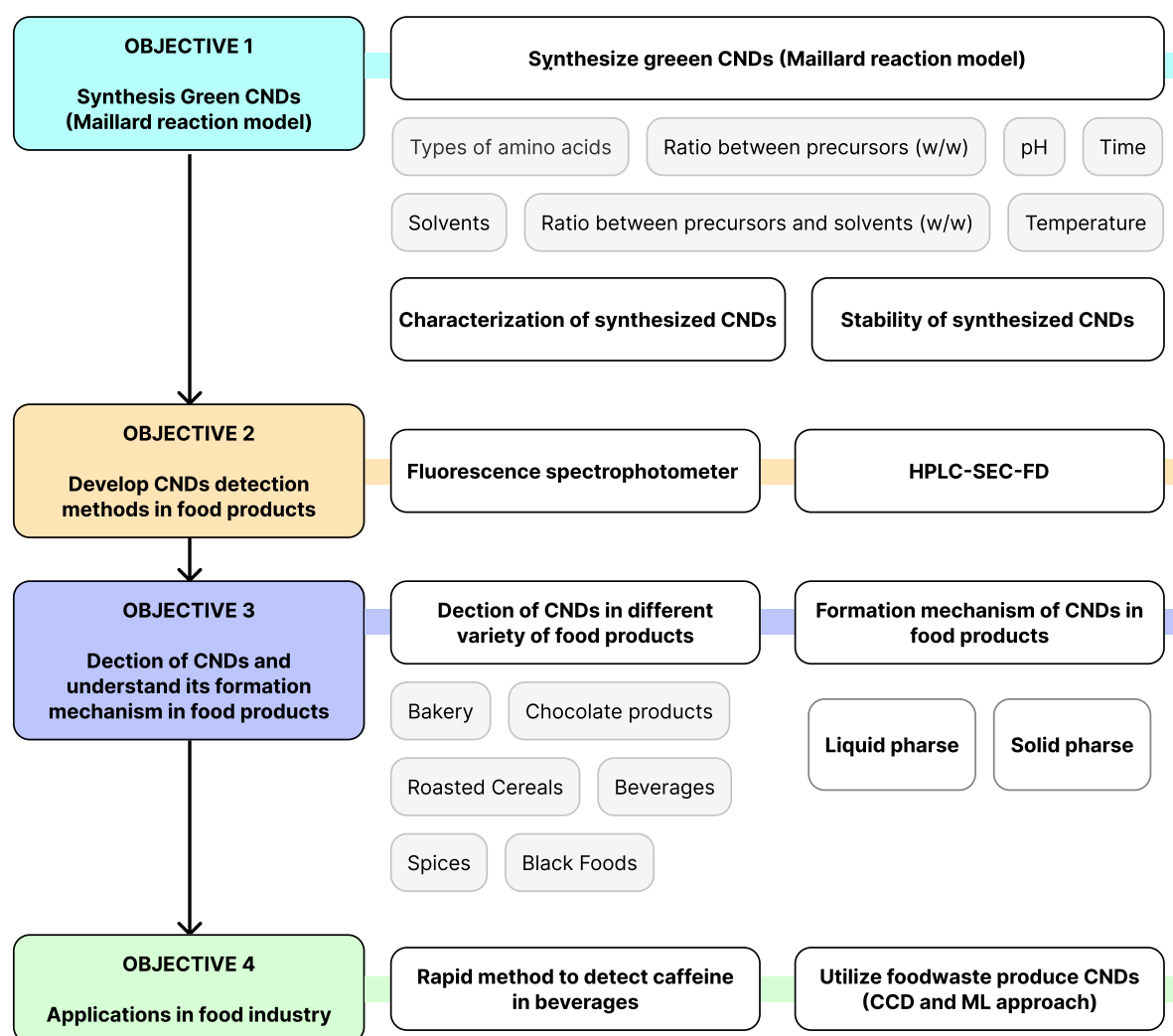


Figure 2. The overall experimental design of this PhD dissertation.

2.1. Objective 1: Synthesis and characterize green CNDs using the Maillard reaction model

2.1.1. Materials

Amino acids (L-arginine, glycine, L-tyrosine, L-proline, L-glutamine, L-phenylalanine), sucrose, hydrochloric acid, sodium hydroxide, and ethanol were obtained from various suppliers. All experiments used ultrapure water.

2.1.2. Method

The synthesis of CNDs followed a three-stage process:

1. **Optimization of Conditions:** Six amino acids were reacted with sucrose to determine the optimal precursor for CND synthesis.
2. **Characterization:** The CNDs were characterized using TEM, Raman spectroscopy, UV-visible spectrophotometry, FTIR, and fluorescence spectroscopy.
3. **Stability Evaluation:** The CNDs' stability was tested under different ionic strengths, pH conditions, and over time. Fluorescence intensity was the primary measure of stability.

Experimental Phases:

- **Phase 1:** Identified the optimal amino acid and sucrose ratio for CND production.
- **Phase 2:** Characterized synthesized CNDs using multiple techniques to ensure strong fluorescence and small particle size.
- **Phase 3:** Evaluated solvent systems for efficient extraction and fluorescence intensity.

2.2. Objective 2: Develop an analytical method to detect CNDs in food products

2.2.1. Quantitative Measurement of CNDs Using Fluorescence Spectroscopy

- **Materials:** Fresh bread was purchased from a local supermarket.
- **Method:** A fluorescence-based method was developed to measure CNDs concentration. Standard curves were generated using Maillard reaction-derived CNDs, and the method was validated for reproducibility and detection limits. Food samples were sonicated, centrifuged, and analyzed for CND content using fluorescence spectroscopy.

2.2.2. Quantitative Measurement of CNDs Using HPLC-SEC-FD

- **Materials:** Analytical-grade solvents and CND standards were used.
- **Method:** HPLC with size-exclusion chromatography and fluorescence detection quantified CNDs in food matrices. Calibration curves were created using reference CNDs, and the method was validated for precision and recovery.

2.3. Objective 3: Detection of CNDs and understand formation mechanisms in food products

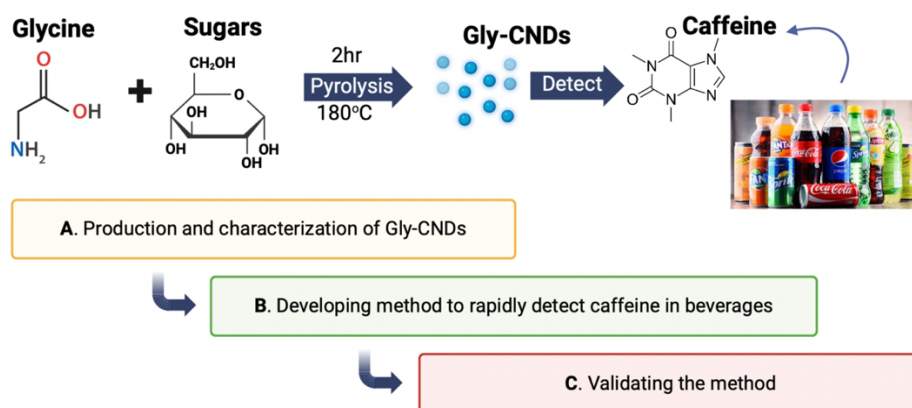
2.3.1. Detection of CNDs in different types of food products

- **Materials:** A variety of thermally processed food products, including bakery products, chocolates, beverages, and spices, were analyzed.

- ### ***2.3.2. Understanding Formation Mechanisms in Food Products: Case study of coffee brewing***

- #### 2.4. Objective 4: Applications of CNDs in food industry

- **Materials:** Roasted *Coffea arabica* and commercial energy drinks were analyzed.
- **Method:** Glycine-sucrose-based CNDs were used to develop a method for detecting caffeine in beverages. Calibration curves were generated, and the method's accuracy was validated against HPLC.



2.4.2. Valorization of food wastes to produce CNDs: CCD and ML approach

- 8

on fluorescence intensity were studied. A machine learning (ML) model was developed to predict CND fluorescence from these variables.

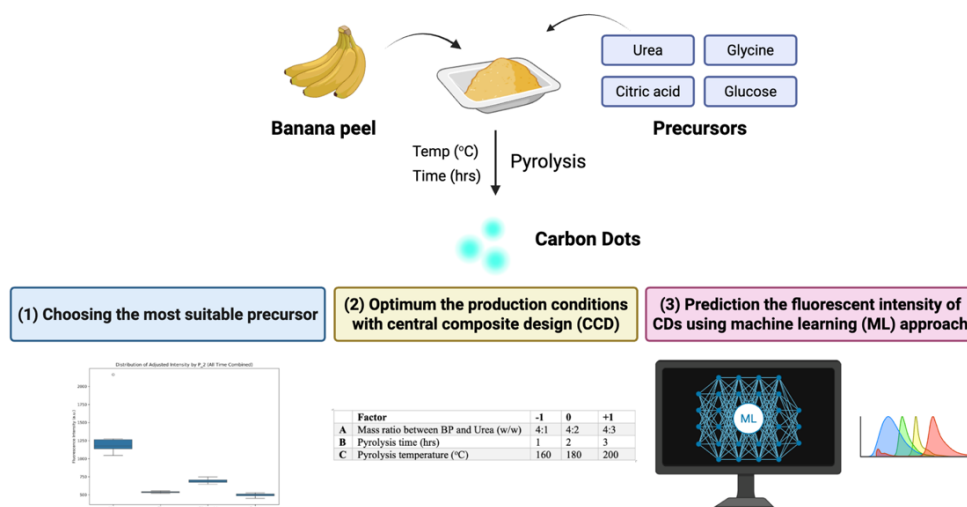


Figure 4. Schematic diagram of a 3-stage experimental design to optimize the production conditions of carbon nanodots from banana peel (BP-CNDs) using central composite design (CCD) and machine learning (ML) approach.

2.5. Analytical methods to study the properties of CNDs in foods.

2.5.1. Fluorescence Spectroscopy

Fluorescence intensity was used to assess quantum yield and determine the optimal synthesis conditions

2.5.2. UV-Visible Spectroscopy

UV-Vis absorbance spectra were measured to identify the typical transitions in CNDs and confirm their graphitic carbon structure.

2.5.3. Transmission Electron Microscopy (TEM)

TEM was used to examine the morphology and particle size distribution of synthesized CNDs.

2.5.4. X-ray Diffraction (XRD)

XRD was employed to analyze the crystallographic structure of CNDs, confirming their amorphous or semi-crystalline nature.

2.5.5. Raman Spectroscopy

Raman spectra were recorded to evaluate the graphitic quality and defect density of CNDs.

2.5.6. Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR was used to analyze surface functional groups present on the CNDs.

2.5.7. Elemental analysis

Carbon and nitrogen content were determined using an elemental analyzer to quantify CND concentration.

2.5.8. High-Performance Liquid Chromatography with Fluorescence Detection (HPLC-SEC-FD)

HPLC-SEC-FD was used to separate and quantify CNDs in food matrices.

2.5.9. High-Performance Liquid Chromatography

Caffeine content in beverages was measured using HPLC with UV–Vis detection.

2.5.10. Quantum yield determination

The photoluminescence quantum yield of CNDs was determined by comparing the sample to quinine sulfate as a reference.

2.5.11. Other methods

Additional methods like pH measurement and browning color development were used to assess food-related properties of CNDs.

2.6. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 25, with Python and GraphPad Prism for data visualization and modeling. Statistical significance was defined as $p < 0.05$.

3. RESULTS

3.1. Synthesis and characterization of green carbon nanodots (CNDs) using Maillard reaction model

3.1.1. Synthesis of green carbon nanodots (CNDs) using the Maillard reaction model

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

The Maillard reaction between sucrose and six amino acids (tyrosine, proline, glycine, arginine, glutamine, phenylalanine) produced CNDs with distinct fluorescence emissions. Glycine-based CNDs (Gly-CNDs) exhibited the highest fluorescence intensity, likely due to glycine's small, non-aromatic structure that facilitates Schiff base formation. Although phenylalanine had higher native fluorescence, Gly-CNDs outperformed it in fluorescence output (2024.0 ± 3.7 a.u. vs. 1900.0 ± 9.1 a.u.).

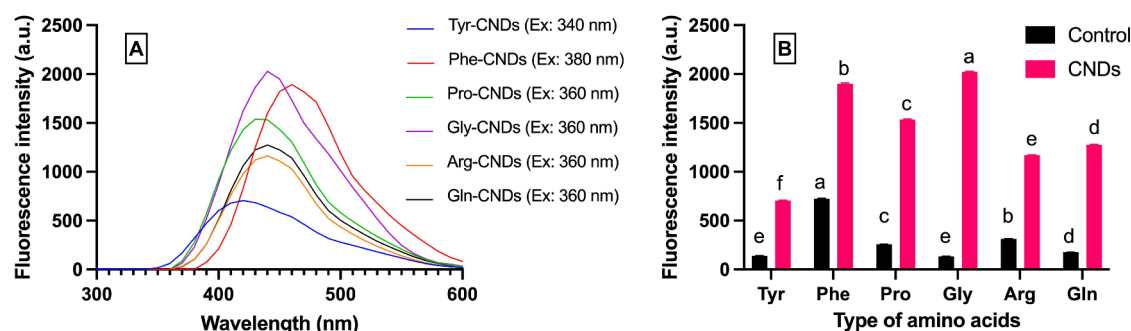


Figure 5. **A.** Fluorescence emission spectra of CNDs synthesized from sucrose and various amino acids: tyrosine (Tyr), proline (Pro), glycine (Gly), arginine (Arg), glutamine (Gln), and phenylalanine (Phe). Excitation wavelengths were adjusted per amino acid: 340 nm for Tyr, 360 nm for Pro and Gly, and 380 nm for Arg, Gln, and Phe. **B.** Fluorescence intensity of amino acid-based CNDs compared with native amino acids. Error bars represent standard deviation ($n = 3$); different letters indicate significant differences at $p < 0.05$ (Duncan's test) (D. Nguyen, Muthu, El-Ramady, Daróczy, et al., 2024).

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

The fluorescence intensity of Gly-CNDs increased with the glycine-to-sucrose ratio, peaking at a 1:1 ratio. Excess glycine beyond this point led to a decrease in fluorescence, suggesting that an optimal precursor balance is crucial for maximizing CND fluorescence.

3.1.1.3. Effect of extraction solvents on the formation of CNDs

Fluorescence intensity varied with solvent type and pH. Alkaline conditions (pH 9.0) and moderate ethanol concentrations (40%) produced the highest fluorescence. Acidic conditions (pH 2.0) suppressed CND formation. These findings suggest that solvent pH and ethanol concentration are critical factors in optimizing fluorescence output.

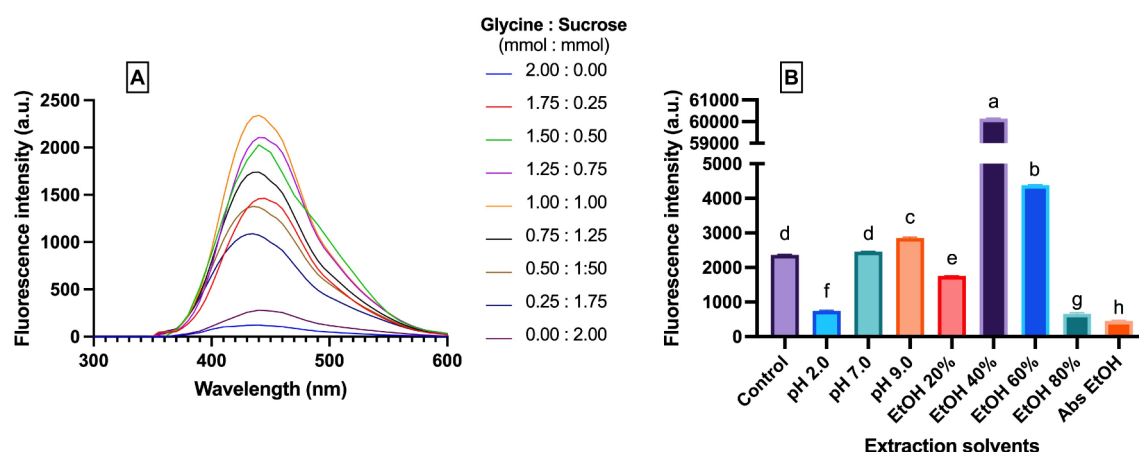


Figure 6. **A.** Fluorescence emission spectra of CNDs synthesized using different glycine-to-sucrose ratios (mmol/mmol), all measured at 360 nm excitation. The ratios tested included: 2:0, 1.75:0.25, 1.5:0.5, 1.25:0.75, 1:1, 0.75:1.25, 0.5:1.5, 0.25:1.75, and 0:2. **B.** Fluorescence intensity of CNDs extracted using solvents with varying pH (2.0, 7.0, 9.0) and ethanol concentrations (20% to 100%). The control was DI water at pH 6.0. Data represent mean $\pm$ SD ($n = 3$). Duncan letters indicate significant differences at $p < 0.05$ (D. Nguyen, Muthu, El-Ramady, Daróczy, et al., 2024).

3.1.2. Characterization of synthesized Carbon nanodots derived from glycine and sucrose (Gly-S-CNDs)

Characterization using TEM, UV-Vis, fluorescence, Raman spectroscopy, XRD, and FTIR revealed that Gly-S-CNDs were spherical with an average size of 4.2 ± 1.2 nm. Fluorescence spectra showed emission at 448 nm when excited at 360 nm, and UV-Vis spectra confirmed the presence of graphitic structures. Raman spectra indicated nitrogen doping, and XRD revealed an amorphous carbon structure. FTIR analysis showed functional groups like C=O and C–N, confirming surface functionalization

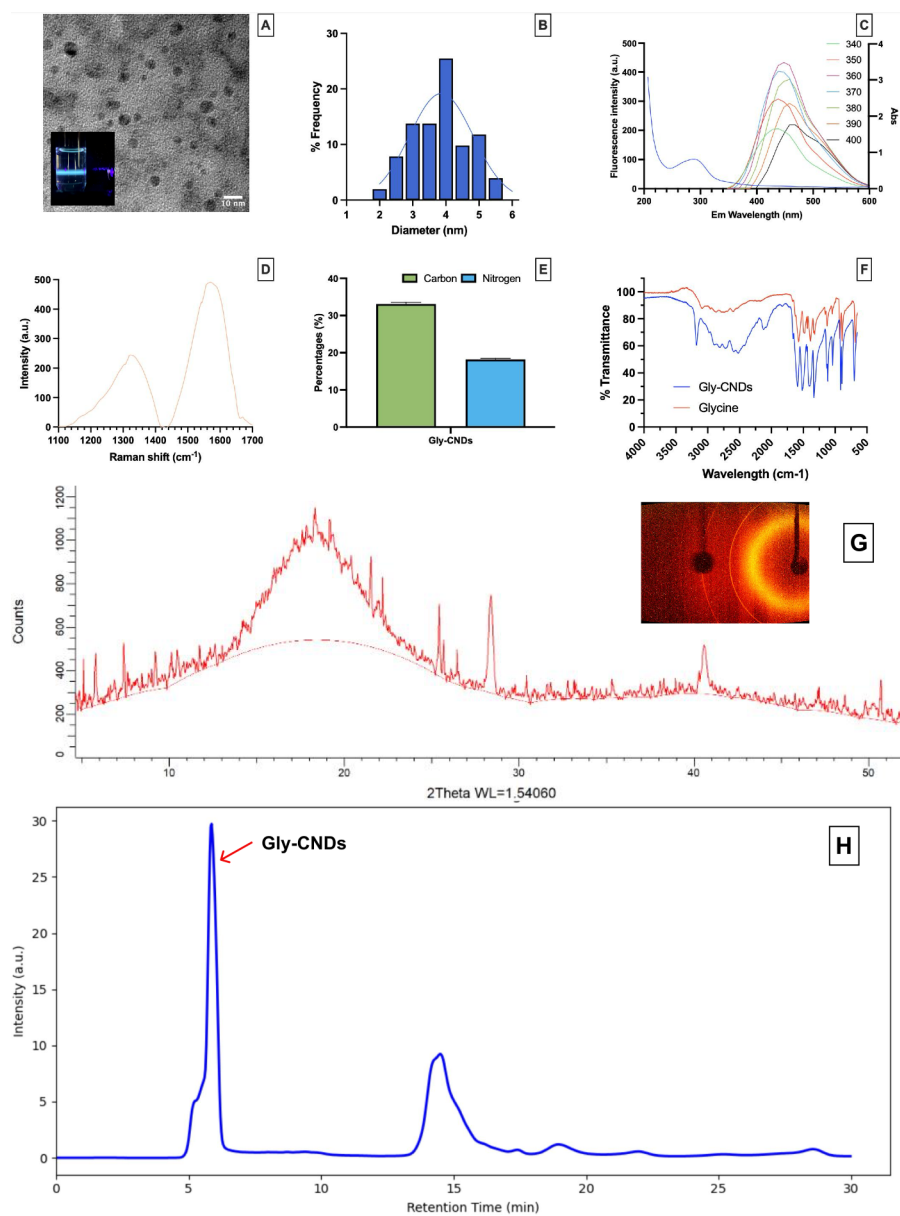


Figure 7. Characterization of carbon nanodots derived from glycine and sucrose (Gly-S-CNDs) including: **A**, TEM images with 10 nm scale. **B**, size distribution histogram with the average size at 4.2 ± 1.2 nm; **C**, the emission fluorescent spectra at different excitation wavelengths (right) and UV spectra with real sample under UV lamp (left). **D**, Raman spectra. **E**, Carbon and nitrogen percentage. **F**, FTIR spectra of Gly-CNDs (blue line) and glycine (red line). **G**, X-ray diffraction (XRD) spectra. **H**, HPLC- SEC-FD chromatogram of isolated Gly-S-CNDs before the final purification (D. H. H. Nguyen, El-Ramady, & Prokisch, 2025).

3.1.3. Stability of synthesized Carbon nanodots derived from glycine and sucrose (Gly-S-CNDs)

Gly-S-CNDs showed strong stability in various NaCl concentrations (0.1–1.0 M), with no significant fluorescence change over time (60 minutes). pH stability tests revealed the highest fluorescence at pH 4.0, while alkaline conditions reduced fluorescence, indicating the importance of pH in maintaining optical stability.

3.2. Development method to detect and measure CNDs in foods.

3.2.1. Quantitative measurement of CNDs using fluorescence spectroscopy

A quantitative fluorescence method was developed to measure CNDs in food matrices. Calibration curves were constructed with Gly-S-CNDs, yielding an excellent linearity ($R^2 = 0.9995$) and detection limits of 0.14 mg/kg. The method was used to quantify CNDs in bread, where the crust contained the highest CND concentration ($5120 \pm 1 \mu\text{g/kg}$), while dough showed no detectable CNDs.

3.2.2. Quantitative Measurement of CNDs using high-performance liquid chromatography with size exclusion and fluorescence detection (HPLC-SEC-FD)

HPLC-SEC-FD was used to quantify CNDs in food matrices. Gly-S-CNDs served as the reference standard, and the method demonstrated high sensitivity with a limit of detection (LOD) of 0.068 mg/L and a limit of quantification (LOQ) of 0.206 mg/L. CNDs concentrations were measured in coffee, black banana, and other processed foods, with black banana showing the highest CNDs levels.

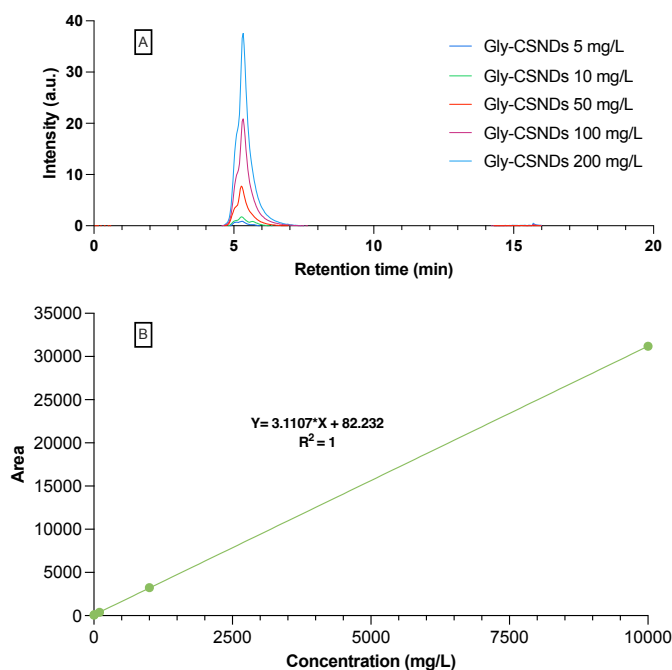


Figure 8. Calibration of HPLC-SEC-FD method using Gly-S-CNDs standards. **A**, Fluorescence chromatograms of Gly-S-CNDs at concentrations from 5 to 200 mg/L, showing consistent retention time and intensity. **B**, Calibration curve constructed from peak area versus concentration (0.1 – 10000 mmg/L), with high linearity ($R^2 = 1$), and method sensitivity defined by LOD = 0.068 mg/L and LOQ = 0.206 mg/L.

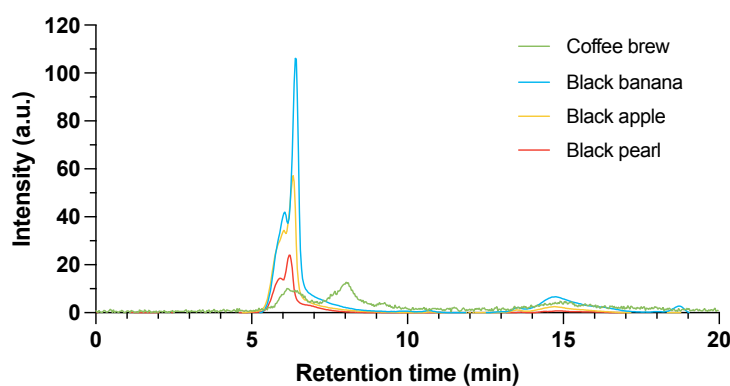


Figure 9. HPLC-SEC-FLD chromatograms of carbon nanodots (CNDs) detected in selected processed food samples.

3.3. Detection of CNDs and understanding formation mechanisms in food products

3.3.1. Detection of CNDs in food products

CNDs were quantified in a wide range of processed foods using a validated HPLC-SEC-FD method, confirming their widespread occurrence in thermally treated food systems. Concentrations varied strongly with processing intensity and matrix composition, reaching the

highest levels in coffee (3,860 mg/kg) and other highly processed/roasted products (e.g., pretzel and roasted beans). In bakery products, a clear heat-dependent gradient was observed (bread crust 1,829 mg/kg > bread crumb 501 mg/kg > dough 15 mg/kg), supporting thermal processing—particularly non-enzymatic browning such as the Maillard reaction—as a key driver of CND formation. Overall, these results indicate that food-intrinsic CND levels primarily reflect processing history rather than intentional formulation.

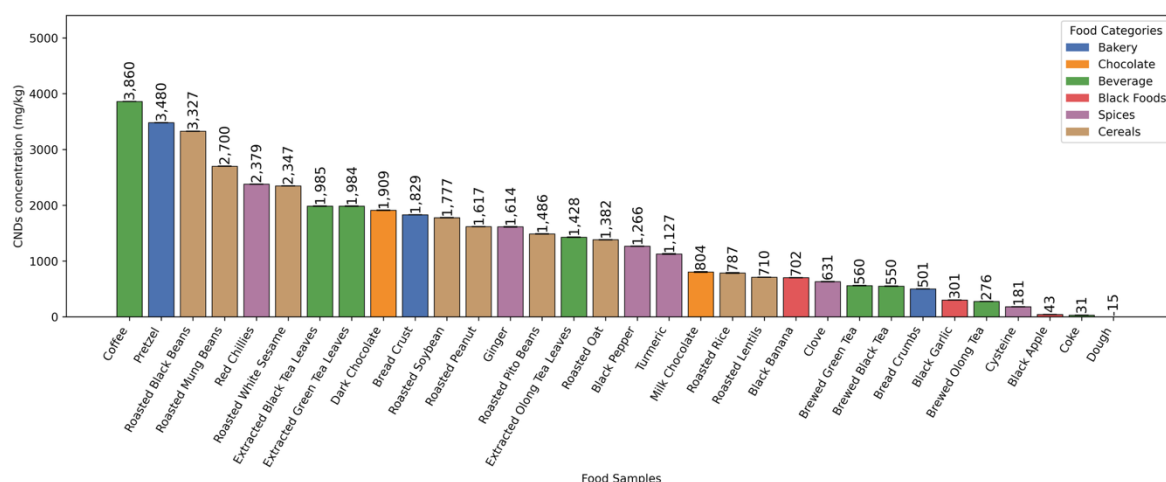


Figure 10. Concentration of carbon nanodots (CNDs) in selected food products grouped by category. CNDs levels (mg/kg) were quantified using a validated fluorescence spectroscopy method. Error bars represent standard deviation from triplicate measurements.

3.3.2. Characterization of food-derived carbon nanodots (F-CNDs)

Food-derived CNDs (F-CNDs) from coffee and baked products exhibited spherical morphology with average diameters of 3.2 nm (coffee) and 3.5 nm (pretzels). Fluorescence spectra confirmed excitation-dependent fluorescence, with C-CNDs showing strong emission at 438 nm, similar to other CNDs derived from food processing.

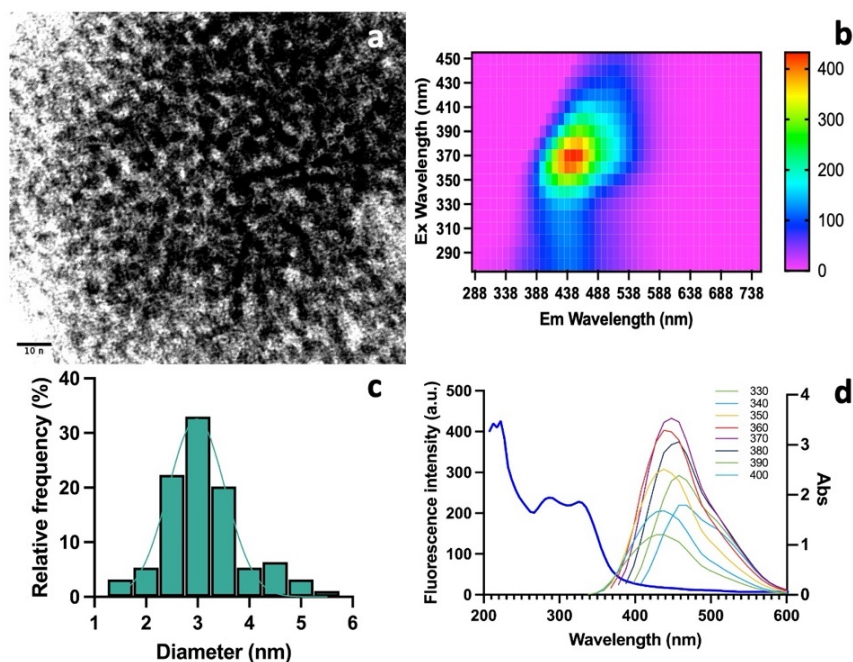


Figure 11. Optical Characterization of Carbon Nanodots Extracted from Brewed Coffee (C-CNDs). **A**, TEM image at 10 nm scale showing spherical morphology. **B**, 3D fluorescence excitation–emission spectra. **C**, Size distribution histogram (average diameter: 3.2 ± 0.9 nm). **D**, UV–Vis absorption spectrum (left) and 2D fluorescence emission spectra at varying excitation wavelengths (right).

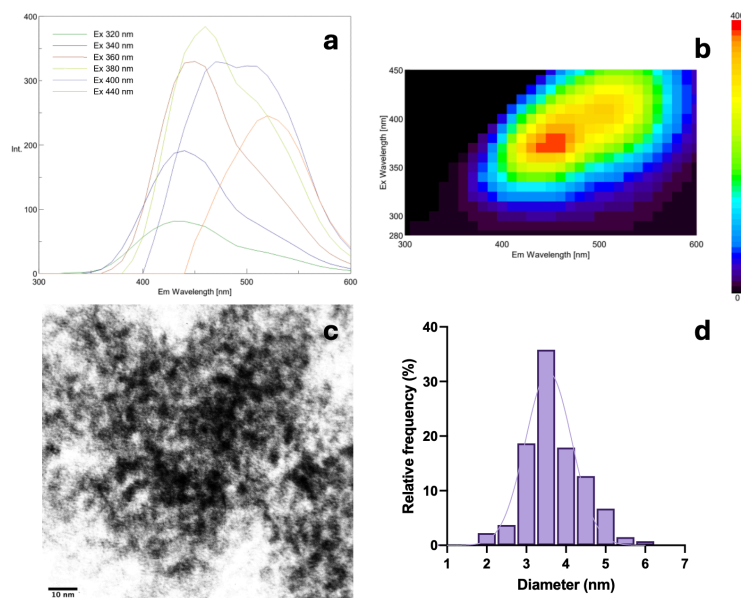


Figure 12. Characterization of carbon nanodots from bakery products (CNDs). **A**, The emission wavelength 300 – 600 nm of CNDs with different excitation wavelengths. **B**, 3D fluorescent spectra of CNDs. **C**, TEM images of CNDs with 10 nm scale. **D**, size distribution histogram of CNDs with an average size of 3.5 nm (Semsey et al., 2024).

3.3.3. Understanding the formation mechanisms of carbon nanodots (CNDs) in food products

Carbon nanodots (CNDs) were found to form as a general consequence of thermal food processing. Quantitative analysis revealed concentrations ranging from low tens of mg/kg in minimally heated products (e.g., dough, ~15 mg/kg) to several thousand mg/kg in highly processed foods such as coffee (~3,860 mg/kg). These results demonstrate that CNDs formation depends strongly on processing intensity and precursor composition rather than a single universal pathway.

In bakery products, a clear heat-dependent gradient was observed (dough < bread crumb < bread crust; ~15 < 501 < 1,829 mg/kg). This pattern confirms the dominant role of non-enzymatic browning reactions, particularly the Maillard reaction, in protein–sugar-rich systems. During baking, increasing thermal exposure promotes the formation of advanced Maillard reaction products and melanoidins, which likely act as precursors for CNDs generation.

Caramelization and high-temperature roasting also contributed to CNDs formation in sugar-rich and plant-based matrices. Spices, roasted cereals, and caramelized products exhibited moderate to elevated CNDs levels, consistent with thermal degradation and carbonization processes occurring during drying or roasting.

Interestingly, coffee contained the highest CNDs concentration despite undergoing wet-heat extraction rather than direct dry heating. This finding demonstrates that high dry temperatures are not strictly required; instead, precursor availability (sugars, amino acids, polyphenols) combined with roasting history and extraction conditions governs CNDs formation and release into the brew. The coffee case study further showed that bean type, grinding size, brewing ratio, and temperature–time combinations significantly affect CNDs yield. Arabica beans produced substantially higher fluorescence than Robusta, and fluorescence was inversely correlated with caffeine content ($r = -0.8$), suggesting caffeine-induced quenching effects. These results indicate that apparent CNDs levels reflect both formation chemistry and post-extraction molecular interactions.

Overall, the study identifies Maillard reactions, caramelization, and thermal degradation processes as the primary mechanisms underlying CNDs formation in foods. Temperature, exposure time, moisture, precursor composition, and extraction dynamics collectively determine CNDs yield and characteristics. These findings provide mechanistic insight into how common cooking and processing methods influence the generation of food-derived nanomaterials.

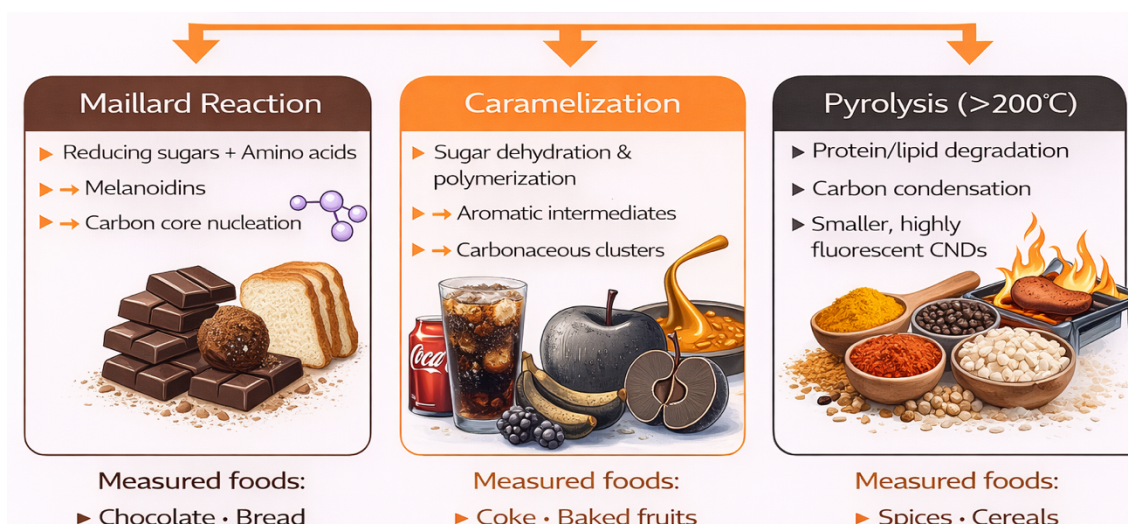


Figure 13. Schematic representation of major thermal reaction pathways (Maillard reaction, caramelization, and pyrolysis >200 °C) contributing to carbon nanodots (CNDs) formation in representative food categories.

3.4. Application of Carbon Nanodots in the Food and Agriculture Industry

3.4.1. Develop a Rapid Detection method to detect Caffeine in Beverages using glycine-sucrose derived carbon nanodots (Gly-S-CNDs)

3.4.1.1. Selectivity of this methods

The Gly-S-CNDs fluorescence response remained specific to caffeine, even in the presence of common beverage constituents like sugars, acids, and ions.

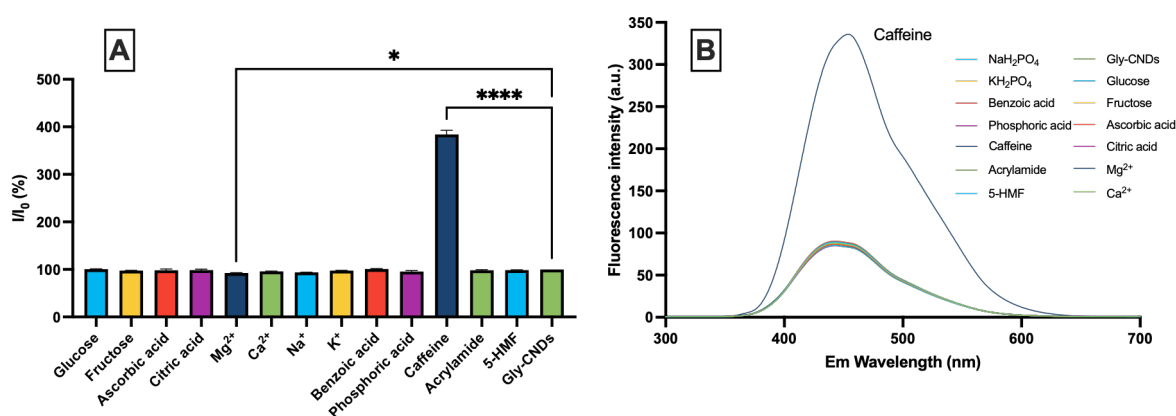


Figure 14. Selectivity of Gly-S-CNDs toward caffeine. **A**, Relative fluorescence intensity (I/I_0) in the presence of common interferents at 500 μ M. **B**, Representative emission spectra at excitation 360 nm. Data represent triplicate measurements; error bars show standard deviations. Significant differences relative to controls are marked (* $p < 0.05$; **** $p < 0.001$).

3.4.1.2. Fluorescence Assay for Caffeine Measurement

The fluorescence intensity of Gly-S-CNDs increased by over 50% in the presence of 5000 μM caffeine, demonstrating strong sensitivity to caffeine. The calibration curve showed a high linearity ($R^2 = 0.9929$), with an LOD of 36.5 nM.

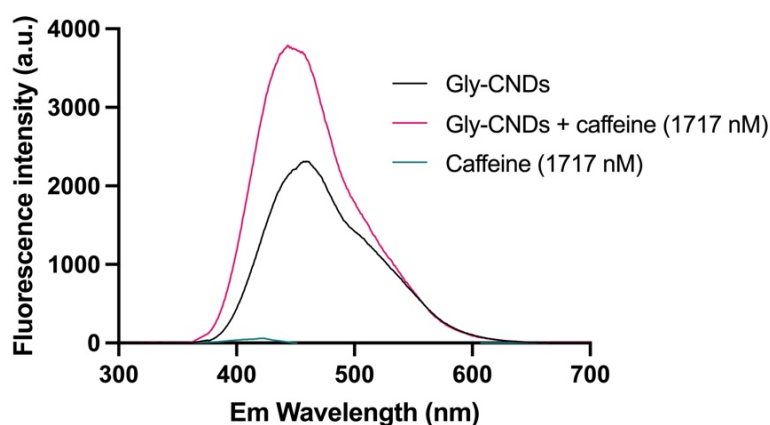


Figure 15. Fluorescence spectra of Gly-S-CNDs (black), Gly-S-CNDs + caffeine (red), and caffeine alone (green) at excitation 360 nm.

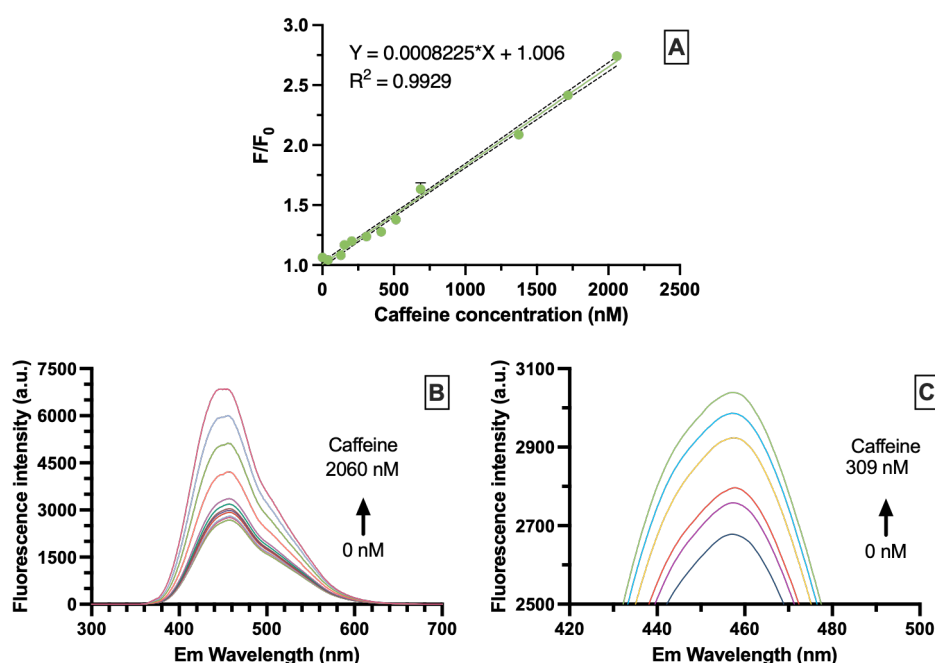


Figure 16. (A) Linear calibration curve of fluorescence enhancement (F/F_0) versus caffeine concentration (0–2060 nM). (B, C) Fluorescence spectra at increasing caffeine levels.

Table 1. Comparison of CNDs-based caffeine sensors.

Types of CNDs	Precursors	Linear ranges	LOD	References
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Sulfur-doped CNDs	Citric acid, $\text{Na}_2\text{S}_2\text{O}_3$	0.2-70 μM	0.05 μM	(Nemati et al., 2018)
Fe-doped red CNDs	Neutral red, FeCl_3	1-40 $\mu\text{g/mL}$	0.024 $\mu\text{g/mL}$	(Othman, 2024)
Gly-S-CNDs	Glycine, sugars	50-2060 nM	0.0403 μM	This work

3.4.1.5. Validation with Commercial Beverages

The method was applied to commercial energy drinks and teas, showing excellent agreement with HPLC results. The recovery rates were between 95.9% and 102.7%, confirming the method's accuracy.

3.4.2. Sustainable valorization of food waste into CNDs

3.4.2.1. Screening of Food Waste Precursors for Carbon Nanodot (CNDs) Production

A variety of food waste categories, including tea leaves, coffee grounds, and banana peels, were tested for CND production. Protein-rich and polyphenol-rich wastes produced the most fluorescent CNDs, with citric acid significantly improving yields in most categories.

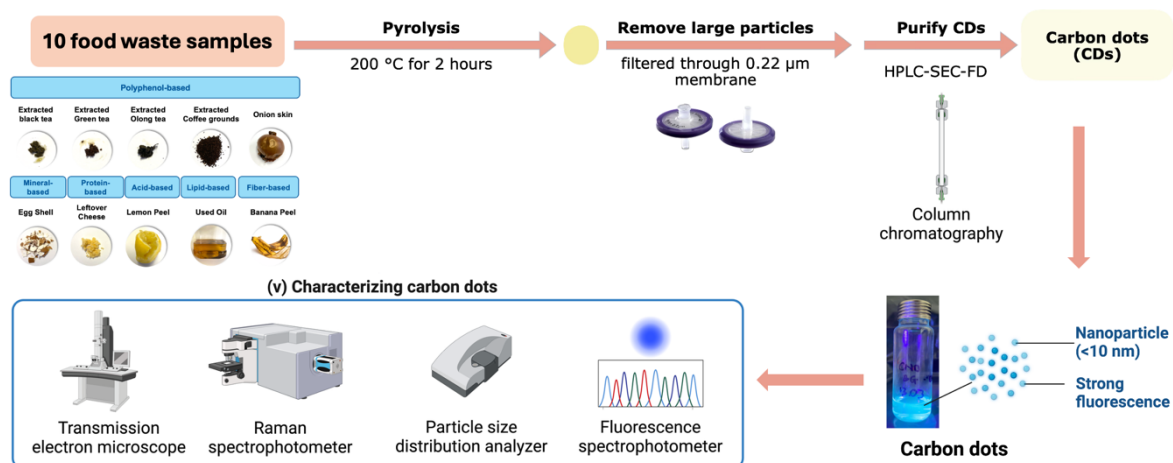


Figure 17. Workflow for synthesizing carbon nanodots (CNDs) from food waste via pyrolysis and subsequent purification and characterization steps.

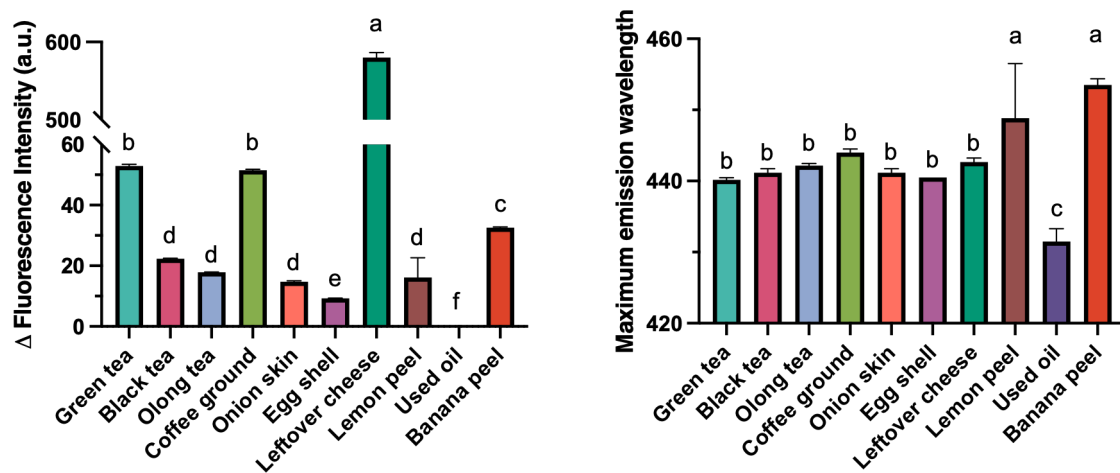


Figure 18. Comparative fluorescence intensities of CNDs synthesized from ten different food waste categories under identical pyrolysis conditions.

3.4.2.2. A Case Study: Production of Carbon Nanodots from Banana Peel Using CCD and ML Approaches

Banana peel (BP) was used as a sustainable source for carbon nanodots (CNDs) production through pyrolysis. A two-phase approach was employed: (i) precursor screening and (ii) optimization of synthesis conditions using Central Composite Design (CCD) and machine learning (ML). Among various precursors, urea was found to be the most effective, yielding the highest fluorescence intensity. The optimal conditions for BP-CND synthesis were a urea:BP mass ratio of 4:2, pyrolysis at 200°C, and a reaction time of 3 hours.

To predict fluorescence intensity based on synthesis conditions, seven ML models were tested, with Random Forest (RF) delivering the best results ($R^2 = 0.843$, RMSE = 1209.37). Feature importance analysis confirmed that the urea:BP ratio was the most critical factor in optimizing fluorescence. The RF model accurately predicted fluorescence intensity, validating the efficiency of combining CCD and ML to optimize CND synthesis.

This approach not only optimized the production of BP-CNDs but also provided valuable insights into the role of nitrogen doping and temperature in enhancing fluorescence, paving the way for scalable, sustainable nanomaterial production from food waste.

Table 2. RMSE and R^2 for seven machine learning models predicting fluorescence intensity of BP-CNDs

Model	RMSE	R^2
Random Forest	1209.365831	0.843477

Gradient Boosting	1446.158552	0.776182
Decision Tree	1872.747239	0.624663
Linear Regression	1996.618776	0.573368
K-Neighbors Regression	2080.528265	0.536756
Support Vector Regression	3225.932897	-0.113714
ANN (MLPRegressor)	6257.512279	-3.190505

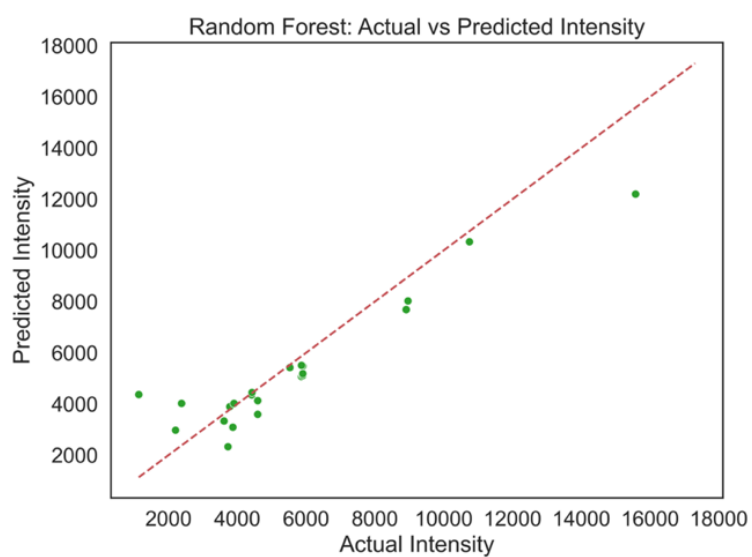


Figure 19. Random Forest regression model performance: predicted vs. experimental fluorescence intensities of BP-CNDs.

4. NEW CONTRIBUTIONS TO ACADEMIC KNOWLEDGE

1. I found that six amino acids - tyrosine, proline, glycine, arginine, glutamine, and phenylalanine - reacting with sucrose under Maillard-reaction conditions form fluorescent CNDs with distinct excitation-dependent emission spectra, using excitation wavelengths of 340 nm (Tyr), 360 nm (Pro, Gly), and 380 nm (Arg, Gln, Phe). Glycine–sucrose-derived CNDs (Gly-CNDs) showed the highest fluorescence intensity (2024.0 ± 3.7 a.u.), identifying glycine as the optimal amino acid precursor.
2. I synthesized and characterized Gly-S-CNDs from glycine and sucrose as ~ 4 nm spherical, nitrogen-doped nanodots with blue fluorescence ($\lambda_{em} \approx 448$ nm at 360 nm excitation) and a UV–Vis band near 280 nm extending into the visible region. Raman/XRD confirmed semi-graphitic, mainly amorphous carbon, while elemental/FTIR analyses showed nitrogen- and oxygen-containing surface groups; HPLC-SEC-FD isolated a single fluorescent fraction, confirming identity and purity. The nanodots retained fluorescence in 0.1–1.0 M NaCl and over time, with maximal intensity at pH 4 and a nearly constant emission maximum (~ 440 nm) from pH 4–11, indicating a stable emissive core with pH-sensitive surface states.
3. I developed and validated two complementary methods for quantifying CNDs in foods using purified Gly-S-CNDs as the calibration standard. A fluorescence spectroscopy method ($\lambda_{ex} = 360$ nm) enabled bulk CNDs quantification with excellent linearity ($R^2 = 0.9995$), low LOD/LOQ (down to ~ 0.14 mg/kg), and high precision ($RSD < 0.1\%$), revealing that CNDs were absent in dough but formed during baking, reaching 501 mg/kg DW in breadcrumbs and 1,829 mg/kg DW in bread crust. In parallel, I established an HPLC-SEC-FD method that separates CNDs by size and quantifies them with very high sensitivity ($R^2 = 1.000$, LOD = 0.068 mg/kg), accuracy (coffee spike-recovery ~ 98 –101%), and robustness (low inter- and intra-laboratory variability), demonstrating a practically superior approach for selective CNDs monitoring in complex food matrices.
4. Using the validated HPLC-SEC-FD method, I showed that CNDs are widespread in thermally processed foods, with the highest levels in coffee ($\sim 3,860$ mg/kg), followed by extracted tea leaves, dark chocolate, and bread (501 mg/kg in breadcrumb, 1,829 mg/kg in bread crust), confirming the key role of heating. By synthesizing four model nanodots (Gly-S-CNDs, Gly-C-CNDs, U-C-CNDs, Lys-C-CNDs), I demonstrated that precursor composition and citric acid strongly modulate optical properties, supporting distinct formation pathways. Integrating model and food data, I proposed a unified

framework in which Maillard reactions, caramelization, pyrolysis, and wet-heat brewing (coffee, ≥ 90 °C, ~ 3.2 nm C-CNDs) all contribute to CNDs formation. I further showed that coffee brewing parameters markedly affect C-CNDs yield (higher for Arabica, coarse grinds, and a 1:15 ratio at 80 °C for 300 s) and that fluorescence is inversely correlated with caffeine ($r = -0.8$), indicating caffeine-induced quenching and the potential of coffee-derived CNDs as caffeine sensors.

5. I developed a rapid, highly selective caffeine sensing system based on Gly-S-CNDs. The method showed a linear response from 50–2060 nM ($R^2 = 0.9929$) with LOD = 36.5 nM and LOQ = 122.1 nM, outperforming reported CND-based caffeine sensors. Optimized conditions (pH 5.0, 25 min incubation, 40 °C) enabled accurate caffeine quantification in commercial energy drinks, teas, and a “fake coffee” sample, with 8-fold fluorescence enhancement, good agreement with HPLC ($p > 0.05$), and recoveries of 95.9–102.7% (RSD < 2%, $n = 5$), confirming suitability for real-world applications.
6. I screened ten food-waste categories as precursors for CNDs via pyrolysis (200 °C, 2 h) and HPLC-SEC-FD and found that all produced CNDs, with protein- and polyphenol-rich wastes giving the highest fluorescence and lipid-rich the lowest. Citric acid co-treatment strongly enhanced yields, especially for polyphenol-, mineral-, and fiber-rich wastes, while sucrose had limited or negative effects. Thus, protein/polyphenol-rich wastes plus citric acid were identified as key levers for maximizing food-waste-derived CNDs production.
7. I upcycled banana peel into highly fluorescent CNDs by optimizing co-pyrolysis conditions with nitrogen/acid precursors using Central Composite Design and machine learning. Urea produced the brightest BP-CNDs, with a dominant SEC peak, elevated nitrogen ($\sim 10.3\%$), and a quantum yield of 40%, outperforming glycine, citric acid, and sucrose ($< 5.2\%$ N). CCD identified an optimal urea:banana peel ratio of 4:2 (w/w), 200 °C, 3 h, yielding $\sim 16,700$ a.u. fluorescence, while Random Forest models ($R^2 = 0.843$) highlighted mass ratio and temperature as key predictors. This integrated strategy enables efficient, data-driven upcycling of banana peel into CNDs with reduced experimental workload.

5. PRACTICAL USE OF THE RESULTS

- I developed an environmentally friendly and cost-effective process to convert banana peel and other food waste into carbon nanodots (CNDs), using pyrolysis and food-grade precursors like urea and glycine. This sustainable approach not only addresses food waste disposal issues but also creates high-quality nanomaterials that can be used in various applications, ranging from food safety to biomedical fields.
- I quantified CND concentrations in various everyday foods such as bread crust, coffee, tea, and chocolate, providing critical baseline data for food safety assessments. This data can be leveraged by food scientists, nutritionists, and regulatory bodies to assess the potential dietary exposure to CNDs, enabling the development of standards and guidelines for safe consumption in food products.
- I validated a fast, cost-effective fluorescence-based assay using glycine-sucrose derived CNDs, which can be used as an alternative or complement to traditional HPLC methods in food quality control laboratories. This assay demonstrated high selectivity, sensitivity, and reproducibility for caffeine detection in various beverage matrices, making it a practical tool for routine monitoring of caffeine levels in commercial beverages such as energy drinks, teas, and coffee.
- I identified key parameters such as temperature, precursor composition, and water ratio that influence the formation of CNDs during food processing. This provides valuable insights into how food preparation techniques, such as baking or brewing, can be optimized to either enhance or mitigate CND formation. These findings offer practical strategies for food manufacturers to control the formation of nanomaterials in their products, with implications for both product safety and functionality.
- I applied a robust experimental design combined with machine learning models to optimize the synthesis of CNDs from food waste. This framework not only improved the efficiency of the process but also minimized experimental workload by reducing the need for extensive trial-and-error. This data-driven approach can be applied to the production of other nanomaterials, accelerating innovation in sustainable food nanotechnology and providing a scalable model for future applications in food and agriculture.

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PUBLICATIONS IN THE FIELD OF RESEARCH



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Registry number: DEENK/543/2025.PL
Subject: PhD Publication List

Candidate: Huu Huong Duyen Nguyen
Doctoral School: Doctoral School of Nutrition and Food Sciences, Doctoral Programme of Food Sciences
MTMT ID: 10093013

List of publications related to the dissertation

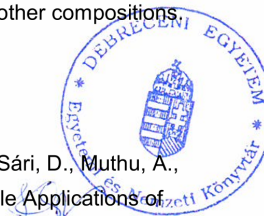
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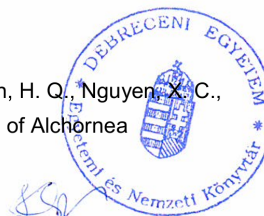




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Foreign language abstracts (1)

28. Muthu, A., **Nguyen, H. H. D.**, Prokisch, J., Béni, Á.: Biosynthesis of Selenium and Tellurium nanoparticles and their alloys using *Lactobacillus casei*: a sustainable approach to functional nutraceuticals.
In: 1st International Congress on Sustainable Food, Green Chemistry and Human Nutrition : GreenNutrifood2025 : Book of abstracts. Eds.: Filip Dujmić, Sven Karlović, Antonela Ninčević Grassino, University of Zagreb, Zagreb, 38, 2025. ISBN: 9789536893225

Total IF of journals (all publications): 101,422

Total IF of journals (publications related to the dissertation): 74,7

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

07 October, 2025

