

Green Synthesis of Carbon Quantum Dots from Buttermilk for Antimicrobial Preservation and Shelf-Life Enhancement of Paneer

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Abstract

The major objective of this study was to synthesize and characterize carbon quantum dots (CQDs) from buttermilk using hydrothermal technique and to evaluate their antimicrobial efficacy as a natural preservative for paneer. The sample with the highest fluorescence intensity among the three prepared samples (10%, 20%, and 30% buttermilk dilution) was chosen for in-depth characterization. The buttermilk-derived CQDs (BM-CQDs) were quasi-spherical with an average size of 6.14 nm as observed by High Resolution Transmission Electron Microscopy. Ultraviolet-Visible spectroscopy showed an absorption peak at 283 nm, corresponding to the π - π^* transition of aromatic C=C bonds, while photoluminescence (PL) analysis revealed a strong emission peak at 422 nm when excited at 340 nm. Elemental analysis indicated the presence of C (67 %), N (6.1 %), and O (14 %), as confirmed by the Energy Dispersive X-ray, suggesting natural doping. X-ray Diffraction analysis revealed a broad peak at $\sim 20^\circ$ (2θ), indicative of amorphous nature, and Fourier Transform-Infrared spectroscopy confirmed the presence of -OH, -NH, -C=O, and other functional groups. The developed BM-CQDs were used for enhancing the shelf-life of paneer. Paneer samples treated with 2,500 mg/L and 5,000 mg/L BM-CQDs showed a significant reduction in microbial counts, total viable, coliform, and yeast and mould count compared to untreated control during storage study. Twenty-one days increase in the shelf-life of paneer was recorded with the BM-CQDs. These findings highlight the potential of BM-CQDs as a novel, eco-friendly solution for dairy products preservation and shelf-life enhancement, aligning with clean-label food trends and waste valorization strategies.

Categories: Food processing, Food quality, Nanotechnology for sustainable agriculture

Keywords: carbon quantum dots, buttermilk, fluorescence, paneer preservation, microbiological quality

Introduction

Nanotechnology has emerged as a transformative discipline across numerous fields, including biomedical sciences, agriculture, and the food industry. One of the most promising areas of nanotechnology is the development of carbon quantum dots (CQDs) - a novel class of zero-dimensional, quasi-spherical carbon-based nanomaterials exhibiting extraordinary optical, electronic, and physicochemical properties. Since their discovery in 2004, CQDs have attracted considerable attention due to their photoluminescence, biocompatibility, low toxicity, water solubility, and ease of functionalization [1]. Unlike metal-based quantum dots such as CdSe or PbS, CQDs do not pose significant environmental or biological toxicity, which positions them as safer alternatives in applications ranging from bioimaging to environmental remediation [2]. Their tunable fluorescence, high quantum yield, and surface functional groups make them highly versatile for use in biosensors, antibacterial agents, and food preservation techniques, food safety, food

packaging, production of composite polymers, the treatment of various diseases, and for detection of food-borne pathogens [3]. A growing research interest supports the use of green synthesis methods for CQDs, emphasizing eco-friendly, cost-effective, and sustainable routes utilizing natural carbon sources such as fruits, vegetables, milk, and other biowastes. This aligns with the principles of green chemistry and sustainable development, minimizing the reliance on hazardous chemicals and energy-intensive processes [4]. Even if the synthesis of CQDs utilizing inexpensive materials and a wide range of practical applications is becoming more and more popular, CQDs remain an intriguing challenge that motivates extensive research in this field. Environmentally friendly CQDs can now be created using a variety of techniques, such as hydrothermal treatment, laser ablation, and electrochemical processes [5].

Buttermilk, a by-product of curd churning in dairy processing, is rich in lactose, proteins (casein and whey), lactic acid and minerals. In the Indian subcontinent, 15-20 kg of sour buttermilk are produced for every kilogram of ghee [6]. Sour buttermilk typically has a brownish hue since the milk is heated for a lengthy time before being inoculated with an unidentified starter culture, which results in uncontrolled fermentation. Buttermilk contained 0.8% fat, 1.29% protein, 3.8% total solids, 0.44% lactic acid, 1.2% lactose, and 0.4% ash. Its short shelf-life, high acidity, low heat stability, large bulk with less total solids content, and inadequate collection and processing system are the main issues making its use difficult [7]. These are the possible reasons for why buttermilk is not used in conventional dairy processing and as a result, it drains into the surroundings. Buttermilk has similar kind of composition that of sour whey which has been used as carbon precursor by many researchers for synthesis of carbon quantum dots. Utilizing buttermilk for the synthesis of BM-CQDs presents a sustainable and economical avenue, where the dairy industry is both massive and under pressure to reduce waste. Through hydrothermal carbonization, the organic constituents of buttermilk undergo dehydration, condensation, and aromatization under high pressure and temperature, leading to the formation of nanoscale carbon dots. The inherent nitrogen and oxygen functionalities in buttermilk proteins and sugars contribute to the surface passivation and fluorescence of the synthesized CQDs.

In this study, buttermilk serves as both a carbon source and a natural dopant, enhancing the antimicrobial and optical properties of the resultant CQDs. The main objectives of this study were synthesis of CQDs from buttermilk using a hydrothermal method and the application of these CQDs for preservation of paneer. This study investigates the use of BM-CQDs as an antibacterial nano-bio-preservative in paneer and offers a novel method for converting buttermilk into CQDs through a green technique. In contrast to earlier research that mostly concentrated on plant-derived CQDs, this research work combines sustainable nanotechnology with dairy by-product valorization to create functional CQDs intended for preservation of dairy products. By turning dairy waste into a valuable functional nanomaterial for food preservation, this method not only provides a sustainable substitute for artificial preservatives but also advances the ideas of the circular bioeconomy.

Materials And Methods

Buttermilk (Chhach) was purchased from the Mother Dairy Milk Parlor for synthesis of carbon quantum dots.

Preparation of buttermilk carbon quantum dots

Buttermilk was properly mixed by shaking and then diluted with distilled water in three different concentrations. Sample 1: 10% diluted buttermilk; Sample 2: 20% buttermilk and sample 3: 30% diluted buttermilk. Then the samples were mixed with the help of magnetic stirrer at 400 rpm for 15 minutes at 27°C. Furthermore, the samples were transferred into 100 mL Teflon-lined autoclave and filled up to 2/3 of the volume and then packed into stainless steel hydrothermal reactor. Hydrothermal reactor was placed into the muffle furnace at 180°C for 8 hours at a rate of heating 5°C/minute as per the method of Kalyani et al. [8] with slight modifications. Then, cooled it to room temperature and a brown-yellow color solution was obtained. Sample was filtered with Whatman No. 1 filter paper, then the permeate was centrifuged in centrifuge machine at 8,000 rpm for 15 minutes. After centrifugation, the sample was filtered with the help of 0.2 µm syringe filter to obtain the butter milk carbon quantum dots (BM-CQDs) and then stored at 4°C.

Optimization of the BM-CQDs

All three samples of synthesized BM-CQDs were observed under UV-light chamber and the BM-CQDs sample with best fluorescence was selected for further study.

Characterization of optimized BM-CQDs

UV-Visible Spectroscopy

The optical absorption properties of the synthesized BM-CQDs were analyzed using a UV-Visible spectrophotometer (Model: Cary 60 UV-Vis, Agilent Technologies, USA). Prior to analysis, the BM-CQDs were uniformly dispersed in deionized water. The absorption spectrum was recorded in the range of 200-800 nm. A 1-cm path length quartz cuvette was used for all measurements, with deionized water serving as the reference blank. The obtained spectra were used to identify characteristic absorption bands corresponding to π - π^* transitions of C=C bonds and n - π^* transitions of C=O groups, indicating the presence of conjugated structures and surface functionalities in the BM-CQDs.

Photoluminescence Spectroscopy

Photoluminescence (PL) characteristics of the synthesized CBDs were examined using a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, USA). The measurements were carried out at room temperature in the wavelength range of 300-600 nm. The excitation wavelength was set at 310 to 400 nm, and emission spectra were recorded to analyze the optical emission behavior of the CQDs. Quartz cuvette with a path length of 1 cm was used for all measurements.

Transmission Electron Microscopy

The morphology of the BM-CQDs was characterized by Transmission Electron Microscopy (TEM). It is widely used as a soft matter characterization tool and offers direct structural information on nanostructured materials. Liquid sample was ultrasonicated and spread on TEM grid and dried in hot air oven for 2 hours at 60°C and observed under TEM for image and average size of CQDs was calibrated.

Energy Dispersive X-ray Spectroscopy (EDX) Analysis

Energy Dispersive X-ray Spectroscopy (EDX) spectroscopy was carried out to analyze the elemental composition of the derived BM-CQDs. The analysis was conducted using an EDX system (Model: Zaluzec), equipped with a SUTW-Sapphire (Super Ultra Thin Window) silicon drift detector (SDD). The system was integrated with a Field Emission Scanning Electron Microscope (FE-SEM) and operated using EDAX Genesis or TEAM™ software for data acquisition and analysis.

Sample preparation: For sample preparation, a small quantity of the dried BM-CQDs sample was placed on a carbon-coated double-sided adhesive tape mounted on an aluminum stub. To avoid charging during electron beam interaction, the sample surface was sputter-coated with a thin gold layer using a vacuum coater. The EDX measurements were performed under high vacuum conditions with an accelerating voltage of 15 kV. Spectra were collected over an energy range of 0 to 20 keV. The system identified and quantified the elemental composition based on the characteristic X-ray emissions of the elements present. Quantitative elemental data (both weight % and atomic %) were processed using the EDAX software.

X-Ray Diffraction

The amorphous or crystalline structure of BM-CQDs was determined using the EMPYREAN X-ray diffraction (XRD) advanced diffractometer (Bruker model-D8 Advance (Eco), Germany). The small amount of liquid sample of BM-CQDs was casted drop by drop over a 1cm² glass slide and dried by placing it over the heating plate and the sample was casted over the glass slide by drop casting. Then the dried sample was examined under XRD for the confirmation of crystalline structure of prepared BM-CQDs.

Attenuated Total Reflectance- Fourier Transform Infrared

The functional groups present in the synthesized BM-CQDs were identified using Attenuated Total Reflectance- Fourier Transform Infrared (ATR-FTIR). The analysis was carried out using JASCO model-FT/IR-4700 spectrometer equipped with an ATR accessory containing a diamond crystal. 2 mg of the liquid BM-CQD sample was placed directly on the ATR crystal without any further sample preparation. The spectrum was recorded in the range of 4000-650 cm^{-1} at a resolution of 1.86 cm^{-1} . Before each scan, the crystal was cleaned thoroughly with ethanol and allowed to dry to avoid contamination. The obtained FT-IR spectra were analyzed to determine the presence of characteristic functional groups.

Application of BM-CQDs in paneer

Paneer was prepared at pilot plant of Department of Dairy Science and Food Technology, Banaras Hindu University, using standardized milk with 4.5% fat and 8.5% SNF. Then paneer was cut into pieces and weighted 11.0g each and categorized in three groups, i.e., A: Control (Paneer dipped in chilled water), B: Paneer (dipped into chilled water having 2,500 mg/L BM-CQDs) and C: Paneer (dipped into chilled water having 5,000 mg/L BM-CQDs). After dipping for 15 minutes, paneer samples were packed and kept in refrigerator at 4°C.

Microbiological analysis

All samples were examined for total viable count, yeast and mould count, and coliform count during storage. Plate Count Agar, Potato Dextrose Agar and Violet Red Bile Agar was used for determination of total plate count, yeast and mould count and coliform count, respectively.

Statistical analysis

Data were expressed as mean $\pm$ standard deviations. All experiments were conducted in triplicates and the data were analyzed using SPSS software. All figures were depicted through Origin software version 8.0.

Results And Discussion

UV-Vis spectroscopy

The BM-CQDs synthesis was first confirmed visually and through spectrophotometric investigation (Figure 1 and Table 1). Sample C (30% diluted BM-CQDs) displayed the highest fluorescence intensity among the three, indicating a greater concentration of emissive carbonaceous domains. This enhanced luminescence suggests superior optical properties, likely due to the higher presence of carbon-rich precursors and surface functional groups. The UV-visible absorption spectrum of carbon quantum dots synthesized from buttermilk using the hydrothermal method, reveals significant insights into their optical characteristics. As depicted in (Figure 2), a sharp absorption peak appears at 283 nm, attributed to the $\pi-\pi^*$ transition of aromatic carbon-carbon double bonds

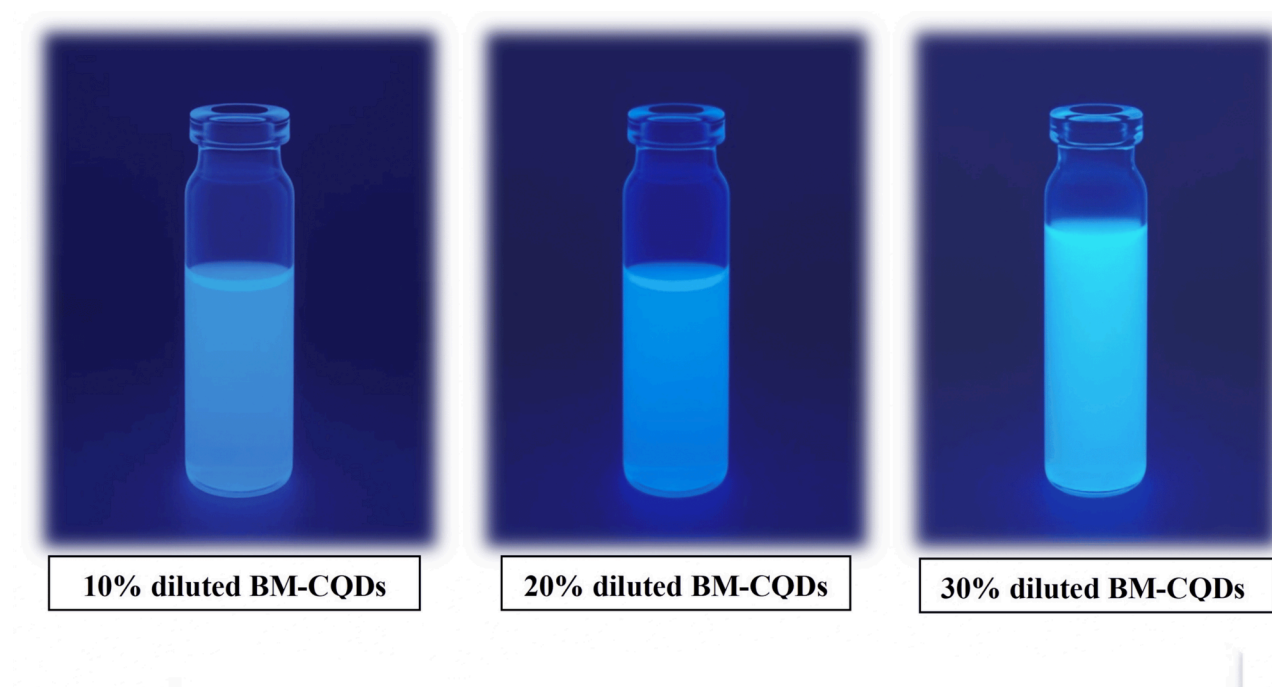


FIGURE 1: Fluorescence pictures of different concentrations BM-CQDs in UV chamber.

BM-CQDs, Buttermilk-Derived Carbon Quantum Dots; UV, Ultraviolet

(C=C). This peak was a typical signature of conjugated carbon structures present in carbon-based nanomaterials [9]. The presence of this absorption peak indicates the successful formation of graphitic domains within the CQD structure. Furthermore, the absorbance decreases sharply beyond 300 nm and remains nearly flat up to 600 nm. The absence of any significant absorption beyond 350 nm indicates that there are no large graphitic particles or metallic impurities present, which would otherwise contribute to red-shifted absorption in the visible region. This clean spectral profile signifies the high purity and small size of the synthesized BM-CQDs.

Sl. No.	Area (nm ²)	Mean	Min.	Max.	Angle	Diameter (nm)
1	1.388	145.704	97.493	193.888	59.153	7.533
2	1.42	143.241	107.447	170	42.184	7.758
3	1	124.885	81.447	170	46.332	5.464
4	1.065	155.36	133.555	172.227	21.801	5.804
5	0.904	145.624	110.843	172.907	45.498	4.869
6	1.323	144.068	99.659	173.25	38.884	7.154
7	0.807	151.123	120.083	172.833	41.634	4.326
8	1.194	153.573	123.79	174.014	46.123	6.48
9	0.839	143.991	107.168	174.766	40.101	4.462
10	1.097	152.542	114.162	181.018	58.736	5.884
11	1.323	146.175	92.628	170	51.116	7.154
12	1.549	150.302	121.643	170	62.354	8.517
13	1.388	137.429	97.605	170	44.029	7.496
14	1	143.205	109.881	170	41.009	5.475
15	1.226	151.277	125.405	170	45	6.605
16	1.097	150.458	102.959	170	-121.264	5.884
17	1.517	146.89	103.631	211.14	38.273	8.314
18	1.062	135.529	15	230	49.128	6.032
19	0.985	150.336	61	195	64.231	5.65
20	1.062	144.217	30	243	47.951	6.025
21	1.18	140.59	25	201	51.801	6.75
22	1.391	123.484	15	226	56.31	7.211
23	1.074	110.141	0	197	55.864	6.198
24	0.93	118.512	3	171	52.943	5.339

25	0.892	111.034	0	203	53.902	5.166
26	1.013	120.739	0	170	59.621	5.846
27	1.202	150.604	65	231	63.759	6.883
28	0.802	144.774	44	208	69.193	4.651
29	1.193	128.252	0	195	56.514	6.836
30	1.154	116.26	0	232	43.363	6.515
31	1.293	120.018	9	170	60.945	7.225
32	1.046	137.331	22	215	53.471	5.895
33	0.97	119.341	0	170	58.57	5.551
34	1.123	133.815	0	245	68.344	6.418
35	1.139	129.547	7	251	58.903	6.454
36	1.17	127.704	14	194	52.98	6.702
37	0.831	135.587	0	195	60.124	4.734
38	0.763	142.54	15	252	65.556	4.221
39	1.136	143.497	32	255	67.203	6.537
40	0.595	133.385	0	198	49.086	3.467

TABLE 1: Measurement of buttermilk carbon quantum dots diameter using imageJ software.

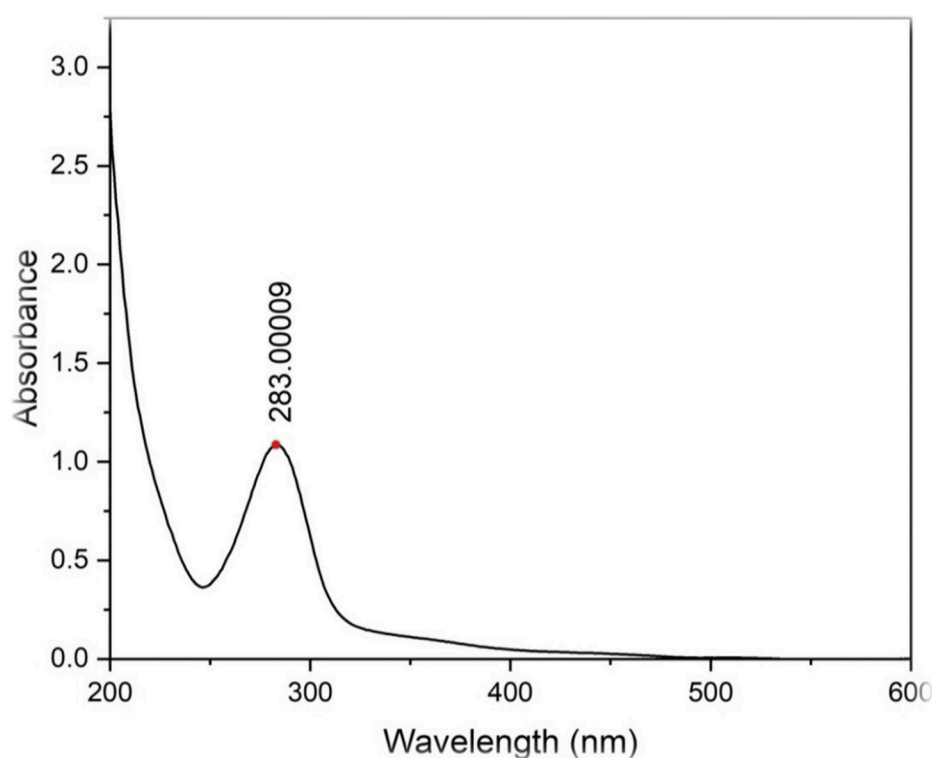


FIGURE 2: UV-visible absorption spectra of buttermilk carbon quantum dots prepared by hydrothermal method.

The UV-Vis profile also confirms that the CQDs possess strong π - π^* transitions but lack significant n - π^* transitions of C=O bonds, usually found between 320 and 380 nm, suggesting a minimal presence of oxygen-containing groups. This observation was consistent with previous reports indicating that hydrothermally synthesized CQDs often exhibit dominant π - π^* transitions due to aromatic structures [10]. Similarly, Kumar et al. [11] reported carbon dots synthesized via hydrothermal treatment of cow milk for potential sensing application showed peak at 275 nm. Carbon quantum dots synthesized from dairy wastes also exhibited a distinct absorption peak around 280 nm, corresponding to π - π^* transitions, confirming that dairy-derived precursors were capable of producing optically active CQDs with comparable electronic behavior. Nataraj et al. [12] also reported that the synthesis of CQDs from milk derivatives exhibited a UV-Vis peak between 280 and 290 nm, aligning well with the findings of present study. Overall, the sharp and intense absorption peak at 283 nm, along with a flat baseline at higher wavelengths, confirms the successful synthesis of well-dispersed, graphitic, and oxygen-lean CQDs with excellent optical purity. These features make the BM-CQDs suitable for further photoluminescent and antimicrobial applications.

Photoluminescence

PL spectrum of BM-CQDs, excited at 340 nm, exhibited a strong emission peak at 421.9 nm (Figure 3A). The observed sharp and symmetric fluorescence peak indicates a narrow size distribution and well-passivated surface of the synthesized CQDs. The intense blue fluorescence was mainly due to π - π transitions in the conjugated sp^2 carbon domains and n - π transitions involving surface functional groups such as carbonyl and amide groups. These functional groups are likely formed during the hydrothermal processing of buttermilk, which contains casein, lactose, and proteins that

naturally provide nitrogen and oxygen-based dopants. The emission aligns well with earlier findings from green-synthesized CQDs, where blue PL was commonly reported due to surface states and size-related quantum confinement effects. Blue PL centered approx. at 420 nm was characteristic of many green-synthesized CQDs. Similar maxima have been reported for hydrothermally prepared dots from sugarcane bagasse at 425 nm [13]. Blue light-emitting carbon dots (emission range: 420-450) were synthesized from casein micelles, a milk protein using microwave exposure.

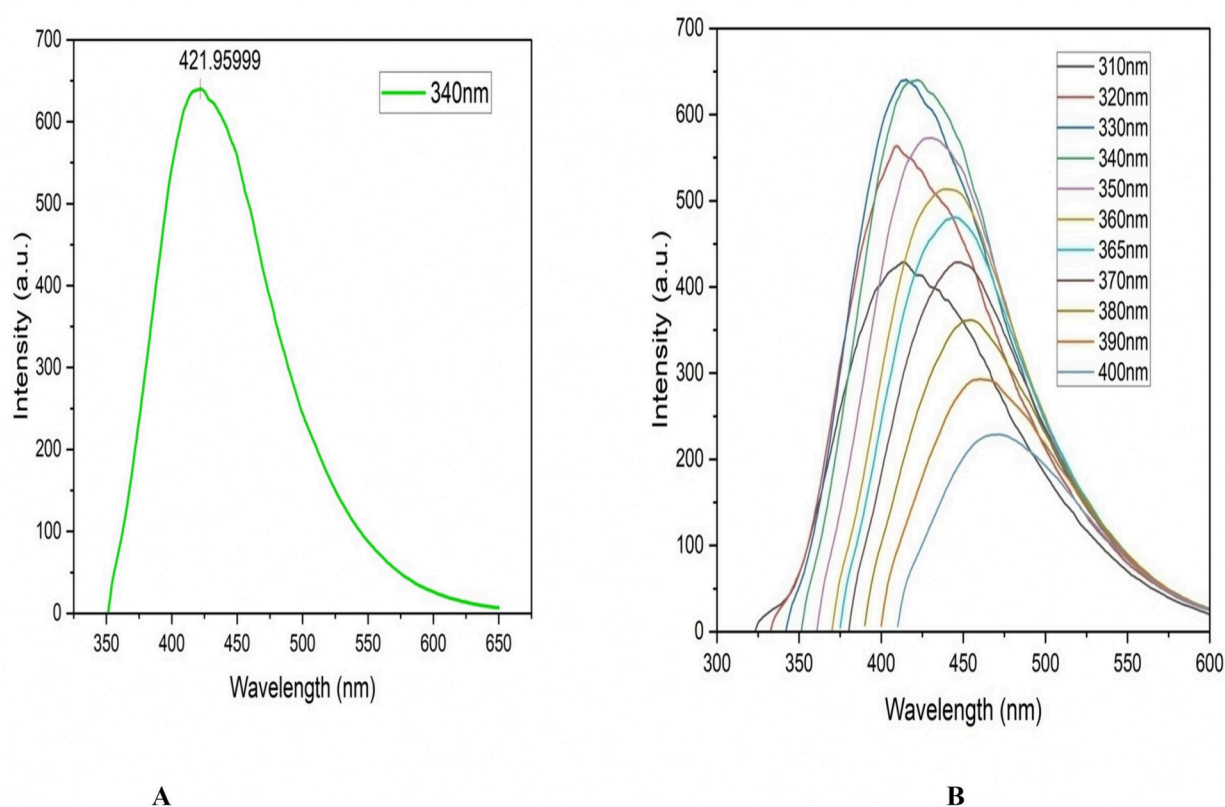


FIGURE 3: (A) Photoluminescence spectra for buttermilk carbon quantum dots excited at 340nm. (B) PL emission spectra at different excitation from 310 nm to 400nm.

Excitation-Dependent Behavior

Furthermore, excitation-dependent PL behavior observed in similar studies suggests the presence of various emissive traps. However, in this study, the emission peak remains relatively stable near 421 nm, indicating a well-controlled synthesis with uniform emissive centers an indicator of consistent optical properties [11]. Milk-derived CDs prepared at 200°C/8 hours that remain centered around 420 nm yet broaden slightly with higher λ_{ex} [14]. Excitation-dependent luminescence typically reflects multiple emissive sub-states; the limited shift in BM-CQDs indicates a dominant, well-passivated state, desirable for optical sensing and bio-imaging.

In this study, PL data confirm that BM-CQDs emit efficiently within the 400-430 nm window ideally suited for photoactivation by low-cost violet/blue LEDs already employed in dairy processing lines. Therefore, the strong emission at 421.9 nm under 340 nm excitation confirms that the BM-CQDs synthesized via a green hydrothermal method possess

desirable fluorescence characteristics suitable for antimicrobial activity and as a potential optical sensor in paneer preservation systems. Figure 3(B) showed the brightness of light (PL intensity) from the CQD solution changes with different light wavelengths used to excite it. As the excitation wavelength increases from 310 nm to 340 nm, the brightness becomes more pronounced. But if the wavelength goes above 350 nm, the brightness starts to fade. Figure 3(B) also showed a red shift in emission peaks, indicating that the peak emission wavelength shifts toward longer wavelengths as the excitation wavelength increases from 310 nm to 400 nm. This shift happens because lower wavelengths carry more energy, so the emitted light comes at shorter wavelengths and vice-versa.

Characterization of BM-CQDs

High-Resolution-Transmission Electron Microscopy

High-Resolution-Transmission Electron Microscopy (HR-TEM) was employed to evaluate the morphological characteristics and size distribution of the synthesized CQDs. Multiple micrographs were captured at different magnifications (200 nm, 100 nm, 50 nm, and 20 nm), providing a detailed visualization of the nanostructures (Figure 4A). These images clearly indicated that BM-CQDs were uniformly dispersed, spherical in shape, and free from aggregation. The particle size analysis revealed that the diameters of the synthesized BM-CQDs ranged between 3 and 9 nm, with an average size of 6.14 nm (Figure 4B). The measurements were obtained by analyzing TEM images using ImageJ software for precision. The scale was calibrated to 20 nm, and the diameter of 40 individual quantum dots were measured. The data presented in Table 1 showcased a narrow and consistent size distribution and further confirming the high mono dispersity of the synthesized CQDs. Hui et al. [15] reported a mean diameter of 2.5-3 nm for CQDs synthesized from coniferous woods using a green hydrothermal method, exhibiting similar spherical morphology. Furthermore, Aydin et al. [16] reported an average diameter of 7.7 nm for heteroatom-doped CQDs synthesized from whey and urine, corroborating the relevance of biowaste sources in CQD synthesis. HR-TEM in this study confirmed the spherical morphology and nanometric scale of the synthesized CQDs. The uniform size and morphology are indicative of a successful hydrothermal green synthesis process. These physico-chemical characteristics significantly enhance the potential of N-CQDs for applications in bioimaging, biosensing, and antimicrobial packaging, where consistent size and shape contributes to the stability and functional performance.

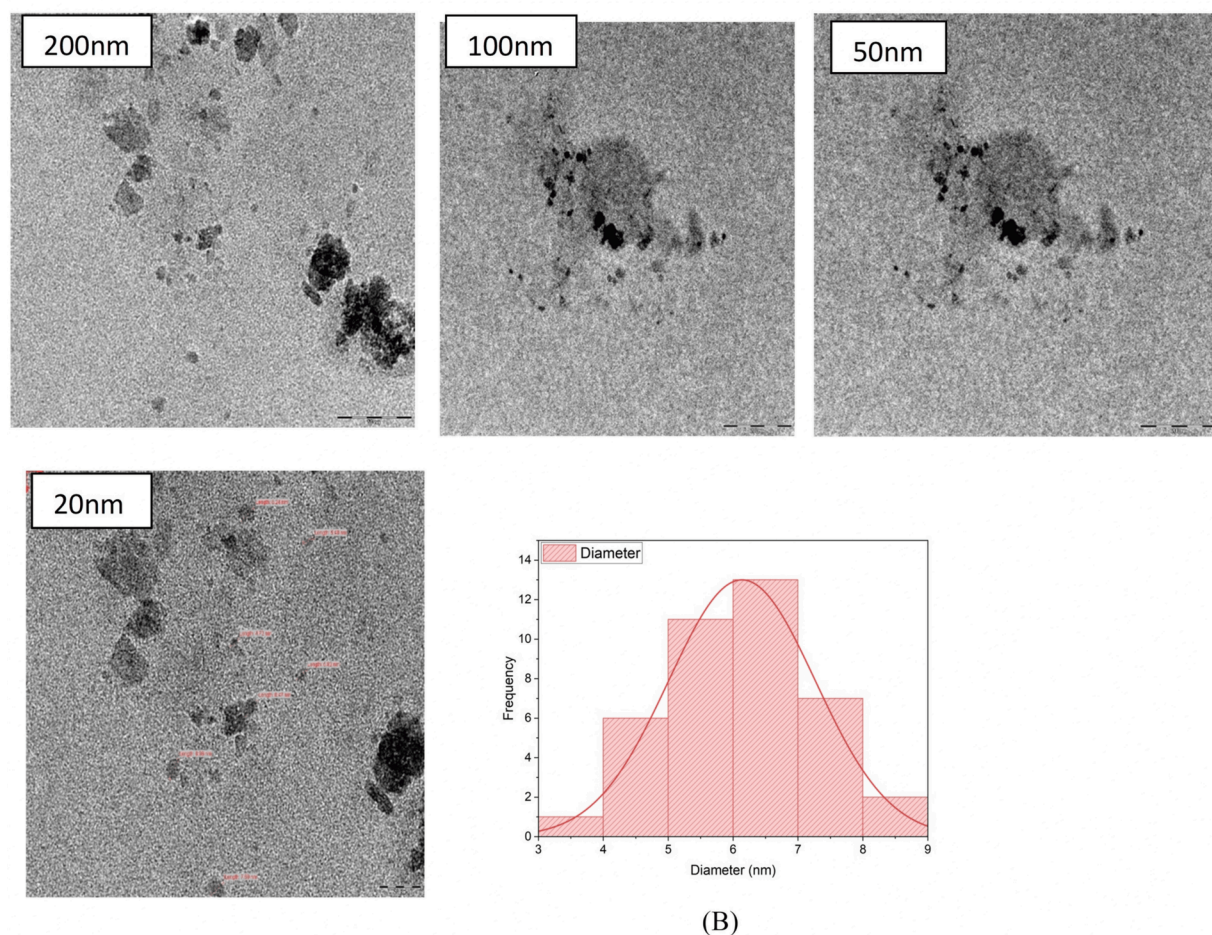


FIGURE 4: HR-TEM images of buttermilk carbon quantum dots at 200 nm, 100 nm, 50 nm, and 20 nm resolution; (B) size distribution histogram of BM-CQDs prepared by hydrothermal method.

HR-TEM, High-Resolution Transmission Electron Microscopy

Energy Dispersive X-Ray

EDX analysis was conducted to verify the elemental composition of the synthesized BM-CQDs. The EDX spectrum and the corresponding elemental composition data are presented in Table 2 and Figure 5A, respectively. High carbon content (67.90 wt %) and oxygen (14.00 wt %) in the EDX analysis strongly confirms the carbonaceous nature of the synthesized material, which was expected in CQDs. Comparable values of carbon content (C-67.4%) and (O-22.7%) were also reported from cow milk-derived CQDs by Ding et al. [17]. Similar results were reported by Zhang et al. [18], i.e., C = 67%, O = 23%, N = 10% during synthesis of CQDs using cow milk via hydrothermal treatment. This high carbon content was consistent in this study also with the use of buttermilk which was rich in organic matter such as lactose, proteins, and fats as a green carbon source. These organic compounds serve as an excellent precursor for CQD synthesis due to their richness in

carbon and other heteroatom. The detection of oxygen (12.00 atomic %) and nitrogen (5.90 atomic %) indicates the incorporation of surface functional groups such as hydroxyl (-OH), carbonyl (C=O), and amine (-NH₂) on the CQDs. These groups enhance the water dispersibility and stability of CQDs and are likely introduced during the hydrothermal carbonization process. The nitrogen content also implies partial doping, which can improve the optical and electronic properties of the quantum dots, particularly PL. Additionally, trace amounts of elements such as sodium (Na), potassium (K), calcium (Ca), chlorine (Cl), and phosphorus (P) were also detected. These may originate from the inherent mineral composition of buttermilk or residual impurities that were not completely removed during post-synthesis purification. Their presence in minor quantities suggests that they do not significantly alter the core structure but may influence surface charge, zeta potential, or bio-interactions of CQDs, which can be beneficial for applications like antimicrobial activity or food preservation.

Elements	Weight %	Atomic %
Carbon (C)	67.90	77.30
Nitrogen (N)	06.10	05.90
Oxygen (O)	14.00	12.00
Sodium (Na)	01.60	01.00
Phosphorus (P)	01.60	00.70
Chlorine (Cl)	02.80	01.10
Potassium (K)	04.60	01.60
Calcium (Ca)	01.40	00.50

TABLE 2: Elemental composition of buttermilk carbon quantum dots.

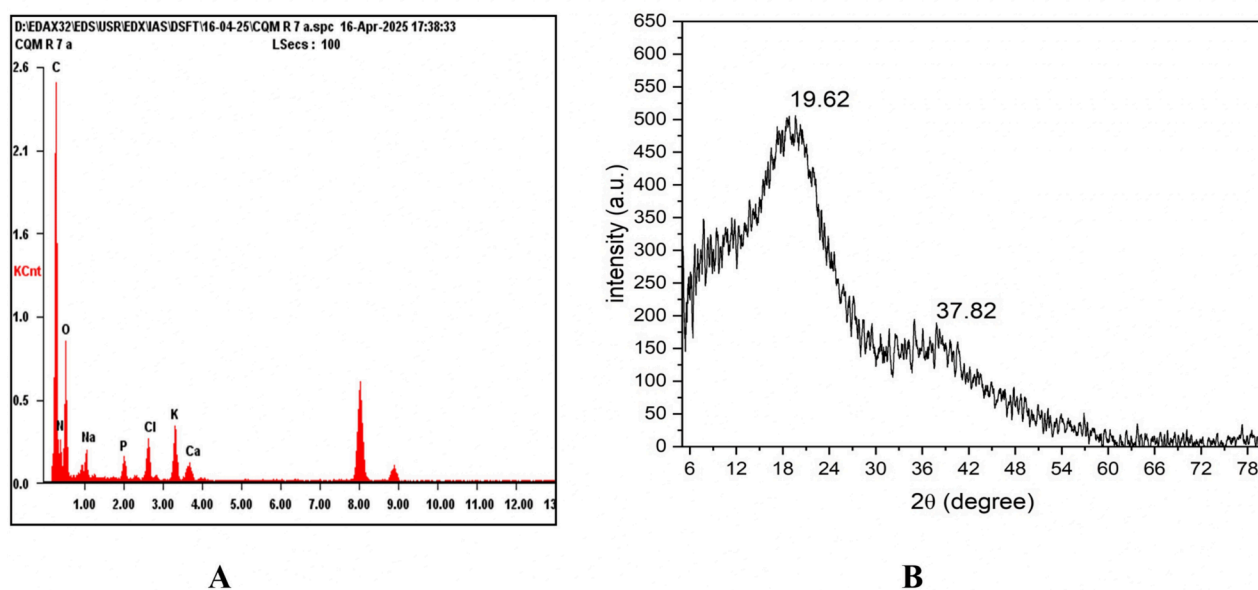


FIGURE 5: (A) EDX spectra of butter milk carbon quantum dots; (B) XRD pattern of BM-CQDs.

EDX, Energy Dispersive X-Ray; XRD, X-Ray Diffraction; BM-CQDs, Buttermilk-Derived Carbon Quantum Dots

X-Ray Diffraction

XRD pattern decides whether a substance is crystalline or amorphous in nature. BM-CQDs showed two very broad, low-intensity diffraction peak at $2\theta=19.62$ and 37.82 on the graph plotted following XRD diffraction, indicating an amorphous structure (Figure 5B). The intense peak at 19.62° and the absence of sharp crystalline lines confirm a largely amorphous, turbo static carbon framework with small sp^2 domains (Table 3). Similar broad diffraction peak indicated as (002) and characteristics of graphitic carbon materials were observed in the XRD patterns of milk-derived CQDs and the peak centered among 20 - 25 degrees 2θ was a common feature of CQDs [19]. The calculated d-spacing for C 002 of 0.452 nm was markedly larger than that of bulk graphite (0.335 nm), indicating enlarged galleries caused by abundant oxygen and nitrogen-containing functional groups introduced during hydrothermal carbonization of buttermilk proteins and lactose. Similar lattice expansion was observed in green-synthesized CQDs from dairy precursors (milk) and attributed to nitrogen doping and edge defects [20]. The presence of defective sp^2 carbon cores, a high surface-to-volume ratio and heteroatom containing functional groups ($-OH/-COOH/-NH_2$) favor charge transfer and generation of reactive-oxygen-species (ROS) generation under visible/UV excitation-mechanisms recognized as major mechanism behind the antimicrobial action of CQDs. Food waste-derived CQDs emphasize that such structure-property attributes translate to effective inhibition of both Gram-positive and Gram-negative spoilage flora [21].

Peak	2 θ °	Assigned plane	d-spacing (nm)*	Full -width at half maximum
P1	19.62	C 002 graphitic	0.452	$\approx 7^\circ$
P2	37.82	C 100/C 101	0.238	-

TABLE 3: XRD data of buttermilk carbon quantum dots.
XRD, X-Ray Diffraction

Fourier Transform Infrared

Fourier Transform Infrared (FT-IR) spectrum of BM-CQDs, confirmed the presence of diverse functional groups on the particle surface (Figure 6). A broad band observed at 3,306 cm⁻¹ was indication of hydrogen-bonded hydroxyl (-OH) groups and overlapping N-H stretches, likely originating from residual peptide structures. Comparable spectral features have been reported for CQDs synthesized from milk using hydrothermal techniques, where similar -OH peaks appeared around 3,289 cm⁻¹ [20] as well as in CQDs derived from food waste sources [22] reinforcing the presence of abundant surface hydroxyl and amide functionalities. These polar groups play a crucial role in enhancing water solubility without the need for additional surface passivation and provide proton-binding sites that influence both photoluminescence and antimicrobial performance. A noticeable shoulder at 2,112 cm⁻¹ was assigned to the stretching vibration of nitrile (C≡N) groups. Although uncommon in CQDs produced from citric acid, absorption bands near 2,100 cm⁻¹ have been attributed to nitrile or graphitic C=C bonds in nitrogen-doped carbon structures [23]. This signal in BM-CQDs likely arises from the thermal dehydration of amide side chains (-CONH2 to -CN) during the 180°C hydrothermal synthesis step, introducing nitrogen atoms into the carbon matrix. Nitrogen doping has been shown to enhance quantum yield and facilitate the generation of ROS under light exposure, improving antimicrobial potential.

The pronounced peak at 1630 cm⁻¹ can be attributed to both the stretching vibrations of carbonyl (C=O) groups in amides and the C=C bonds within sp²-hybridized domains. CQDs derived from milk sources typically showed similar absorption in 1,640-1,650 cm⁻¹ range, associated with peptide carbonyls and heterocyclic aromatic structures formed via Maillard reactions [20]. Additionally, the appearance of minor peaks in 1,400-1,100 cm⁻¹ region corresponds to C-N and C-O stretching vibrations (Table 4). This spectral evidence supports the presence of a chemically rich surface incorporating hydroxyl, carboxyl, carbonyl, and nitrogen-containing groups. FTIR profile confirmed that hydrothermal carbonization of buttermilk furnishes heteroatom-rich, highly oxygenated CQDs without external dopants. The co-presence of -OH (hydrophilicity, metal-chelating), nitrile (electron delocalization), and carbonyl/aromatic C=C (π-π* transitions) produces a multifunctional surface that (i) raises quantum yield and photodynamic antimicrobial activity (ii) affords colloidal stability in the paneer matrix (iii) no toxic residue left, aligning with “green” processing expectations.

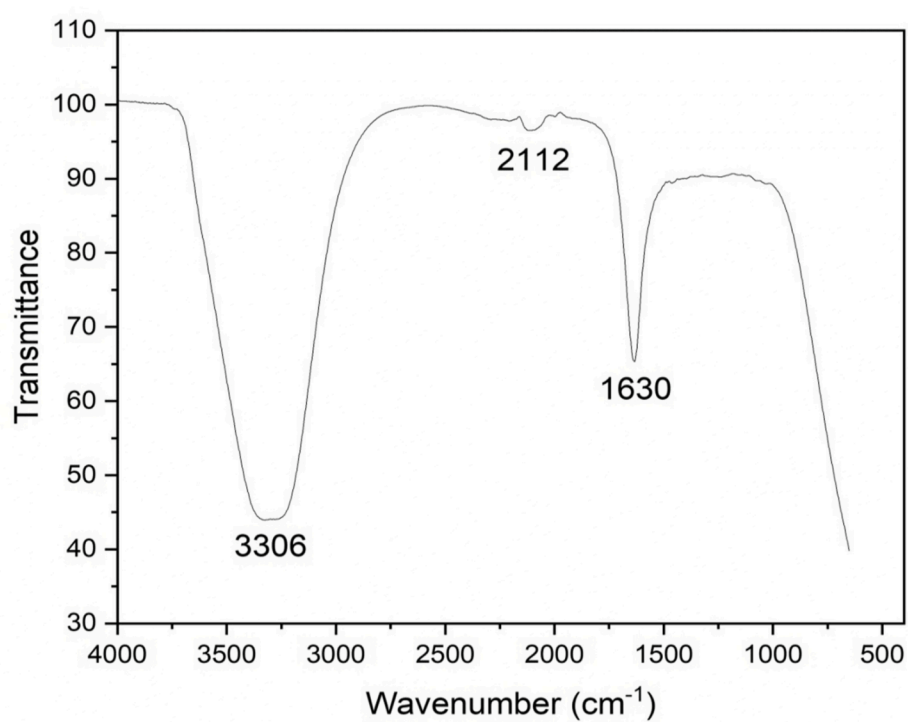


FIGURE 6: FT-IR spectrum of buttermilk carbon quantum dots.

FT-IR, Fourier Transform Infrared Spectrum

Wavenumber (cm ⁻¹)		Appearances	Bond	Functional Group	Possible compound in BM-CQDs
3,600-3,100	3,306	BROAD, intense	O-H	Hydroxyl	Lactose, lactic acid, whey protein
3,330-3,250			N-H	Amide	
2,160-2,100	2,112	Weak shoulder	C≡N	Nitrile	Dehydrated amino-acid side chains; N-doping during pyrolysis
1,660-1,600	1630	sharp	C=O & C=C	Carbonyl & alkene	Peptide backbone, Maillard-derived aromatics
1,400-1,100		Shallow multiplet	C-N, C-O-C	Ether, Carboxyl, amine	Lactose fragments, peptides

TABLE 4: FT-IR results for buttermilk-derived carbon quantum dots.

FT-IR, Fourier Transform Infrared Spectrum; BM-CQD, Buttermilk-Derived Carbon Quantum Dots

Effect of BM-CQDs on Microbiological Quality of Paneer

This study examined the effect of BM-CQDs on the microbiological quality of paneer. BM-CQDs were directly added to dipping water in two different doses, i.e., 2,500 mg/L and 5,000 mg/L and paneer was dipped in it for 15 minutes. Changes in the number of total plate count, yeast and mould count, and coliform count over time were reported in (Table 5). The data showed that these carbon dots have interesting effects on microbial growth that were monitored during their storage. Table 5 shows that the total plate count proliferated in all samples during storage; however, there were different patterns among the various samples of tested paneer. In the control sample, the microbial count increased beyond the minimum threshold (3,00,000 CFU/g) after 7 days of storage. But when the BM-CQDs at 2,500 mg/L and 5,000 mg/L were dissolved directly in dipping water for paneer, the microbial growth inhibition was more prominent, and the samples reached to minimum threshold after 21 days. Delayed microbial growth in sample B and C was also observed for coliforms. The treated samples kept the microbial count below 5.1 log₁₀ CFU/g at 21 days, while the control paneer exceeded 5.60 log₁₀ CFU/g during 7 days of storage. These findings align with previous reports indicating that carbon-based nanomaterials inhibit microbial growth by disrupting membrane integrity and generating ROS [24]. Furthermore, Kumar et al. [25] noted that food-grade CQDs derived from citrus waste significantly inhibited aerobic mesophilic bacteria, similar to the total plate count trends observed in this study also. Zhao et al. [26] reported that ROS generation, bacterial cell wall/membrane breakdown, oxidative stress induction, photodynamic activity, DNA/RNA damage, and C-Dots adhesion to bacterial surfaces are all linked to their antibacterial qualities. Similar results were reported by Chatzimitakos and Stalikas, and Surendran et al. [27,28] when exposed to C-Dots, both gram-positive and gram-negative bacteria had a fully disrupted and rough cell surface; however, *Salmonella typhimurium* displayed a higher degree of deformation. Notably, N-doping increases C-Dots antibacterial efficacy. Sample C was the best paneer sample, most

probably owing to the enough concentration of BM-CQDs, compared to the sample B with comparatively less concentration of 2,500 mg/L of BM-CQDs. The overall microbial results showed that the increased concentration of C-dots in dipped water has fostered the increased antimicrobial efficacy on spoilage microbes, compared to control sample.

Standard Plate count (log ₁₀ CFU/g)			
Days	Treatment		
	Control (Sample A)	Sample B (2500mg/L)	Sample C (5000mg/L)
0	2.40±0.20 ^a	2.1±0.30 ^a	2.0±0.22 ^a
7	5.60±0.23 ^{bc}	2.9±0.32 ^a	2.3±0.12 ^a
14	6.10±0.14 ^c	3.5±0.34 ^b	2.6±0.32 ^a
21	7.80±0.78 ^d	5.1±0.52 ^c	3.4 ±0.41 ^b
Coliform count (log ₁₀ CFU/g)			
Days	Treatment		
	Control Sample(A)	Sample B (2500mg/L)	Sample C (5000mg/L)
0	ND	ND	ND
7	1.8±0.11 ^a	1.5±0.12 ^a	1.0±0.10 ^a
14	2.2±0.14 ^b	1.8±0.31 ^a	1.2±0.16 ^a
21	3.6±0.18 ^b	2.1±0.24 ^a	1.7±0.22 ^a
Yeast and Mould count (log ₁₀ CFU/g)			
Days	Treatment		
	Control Sample(A)	Sample B (2500mg/L)	Sample C (5000mg/L)
0	ND	ND	ND
7	1.2±0.22 ^a	0.7±0.21 ^a	0.4±0.11 ^a
14	2.3±0.14 ^b	1.3±0.21 ^a	0.7±0.11 ^a
21	3.6±0.35 ^c	2.1±0.23 ^b	1.0±0.14 ^a

TABLE 5: Effect of butter milk carbon quantum dots on shelf- life of paneer

Conclusions

The purpose of this research was to synthesize carbon dots using buttermilk and the application of C-dots for extension of the shelf-life of paneer. BM-CQDs in different concentration were added in dipping water for paneer. The application of BM-CQDs on paneer significantly inhibited the growth of spoilage microorganisms, including coliforms, yeast and mold, and total viable bacteria. Thus, BM-CQDs proved to be an effective natural antimicrobial agent capable of increasing the storage life of paneer for 21 days under refrigeration, without affecting its quality. This work contributes to the growing field of green nanotechnology in food preservation. It offers a sustainable solution for two major concerns: the valorization of dairy waste and the reduction of microbial spoilage in fresh dairy products. The suggested solution is a promising, easy to apply in an industrial setting, and supports a sustainable approach because buttermilk is a basic substrate for the synthesis of carbon dots. The research findings could interest the industrial sector for a number of reasons. Moreover, the process aligns with current trends in food safety, clean-label preservation methods, and circular economy principles.

Additional Information

Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen. **Human subjects:** All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

The datasets (and/or code) supporting this study are available from the corresponding author upon reasonable request.

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