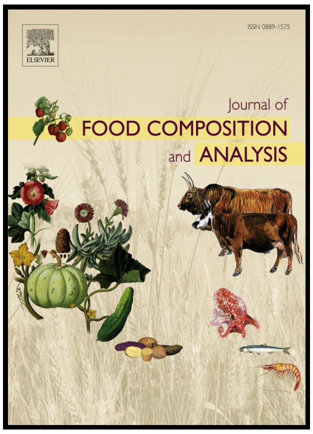

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Detection and formation of fluorescent carbon nanodots in coffee brews and its relationship with other compositions

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Abstract

Coffee, a widely consumed beverage, offers health benefits alongside enjoyment. While coffee chemistry has been studied for years, the existence and formation of nanoparticles in coffee remain incompletely understood. This research explores how carbon nanodots (C-CNDs) form in coffee under various conditions of brewing, including (1) bean types, (2) grind sizes, (3) the coffee-to-water ratios, and (4) brewing time and temperatures. The results show that the C-CNDs formation increased with Arabica beans, coaster grinds, and a 1:15 coffee-to-water ratio (g/g). Higher temperatures (over 90 °C) and longer brewing times also yielded more C-CNDs. Our findings revealed that the isolated C-CNDs have an average size of 3.2 ± 0.9 nm and are characterized using fluorescence spectrophotometer, UV-Vis, and TEM techniques. We also examined fundamental coffee compositions like caffeine content, pH, total dissolved solids (TDS), and browning color, finding the correlation with C-CNDs. While C-CNDs show a strong negative correlation with caffeine content (-0.8), there is a weak negative correlation with pH (-0.33), TDS (-0.48), and browning color (-0.62). This establishes a new link between C-CNDs and caffeine content. These discoveries, backed by robust scientific methods, enhance our understanding of coffee's composition and have potential health implications.

Keywords: carbon dots, extraction, Maillard reaction, nanoparticles, beverages, brewing coffee.

1. Introduction

Coffee, renowned as one of the most consumed beverages globally, is considered a functional food because it contains high bioactive compounds and antioxidants, which can have potential health benefits (Butt and Sultan, 2011; Farah, 2012). Coffee has a complex chemical composition comprising caffeine, chlorogenic acids, aliphatic acids, polysaccharides, proteins, lipids, and minerals, directly linked to cup quality (Farah et al., 2006; Petracco, 2005). Several factors significantly influence the composition of coffee, like bean varieties, roasting, grinding, storage, and brewing conditions (Illy and Viani, 2005). Although coffee chemistry has been studied for several years, the presence of nanoparticles in coffee and their formation in coffee brewing has yet to be fully understood.

Recently, carbon nanodots (CNDs), fluorescent nanoparticles, have drawn attention due to their broad range of applications, including bioimaging (Jing et al., 2023; Khan et al., 2021), optoelectronics (Hola et al., 2014), food packaging (Chen et al., 2023; Ezati et al., 2022) and monitoring of food components (Ezati et al., 2023; Lin et al., 2022; Zheng et al., 2017). CNDs were not only synthesized in the laboratory but also naturally formed in food processing. CNDs have been found in several heat-treated foods, like bread (Sk et al., 2012), caramelized sugar (Sk et al., 2012), roasted chicken (Song et al., 2018), and grilled salmon (Song et al., 2019). It was stated that there is a close relationship between the heat treatment process and the formation of CNDs in processed foods. While CNDs are generally recognized for their biocompatible properties (Hola et al., 2014; Sahu et al., 2012), their potential toxicity upon consumption must be carefully evaluated depending on the dosage. 80 % of cells survived with CNDs at 1 mg/mL, while 90 % died when CNDs were increased to 10 mg/mL (S. Li et al., 2018). The presence of nanoparticles in food raises concerns about their potential health effects. Studies have shown that these particles, due to their small size, can penetrate biological membranes, potentially leading to adverse health effects (Tran and Chaudhry, 2010).

Notably, Jiang et al. (2014) successfully isolated CNDs from instant coffee, suggesting their potential as biocompatible fluorescent compounds for bioimaging applications. Later, the presence of CNDs in coffee grounds and how roasting time affects their sizes were reported (Chu et al., 2023). Although the presence of CNDs in coffee has been proven, the effects of bean types, grinding sizes, and brewing conditions on the formation of CNDs in the brewing process still need to be acknowledged.

Therefore, this work contributed to a comprehensive understanding of coffee chemistry by exploring the formation of fluorescent CNDs during brewing. In coffee brews, CNDs were detected, isolated, and characterized. To investigate how different conditions, including (1) bean type, (2) grinding size, (3) coffee-to-water ratio, and (4) brewing duration and temperature, affected the formation of CNDs. It also investigated the possible links between CNDs formation and other significant coffee properties, such as caffeine content, pH, total dissolved solids, and browning color.

2. Materials and methods

2.1. Materials

Three types of roasted coffee beans were used for the experiment, including (1) 100 % Arabica (*Coffea arabica* L.) bought from ALDI supermarket (Essen, Germany) with a roasting level 8/10 originated from Peru; (2) 100 % Robusta (*Coffea canephora* L.) with the roasting level 8/10 originated from Vietnam from Laviet Coffee factory, Da Lat, Vietnam; and (3) mixed coffee beans (50 % Arabica: 50 % Robusta) with roasting level 8/10 provided from Laviet Coffee factory, Da Lat, Vietnam. All samples were analyzed within one month of production's date. Chemicals used in all experiments were listed. All of the experiments were conducted using ultrapure water. Ethanol ≥ 99.5 %, hydrochloric acid solution 0.01 mol/L, sodium hydroxide solution 0.01 mol/L, and HPLC-grade acetonitrile ≥ 99.5 % were provided by VWR International company from Pennsylvania, USA.

2.2. Experimental design

The experimental design of this study is illustrated in **Figure 1**, which includes three experimental stages. First, carbon nanodots in coffee brews (C-CNDs) were detected, isolated, and characterized to understand their properties using transmission electron microscopy (TEM), fluorescence spectroscopy, and UV-vis spectrophotometry (**Figure 1A**). Then, the formation of C-CNDs in brewing coffee was studied with four separate experiments to understand the effect of bean type, grinding size, sample-to-water ratio, and brewing conditions (**Figure 1B**). Finally, the correlation between C-CNDs and other components was analyzed (**Figure 1C**).

Coffee brews were prepared following the below procedure where the parameters were changed depending on each experiment described in **Figure 1.** Coffee beans were ground immediately before the brewing process using Timemore Chestnut C2 (Timemore Co. Ltd, Shanghai, China), 0.3 mm (fine size), 0.7 mm (medium size), and 1.1 mm (coarse size). Ground coffee was brewed following the described method with a slightly modified (Bekedam et al., 2006). First, 100% Arabica coffee beans were ground in 0.7 mm (medium-sized coffee grounds). Then, 10 g of ground coffee was brewed with 150 mL of deionized water at 100 ^{o}C. The extraction was performed in 180 seconds and filtered with 0.45 μm filter paper (VWR International Co., Ltd., Pennsylvania, USA) on a V60 coffee dripper (HARIO Co., Ltd., Japan). The obtained coffee brews were filtered with a 0.45 μm syringe filter. Samples were measured for the caffeine content, pH, TDS, and browning color within 15 minutes of brewing. C-CNDs were isolated and measured within one day of brewing using the procedure described in section 2.3.

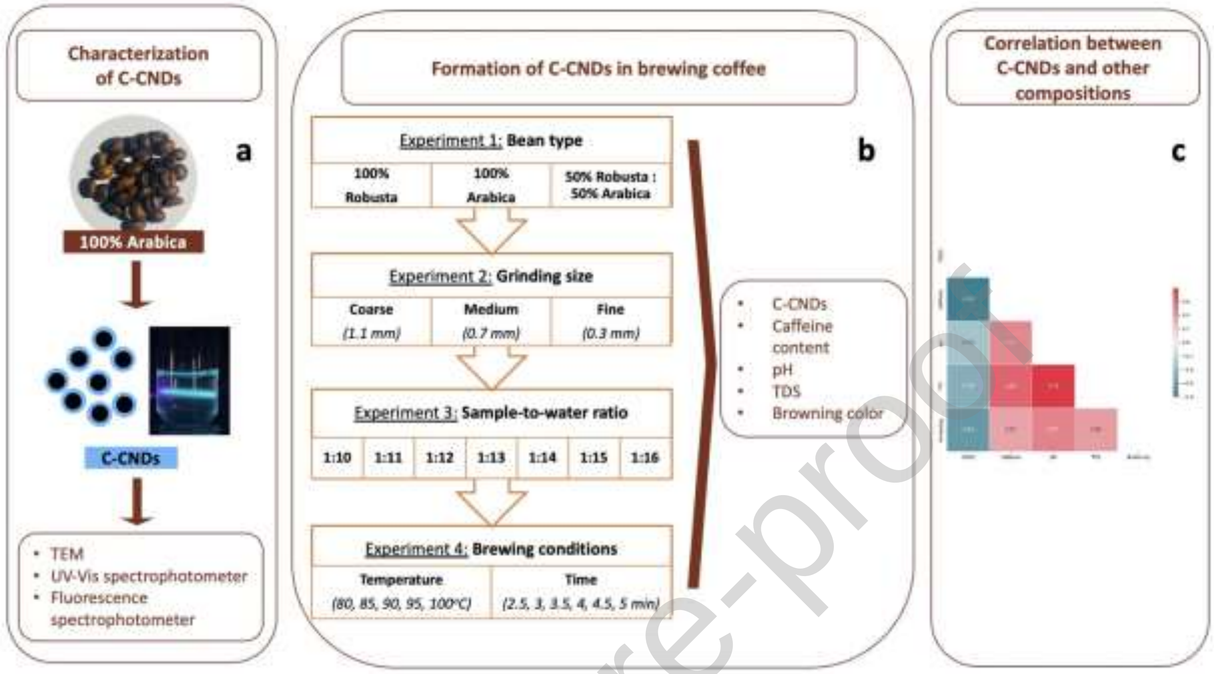

Figure 1. Schematic diagram of the experimental design outlining three stages of the study on forming carbon nanodots (C-CNDs) in coffee brews: **(a)** C-CNDs isolation and characterization with TEM, fluorescence, and UV-Vis spectrophotometers; **(b)** investigation of C-CNDs formation in brewing coffee with four experiments including (1) Bean type, (2) grinding size, (3) sample-to-water ratio, and (4) brewing conditions measuring C-CNDs, caffeine content, pH, TDS, and browning color; and **(c)** correlation analysis of C-CNDs and other coffee components. Real-time UV lamp images of C-CNDs are included.

2.3. Measurement and characterization of carbon nanodots in coffee brews (C-CNDs)

The isolation and extraction method of CNDs was followed in the previous publication with modifications (Nguyen et al., 2024). The coffee brews were precipitated with cold ethanol (96%) in a ratio of 1:1 (v/v). The mixture was centrifuged at 4032 x g for 15 min; then, the supernatant was collected, passed through the macroporous cellulose column, and filtered with a 0.22 μm syringe filter. The isolated CNDs solution from coffee extract (C-CNDs) was characterized with an FP-8500 fluorescent spectrophotometer (Jasco Co., Oklahoma, USA) and a Lambda 35 UV-Vis spectrophotometer (Norwalk, USA). The obtained samples were lyophilized to get the powder for further characterization of samples with the transmittance electron microscope (TEM) (JEM-2000FXII model, JEOL Ltd, Tokyo, Japan).

2.4. Measurement of caffeine content using the HPLC system

The method to measure caffeine in coffee was built based on ISO 20481 – 2008 (HPLC, reference method) with slight modifications (ISO 20481:2008, 2017). The caffeine of samples was measured using the ECS05 Gradient Analytical HPLC System and the ECDA2800 UV-VIS PDA detector (ECOM Co., Praha, Czech Republic), with Nucleosil C18 100Å column (5 μm, 150 x 4.6 mm) produced by Phenomenex Corporation in California, United States. All measurements were performed at 25 ± 2 oC with a mobile phase combining 0.01 M phosphate buffer (pH 6.4) and acetonitrile (ACN) in the ratio of 90:10 (v/v). The analyses were operated with a flow rate of 2 mL/min. The run time was 8 min, and the caffeine retention time was around 5.3 – 5.6 min. The standard curve was plotted with caffeine standard bought from Sigma-Aldrich company (Missouri, United States).

2.5. Measurement of pH, total dissolved solid, and browning color in coffee brews

A FE30 pH meter (Mettler-Toledo Group, Schwerzenbach, Switzerland) was used to measure the pH of brewed coffee. The browning color of coffee samples was measured with a Lambda 35 UV-Vis spectrophotometer (Norwalk, USA) at 420 nm after diluting 10 times with distilled water (Meydav et al., 1977). All samples were measured in triplicate.

The methods to measure total dissolved solids (TDS) in the coffee brew followed the procedure of Moreno et al. (2015). The refractometer meter was used to determine Brix degree of brewed coffee using NR-151 digital refractometer (Rogo-Sampaic SA, Deutschland, Germany). TDS was converted from ^{o}Brix using the following equation:

Total dissolved solids (%) = $0.87 \times {}^{o}$Brix

2.6. Statistical analyses

The statistical analysis used SPSS software with 25.0 version (IBM Ltd., New York, USA). Statistical significance was determined for differences between means when $p < 0.05$. ImageJ software version 1.54D, produced by the National Institutes of Health (New York, USA), was used to analyze TEM images and distribute particles. All samples were measured in triplicate, and all charts were created with GraphPad Prism version 9.0 (GraphPad Software Inc., California, USA).

3. Results and Discussion

3.1 Detection and characterization of carbon nanodots in coffee brew

In this study, CNDs were found to be presented naturally in coffee brews. The CNDs in the coffee brew (C-CNDs) were extracted following the procedure described in section 2.3 and characterized with TEM, fluorescence spectroscopy, and UV-vis spectrophotometry (**Figure 2**).

The TEM image at 10 nm scale (**Figure 2A**) reveals dark, spherical C-CNDs with an average size of 3.2 ± 0.9 nm. Fluorescence spectra were obtained with excitation and emission wavelengths ranging from 280-460 nm and 288-730 nm, respectively (**Figure 2B** and **Figure 2D**). The highest fluorescence intensity was observed at an excitation wavelength of 370 nm and an emission wavelength of 438 nm, similar to the CNDs extracted from coffee grounds (Chu et al., 2023). However, this result shows a slight difference compared to CNDs from instant coffee (Ex: 390 nm, Em: 465 nm) (Jiang et al., 2014). Chu et al. (2023) noted that the roasting time can affect the fluorescent properties of C-CNDs (Chu et al., 2023). The UV-vis spectrum (**Figure 1C**) displayed a similar trend to the CNDs from instant coffee and coffee ground around 300 nm, further indicating the presence of the π-π electronic transition in C=C bonds at 275 nm (Chu et al., 2023; Ding et al., 2016).

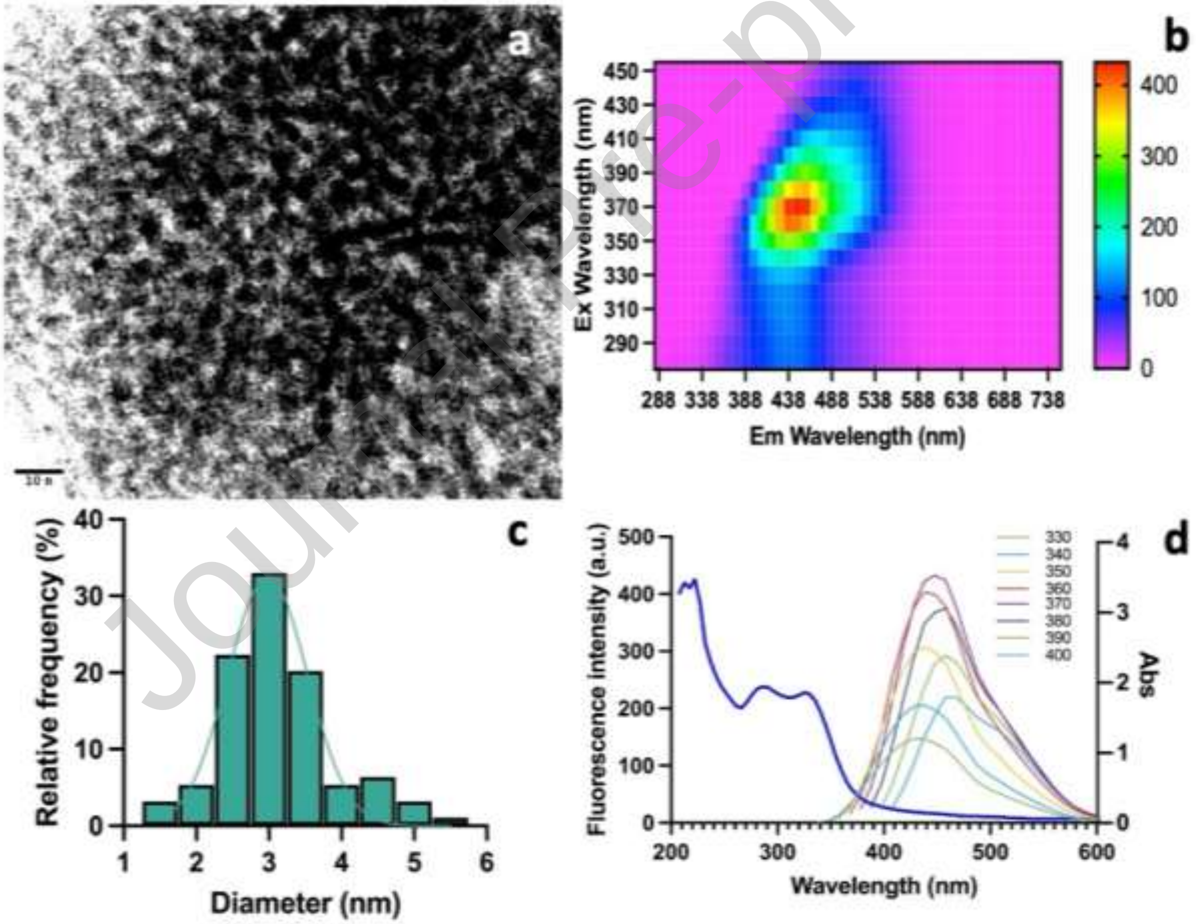

Figure 2. Characterization of carbon nanodots isolated from coffee (C-CNDs) with **(a)** transmittance electron microscopic (TEM) images at 10 nm scale; **(b)** 3D-fluorescent spectra; **(c)**

size distribution histogram with average size of 3.2 ± 0.9 nm; **(d)** the UV-Vis spectra (on the left) and 2D-emission fluorescent spectra at different excitation wavelength (on the right).

3.2. Changes of carbon nanodots content in the brewing process of coffee

After confirming the presence and characterizing of C-CNDs, the study investigated the formation in coffee brewing through four experiments presented in **Figure 3**. **Figure 3A** demonstrates a significant impact of bean types on C-CNDs formation. 100% Arabica beans (*Coffea arabica* L.) exhibited fluorescence over three times higher than the C-CNDs from 100 % Robusta beans (*Coffea canephora* L.), with fluorescent intensities of 5046 ± 15.29 (a.u) and 1444.01 ± 28.39 (a.u), respectively. This difference might be due to the varying chemical compositions of Arabica and Robusta coffee beans. Robusta beans have been reported to contain lower levels of sucrose and higher levels of amino acids, particularly alanine and asparagine, compared to arabica beans (Murkovic and Derler, 2006). During the roasting stage, differences in the initial chemical makeup of coffee beans, especially amino acids, and sugar content, might lead to variations in Maillard reaction rates. This is closely related to the formation of CNDs differently (D. Li et al., 2018; Ma et al., 2020; Nguyen et al., 2024). Furthermore, Bekedam et al. (2006) reported that melanoidins were formed in the roasting step and then diffused with a large amount of high molecular weight melatonin, leading to the coffee color. Interestingly, CNDs were also stated to have a close direct relationship with melatonin compounds (Li et al., 2019).

Figure 3B explores the effect of grinding size on C-CNDs concentration using three sizes: 1.1 mm (coarse), 0.7 mm (medium), and 0.3 mm (fine). The larger size of the coffee ground, the higher C-CNDs levels are. Coarse grounds yielded the highest fluorescent intensity (6562.1 ± 32.7 a.u), while fine grinds exhibited over 36% lower intensity (4183.4 ± 22.0 a.u). This contradicts the trend observed with caffeine content, where Bell et al. (1996) reported that higher caffeine extraction was achieved with finer grinding. This difference suggests a possible negative relationship between caffeine and C-CNDs. CNDs are nanoparticles known for their intense fluorescence (Sk et al., 2012). However, various quenching mechanisms can diminish CNDs fluorescence depending on interacting compounds (Zu et al., 2017). Here, the presence of caffeine might act as a quencher, reducing C-CNDs fluorescence. Furthermore, larger grind sizes present a lower surface area for water interaction compared to finer grinds. Interestingly, this might enhance the C-CNDs extraction process. With larger grinds, water penetration is deeper, potentially facilitating more thorough extraction and C-CND formation within the coffee grounds. In other words, C-CNDs might be distributed throughout the grounds rather than solely on the surface.

Figure 3C shows the effect of the sample-to-water ratio on the C-CNDs level in the coffee brew. It is observed that the ratio of 1:15 results in the highest amount of C-CNDs in samples. Temperature and water/coffee ratio were critical factors influencing extraction behavior (Nicoli et al., 1990). However, adding more water leads to a significant reduction in the formation of C-CNDs. This trend could be explained by the limited availability of precursors for C-CNDs formation. While a higher water ratio might improve the extraction of some coffee components, an excessively high amount of water might limit the interaction between coffee solutes that serve as precursors for C-CNDs during brewing. As the water ratio increases, the concentration of these precursors potentially becomes too dilute, hindering their interaction and subsequent C-CNDs formation (Barros et al., 2019).

The brewing temperature and time significantly impact the composition of coffee during extraction. Two opposite trends of C-CNDs formation are observed in **Figure 3D**. At lower extraction temperatures (80 ^{o}C—90 ^{o}C), C-CNDs content was increased along with increased brewing time. However, when the temperature reaches 95^{o}C, almost all the C-CNDs are extracted after 150 seconds; the prolonged time might cause some structure changes, leading to a drop in C-CNDs levels (**Figure 3D**). Lower brewing temperatures and longer time showed a more significant effect on forming C-CNDs, where the highest value was found at 80 ^{o}C and 300 seconds (5163.5 ± 25.8 a.u).

This experimental data suggested that the bean types, grinding size, sample-to-water ratio, and brewing time and temperature significantly impact the formation of C-CNDs in brewing coffee. The highest fluorescent intensity was detected with Arabica beans, medium ground size, and sample-to-water ratio at 1:15 (v/v). Based on this result, the synthesis or mitigating strategies could be applied depending on the initial purpose, such as the production of CNDs or the removal of CNDs from foods. A pulsed electric field was recorded to significantly remove C-CNDs from coffee grounds (Chu et al., 2023). C-CNDs collected from coffee could be considered green fluorescent nanoparticles, which are safer than CNDs synthesized from chemical sources.

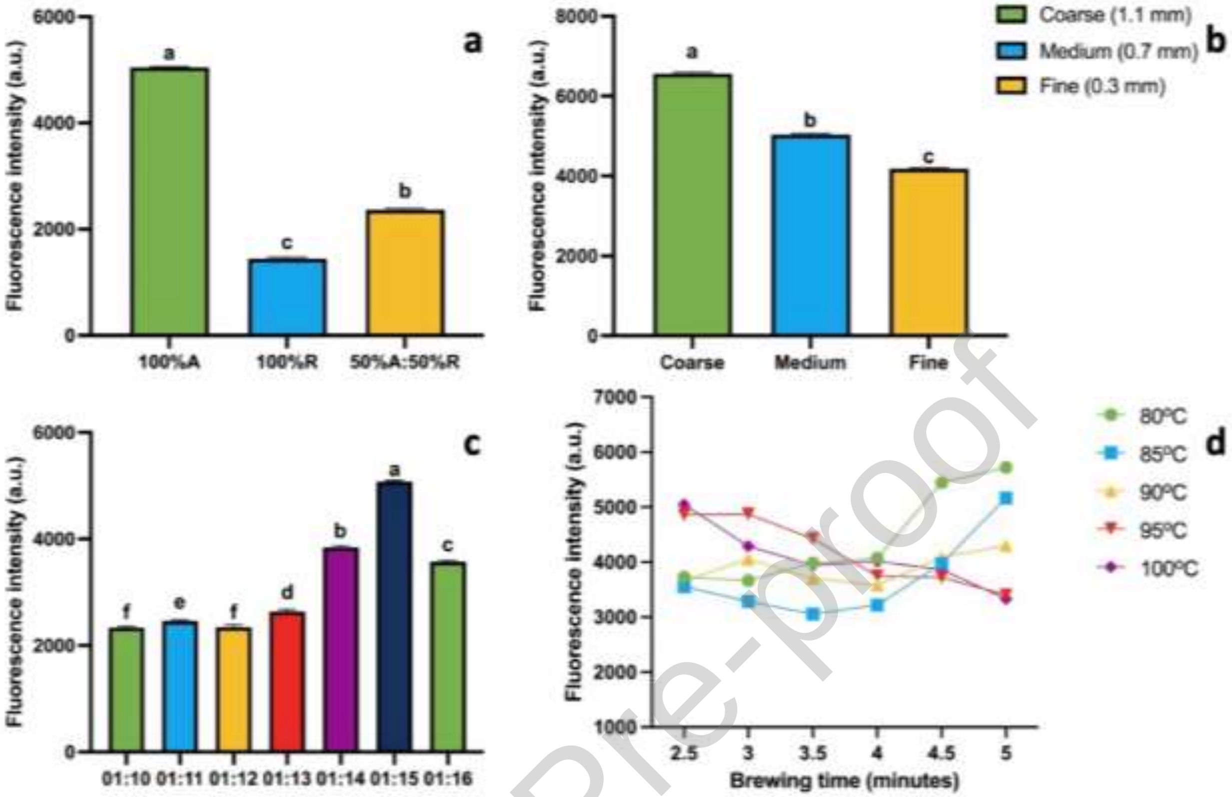

Figure 3. The fluorescence intensity (a.u.) of carbon nanodots in coffee (C-CNDs) at the excitation wavelength 370 nm in four experiments: **(a)** difference types of the coffee bean where 100 % Arabica (100%A); 100 % Robusta (100%R); 50 % Arabica: 50 % Robusta (50%A: 50%R); **(b)** difference coffee ground size; **(c)** coffee-to-water ratio ranging from 1:10 to 1:16 (g/g); **(d)** difference brewing time (150, 180, 210, 240, 270, and 300 seconds) and temperatures (80 °C, 85 °C, 90 °C, 95 °C, and 100 °C). The error bars illustrate the standard deviations of triplicate samples. Different letters (a-f) indicate a significant difference ($p < 0.05$) compared to samples at the same treatment.

3.3. Changes of caffeine content (mg/mL) in the brewing process of coffee

Figure 4 illustrates the outcomes of four experiments designed to examine various factors influencing the caffeine concentration of coffee measured via the HPLC system. The impact of different coffee bean types on caffeine concentration is revealed in **Figure 4A**. 100 % Arabica beans exhibited the lowest concentration (0.25 ± 0.02 mg/mL), followed by a blend of 50 % Arabica and 50 % Robusta (0.46 ± 0.02 mg/mL), and then 100 % Robusta beans (0.56 ± 0.03 mg/mL), indicating a correlation between bean type and caffeine concentration. Previous studies consistently show that robusta beans contain higher levels of caffeine than arabica beans (Caracostea et al., 2021; Purwoko et al., 2022; Yu et al., 2021). This is further supported by the finding that robusta coffee beans yield more caffeine during extraction than arabica beans (Yu et al., 2021). Furthermore, **Figure 4B** investigates the influence of coffee grind size on caffeine concentration, showing that finer grinds yielded higher concentrations than coarser grinds with the caffeine content at 0.32 ± 0.02 mg/mL with fine size and 0.24 ± 0.03 mg/mL with coarse size. The effect of coffee grind size on caffeine concentration can be explained by the surface area exposed to the solvent during extraction. Finer grinds have a larger surface area, facilitating the more efficient extraction of caffeine and other compounds from the coffee grounds (Castañeda-Rodríguez et al., 2022; Spiro and Selwood, 1984; Wang et al., 2016). This phenomenon is consistent with principles of solid-liquid extraction, where smaller particle sizes lead to increased extraction rates (Oshita et al., 1994). **Figure 4C** analyzed the effect of the coffee-to-water ratio on caffeine concentration, with a ratio of 1:10 resulting in the highest concentration, suggesting a direct relationship between grounds-to-water ratio and caffeine concentration. **Figure 4D** examined the impact of brewing time and temperature on caffeine concentration, revealing that higher temperatures and longer brewing times led to increased caffeine extraction.

These results highlight the multifaceted nature of factors influencing the caffeine concentration of coffee brews including coffee bean type, grind size, coffee-to-water ratio, brewing time, and temperature. Research indicates that the caffeine concentration in coffee generally increases with longer brewing time, up to a certain point, due to the extraction of caffeine from the coffee grounds by hot water (Musilová and Kubíčková, 2018; Olechno et al., 2021; Sharma and Paul, 2015). However, after this point, the concentration plateaus or even decreases, possibly due to the breakdown of caffeine molecules at higher temperatures (Sharma and Paul, 2015). The specific factors influencing caffeine content in coffee include brewing time, water temperature, and pressure (Olechno et al., 2021). Research has shown that the brewing temperature significantly impacts caffeine concentration in coffee. At lower temperatures, caffeine concentration increases gradually with longer brewing time, while at higher temperatures, caffeine is extracted more quickly, reaching its peak sooner (Telis-Romero et al., 2000). This is due to the more efficient extraction of coffee compounds by hotter water. The impact of roasting temperature on the formation of aroma compounds in coffee beans has also been studied, with different time-temperature histories leading to distinct aroma compound profiles (Schenker et al., 2002).

Surprisingly, these findings clearly show that coffee brews with less caffeine content have more C-CNDs, providing compelling evidence for an inverse correlation between the two compounds (**Figure 3** & **Figure 4**). While further investigation is warranted, the findings suggest a definitive role for caffeine as a potential inhibitor in C-CNDs formation. These results contribute significantly to our understanding of the influence of caffeine on C-CNDs properties, highlighting the need for continued exploration within this intriguing research avenue.

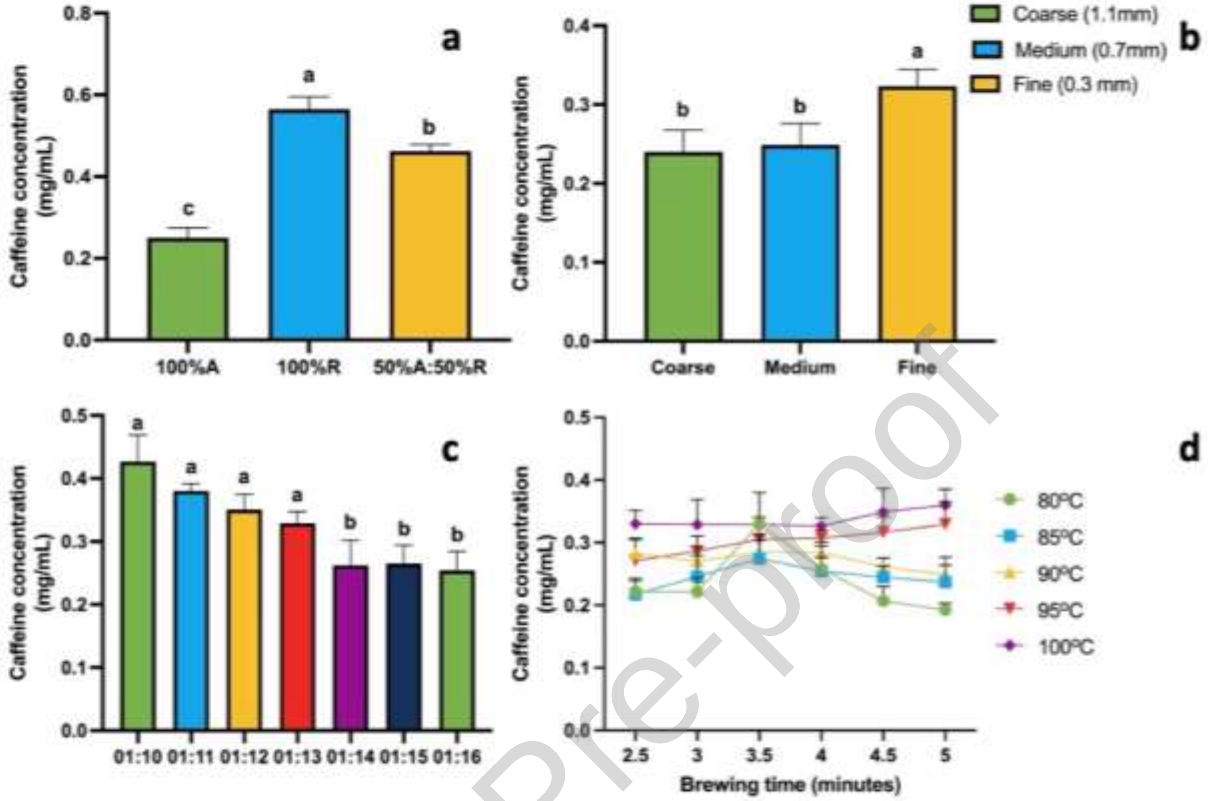

Figure 4. The caffeine concentration (mg/mL) of coffee brew measuring by HPLC system in four experiments: **(a)** difference types of the coffee bean where 100 % Arabica (100%A); 100 % Robusta (100%R); 50 % Arabica: 50 % Robusta (50%A: 50%R); **(b)** difference coffee ground size; **(c)** coffee-to-water ratio ranging from 1:10 to 1:16 (g/g); **(d)** difference brewing time (150, 180, 210, 240, 270, and 300 seconds) and temperatures (80 °C, 85 °C, 90 °C, 95 °C, and 100 °C). The error bars illustrate the standard deviations of triplicate samples. Different letters (a-f) indicate a significant difference ($p < 0.05$) compared to samples at the same treatment.

3.4. Changes in pH and total dissolved solids in the brewing process of coffee

Figure 5 illustrates the significant impact of various brewing parameters on the pH of coffee brews. Notably, the type of coffee bean played a crucial role. Brews made from 100 % Robusta (pH 5.22 ± 0.02) and a blend of 50 % Arabica and 50 % Robusta beans (pH 5.16 ± 0.04) consistently exhibited higher pH levels compared to those sourced solely from 100 % Arabica (pH 4.97 ± 0.03) beans (**Figure 5A**). A similar trend is found in **Figure 6A** with total dissolved solids (TDS) content. This could be the explanation for the Robusta-based brews, which tend to yield higher pH levels as well as TDS content than those derived from Arabica beans (Bicho et al., 2011; Chindapan et al., 2019; Defernez et al., 2017). This difference also can be attributed to the denser cellular structure of Robusta beans, which fosters an excellent release of solubles during brewing (Fischer et al., 2001). These results reveal that C-CNDs concentrations are higher in lower pH environments with lower TDS content. **Figure 3A**, **Figure 5A**, and **Figure 6A** visually demonstrate this trend. Indeed, the pH of the environment significantly affects the optical properties of CNDs, particularly their photoluminescence and absorption spectra (Dutta Choudhury et al., 2017). This effect is attributed to the protonation/deprotonation of surface groups, such as carboxyl and hydroxyl, which are directly involved in determining the electronic energy levels and optical transitions (Kong et al., 2014). CNDs derived from coffee brews exhibited a preferential formation or stability at lower pH values.

Interestingly, grind size did not significantly affect pH (**Figure 5B**). However, there is a significant difference in the TDS of coffee brew compared between different grinding sizes (**Figure 6A**). Brewing coffee with finer grinds (300 µm) resulted in higher TDS content (2.134 ± 0.049 %) compared to coarser grinds (1100 µm) with TDS content (1.395 ± 0.003 %), likely due to increased surface area exposure to water, enhancing extraction efficiency, as demonstrated by a similar study (Wang et al., 2016). Conversely, the coffee-to-water ratio had an evident influence, with lower ratios yielding more concentrated brews and, consequently, higher pH levels (**Figure 5C**) and higher TDS content (**Figure 6C**).

Lastly, a consistent trend of increasing pH and TDS with increasing brewing time and temperature was observed (**Figure 5D** for pH and **Figure 6C** for TDS). Longer brewing durations and elevated temperatures likely lead to greater extraction of solubles from the coffee grounds, thereby increasing the overall pH and TDS (Lee et al., 2023).

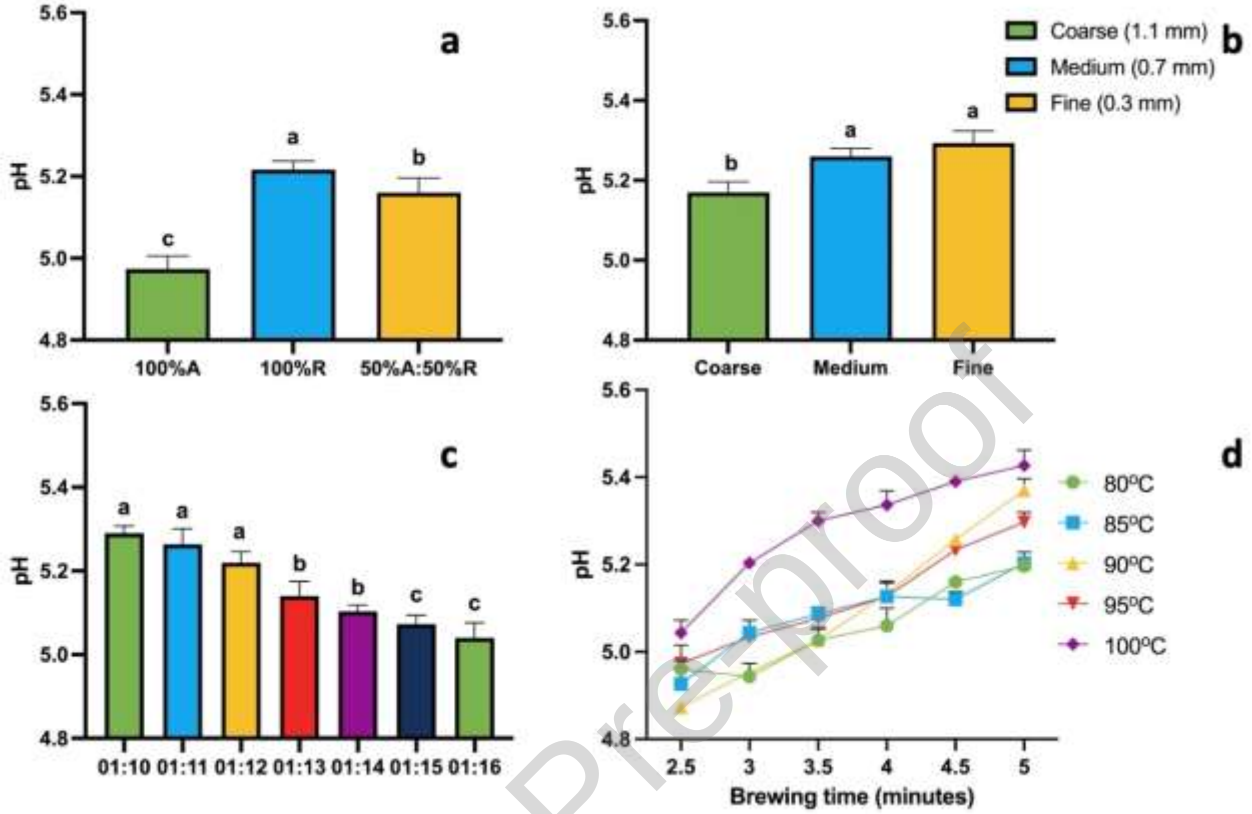

Figure 5. pH of coffee brew in four experiments: **(a)** difference types of the coffee bean where 100 % Arabica (100%A); 100 % Robusta (100%R); 50 % Arabica: 50 % Robusta (50%A: 50%R); **(b)** difference coffee ground size; **(c)** coffee-to-water ratio ranging from 1:10 to 1:16 (g/g); **(d)** difference brewing time (150, 180, 210, 240, 270, and 300 seconds) and temperatures (80 ^{o}C, 85 ^{o}C, 90 ^{o}C, 95 ^{o}C, and 100 ^{o}C). The error bars illustrate the standard deviations of triplicate

samples. Different letters (a-f) indicate a significant difference ($p < 0.05$) compared to samples at the same treatment.

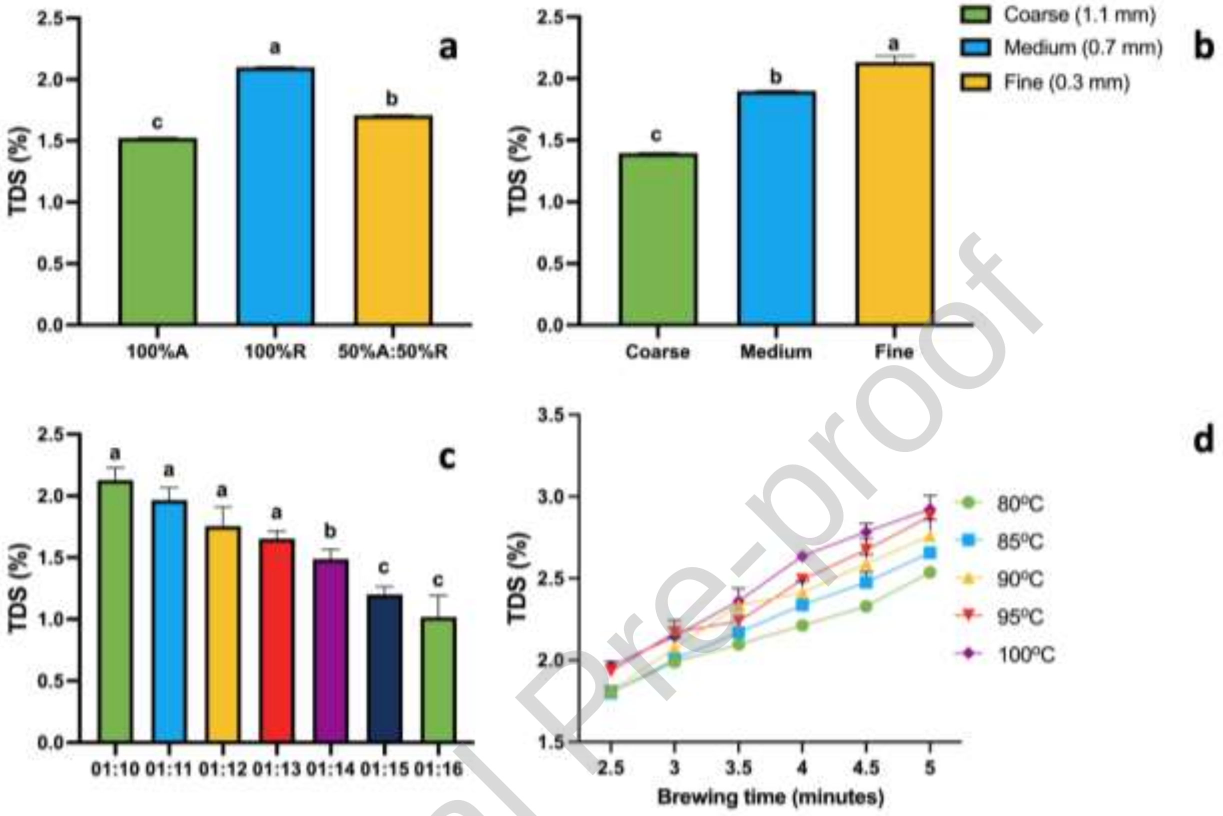

Figure 6. Total dissolved solids (%) of coffee brew in four experiments: **(a)** difference types of the coffee bean where 100 % Arabica (100%A); 100 % Robusta (100%R); 50 % Arabica: 50 % Robusta (50%A: 50%R); **(b)** difference coffee ground size; **(c)** coffee-to-water ratio ranging from 1:10 to 1:16 (g/g); **(d)** difference brewing time (150, 180, 210, 240, 270, and 300 seconds) and temperatures (80 ºC, 85 ºC, 90 ºC, 95 ºC, and 100 ºC). The error bars illustrate the standard deviations of triplicate samples. Different letters (a-f) indicate a significant difference ($p < 0.05$) compared to samples at the same treatment.

3.5. Changes of browning color in the brewing process of coffee

The degree of caramelization and Maillard reactions can be evaluated by observing the formation of browned compounds as products of reactions at 420 nm (Meydav et al., 1977). **Figure 7** illustrates how various coffee brewing parameters influence the browning color of coffee brews, as measured by absorbance at 420 nm. Absorbance at this wavelength is associated with the concentration of melanoidins, which are high molecular weight nitrogenous and brown-colored compounds formed during the Maillard reaction (Bekedam et al., 2008; Moreira et al., 2012). The Maillard reaction is a complex series of chemical reactions between sugars and amino acids during heating. The roasting degree of coffee beans affects the properties and formation mechanisms of melanoidins, with proteins and chlorogenic acids playing a primary role (Bekedam et al., 2008). First, the types of coffee beans did not appear to have a significant effect on the browning color of the coffee brew (**Figure 7A**). The previous study suggests that the kind of coffee bean used, whether Arabica or Robusta, does not significantly impact the browning color of the coffee brew, indicating similar Maillard reaction processes (Nebesny and Budryn, 2006). Regarding the size of the coffee grounds, grind size also did not appear to impact the browning color significantly (**Figure 7B**). This contradicts the expectation that a finer grind size would result in more browning due to increased surface area exposure and potentially faster extraction (Demir et al., 2010). However, as mentioned in the previous studies, the relationship between grind size and browning color may be more nuanced than previously thought (Rowe and Chen, 1997). Further research is needed to fully understand the impact of grind size on browning color. A minimal effect on the browning color was found when changing the coffee-to-water ratio across the range investigated (**Figure 7C**). This suggests that the concentration of coffee solubles did not significantly influence the extraction of Maillard reaction by-products under these brewing conditions. No clear trend was observed between browning color and brewing time or temperature. While the unchanged browning color is found at the brewing temperatures of 85 ^{o}C and 90 ^{o}C, there is an increment of browning color over time with higher temperatures (**Figure 7D)**. It could be concluded that higher temperatures (above 90 °C) facilitate the effective extraction of melatonin into an aqueous solution, which consequently produces a darker color. While increased reaction temperature and time in the roasting processes are directly linked to browning intensity in the Maillard reaction of D-psicose and glycine mixtures (Baek et al., 2008), extraction time and temperature also significantly affect the diffusion of Maillard reaction products into the solution.

Overall, the findings suggest that under the conditions of this experiment, the brewing parameters investigated had a minimal effect on the browning color of coffee brews as measured by absorbance at 420 nm. Further investigation is needed to determine how these parameters influence the formation of specific browning compounds and their impact on overall coffee quality.

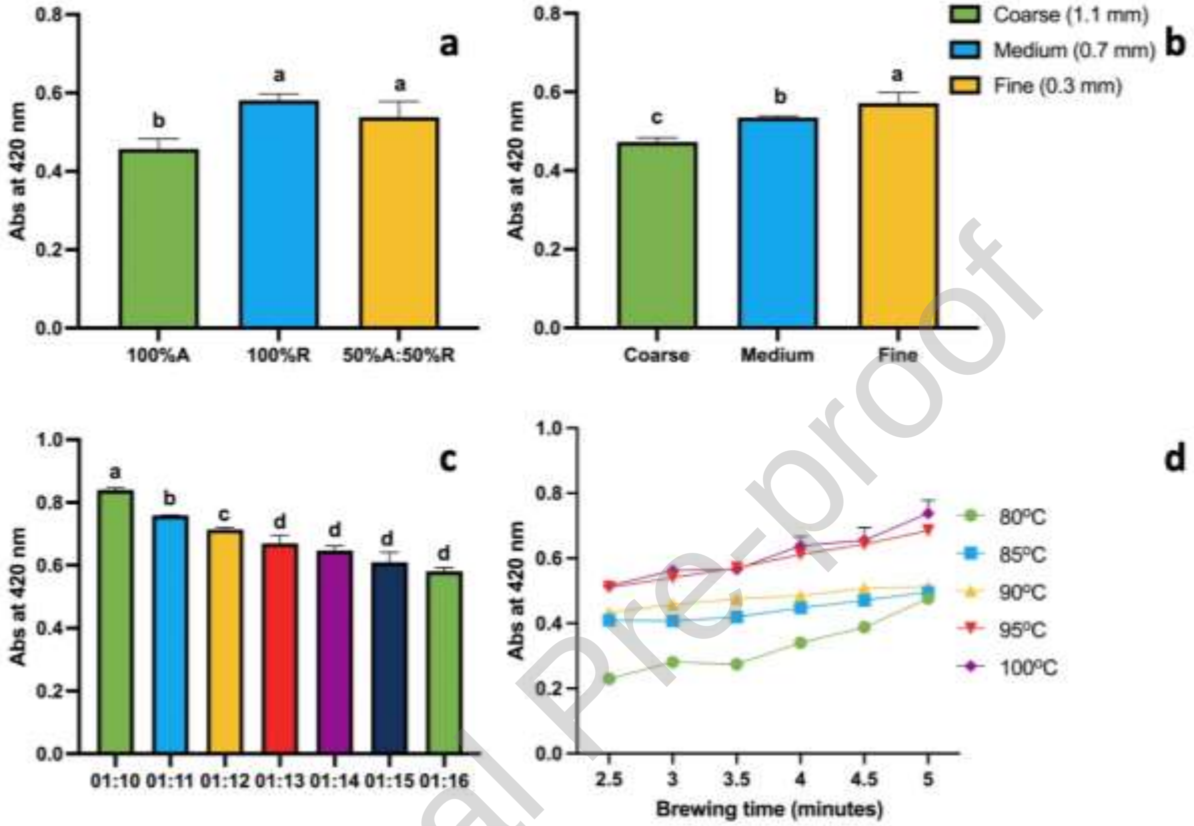

Figure 7. Browning color of coffee brew at the absorbance 420 nm (Abs) in four experiments: **(a)** difference types of the coffee bean where 100 % Arabica (100%A); 100 % Robusta (100%R); 50 % Arabica: 50 % Robusta (50%A: 50%R); **(b)** difference coffee ground size; **(c)** coffee-to-water ratio ranging from 1:10 to 1:16 (g/g); **(d)** difference brewing time (150, 180, 210, 240, 270, and 300 seconds) and temperatures (80 °C, 85 °C, 90 °C, 95 °C, and 100 °C). The error bars illustrate the standard deviations of triplicate samples. Different letters (a-f) indicate a significant difference ($p < 0.05$) compared to samples at the same treatment.

3.6. Correlations between carbon nanodots with caffeine, pH, TDS, and browning color

Our findings on C-CNDs formation and its relationship with caffeine content, pH, TDS, and browning color reveal a key role for caffeine in influencing C-CNDs formation. Higher caffeine content correlates with lower C-CNDs levels, suggesting a direct association between caffeine and inhibition of C-CNDs production (**Figure 3** & **Figure 4**). Furthermore, our analysis shows that C-CNDs tend to form more readily in acidic conditions, highlighting the complex interplay between brewing factors and C-CNDs formation (**Figure 3** & **Figure 5**). To further elucidate these relationships, we conducted a comprehensive correlation analysis of five key factors in coffee brews, including C-CNDs, caffeine content, pH, TDS, and browning color (**Figure 8A**). Notably, a negative correlation coefficient was observed between C-CNDs and all components. Particularly noteworthy is the strong negative correlation between C-CNDs and caffeine content (-0.8), indicating a significant impact of caffeine on C-CNDs levels. Weaker negative correlations were also found between C-CNDs and pH (-0.33), TDS (-0.48), and browning color (-0.62), underlining the multifaceted nature of C-CNDs formation.

These findings suggest that caffeine may act as a quencher, binding to functional groups on the surface of CNDs and hindering their fluorescence. While the precise mechanism requires further investigation, the observed interaction between caffeine and CNDs presents a promising avenue for rapid caffeine detection in foods, leveraging the unique properties of CNDs. The quenching of C-CNDs fluorescence can occur through various mechanisms, including static quenching, dynamic quenching, Förster resonance energy transfer (FRET), photoinduced electron transfer (PET), surface energy transfer (SET), Dexter energy transfer (DET), and inner filter effect (IFE) (Zu et al., 2017). Furthermore, research has shown that CNDs fluorescence can be manipulated. For example, C-CNDs exhibit quenching phenomena in the presence of various compounds such as metal ions (Laptinskiy et al., 2022), mercury ions (Hg^{2+}) (Xavier et al., 2018) , and cyanide (Hu et al., 2016). This reversible and selective fluorescence switching in CNDs holds immense promise for developing highly sensitive and specific sensors, potentially revolutionizing detection technologies across various industries.

Figure 8B focuses specifically on the correlation between CNDs and caffeine content. As mentioned earlier, the data suggests a strong negative correlation (-0.8). Therefore, there is an upward trend; the data points are scattered, indicating a negative relationship. Previous studies have been developed electrochemical sensors for caffeine using carbon-based electrodes (Carolina Torres et al., 2014; Habibi et al., 2012). These studies demonstrate the potential of carbon-based materials in caffeine detection. According to our knowledge, no research worked on the relationship between CNDs and caffeine. Therefore, this finding could lead to developing CNDs-based sensors to rapidly detect caffeine content in foods. Further investigation is needed to understand the potential interactions between carbon nanodots, caffeine, and other coffee components during brewing.

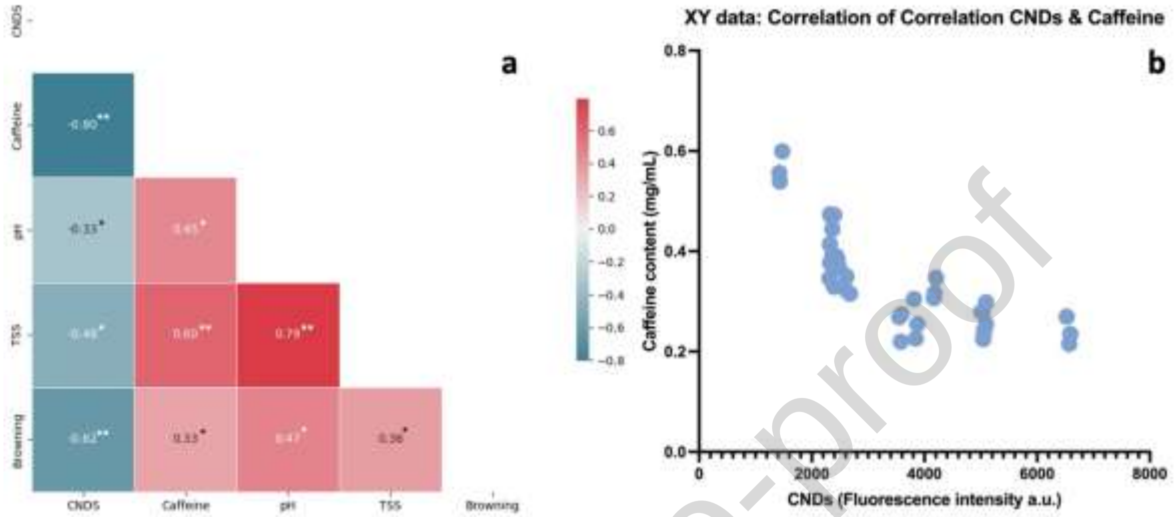

Figure 8. (a) Heatmap of Pearson correlations among C-CNDs (fluorescence intensity (a.u.), caffeine content (mg/mL), pH, total dissolved solids (TDS), and browning color (Abs at 420 nm) in coffee brews and **(b)** the correlation between C-CNDs and caffeine content in coffee brews. *Correlation coefficient at $p < 0.05$. **Correlation coefficient at $p < 0.001$.

4. Conclusions

Our research has shed light on a previously unexplored aspect of coffee: the formation and characteristics of carbon nanodots (C-CNDs) during brewing. We observed that C-CNDs formation is influenced by factors like bean type (higher with Arabica), grind size (higher with coarser grinds), coffee-to-water ratio (stronger at 1:15), brewing temperature (over 90°C), and brewing time (longer times). Isolated C-CNDs with an average size of 3.2 ± 0.9 nm were found using TEM analysis. The optical properties of isolated C-CNDs were identified through fluorescence and UV-Vis spectroscopy with the optimal excitation wavelength at 370 nm. Notably, C-CNDs exhibited a strong negative correlation with caffeine content (-0.8), where the higher caffeine content inhibited the formation of C-CNDs. Furthermore, C-CNDs found weak relationships with pH, TDS, and browning color. This study offers compelling evidence for the presence of C-CNDs in coffee and establishes a critical relationship between their formation and caffeine content. Future research should explore the impact of C-CNDs on coffee quality and potential health effects. Additionally, using C-CNDs could have the potential for rapid detection of caffeine content in foods.

CRediT authorship contribution statement

Duyen H.H. Nguyen: Writing – original draft, Writing –review & editing, Visualization, Investigation, Formal analysis, Conceptualization. Hassan El-Ramady: Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. Arjun Muthu: Writing –review & editing, Writing – original draft, Resources, Investigation, Conceptualization. József Prokisch: Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. Áron Béni: Writing –review & editing, Resources, Investigation.

Declaration of competing interest

No competing interests.

Data availability

Data will be made available on request.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights

- Coffee brewing created tiny nanoparticles called carbon nanodots (C-CNDs), previously unknown in this popular beverage.
- Brewing conditions significantly impact C-CND formation, with Arabica beans, finer grinds, stronger ratios (1:15), hotter temperatures (>90^{o}C), and longer brewing times yielding more.
- Isolated C-CNDs have an average size of 3.2 nm and were thoroughly characterized.
- A surprising link emerged between C-CNDs and caffeine content, with C-CNDs increasing as caffeine levels decrease.
- Weaker negative correlations were also found with pH, total dissolved solids, and browning color.