



## OPEN Nano packaging with carbon quantum dots and essential oil nanocapsules prolongs shelf life of fresh cut Kale

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Postharvest handling of fresh-cut leafy vegetables such as kale poses significant challenges due to rapid spoilage and nutrient degradation. This study explores the effectiveness of two nano-packaging strategies, carbon quantum dot (CQD) coatings and essential oil-based nano-capsules (EO-NCs), in preserving the quality and extending the shelf life of fresh-cut kale up to 40 days of refrigerated storage. CQD-treated samples demonstrated superior performance, exhibiting the lowest weight loss (5.50 g) compared to EO-NC treatments (11.92 g for *Thymus daenensis* Celak, 12.49 g for *Bunium persicum*) and the control (27.29 g). CQDs also retained higher levels of chlorophyll (12.61 mg g<sup>-1</sup> FW) and ascorbic acid (50.33 mg g<sup>-1</sup> FW), surpassing both EO-NC-treated and control samples. Microbial counts were significantly lower in CQD fresh-cut kale (130 CFU/ml), affirming its potent antimicrobial effect compared to EO-NCs and the untreated group (946 CFU/ml). CQDs effectively inhibited oxidative enzymes such as catalase (0.81 U/mg g FW) and polyphenol oxidase (0.79 U/g FW), contributing to reduced browning and spoilage. Antioxidant metrics, including total phenolic content (251.97 µg/ml) and DPPH radical scavenging activity (39.64%), were also enhanced with CQD treatment, highlighting its role in maintaining nutritional quality. While EO-NCs particularly those based on *T. daenensis* provided moderate preservation benefits, CQDs consistently outperformed them across all parameters. These findings underscore the potential of CQD-based nano-packaging as a promising strategy for enhancing food preservation, reducing waste, and supporting sustainability. Further studies are needed to evaluate commercial scalability, safety, and cost-effectiveness.

**Keywords** Carbon quantum dots, Essential oil nano-capsules, Fresh-cut kale, Nano-packaging, Shelf life

Kale (*Brassica oleracea* var. *sabellica*) is a highly nutritious leafy green vegetable, often considered a superfood due to its impressive nutritional profile and medicinal properties. Since kale is one of those leafy vegetables that are highly perishable and nutritious, efficient packaging helps it maintain freshness, quality, and safety. The packaging maintains proper humidity and temperature, and plays a very important role in preserving the texture, taste, and nutritional value of kale<sup>1,2</sup>. Advanced packaging technologies period store and protect food from health-affecting microorganisms, hence making sure it is safe<sup>3,4</sup>. According to previous studies, this makes foods safe<sup>5,6</sup>. Moreover, ecological problems will be properly solved by reducing plastic waste and limiting ecological impact with sustainable packaging by making use of biodegradable materials and compostable ones<sup>7,8</sup>.

The dynamic nature of the food industry demands the innovation of packaging methodologies because of product quality, food safety, and its increasing market appeal<sup>9,10</sup>. Nano-packaging and nano-packaging are disruptive technologies, especially those that are coming today for vegetable packaging and marketing<sup>11,12</sup>.

The primary advantages of nano-packaging stem from its ability to substantially prolong the storage duration of vegetables<sup>13</sup>. This occurs mostly owing to the integration of nanoparticles possessing antibacterial capabilities into the packaging material<sup>14,15</sup>. Particularly, silver, zinc oxide, and titanium dioxide may inhibit the growth of a wide range of bacteria and fungi which are often responsible for vegetable decay<sup>16</sup>. Besides, nano-packaging can be designed to have a controlled release of preservatives<sup>17</sup>. The slow-release system maintains preservative efficacy for longer periods, thereby enhancing the shelf life of veggies that are packaged<sup>18,19</sup>. Packaging material incorporated with nanocapsules imparts gradual release of the antimicrobial agent, ensuring continuous action

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against spoilage and contamination. Synthesis of lignocellulose nanofibers (LCNFs), cellulose nanofibers (CNFs), chitosan, and alginate has led to reduced moisture loss and blueberry fruit quality for 15 days storage<sup>20</sup>. Application of bio-based process in combination with plant-based nanomaterials and non-thermal technologies is a novel and lucrative process for increased shelf life and quality of fresh cut vegetables and fruits<sup>21</sup>. This release technology is particularly applied in the case of perishable commodities like vegetables, for whose maintenance, freshness is of prime importance<sup>22</sup>.

Nano-packaging can also control odors: certain nanoparticles can absorb and neutralize odors released from plants due to deterioration with time<sup>23</sup>. This function of odor management not only preserves the sensory appeal, but it also makes the experience of the fruit in question even more heightened for the consumer<sup>24</sup>. The material usage on a reduced scale leads to less packaging waste, along with low transportation costs and energy use<sup>13</sup>. Intelligent nano-packaging can be very well used for supply chain management optimization<sup>25</sup>. Its analytics capability and packaged items attached to produce allow continued monitoring of the conditions of the produce while moving and storing it. Logistics could be optimized by taking a data-driven approach, and the tendency to rot is minimized. Increased efficiency of the supply chain means a reduction in operational costs and competitiveness in the market<sup>26</sup>. Nano-packing for marketing and cost-effectiveness contributes to consumer confidence and brand differentiation, holistic profitability, which it is projected to offer. In the future, the food industry will continue to depend on the use and adaptation of nano-packaging to effectively meet all the challenges and opportunities coming up in the future<sup>9</sup>.

The current study tackles a clear gap in the scientific literature by comparing CQD and EO-NCs as two active packaging agents for fresh-cut kale stored under refrigeration. In contrast to previous studies that focused either on single agents or on short periods of storage, we tested both technologies under identical conditions up to 40 days, monitoring physiological, biochemical, and microbiological parameters. This study provides novelty on: (i) the first direct comparison between CQD vs. plant EO-NCs on fresh-cut kale; (ii) the extended duration of storage with repeated sampling, and (iii) the combination of barrier, enzymatic, and nutrient-retention parameters.

## Materials and methods

### Plant materials and treatments

Fresh-cut kale was harvested from a commercial greenhouse in the Alborz province of Iran. The leaves were promptly transported to the laboratory, where the petioles were removed. The leaves were then cut into 40 × 40 mm sections using a sharp knife, and the fresh-cut produce was prepared. *B. persicum* (Herbarium code: MPH-1398, Tehran province, Iran, 35°48'N, 51°49'E, 2190 m) and *T. daenensis* Celak (Herbarium code: MPH-1742, Fars province, Iran, 28°41'N, 57°42'E, 710 m) were collected and authenticated by Prof Ali Sonboli at the Herbarium of Medicinal Plants and Drugs Research Institute, Shahid Beheshti University. The essential oils were extracted using a Clevenger apparatus for 5 h and subsequently stored at −20 °C until further use in nanocapsule (NC) synthesis<sup>27</sup>.

For the purpose of conducting this research, kale seeds (Pakan Seed Co.) were grown under controlled conditions to maturity. Homogenous leaves were harvested for post-harvest analysis. A factorial experiment was designed as a completely randomized design (CRD) with 24 treatments, three replications, and five observations per treatment to evaluate the effect of nano-packaging on fresh-cut kale quality and shelf life (Table 1). Experimental variables included six types of nanoparticles CQD and nanoparticles loaded with essential oils of *B. persicum* and *T. daenensis* Celak for four storage periods (0, 10, 20, 30, and 40 days). Carbon dot nanoparticles were synthesized, and essential oils were loaded and encapsulated into nanoparticles that will be added to absorbent pads used in packaging. A 10 × 5 cm pad was sprayed on the bottom of the container, on which 10 ml of solution containing essential oil/nanocapsule/quantum dot was placed. Then the container was closed and covered with plastic wrap to prevent air from entering. Ten pieces per treatment were stored in storage containers with the pads impregnated with nanoparticles. Storage quality attributes were assessed on specific days, and the data recorded will be subjected to analysis using appropriate statistical software. The control condition consisted of uncoated packaging pads sprayed with water. Also, all treatments were stored at 4–6 °C in the same refrigerated chamber.

### Nanostructures synthesis

#### Essential oil nano capsules

The EO-NC were prepared using a homogenization method with a cylindrical ceramic container (10 cm × 6 cm × 3 mm). A 40 mL chitosan (0.5% W/V)-PVA (0.1% W/V) aqueous solution was transferred to the test container, and the homogenizer head was immersed 1 cm above the container's base. After 10 s of homogenization, 5 mL of an ethyl acetate solution containing 30% essential oil was added, and the process continued for 20 min<sup>28</sup>. The

Code	Type	Concentration	Application	Storage (°C)	Sampling days	Replicates
T1	Control	Water	10 mL on a pad	4–6 °C	0, 10, 20, 30, 40	n = 3
T2	CQD	5% V/V				
T3	<i>T. daenensis</i> EO-NC					
T4	<i>B. persicum</i> EO-NC					

**Table 1.** Experimental treatments and parameters used in the study.

resulting emulsion was transferred to glass containers, covered with aluminum sheets, and stirred overnight at room temperature at 500 rpm to evaporate the organic solvent. To separate and settle the particles, 10 mL of the sample was centrifuged at 6000 rpm for 45 min at  $-4\text{ }^{\circ}\text{C}$ . The supernatant was discarded, and the pellet was resuspended in HPLC-grade water. The mixture underwent ultrasonication, followed by three centrifugation-resuspension cycles before analysis.

#### *Carbon quantum dot*

To synthesize carbon quantum dots (CQDs), 1 g of ascorbic acid and 0.5 g of anhydrous citric acid were dissolved in 30 mL of water. The solution was then transferred to a synthesis reactor with a stainless-steel outer body and a Teflon-lined inner chamber (50 mL capacity). The reactor was placed in a furnace preheated to  $360\text{ }^{\circ}\text{C}$  and maintained for 6 h. After the reaction, the reactor was allowed to cool, yielding 26 mL of a yellow CQD-containing solution with an initial pH of 2.0–2.6. The pH was adjusted to 7.0 by adding 4 mL of 1 M NaOH. The solution was then purified by centrifugation at 6000 rpm for 25 min, filtered through a  $0.22\text{ }\mu\text{m}$  membrane, and stored at  $4\text{ }^{\circ}\text{C}$  until further use<sup>29</sup>.

#### **Nanostructure characterization**

Following nanoparticle synthesis, their physical and chemical properties were analyzed using various techniques. Each characterization technique was performed on three independent samples and averaged. The absorption pattern was examined using a UV-2501PC ultraviolet absorption spectrophotometer (Shimadzu Co., Japan) equipped with tungsten and deuterium lamps, with measurements conducted in a 3 mL quartz cuvette. The morphology and structure of the nanoparticles were studied using a Hitachi SU3500 scanning electron microscope (Japan) after coating the samples with a 2–5 nm gold layer. Particle size distribution was determined via a Nanophox dynamic light scattering (DLS) system (SympaTEC Co., Pulverhaus, Germany), featuring a 10 mW He-Ne dual-beam laser (632.8 nm wavelength), automatic sample positioning (50–350  $\mu\text{L}$  chamber), and temperature control ( $5\text{--}35\text{ }^{\circ}\text{C}$ ), enabling size measurements in the range of 0.1–50,000 nm. The crystalline structure was characterized using a STOE STADI P X-ray powder diffraction (XRD) system with a Cu  $\text{K}\alpha_1$  source ( $\lambda = 1.5406\text{ \AA}$ ) and a  $2\theta$  scanning range of  $1^{\circ}\text{--}80^{\circ}$ , with samples placed in aluminum holders. Infrared absorption characteristics of polymer nanoparticles were analyzed using an FTIR-Tensor27 spectrometer (Bruker Co., Ettlingen, Germany)<sup>28,29</sup>.

The loading capacity of the nanoparticles was determined using an ultraviolet-visible spectrophotometer<sup>30</sup>. First, the solution was centrifuged at 24,000 rpm for 45 min. Subsequently, 1 mL of the supernatant was collected and mixed with 5 mL of acetate buffer (pH = 5). The absorption was then measured at 295 nm using a spectrophotometer, and the loading capacity was calculated. Encapsulated essential oil content was quantified after digesting the nanoparticles with a hydrochloric acid and alcohol solution. The concentration of essential oil in alcohol was measured at 295 nm using a spectrophotometer, allowing for the calculation of encapsulation efficiency. The Loading was calculated using below equation.

$$\text{Loading capacity (\%)} = \frac{\text{Abs}_{295} \text{ after digesting}}{\text{Abs}_{295} \text{ of nanocapsul}} \times 100 \quad (1)$$

The antimicrobial effects of nanoparticles were evaluated using *Staphylococcus aureus* ATCC 25,923 and *Escherichia coli* ATCC 22,312 (provided by the Iranian Research Organization for Science and Technology). Aerobic cultivation was carried out in Mueller-Hinton broth at  $37\text{ }^{\circ}\text{C}$  with continuous shaking at 450 rpm. Bacterial growth curves, both with and without nanoparticle exposure, were determined by measuring optical density at 600 nm (OD<sub>600</sub>). Since no specific antibacterial guidelines exist for nanoparticles, their antistaphylococcal activity was assessed using the microbroth dilution method, following the National Committee of Clinical Laboratory Standards (NCCLS, 2005) guidelines with modifications. Bacterial inocula were prepared from freshly cultured colonies, adjusted to 0.5 McFarland standard turbidity using normal saline, and further diluted (1:100) in sterile Mueller-Hinton broth before being added to the test trays. Nanoparticles were serially diluted in concentrations ranging from 0.5 to 256 mg/mL in sterile 96-well microdilution plates containing Mueller-Hinton broth. After incubation at  $37\text{ }^{\circ}\text{C}$  for 22 h, the minimum inhibitory concentration (MIC) was recorded. The minimum bactericidal concentration (MBC) was determined by subculturing 100  $\mu\text{L}$  from wells showing no visible growth onto nutrient agar plates. The MBC was defined as the lowest concentration that killed 99.9% of bacterial strains. All experiments were conducted in triplicate<sup>31</sup>.

The Clinical and Laboratory Standards Institute (CLSI) guidelines were followed to evaluate the inhibition zone of nanoparticles. A bacterial suspension, adjusted to 0.5 McFarland turbidity standard, was prepared in normal saline and uniformly spread on Mueller-Hinton agar using a sterile swab in three directions. After 10 min, discs containing 10  $\mu\text{g}$  of different nanoparticle samples were placed on the inoculated plates. The plates were then incubated at  $37\text{ }^{\circ}\text{C}$  for 20 h. After incubation, the diameter of the inhibition zone around 5 mm discs was measured and recorded using a ruler.

#### **Physiological study or traits**

##### *Color measurement*

The entire surface of the fresh-cut kale leaf was analyzed for color measurement using the CIE Lab color space. Color was measured by a 3nh Mini Digital Portable Colorimeter, CIE Lab values<sup>32</sup>.

##### *Ion leakage and electrolytic content*

To assess electrolyte leakage, 0.2 g of fresh-cut kale from each replicate was carefully washed and placed in glass containers with lids containing 10 mL of ionized water. The containers were kept at room temperature

for 26 h, after which the electrical conductivity (EC) was measured using a HI2315 desktop EC meter (Hanna Instruments Ltd, Italy) with a measurement range of 0.0 to 199.9 mS<sup>33</sup>.

## Elemental and metabolites analysis

### Mineral and elemental analysis

Elemental composition, including potassium, phosphorus, calcium, iron, copper and manganese, was determined using the acid digestion method with concentrated hydrochloric acid and nitric acid at 300 °C. The elemental content in plant tissue was analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, ELAN 6100 DRC-e, Perkin Elmer Co.). This method allows for the quantification of over 70 elements in the parts per billion (ppb) range while utilizing Dynamic Reaction Cell (DRC) technology to minimize polyatomic interferences<sup>3</sup>.

### Pigment concentration analysis

To determine total chlorophyll content, 20 mg of dried sample (dried for 3 days at 60 °C) was mixed with 2 mL of DMSO and heated at 70 °C for 60 min. After cooling, the sample was centrifuged at 8000 rpm for 5 min, and the absorbance of the supernatant was measured at 470, 646, and 663 nm. The concentrations of chlorophyll a (Cla), chlorophyll b (Clb), total chlorophyll (ClTotal), and total carotenoids were calculated using the following equations, where DW represents the initial dry weight<sup>31</sup>.

$$\text{Chlorophyll a} = [(13.36 \times A_{663}) - (5.19 \times A_{646})] \times 8.1 / DW \quad (2)$$

$$\text{Chlorophyll b} = [(27.43 \times A_{646}) - (8.12 \times A_{663})] \times 8.1 / DW \quad (3)$$

$$\text{Total chlorophylls} = [(5.24 \times A_{663}) + (22.24 \times A_{646})] \times 8.1 / DW \quad (4)$$

$$\text{Total carotenoids} = [(4.785 \times A_{470}) + (3.657 \times A_{663}) - (12.76 \times A_{646})] \times 8.1 / DW \quad (5)$$

### Ascorbic acid concentration analysis

Ascorbic acid content was measured using an analysis kit (Kiazist Co., Iran). 300 mg of plant tissue was homogenized in 500 µL of Lysis Buffer, followed by centrifugation at 15,000×g for 10 min. The supernatant was collected, and 20 µL of concentrated sulfuric acid was added and thoroughly mixed. The mixture was then centrifuged again at 15,000×g for 10 min. 200 µL of the prepared sample and standard solutions were combined with 12 µL of the oxidizing Agent and incubated at room temperature for 5 min. Subsequently, 70 µL of developing agent was added, and the mixture was centrifuged at 15,000×g for 1 min. 130 µL of the resulting supernatant was mixed with 20 µL of chromogen and incubated at 37 °C for 4–5 h. Finally, 100 µL of 85% sulfuric acid was added, and the solution was shaken for 5 min until the red precipitate dissolved. The absorbance of the sample was measured in the 500–540 nm spectrum<sup>3</sup>.

### Total polyphenol concentration analysis

Total phenolic content was determined using gallic acid as the standard. 100 µL of plant extract (2 mg/mL) was mixed with 500 µL of 10% (v/v) Folin-Ciocalteu reagent. After 5 min, 500 µL of 7% sodium carbonate was added to the mixture. The samples were incubated in the dark for 2 h, and the absorbance was measured at 765 nm<sup>34</sup>.

### Antioxidant capacity measurement

The DPPH radical scavenging capacity of each extract was determined using a modified Brand-Williams method. DPPH radicals, which exhibit maximum absorption at 515 nm, lose their absorbance upon reduction by an antioxidant compound. A DPPH<sup>•</sup> solution in methanol ( $6 \times 10^{-5}$  M) was freshly prepared daily. 200 µL of this solution was mixed with 100 µL of plant extract solution in a 96-well plate. The samples were incubated at 25 °C for 20 min under 450 rpm stirring, and the absorbance decrease at 515 nm was recorded. All experiments were performed in triplicate<sup>34</sup>. Radical scavenging activity was calculated using:

$$\text{Inhibition (\%)} = \frac{1 - Abs_S - Abs_B}{Abs_C} \times 100 \quad (6)$$

Where  $A_S$ ,  $A_B$ , and  $A_C$  were the absorbance of the samples, blank absorbance (extract solution), and absorbance of the control (DPPH solution), respectively.

### Total carbohydrate concentration analysis

The phenol-sulfuric acid colorimetric method, one of the simplest and most reliable techniques for measuring reduced sugar content in a liquid medium, was used. Glucose served as the standard. 50 µL of the sample solution was added to each well, followed by 150 µL of concentrated sulfuric acid (98%). Immediately, 30 µL of 5% phenol aqueous solution was added. The mixture was then incubated in a  $90 \pm 1$  °C water bath for 5 min, followed by cooling to room temperature in another water bath for 5 min. The color intensity, indicative of carbohydrate concentration, was measured using a Powerwave spectrophotometer at 490 nm. A 50 µL glucose standard solution was used to generate the standard curve<sup>31</sup>.

## Enzymatic variations analysis

### Catalase enzyme activity

Catalase (CAT) enzyme activity was measured using an analysis kit (Kiazist Co., Iran). 20 µL of substrate catalase was added, and the reaction mixture was incubated at 25 °C for 20 min. After incubation, 30 µL of stop solution

and 30  $\mu\text{L}$  of chromogen were added to each well. The wells were incubated at 25  $^{\circ}\text{C}$  in the dark for 10 min. Subsequently, 10  $\mu\text{L}$  of periodate catalase was added, and after 5 min, the absorbance was measured at 520–560  $\text{nm}$ <sup>3</sup>.

#### *Superoxide dismutase enzyme activity*

Superoxide dismutase (SOD) enzyme activity was measured using an analysis kit (Kiazist Co., Iran). This kit simultaneously detects both cytoplasmic and mitochondrial SOD forms in eukaryotic cells. 10–20 mg of fresh plant tissue was homogenized using a probe homogenizer in PBS buffer containing a protease inhibitor cocktail. The homogenate was then centrifuged at 12,000 rpm for 15 min at 4  $^{\circ}\text{C}$ , and the resulting supernatant was stored at  $-80^{\circ}\text{C}$  until analysis. For the assay, 20  $\mu\text{L}$  of the sample (from a 100  $\mu\text{L}$  total volume) was mixed with SOD assay reagent and incubated at 37  $^{\circ}\text{C}$  for 20 min before measurement<sup>3</sup>.

#### *Phenylalanine ammonia lyase (PAL) enzyme activity*

To measure phenylalanine ammonia lyase (PAL) enzyme activity, 0.5 mL of 10 mM phenylalanine solution, 0.4 mL of double-distilled water, 0.1 mL of extracted protein, and 1 mL of 50 mM Tris-HCl buffer (pH 8.8) were added to a test tube. A control sample was prepared by including all reagents except the protein extract. The reaction mixture was incubated at 37  $^{\circ}\text{C}$  for 1 h, the optimal temperature for PAL activity. To terminate the reaction, 0.5 mL of HCl was added to inactivate the PAL enzyme. The sample was then washed with 15 mL of ethyl acetate, followed by evaporation under airflow. The residue was dissolved in 3 mL of 0.05 M sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) until fully dissolved. The absorbance of the sample, corresponding to enzyme concentration (mg/mL), was measured at 550  $\text{nm}$ <sup>3</sup>.

#### *Peroxidase (POD) enzyme activity*

Peroxidase enzyme activity was determined by mixing 50  $\mu\text{L}$  of enzyme extract with 3 mL of measuring buffer, 4.51  $\mu\text{L}$  of  $\text{H}_2\text{O}_2$ , and 3.35  $\mu\text{L}$  of guaiacol. The absorbance change at 470 nm was recorded over 2 min. Peroxidase activity was measured based on the formation of tetraguaiacol from guaiacol in the presence of hydrogen peroxide and peroxidase enzyme. The specific activity of peroxidase was expressed as micromoles of tetraguaiacol formed per minute per milligram of protein<sup>3</sup>.

#### *Polyphenol oxidase (PPO) enzyme activity*

PPO activity was measured using a reaction mixture containing 1 mL of 0.05 M phosphate buffer (pH 7.0), 3.3  $\mu\text{L}$  of 30% hydrogen peroxide, and 1  $\mu\text{L}$  of guaiacol solution. The control sample contained 1 mL of 0.05 M phosphate buffer, 1  $\mu\text{L}$  of 30% hydrogen peroxide, 1  $\mu\text{L}$  of guaiacol solution, and 16.6  $\mu\text{L}$  of enzyme extract. Enzyme activity was determined by monitoring absorbance changes at 270 nm over 10 min and expressed as activity per mg of protein<sup>3</sup>.

### Statistical analysis

After data collection, a combined analysis of variance (ANOVA) was conducted on the data collected over two years using SAS statistical software, version 9.1 (SAS Institute Inc., 2009). Prior to ANOVA, the normality of data distribution was assessed using the Shapiro-Wilk test to ensure the validity of parametric statistical analyses. Mean comparisons were performed using Duncan's multiple range test (DMRT) at a 5% significance level.

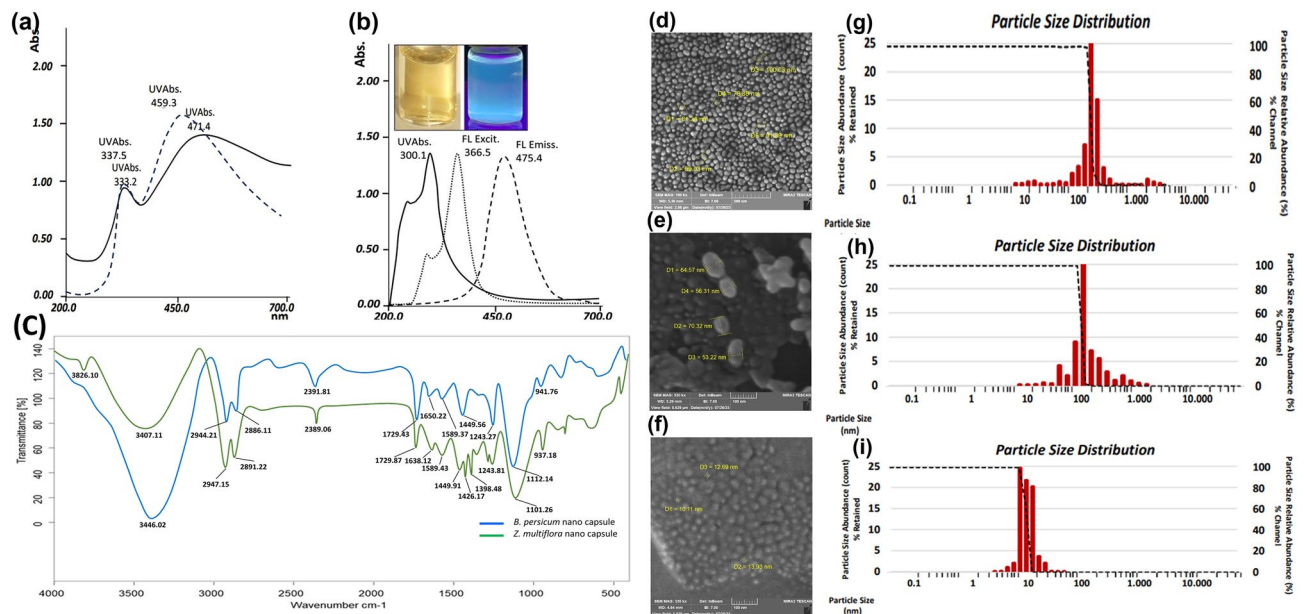
### Results

Kale is a highly perishable leafy vegetable whose nutritional value, visual appeal, and marketability are significantly influenced by post-harvest management practices. Conventional packaging methods often fail to maintain biochemical and physical properties, leading to nutrient loss, microbial spoilage, and weight reduction during storage. To address these challenges, novel active packaging solutions incorporating EO-NCs and CQDs (each individually) were explored as innovative preservation strategies. The study assessed key post-harvest quality parameters, including weight loss, color stability, nutrient retention, enzymatic activity, microbial load, and antioxidant activity. The application of EO-NCs and CQDs demonstrated potential in enhancing fresh-cut kale's shelf life, preserving its biochemical composition, and reducing deterioration effects compared to conventional storage methods.

#### Synthesis and analysis of nanostructures

Following the synthesis of nanomaterials as outlined in Sect. 2, their physical and chemical properties were thoroughly analyzed to confirm successful formation and stability (Fig. 1). UV-visible spectroscopy of EO-NCs revealed two distinct absorption maxima at 330 nm and 460 nm, indicative of nanocapsules with diameters below 100 nm (Fig. 1a and b). The observed absorption bands in the ultraviolet region correspond to  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  electronic transitions, characteristic of aromatic compounds and oxygen-containing functional groups. These spectral features confirm the successful encapsulation of essential oils within the chitosan-PVA nanostructure, ensuring structural integrity and functional stability of the synthesized EO-NCs.

The UV-Vis spectral analysis (Fig. 1b) revealed distinct optical properties associated with quantum effects and specific surface interactions of the synthesized nanoparticles. The CQDs exhibited a strong absorption peak at 300.1 nm, an excitation peak at 366.5 nm, and a fluorescence emission peak at 475.4 nm, confirming their characteristic blue emission properties. The Fourier Transform Infrared (FT-IR) spectroscopy (Fig. 1c) further validated the successful synthesis of the nanostructures by identifying key functional groups. Prominent absorption bands corresponding to C=O (carbonyl), C-O (ether), and OH (hydroxyl) were observed, indicating the presence of carboxylic and ether functionalities, which contribute to enhanced hydrophilicity and stability of the nano-capsules. Figure 1c presents the FTIR spectra for bare chitosan-PVA, EO NCs, and CQDs. The band



**Fig. 1.** (a): The UV-visible analysis of polymeric nano capsule. (b): The UV-Vis absorption, excitation and fluorescent emission of carbon quantum dots. (c): The Fourier transform infrared (FT-IR) analysis of the chitosan-PVA nano capsule formation. The scanning electron microscope (SEM) results of (d): *B. persicum* essential oil loaded nano capsule, (e): *T. daenensis* essential oil loaded nano capsule and (f): carbon quantum dots. The dynamic light scattering (DLS) analysis of (g): *B. persicum* essential oil loaded nano capsule, (h): *T. daenensis* essential oil loaded nano capsule and (i): carbon quantum dots.

at about  $3420\text{ cm}^{-1}$  represents the broad O–H/N–H stretching, and the band at about  $1720\text{ cm}^{-1}$  represents the C=O stretching. A pronounced shift from  $1720$  to  $1712\text{ cm}^{-1}$  and a greater absorption at  $1250\text{ cm}^{-1}$ , which occurred after the EO NC encapsulation, could be ascribed to the hydrogen bonding between the components of the encapsulating agent and the polymeric matrix. In the CQD-treated materials, greater absorption within the band at about  $1600\text{--}1650\text{ cm}^{-1}$  indicates the presence of C=C/C=O bonds.

The Scanning Electron Microscopy (SEM) analysis (Fig. 1d–f) confirmed the morphological characteristics of the synthesized materials. The EO-NCs measured between 50 and 80 nm, while the CQDs were smaller than 12 nm, both exhibiting a highly uniform size distribution. These findings were further corroborated by Dynamic Light Scattering (DLS) analysis (Fig. 1g–i), which indicated that the hydrodynamic diameter of the particles was slightly larger than the sizes observed in SEM. This discrepancy is attributed to the formation of a hydration layer around the nanoparticles due to surface functional groups. The particle size distribution map from DLS analysis strongly emphasizes the importance of pH optimization and the selection of appropriate stabilizing agents to achieve uniform nanoparticle size and dispersion stability.

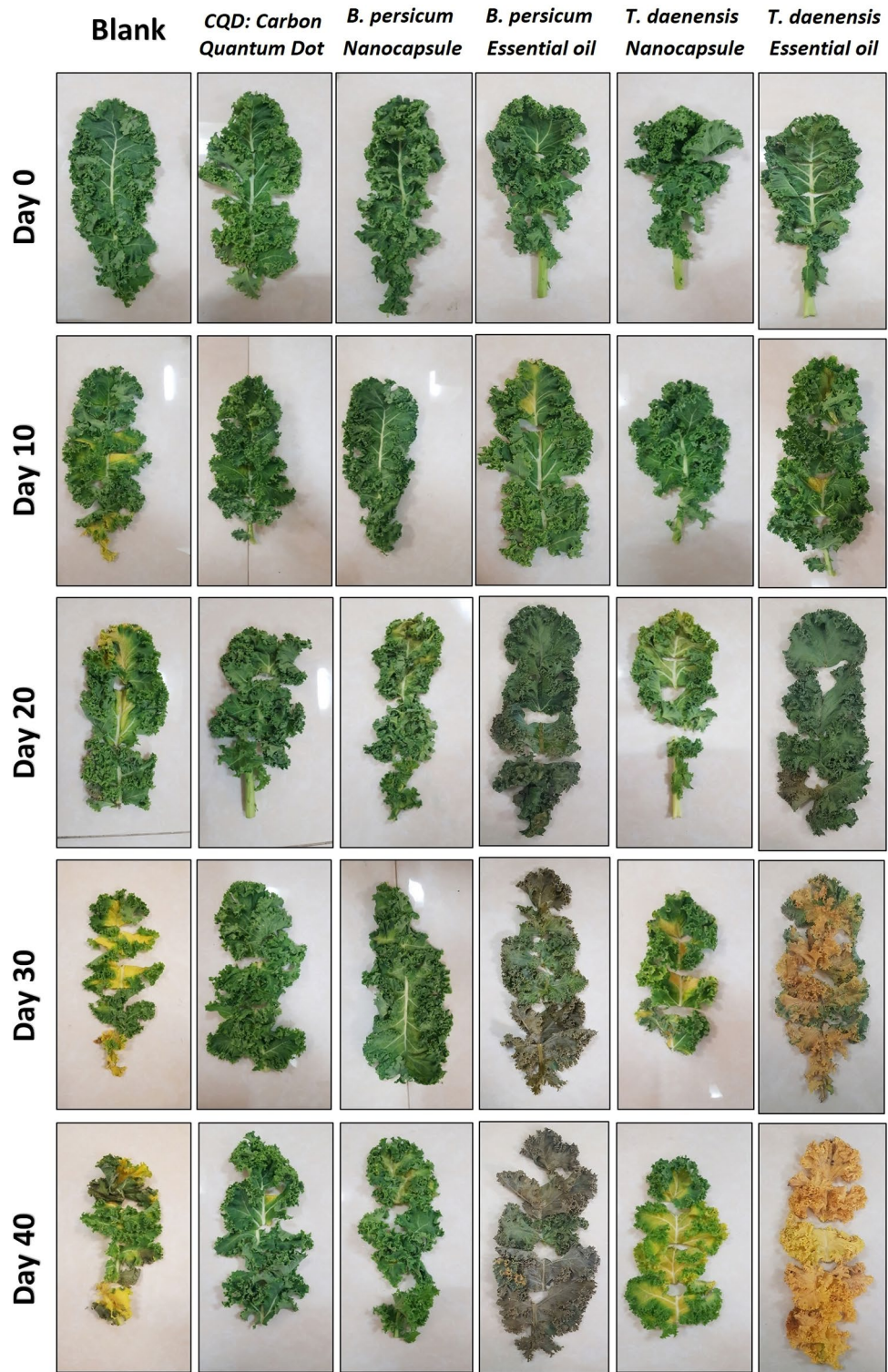
## Physiological study of fresh-cut kale: variations in weight, colors and electrolyte leakage

### Weight loss and moisture retention

Fresh weight loss (Fig. 3A), a key indicator of water retention and overall freshness, progressively increased across all treatments over time. However, the control group (untreated fresh-cut kale) exhibited the highest weight loss (27.29 g), indicating rapid dehydration and spoilage (Fig. 2). In contrast, samples treated with EO-NCs and CQDs showed significantly lower weight loss, demonstrating their effectiveness in moisture retention and preventing excessive desiccation. Among the treatments, CQD-treated samples exhibited the lowest weight loss (5.50 g), showing a statistically significant difference ( $p < 0.01$ ,  $CV = 17.58\%$ ) compared to both EO-NC treatments and the control group. Among EO-NC-treated samples, *T. daenensis* NC (11.92 g) and *B. persicum* NC (12.49 g) displayed moderate weight loss values. The superior performance of CQDs in minimizing weight loss can be attributed to their effective barrier properties, which reduce water vapor transmission and respiration rates. In contrast, while EO-NCs provided some level of moisture retention, they were less effective than CQDs. The control group experienced maximum moisture loss due to unrestricted moisture exchange under atmospheric conditions, as no advanced packaging was applied (Table 1).

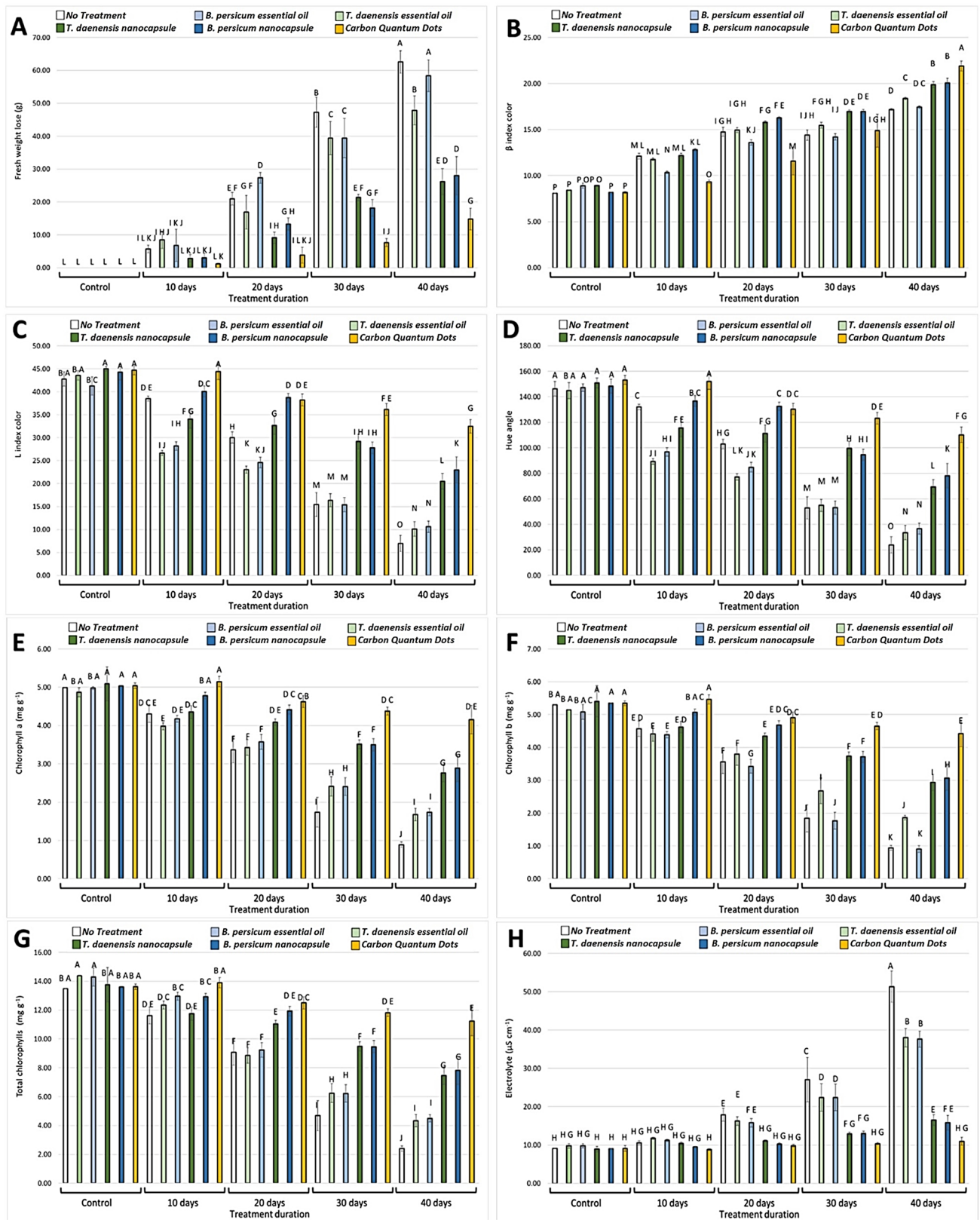
### Color stability and chlorophyll retention

Color is a critical visual parameter influencing consumer perception of freshness and quality in leafy vegetables. Yellowing ( $\Delta\beta$ , Fig. 3B), an indicator of chlorophyll degradation and senescence, was most pronounced in the control group, which experienced severe discoloration. In contrast, EO-NC- and CQD-treated samples retained their green coloration for a longer duration, reflecting their ability to delay chlorophyll breakdown and visible decay.



**Fig. 2.** Color changes of kale leaf samples in control conditions compared to three samples stacked with bread and ingredients over 40 days at 10-day intervals.

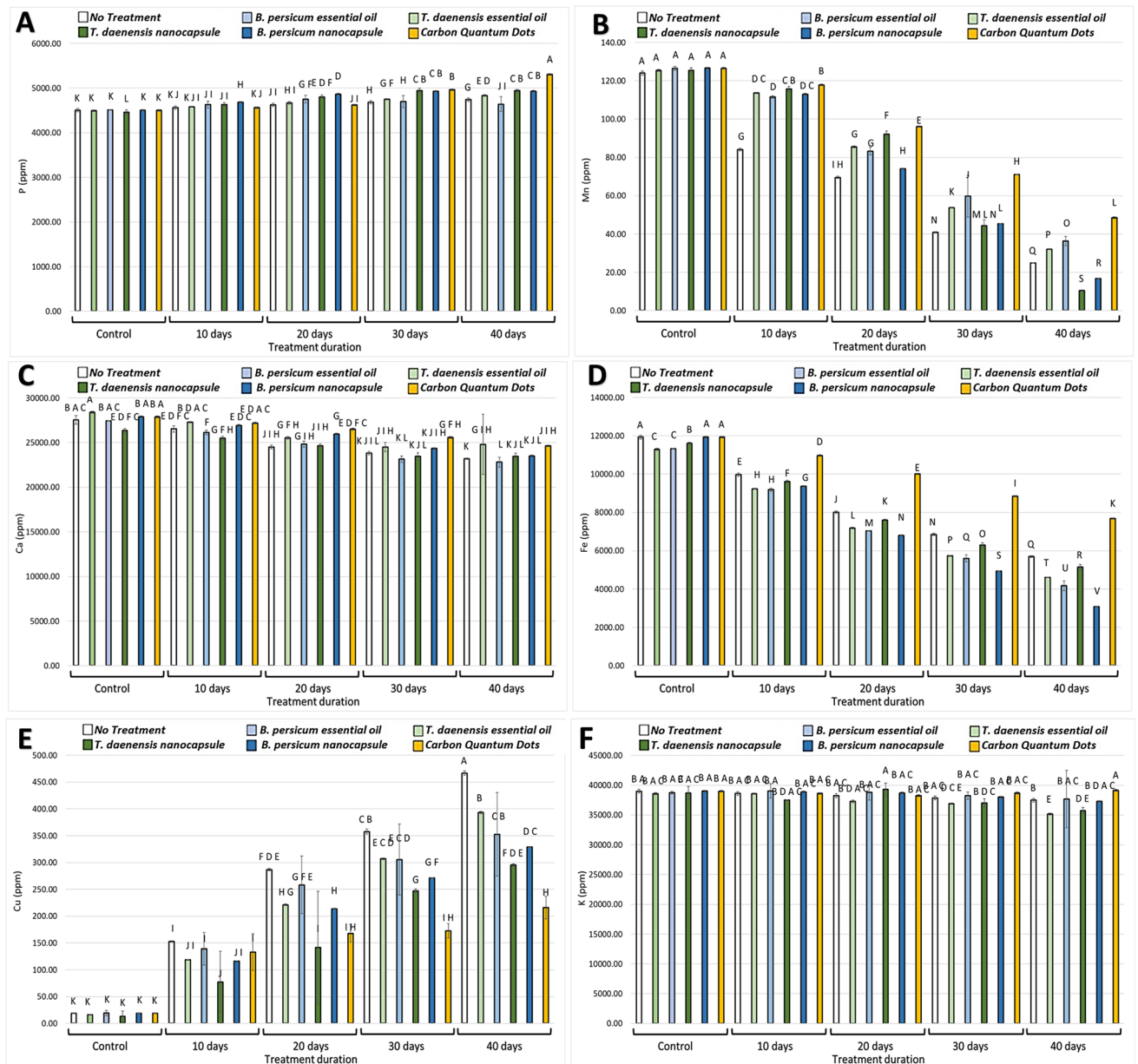
Similarly, lightness (L, Fig. 3C), which quantifies the brightness of fresh-cut kale leaves, declined significantly in untreated samples, making them appear darker and less fresh. In contrast, EO-NC- and CQD-treated samples maintained higher L values, indicating that these treatments effectively delayed darkening and preserved visual appeal. Hue angle (Fig. 3D) further confirmed the protective effects of these treatments. The control group exhibited a sharp increase in hue angle (148.43° after 40 days), indicating accelerated browning and senescence. In contrast, CQD-treated samples exhibited a slower hue angle increase (133.75°), demonstrating their ability to



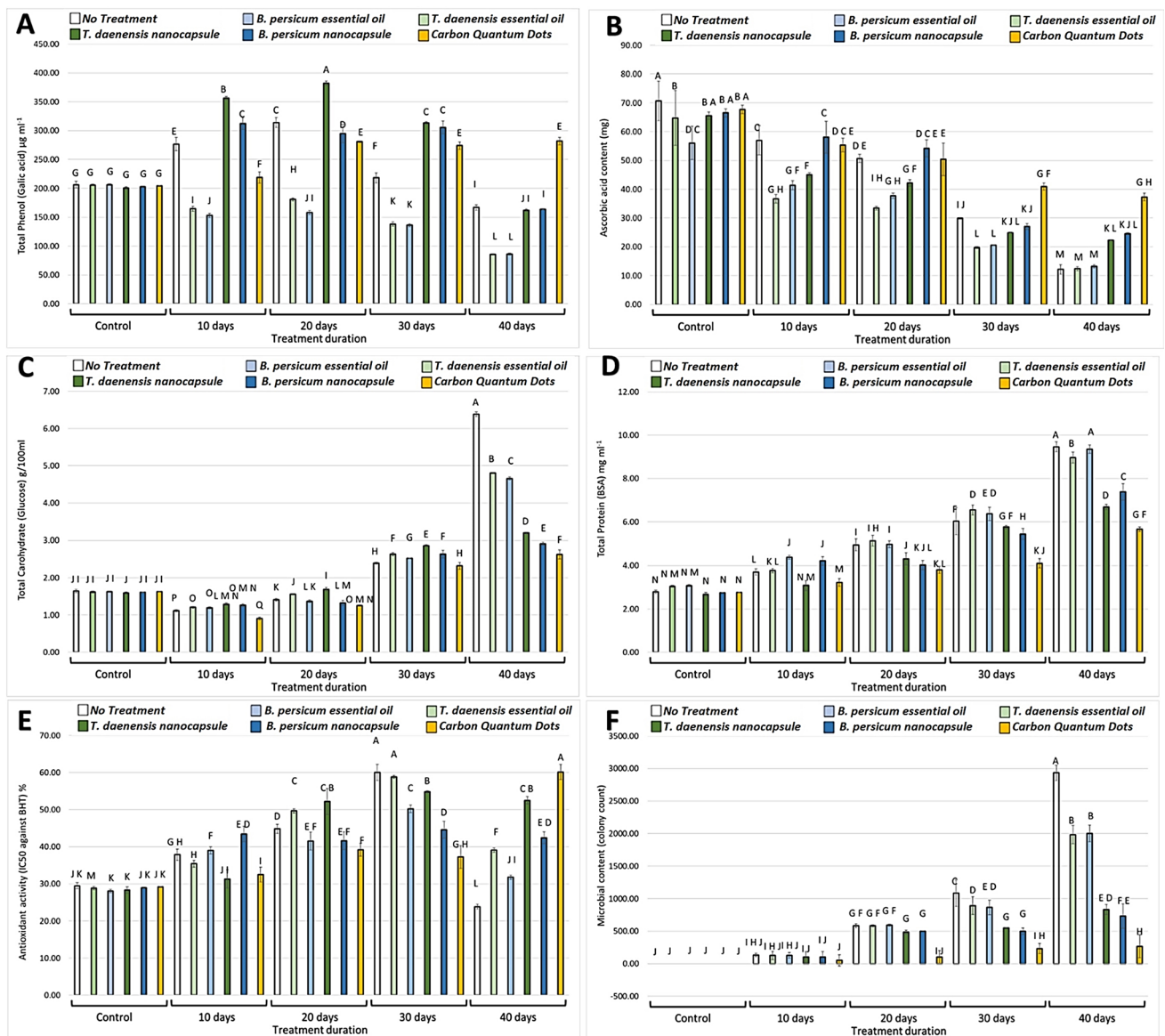
**Fig. 3.** Effect of various treatments on the preservation of fresh-cut kale over different storage durations (0, 10, 20, 30, and 40 days). The figure shows the results for different parameters: (A) Fresh weight loss, (B)  $\Delta\beta$  (yellow color), (C) Lightness ( $L^*$ ), (D) Hue angle, (E) Chlorophyll a content, (F) Chlorophyll b content, (G) Total chlorophyll content, (H) Electrolyte leakage. Treatments include no treatment (control), essential oils (*T. daenensis* and *B. persicum*), nanoencapsulated essential oils (EO-NC), and carbon quantum dots (CQDs). Different letters represent significant differences between treatments.

delay color degradation. While EO-NCs outperformed the control group, they did not surpass CQDs in color preservation.

Chlorophyll content (Fig. 3G), a fundamental biochemical marker of freshness, followed a similar trend. Chlorophyll a (Fig. 3E) and chlorophyll b (Fig. 3F), the primary photosynthetic pigments, degraded most rapidly in the control group. In contrast, CQD-treated samples retained the highest chlorophyll content (12.61 mg g<sup>-1</sup> FW), outperforming *T. daenensis* NC (11.14 mg g<sup>-1</sup> FW) and *B. persicum* NC (10.70 mg g<sup>-1</sup> FW). The control group exhibited the lowest chlorophyll levels (8.25 mg g<sup>-1</sup> FW), indicating accelerated aging and degradation. These results strongly suggest that CQD treatments effectively mitigate oxidative stress, enhance protection against environmental factors, and delay chlorophyll breakdown, contributing to extended freshness and shelf life. The obtained analysis results, their precision and standard deviation are detailed in Tables S1 and S2 (Supplementary file). Based on these results, Figs. 3, 4, 5, 6 and 7 have been drawn.



**Fig. 4.** Effect of various treatments on the preservation of fresh-cut kale over different storage durations (0, 10, 20, 30, and 40 days). The figure shows the results for different parameters: (A) phosphorus content, (B) manganese content, (C) calcium content, (D) Iron content, (E) copper content. Treatments include no treatment (control), essential oils (*T. daenensis* and *B. persicum*), nanoencapsulated essential oils (EO-NC), and carbon quantum dots (CQDs). Different letters represent significant differences between treatments.



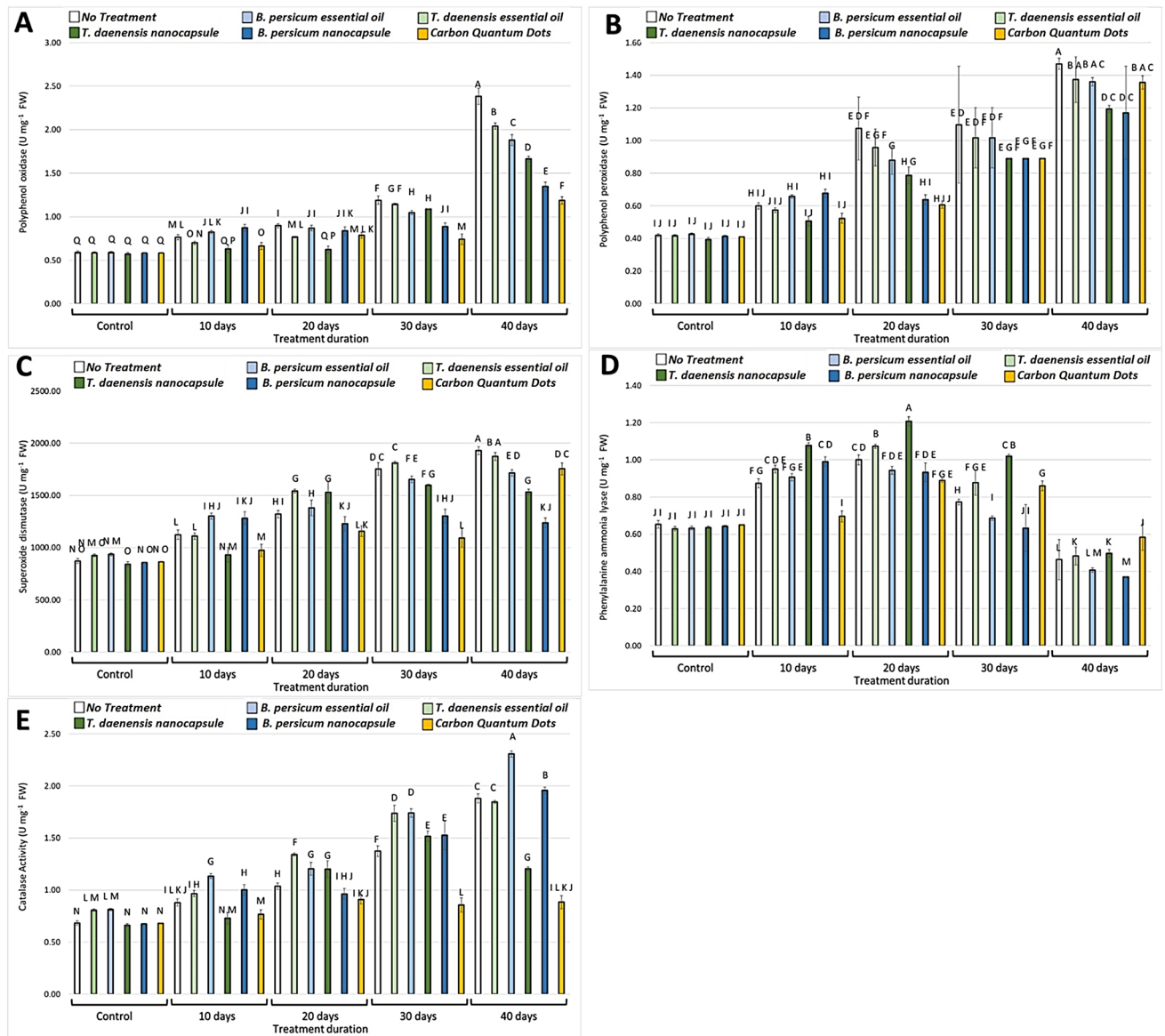
**Fig. 5.** Effect of different treatments on various parameters of fresh-cut kale over different storage durations (0, 10, 20, 30, and 40 days). The figure includes the following measurements: **(A)** Total phenolic content, **(B)** Ascorbic acid content, **(C)** total carbohydrate content, **(D)** total protein content, **(E)** Antioxidant content, **(F)** microbial content. Treatments include no treatment (control), essential oils (*T. daenensis* and *B. persicum*), nanoencapsulated essential oils (EO-NC), and carbon quantum dots (CQDs). Different letters represent significant differences between treatments.

#### Electrolyte leakage and membrane integrity

Electrolyte leakage (Fig. 3H), a key indicator of membrane integrity and cellular stability, was highest in the control group, reflecting widespread cell rupture and structural deterioration. Conversely, EO-NC and CQD treatments significantly reduced membrane leakage, indicating their ability to maintain cellular stability and retard tissue degradation. The reduced electrolyte leakage in treated samples suggests that these treatments not only preserve external quality attributes (e.g., color and moisture retention) but also enhance internal cellular health, ultimately prolonging the usability of fresh-cut kale.

#### Statistical analysis and treatment efficacy

Treatments were statistically analyzed using ANOVA (Table 2), followed by Duncan's test, to determine significant differences among treatments. Weight loss, a critical indicator of postharvest deterioration, progressively increased across all treatments over 40 days. CQD-treated samples exhibited the lowest weight loss (5.50 g), significantly outperforming EO-NC-treated samples and the control group (27.29 g). The statistical analysis confirmed a highly significant difference ( $p < 0.01$ ,  $CV = 17.58\%$ ) among treatments. The superior preservation effect of CQDs is attributed to their effective barrier properties, which reduce transpiration and respiration

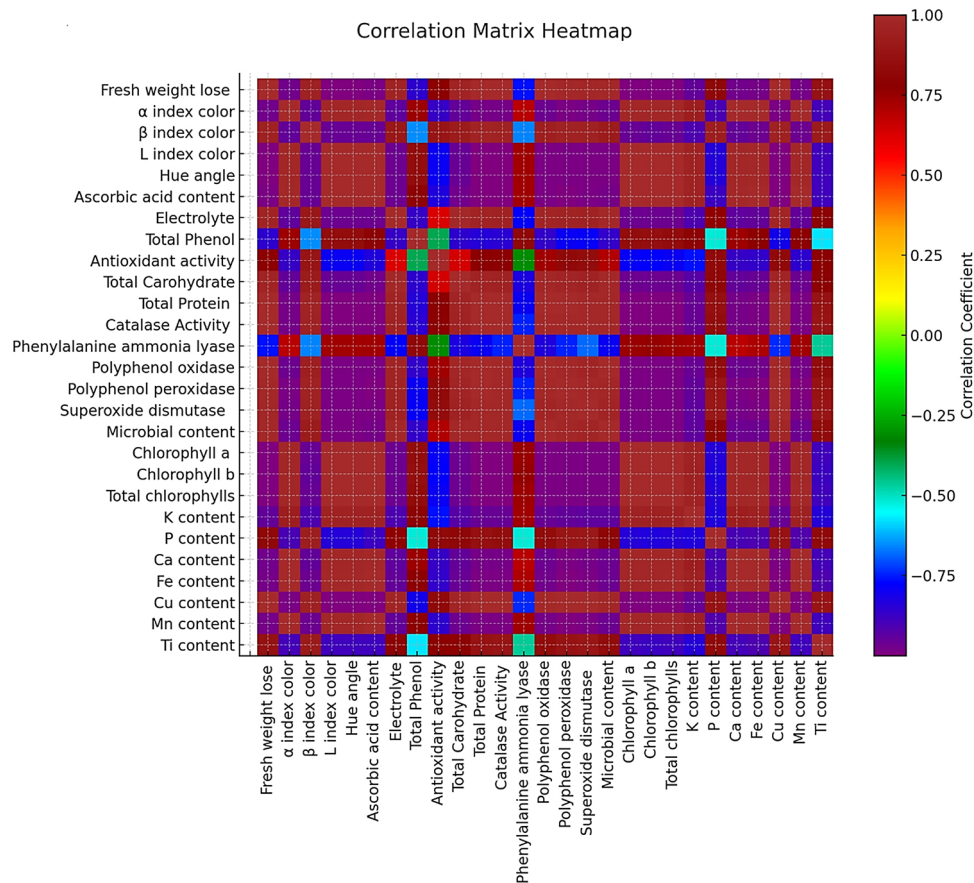


**Fig. 6.** Effect of different treatments on various parameters of fresh-cut kale over different storage durations (0, 10, 20, 30, and 40 days). The figure includes the following measurements: (A) polyphenol oxidase activity, (B) polyphenol peroxidase activity, (C) superoxidase activity, (D) phenylalanine ammonia lyase activity, (E) catalase activity. Treatments include no treatment (control), essential oils (*T. daenensis* and *B. persicum*), nanoencapsulated essential oils (EO-NC), and carbon quantum dots (CQDs). Different letters represent significant differences between treatments.

rates, thereby minimizing weight loss. EO-NCs also contributed to moisture retention, though their effect was less pronounced than CQDs. The control group, which lacked any nano-packaging interventions, experienced maximum weight loss, likely due to unregulated moisture exchange with the environment.

### Mineral content in fresh-cut Kale

Figure 4 illustrates the impact of different treatments on the mineral content of fresh-cut kale up to 40 days of refrigerated storage. The parameters analyzed include phosphorus (P, Fig. 4A), manganese (Mn, Fig. 4B), calcium (Ca, Fig. 4C), iron (Fe, Fig. 4D), copper (Cu, Fig. 4E) and potassium (K, Fig. 4F). The study compares untreated control samples with those treated using essential oils (*T. daenensis* and *B. persicum*), their nanoencapsulated counterparts, and CQDs. The results reveal significant differences in mineral retention, highlighting the effectiveness of these treatments in minimizing nutrient loss and maintaining the nutritional value of fresh-cut kale during storage.



**Fig. 7.** Heatmap analysis showing the changes in various parameters such as weight loss, chlorophyll content, ascorbic acid, antioxidant activity, etc. over time (0, 10, 20, 30, and 40 days). The data highlights the differences between various treatments (CQD, EO-NC, control) and their effects on the preservation of kale quality throughout the storage period.

#### Phosphorus (P)

Phosphorus (Fig. 4A), illustrates the decreased trend in phosphorus levels over time compared to the control in all the treated samples. While the untreated group maintains high P levels, treatments with *B. persicum* EO-NCs and CQDs result in the highest reduction after 20 days. Overall, the various treatments were significantly effective in lowering phosphorus accumulation with increased exposure durations.

#### Manganese (Mn)

Manganese (Fig. 4B), an essential micronutrient involved in enzyme activation and antioxidant defense, declined significantly in untreated fresh-cut kale over time. The control samples exhibited a steep reduction, whereas CQD- and EO-NC-treated samples retained significantly higher Mn levels. These findings suggest that CQDs and EO-NCs stabilize cellular structures and mitigate oxidative stress, thereby reducing mineral depletion and promoting greater nutrient retention.

#### Calcium (Ca)

Calcium (Fig. 4C), a fundamental nutrient for cell wall integrity and structural stability, exhibited a progressive decline across all treatments, with the control group experiencing the most severe deterioration. However, samples treated with nanoencapsulated essential oils and CQDs maintained significantly higher calcium levels, suggesting their role in enhancing cell structure, delaying tissue degradation, and retarding senescence. Although free essential oils provided moderate protection, they were less effective than their nanoencapsulated counterparts, reinforcing the importance of controlled release mechanisms in nutrient stabilization.

#### Iron (Fe)

Iron (Fig. 4D), a key element in enzymatic activity and plant metabolism, showed a marked decline in the control group, indicating substantial nutrient loss over time. In contrast, nanoencapsulated essential oils and CQDs effectively preserved Fe levels, suggesting their role in maintaining metabolic processes and preventing oxidative stress-induced degradation. While free essential oils helped slow Fe depletion, their nanoencapsulated versions were significantly more effective, likely due to their enhanced stability and prolonged protective action.

Source	DF	Fresh weight loss (g)	$\alpha$ index color	$\beta$ index color	L index color	Hue angle	Ascorbic acid content (mg)	Electrolyte ( $\mu\text{S cm}^{-1}$ )	Total Phenol ( $\mu\text{g ml}^{-1}$ Galic acid)	Total Carbohydrate (g/100 ml Glucose)
Rep	2	3.45ns	335285.12ns	1.60*	8.92*	72.88ns	17.65ns	7.21ns	64.70ns	0.85ns
Model	31	969.89**	7573499.50**	43.13**	374.03**	4360.19**	906.32**	295.06**	16529.10**	4.38**
Block	2	3.45ns	335285.12ns	1.60*	8.92*	72.88ns	17.65ns	7.21ns	64.70ns	0.85ns
CKTR	20	217.26**	477525.16ns	4.56**	54.36**	660.06**	74.78**	127.61**	7099.49**	1.29**
Error	58	9.67	448169.3	0.36	2.05	25.87	9.71	3.22	36.16	0.00225
CV (%)	-	17.58	2.63	4.37	4.74	4.95	7.55	11.27	2.71	2.18
Source	DF	Total Protein (BSA) mg ml <sup>-1</sup>	Catalase Activity (U mg <sup>-1</sup> FW)	Phenylalanine ammonia lyase (U mg <sup>-1</sup> FW)	Polyphenol oxidase (U g <sup>-1</sup> FW)	Polyphenol peroxidase (U g <sup>-1</sup> FW)	Superoxide dismutase (U mg <sup>-1</sup> FW)	Microbial content (colony count)	Antioxidant activity (IC50 against BHT) %	
Rep	2	0.09ns	0.01*	1.34ns	0.00282ns	0.00936ns	3543.09ns	23386.94ns	1.95ns	
Model	31	10.86**	0.57**	0.13573**	0.60746**	0.31423**	327639.78**	1342305.76**	340.67**	
Block	2	0.09ns	0.01*	1.34ns	0.00282ns	0.00936ns	3543.09ns	23386.94ns	1.95ns	
CKTR	20	1.20**	0.15**	0.02474**	0.11849**	0.02266ns	89357.89**	527817.41**	223.41**	
Error	58	0.05	0.00227	0.00167	0.00134	0.01234	2286.9	7591.32	2.66	
CV (%)	-	4.41	4.05	5.33	3.75	13.51	3.64	15.98	4.06	
Source	DF	Chlorophyll a (mg g <sup>-1</sup> )	Chlorophyll b (mg g <sup>-1</sup> )	Total chlorophylls (mg g <sup>-1</sup> )	K (ppm)	P (ppm)	Fe (ppm)	Ca (ppm)	Cu (ppm)	Mn (ppm)
Rep	2	0.09ns	0.11ns	0.62ns	402581.78ns	13931.81*	44740.51**	335285.12ns	2716.18ns	16.57*
Model	31	4.02**	5.18**	32.86**	2765168.10**	102553.23**	19111545.10**	7573499.5**	45358.51**	4000.99**
Block	2	0.09ns	0.11ns	0.62ns	402581.78ns	13931.81*	44740.51**	335285.12ns	2716.18ns	16.57*
CKTR	20	0.66**	0.91**	6.33**	1382941.84ns	41474.48**	1127036.26**	477525.163ns	4487.16**	198.61**
Error	58	0.04	0.05	0.3	1048951.2	2325.1	5089.3	448169.3	969.3	4.67
CV (%)	-	5.34	5.84	5.33	2.69	1.02	0.88	2.627627	15.97	2.71

**Table 2.** ANOVA interaction analysis for CQD.

#### Copper (Cu)

Copper (Fig. 4E), an essential cofactor in plant metabolism and antioxidant defense, declined rapidly in untreated samples, indicating accelerated degradation. Among the treatments, CQDs and nanoencapsulated essential oils retained the highest copper levels, reinforcing their protective role in reducing nutrient loss. The superior performance of these treatments may be attributed to their ability to counteract oxidative stress and promote nutrient stabilization, ensuring greater bioavailability of essential minerals throughout the storage period.

#### Potassium (K)

Potassium (Fig. 4F), a critical macronutrient for cell function and osmotic regulation, exhibited a steady decline across all samples over time. However, the control group experienced the sharpest reduction, indicating severe potassium leaching and compromised membrane integrity. In contrast, CQD- and EO-NC-treated samples showed significantly higher potassium retention, suggesting that these treatments effectively preserved membrane stability and reduced nutrient loss. While free essential oils provided some level of protection, their nanoencapsulated forms performed better, likely due to controlled release and improved bioavailability.

#### Metabolite and microbial activity

The results presented in Fig. 5 demonstrate the impact of various treatments on the retention of bioactive metabolites, antioxidant activity, and microbial load in fresh-cut kale up to 40 days of refrigerated storage. The findings indicate significant variations in the maintenance of biochemical components, reinforcing the effectiveness of EO-NCs and CQDs in preserving nutritional quality, mitigating oxidative stress, and limiting microbial proliferation. The details of these results are presented in detail below.

#### Total phenolic content

Total phenolic content (Fig. 5A), a key determinant of antioxidant potential and phytochemical stability, exhibited a progressive decline over time, with the control group experiencing the most severe reduction, indicating rapid oxidative degradation. In contrast, EO-NC and CQD treatments significantly preserved phenolic levels, suggesting their protective role in mitigating oxidative stress and stabilizing bioactive compounds. While free essential oils exhibited moderate protection, their nanoencapsulated counterparts showed superior stabilization, likely due to their controlled release mechanism, which prolongs their bioactivity over time.

#### Ascorbic acid (Vitamin C)

Ascorbic acid (vitamin C, Fig. 5B), another critical antioxidant, also followed a similar declining trend, with the control group exhibiting the most rapid loss due to oxidation over time. However, CQD- and EO-NC-

treated samples retained significantly higher ascorbic acid levels, suggesting their role in inhibiting oxidative degradation and extending the shelf life of vitamin C in fresh-cut kale.

#### Total carbohydrate preservation

Total carbohydrate content (Fig. 5C), an essential energy source and quality determinant, remained relatively stable during the early storage period but showed a steep decline in untreated samples over time. The control group exhibited the highest carbohydrate loss, likely due to accelerated metabolic degradation and microbial consumption. Conversely, EO-NC- and CQD-treated samples effectively preserved carbohydrate content, suggesting that these treatments slow down metabolic degradation and limit microbial activity, thereby maintaining carbohydrate reserves in fresh-cut kale.

#### Total protein

Total protein concentration (Fig. 5D), a critical indicator of nutritional quality, declined markedly in untreated samples, reflecting cellular breakdown and enzymatic degradation over time. However, CQDs and EO-NCs significantly slowed protein degradation, retaining higher protein levels than both free essential oil-treated samples and the control group. These findings suggest that these treatments support cellular integrity, preserve metabolic functions, and delay senescence, thereby improving nutritional stability during storage.

#### Antioxidant activity

Antioxidant activity (Fig. 5E), a crucial factor in suppressing oxidative damage and maintaining food quality, showed a substantial decline in the control group, indicating the progressive loss of bioactive molecules. However, CQD- and EO-NC-treated samples maintained significantly higher antioxidant activity, reinforcing their ability to protect functional compounds, reduce oxidative stress, and enhance postharvest longevity in fresh-cut kale.

#### Microbial load and shelf-life extension

Microbial population dynamics (Fig. 5F), a primary determinant of shelf life and food safety, revealed uncontrolled microbial proliferation in the control group, leading to spoilage and potential health risks. In contrast, CQDs and EO-NCs exhibited the most effective antimicrobial properties, significantly inhibiting microbial growth and extending the microbial safety of stored fresh-cut kale. While free essential oils provided some level of antimicrobial protection, their nanoencapsulated formulations demonstrated superior efficacy, likely due to their sustained and controlled.

### Antioxidant and antibacterial study of fresh-cut Kale

The results indicate that EO-NCs exhibited moderate efficacy, likely due to their antioxidant and antibacterial properties. However, CQD fresh-cut kale demonstrated substantial antioxidant activity, as measured by IC50 against BHT, with a 39.64% retention rate, comparable to *T. daenensis* NC (43.82%) and slightly higher than the control group (39.20%). These findings highlight the ability of CQDs to preserve bioactive molecules, thereby reducing oxidative stress and maintaining fresh-cut kale's antioxidant potential (Fig. 5).

Microbial analysis further underscores the superior efficacy of CQD coatings in limiting bacterial proliferation. Among the treatments, CQD-treated samples exhibited the lowest microbial count (130 CFU/mL), followed by *T. daenensis* NC (393 CFU/mL) and *B. persicum* NC (366 CFU/mL). In contrast, the control group had the highest microbial load (946 CFU/mL), indicating rapid microbial growth and spoilage. The superior antimicrobial activity of CQDs is likely attributed to their ability to disrupt bacterial cell membranes, inhibit microbial proliferation, and extend shelf life.

Ascorbic acid (vitamin C), the primary antioxidant in fresh-cut kale, is essential for nutritional retention, and its degradation significantly impacts overall food quality. The treatments resulted in notable differences in ascorbic acid preservation, with CQD-treated samples exhibiting the highest retention (50.33 mg), followed by *T. daenensis* NC (39.99 mg) and *B. persicum* NC (33.41 mg). The control group displayed substantial ascorbic acid depletion, reinforcing the necessity of protective treatments to preserve vitamin C levels. These findings confirm that CQDs and nanoencapsulated essential oils are highly effective in slowing ascorbic acid degradation, ensuring greater retention of nutritional quality over prolonged storage periods.

The CQD-treated samples exhibited the highest ascorbic acid retention (50.33 mg), followed by *T. daenensis* NC-treated samples (39.99 mg) and *B. persicum* NC-treated samples (33.41 mg). In contrast, the control group exhibited the lowest ascorbic acid concentration (20.33 mg), indicating significant oxidative degradation over the storage period. The superior performance of CQD coatings can be attributed to their ability to mitigate oxidative processes, forming an effective barrier against oxygen and reactive oxygen species (ROS). While EO-NCs provided moderate antioxidant protection, their efficacy remained inferior to CQDs. The retention of ascorbic acid showed statistically significant differences ( $p < 0.01$ ), with a coefficient of variation (CV) of 7.55% (Fig. 5).

Phenolic compounds are crucial for enhancing fresh-cut kale's antioxidant properties and extending its shelf life. The highest levels of phenolic content were observed in samples treated with *T. daenensis* NC (282.89 µg/mL) and CQD coatings (251.97 µg/mL),

while the control group exhibited the lowest phenolic concentration (236.38 µg/mL), indicating substantial oxidative degradation (Fig. 5). These results further highlight the efficacy of nanoencapsulated essential oils and CQDs in preserving bioactive compounds, thereby enhancing antioxidant stability and prolonging the postharvest quality of fresh-cut kale.

## Enzymatic activity

The study also examined the activity of key enzymes associated with oxidative stress and quality degradation, including PAL, SOD, CAT, and PPO. The CQD-treated samples exhibited the lowest catalase (0.81 U/mg g<sup>-1</sup> FW) and polyphenol oxidase (0.79 U/g FW) activity, indicating reduced oxidative stress and delayed enzymatic browning. In contrast, the control group displayed the highest enzymatic activity, reflecting greater oxidative degradation and faster quality deterioration. EO-NC-treated samples also demonstrated lower enzyme activity, reinforcing their effectiveness in preserving postharvest quality. Figure 6 illustrates the changes in enzymatic activity across different treatments during the 40-day storage period.

### Polyphenol oxidase

As shown in Fig. 6A, PPO activity, the enzyme responsible for browning reactions, increased progressively over time in all treatments, with maximum activity occurring at 40 days. However, samples treated with nanoencapsulated essential oils (particularly *T. daenensis* EO-NC) exhibited a significant reduction in PPO activity relative to the control, suggesting their role in delaying enzymatic browning and extending visual freshness.

### Polyphenol peroxidase

Figure 6B presents changes in polyphenol peroxidase (POD) activity, another enzyme linked to oxidative stress. The control group showed a sharp increase in POD activity, indicating heightened oxidative damage. In contrast, samples treated with *T. daenensis* EO-NC and CQDs displayed significantly lower POD activity, supporting their role in reducing oxidative stress and maintaining fresh-cut kale quality over time.

### Superoxide dismutase

As depicted in Fig. 6C, SOD activity, a critical antioxidant enzyme responsible for scavenging reactive oxygen species (ROS), initially increased across all treatments before declining at 40 days. The lowest SOD levels at 40 days were observed in *T. daenensis* EO-NC and *B. persicum* EO-NC samples, suggesting that these treatments provided the strongest antioxidant defense mechanisms, effectively neutralizing ROS and mitigating oxidative stress.

### Phenylalanine ammonia-lyase

Figure 6D highlights PAL activity, an enzyme essential for phenolic compound biosynthesis and plant stress responses. PAL activity peaked at 20–30 days in most treatments before declining. However, *T. daenensis* EO-NC and essential oil-treated samples maintained significantly higher PAL activity, indicating their potential to stimulate secondary metabolite production and enhance stress tolerance, contributing to improved postharvest quality.

### Catalase

As shown in Fig. 6E, CAT activity, which decomposes hydrogen peroxide into water and oxygen, remained highest and most stable in *T. daenensis* EO-NC and *B. persicum* EO-NC-treated samples. This suggests that these treatments effectively reduced oxidative damage, contributing to extended shelf life and improved quality preservation in fresh-cut kale.

## Discussions

This study utilized heatmap analysis to assess the effects of essential oils (*T. daenensis* and *B. persicum*) and EO-NC on the postharvest quality of fresh-cut kale over a 40-day storage period. The findings revealed a progressive decline in fresh weight, chlorophyll content, and ascorbic acid levels, with the control group exhibiting the most significant deterioration by day 40. In contrast, essential oil treatments effectively slowed these losses, preserving higher levels of these key quality indicators. Notably, *T. daenensis* EO-NC outperformed other treatments, demonstrating superior ascorbic acid retention and antioxidant activity, suggesting that certain essential oils are particularly effective in minimizing oxidative degradation. As seen in Fig. 7, there was a positive correlation between K, Ca, and Fe and antioxidant compounds (ascorbic acid, total polyphenols, and DPPH activity) and a negative correlation with loss of fresh weight and microbial matter. These data indicated an inverse relation between the loss of minerals and the loss of freshness and microbial matter. The correlation clusters in Fig. 7 also show that the samples with higher levels of chlorophyll and phenolics mostly had lower levels of enzymatic activity and ion leakage, indicating the positive effect of nano-treatments on retaining tissue integrity. Moreover, Fig. 7 indicated that the above-mentioned antioxidant and mineral properties were closely related, further clarifying the synergistic effect of EO-NC and CQD treatment.

Beyond EOs, CQDs emerged as a highly effective nano-packaging solution for fresh-cut kale, offering multiple postharvest benefits<sup>35</sup>. In this work the CQDs significantly reduced weight loss (< 15% compare to control), preserved chlorophyll content (> 35% compare to control) regulated enzymatic activity, and inhibited microbial growth (> 13% compare to control), all of which contribute to extended shelf life. Javdani et al. have developed a Chitosan/Polyvinyl Alcohol/Carbon Dots film that has both antimicrobial/antioxidant properties and changes color with pH/alkaline spoilage gases. The film formulation contains CDs (about 2%) and anthocyanins (about 4%)<sup>36</sup>. In another study, the chlorogenic acid carbon dots clay nanostructure showed minimal browning and mold, which is closer to a cut vegetable scenario. They also showed that carbon quantum dots have food contact safety and low toxicity in cell tests<sup>37</sup>. These types of packaging can reduce the microbial content more than 80%<sup>38</sup>. A study by Thanawutthiphong et al. showed that nitrogen- and sulfur-doped carbon dots (S, N-doped CDs) can effectively enhance the antimicrobial, antifungal, and antioxidant properties of polymer films by up to 54%<sup>39</sup>.

The findings of this study align with previous research on nano-packaging methods, including CQDs and EOs, and their impact on the physical and nutritional qualities of fresh-cut kale during storage<sup>38</sup>. The comparative assessment of CQDs and EO-NCs indicated that CQDs have better preservation effects than EO-NCs, particularly in the context of weight retention, color stability, ascorbic acid preservation (50.33 mg g<sup>-1</sup> FW), phenolic content (251.97 µg/ml), and antioxidant activity (> 39.64%). The results are in agreement with other studies that demonstrated 80% efficiency for improving antioxidant activity<sup>36</sup>. Specifically, CQD-treated fresh-cut kale exhibited the lowest weight loss (5.50 g) after 40 days, significantly outperforming both EO-NC-treated and control samples, which experienced higher weight losses (< 15% compare to control). Previous reported mentioned that, CQDs serve as an effective barrier against water vapor, thereby slowing respiration and reducing moisture loss<sup>40</sup>.

Additionally, the current study corroborates previous research on color retention in fresh-cut leafy greens. One of the works, by Thanawutthiphong et al., showed that the use of CQDs acts mainly through the inhibition of oxidation and weakening of the membrane of microorganisms, but its effect is highly dependent on the humid and breathable environment of the vegetable<sup>39</sup>. While EO-NC treatments helped preserve color, they were not as effective as CQDs, demonstrated that essential oils such as oregano and thyme contribute to color stability in leafy greens but do not surpass the protective effects of nano-packaging materials like CQDs<sup>41</sup>. Carbon quantum dots primarily work as multi-functional active packaging materials because of their nanometric size, functional groups, and redox properties. Due to a high number of hydroxyl and carboxyl groups existing on the surface of carbon quantum dots, there is strong interaction with water and oxygen, hence restricting water vapor and oxygen diffusion at the produce surface<sup>42</sup>. Moreover, the obtained CQDs possess inherent antioxidant properties in terms of reactive oxygen species scavenging and regulating the activity of oxidative enzymes. This further explains why there is a reduction in activities of both PPO and CAT. It should be noted that their antimicrobial properties are primarily associated with cell damage in microbes and inducing oxidative stress in microbial cells.

While EO-NCs demonstrated moderate efficacy in preserving fresh-cut kale quality, their barrier properties were less effective, likely due to the lower vapor resistance of essential oils. Specifically, the  $\Delta L$  index (brightness) remained stable in CQD-treated fresh-cut kale, and the hue angle changed at a slower rate, indicating superior color retention (> 18%). These results are consistent with previous research on CQD applications, which demonstrated that in a storage test of fresh-cut apples, they report that the active coating containing CGA-CDs prevented the least browning and mold<sup>37</sup>. For instance, it was reported that CQD coatings significantly preserved color stability in leafy greens such as lettuce and spinach by inhibiting oxidative stress-induced degradation. The findings of this study further confirm that EO-NCs contributed to color retention, although their effectiveness was lower compared to CQDs. This aligns with previous studies, which found that essential oils such as oregano and thyme could maintain color stability in leafy greens, but their efficacy was inferior to nano-packaging technologies like CQDs<sup>43,44</sup>.

Ascorbic acid is one of the most crucial antioxidants in kale, playing a key role in maintaining nutritional quality. In this study, CQD-treated fresh-cut kale exhibited the highest ascorbic acid retention (50.33 mg g<sup>-1</sup> FW), followed by EO-NC treatments with *T. daenensis* (39.99 mg g<sup>-1</sup> FW) and *B. persicum* (33.41 mg g<sup>-1</sup> FW). The control group showed the lowest ascorbic acid retention (20.33 mg g<sup>-1</sup> FW), indicating significant oxidative degradation over the storage period. These findings align with previous research, which demonstrated that CQDs effectively preserved ascorbic acid in fresh vegetables by inhibiting oxygen exposure and reducing reactive oxygen species (ROS)-induced degradation<sup>45,46</sup>.

Essential oils, particularly *T. daenensis*, played a role in ascorbic acid preservation, though their efficacy was lower than that of CQD coatings. Previous studies have shown that thyme and oregano essential oils contribute to vitamin C retention, yet their effectiveness remains inferior to synthetic coatings such as CQDs<sup>47,48</sup>. Among all treatments, CQD coatings demonstrated the highest efficacy, preserving phenolic content at 251.97 µg/mL and antioxidant activity at 39.64%, outperforming EO-NC treatments and the control. The application of CQDs to fresh-cut produce has been consistently linked to successful phenolic compound preservation and enhanced antioxidant potential. Although *T. daenensis* EO-NC exhibited notable retention of phenolic compounds and antioxidant activity, its performance did not reach the same level of effectiveness as CQD coatings. Previously reported that despite antioxidant activity of leafy greens preserved in such essential oils as *T. daenensis*, CQDs exhibited enhanced protection against oxidative stress and bioactive compound conservation<sup>41,49</sup>.

Chlorophyll retention is a critical factor influencing the freshness and visual appeal of leafy vegetables. In this study, CQD-treated fresh-cut kale exhibited the highest chlorophyll content (12.61 mg g<sup>-1</sup> FW), significantly outperforming both EO-NC treatments and the control group. While EO-NC treatments demonstrated moderate chlorophyll retention, their efficacy remained lower than that of CQDs. The control group showed the lowest chlorophyll content (8.25 mg g<sup>-1</sup> FW), reflecting accelerated senescence and breakdown due to unregulated oxidative stress. previously, it was reported that the CQDs reduced salicylic acid content, reactive oxygen species generation, lipid peroxidation and antioxidant enzymes activities, while enhanced photosynthetic performance, including relative electron transport rate, maximum efficiency of photosystem II (Fv/Fm), Rubisco activity, chlorophyll and flavonoid contents, glucose content and leaf area<sup>50</sup>. This is in agreement with the previous reports, suggesting that coating with CQDs preserves the chlorophyll content in leafy vegetables by acting as a physical barrier for ROS induced oxidative damage<sup>46</sup>.

Another crucial indicator of postharvest quality is enzymatic activity, including key oxidative enzymes such as CAT, PPO, and other oxidases. In fresh cut vegetables, enzymatic browning (PPO), chlorophyll loss, crispiness, water loss/weight loss, and mold are usually more important than TVB-N. Actually, the Mao et al., results on algin on apples gives exactly these areas that aggravate the cutting, visibility/oxidation, and browning<sup>37</sup>. Herein among all treatments, CQD-treated samples exhibited the lowest enzymatic activity, reinforcing their role in reducing oxidative stress and enzymatic browning. In contrast, the control group demonstrated the highest enzyme activity, indicating greater oxidative degradation and increased browning and spoilage. These findings

align with previous research, which has demonstrated that CQDs effectively inhibit oxidative enzyme activity, thereby minimizing enzymatic browning and prolonging the shelf life of fresh vegetables<sup>40,51</sup>.

The enzymatic activities recorded in EO-NC-treated samples exhibited moderate trends, indicating that essential oils possessed some antioxidant properties, albeit with lower effectiveness compared to CQDs. While EO-NCs contributed to reducing enzymatic degradation, their impact was less pronounced, reinforcing the superior oxidative stress mitigation capabilities of CQDs. The stronger enzymatic inhibition observed in CQD-treated samples further highlights their role in minimizing oxidative browning and enzymatic degradation, contributing to extended freshness and postharvest quality preservation.

## Conclusions

This study highlights the transformative potential of nano-packaging technologies in extending the shelf life, nutritional value, and marketability of perishable vegetables like kale. The findings reinforce the dual advantage of these technologies enhancing food quality while promoting sustainability. The superior barrier properties of CQDs and EO-NCs offer a significant advancement over conventional packaging methods, addressing the pressing need for innovative and eco-friendly food preservation strategies. These results emphasize the potential of CQDs and EO-NCs to inspire future innovations in food packaging methodologies, helping the industry meet evolving consumer demands for high-quality, sustainable products.

The study further establishes that CQDs outperformed EO-NCs in reducing weight loss, maintaining chlorophyll content, and preserving antioxidants such as ascorbic acid and phenolic compounds. The exceptional barrier properties of CQDs significantly reduced water vapor transmission, minimized oxidative stress, and inhibited microbial growth, making them the most effective nano-packaging solution. While EO-NCs provided moderate protection, they show potential as a complementary or alternative preservation technique. Additionally, both CQDs and EO-NCs contributed to enhanced visual appeal, maintaining lightness ( $\Delta L$ ) and reducing hue angle shifts, which are critical for consumer acceptance. These technologies demonstrate significant promise in extending the freshness and nutritional stability of fresh-cut kale, ensuring higher quality produce for longer periods.

Despite the success achieved, there are limitations imposed in this study, which are considered significant. First, the release of CQDs, EO-NCs, and their migratory abilities were not carried out under the permissible conditions of food contact in relation to studying the risks imposed by these samples. Second, although the capability of both the microbial inhibiting agent and the biochemical medium has been confirmed, the fact remains that the sensory evaluation has not yet been conducted, which could make it impossible to interpret the study regarding the pleasantness of the odor, taste, and appearance of the food. Moreover, considering the fact that the conducted study took place at a laboratory, the different manner of refrigerating, the shape of the packages, and the process of handling could make a difference. Additionally, the cost-effectiveness of CQDs and EO-NC mass production cannot be clarified either.

The outcome of this work shows a great potential for active packaging consisting of CQD and EO-NCs within refrigerated chains for fresh cut produce. Further studies should aim for more scalable methods for preparing these materials using carbon sources from biomass, which should then improve costs and eco-friendliness of production. These materials might easily be incorporated with existing packaging materials and methods, for example, pads, sachets, and spraying applications. Detailed migration analyses, in vivo toxicology testing, and techno-economic analyses should also be conducted as further steps towards approval and commercialization. Notably, with growing consumer interest in sustainable and chemical-free preservation methods, the emergence of CQDs and EO-NCs offers hope for the next generation of active packaging materials.

## Data availability

The datasets generated and analysed during the current study supported the PhD thesis and are not publicly available because of the policy of the university before final acceptance.

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### Author contributions

FM: Conceptualization, Methodology, Investigation, Visualization, Funding acquisition, Writing – original draft, RE: Resources, Supervision, Project administration, Writing - review, WW: Data curation, Conceptualization, Methodology, Investigation, Software, Validation, Writing - review & editing, OK: Formal analysis, Methodology.

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### Declarations

### Competing interests

The authors declare no competing interests.

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