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Early physiological and antioxidant responses of amla (*Phyllanthus emblica* L.) to short-term CO_2 exposure

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ABSTRACT

Carbon dioxide (CO_2) is widely used in post-harvest storage systems, yet the immediate biochemical responses of fruit tissues to short-term exposure remain poorly understood. This study investigated the early physiological and biochemical responses of amla (*Phyllanthus emblica* L.) fruit following brief and elevated CO_2 exposure. Whole fruits were treated with 5 kPa CO_2 for 5, 10, and 15 minutes, after which biochemical, antioxidant, and structural parameters were evaluated. Ascorbic acid content increased from 370.83 to 427.23 mg/100 g after 5 minutes of CO_2 exposure and further increased to 458.00 mg/100 g after 15 minutes. Radical scavenging activity increased from 37.89% in the control to 74.35% after 5 minutes of exposure. Total phenolic content exhibited a transient increase, whereas carbohydrate content showed an initial decline followed by partial recovery. Short-term CO_2 exposure also induced transient increases in hydrogen peroxide levels and ascorbate peroxidase activity. Microscopic analysis revealed apparent cellular swelling, while moisture content, Fourier Transform Infrared Spectroscopy spectral profiles, and Commission Internationale de l'Éclairage colour parameters remained largely unchanged following short-term CO_2 exposure. The findings suggest that short-term CO_2 exposure elicits antioxidant-related biochemical responses in amla fruit while producing only minor changes in the measured physicochemical attributes.

1. INTRODUCTION

Carbon dioxide (CO_2) plays a fundamental role in plant physiology by regulating photosynthesis, carbon metabolism, and cellular homeostasis. In postharvest systems, elevated CO_2 (eCO_2) is widely employed in controlled-atmosphere (CA) storage and modified-atmosphere packaging (MAP) to suppress respiration, delay senescence, reduce microbial growth, and preserve the quality of fresh fruits and vegetables. In addition to these physiological effects, increasing evidence indicates that eCO_2 influences antioxidant metabolism and cellular redox balance. A meta-analysis involving multiple vegetable species reported significant increases in ascorbic acid, phenolic compounds, flavonoids, and total antioxidant capacity under eCO_2 conditions [1]. Similarly, studies in horticultural crops such as strawberry and tomato have demonstrated that CO_2 exposure modifies antioxidant enzyme activities and redox-related metabolites [2,3]. However, these studies have largely examined prolonged or continuous CO_2 exposure, whereas the immediate physiological and biochemical responses to short-term CO_2 treatment in whole fruits remain poorly understood.

Amla (*Phyllanthus emblica* L.), commonly known as Indian gooseberry, is an economically important fruit valued for its exceptionally high ascorbic acid content and diverse bioactive phytochemicals. The fruit is rich in gallic acid, ellagic acid, hydrolysable tannins, and other phenolic compounds that contribute to its remarkable antioxidant capacity and therapeutic properties [4,5]. Consequently, amla has gained considerable importance in the food, nutraceutical, and pharmaceutical industries [6]. Despite its nutritional significance, fresh amla is highly perishable and undergoes rapid physiological deterioration after harvest. Moisture loss, tissue softening, progressive losses in ascorbic acid and antioxidant capacity, changes in colour and tissue structure, and oxidative stress all of which result in postharvest losses estimated to reach one-third of the harvested crop [7]. Under ambient storage conditions, the fruit generally remains marketable for only 5–6 days, during which physiological loss in weight increases while firmness, titratable acidity, and ascorbic acid content progressively decline [8–10].

To extend storage life, several postharvest technologies have been investigated for amla, including low-temperature storage, low-density polyethylene packaging, MAP, edible coatings, and other postharvest treatments [11–13]. Among these approaches, eCO_2 has attracted considerable attention because of its ability to retard senescence, suppress respiration, reduce physiological disorders, and maintain fruit quality during storage [14,15]. Beyond these beneficial effects, eCO_2 has also been associated with changes in antioxidant metabolism and cellular redox status [16–18], suggesting that CO_2 may function as a physiological signal capable of activating stress-responsive metabolic pathways [19]. Since cellular signalling events generally

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precede long-term metabolic adaptations, understanding the early responses induced by CO_2 exposure may provide valuable insight into the mechanisms underlying postharvest quality preservation [20].

In commercial postharvest handling, fruits are frequently subjected to transient exposure to eCO_2 during gas flushing, establishment of CA conditions, MAP, transportation, and storage transitions [21,22]. Although these exposures are relatively brief, they may be sufficient to initiate rapid physiological and biochemical responses before longer term acclimation occurs [19,23,24]. Nevertheless, little information is available regarding the immediate effects of short-duration CO_2 exposure on antioxidant metabolism in amla [17]. To date, studies on eCO_2 have predominantly focused on prolonged storage or continuous atmospheric enrichment [19,23], while the biochemical responses occurring within minutes of CO_2 exposure remain largely unexplored [20].

We hypothesize that brief exposure to eCO_2 acts as a mild abiotic stimulus that could induce rapid antioxidant responses and stimulate ascorbic acid accumulation through transient oxidative signalling without causing significant physicochemical deterioration of amla fruit. Since oxidative deterioration in amla is characterized by the decline of ascorbic acid, antioxidant capacity, tissue firmness, and overall fruit quality, these parameters were selected to evaluate the early physiological response to CO_2 exposure. Therefore, the objective of the present study was to investigate the immediate physiological and biochemical responses of amla fruit following short-duration (5–15 minutes) CO_2 exposure. This study provides new insight into the instantaneous biochemical responses of amla fruit to transient eCO_2 exposure.

2. MATERIALS AND METHODS

2.1. Sample Preparation

Commercially mature amla (*P. emblica* L.) fruits (Neelam cultivar) were purchased from a local fruit market in Yendada, Visakhapatnam, Andhra Pradesh, India, and stored at room temperature (29°C–30°C) until further processing. They were subjected to surface decontamination using a 1% (w/v) sodium hypochlorite solution, followed by sequential rinsing with sterile deionized water and ambient air-drying. Atmospheric CO_2 treatments were conducted within a hermetically sealed, custom-engineered expanded polystyrene chamber (20 × 20 × 15 cm) at room temperature. The system was equipped with a gas inlet port coupled to a medical-grade CO_2 cylinder and monitored via a calibrated portable infrared gas analyser. Throughout the experimental duration, a constant CO_2 partial pressure of 5 kPa was maintained. Whole fruits were treated with CO_2 for transient intervals of 5, 10, and 15 minutes. These short exposure durations were selected to evaluate immediate biochemical responses to CO_2 treatment. Following each exposure, the fruits were immediately processed by removing the seeds and isolating the mesocarp (pulp) tissue for biochemical characterization. Control fruits were maintained under identical conditions within the sealed chamber under ambient atmospheric CO_2 (~0.04 kPa).

2.2. Microscopic Examination

To evaluate the impact of transient exposure on cellular architecture, thin transverse sections of the amla mesocarp were prepared via manual microtomy using a sterilized surgical blade. The sections were subjected to histological staining with Eosin Y (0.5% w/v), and excess dye was removed through sequential rinsing with distilled water. Microscopic characterization was performed using a Magnus MLX Plus LED light microscope at 10× and 40× optical magnifications.

2.3. Estimation of Ascorbic Acid (Vitamin C)

Fresh mesocarp tissue was homogenized to a fine pulp. The resulting homogenate was filtered, and the filtrate was brought to a final volume of 100 ml using 4% (w/v) oxalic acid. For the assay, 5 ml aliquots of the diluted sample were acidified with 10 ml of 4% oxalic acid and titrated against a freshly prepared 2,6-dichlorophenolindophenol solution. The titration was conducted until the achievement of a persistent light pink endpoint (lasting ≥15 seconds). A primary standard of L-ascorbic acid (1 mg/ml in 4% oxalic acid) was utilized to calibrate the reagent and determine the dye factor (mg of ascorbate equivalent to 1 ml of dye). Ascorbic acid was expressed as mg/100 g fresh weight (FW) and calculated using:

$$\text{Vitamin C (mg/100 g)} = \frac{D \times V \times DF}{A \times W} \times 100$$

Where D is the dye factor (mg ascorbic acid/ml dye), V is the titre value of sample (ml), DF is the dilution factor, A is the aliquot volume analysed (ml), W is the sample weight (g) [25].

2.4. Total Phenolic Content

Total phenolic content (TPC) was estimated using the Folin–Ciocalteu colorimetric assay with minor modifications. Fresh mesocarp tissue was homogenized and extracted in methanol at a 1:10 (w/v) ratio for 1 hour at ambient temperature. The resulting homogenate was filtered to obtain a clarified methanolic extract. A 1 ml aliquot of this extract was combined with 1 ml Folin–Ciocalteu reagent. After an equilibration period of 5 minutes, the mixture was neutralized by the addition of 2 ml of 2.5% (w/v) sodium carbonate (Na_2CO_3). After 120 minutes of dark incubation at room temperature, absorbance was measured at 760 nm using a UH5300 spectrophotometer. A standard calibration curve was generated using gallic acid concentrations (10–100 µg/ml), and the results were expressed as milligrams of gallic acid equivalents per gram of FW (mg GAE/g FW). TPC was calculated using the following formula:

$$\text{TPC} = \frac{C \times V}{M}$$

where C (mg/ml) = phenolic concentration, which was obtained from the standard curve graph, V (ml) = volume of the extract, and M (g) = mass of the sample [26].

2.5. Total Carbohydrate Content

Fresh mesocarp tissue (1 g) was homogenized and subjected to acid hydrolysis using 2.5 N hydrochloric acid (5 ml) in a boiling water bath for 3 hours. Following hydrolysis, the reaction mixture was cooled to ambient temperature and carefully neutralized with Na_2CO_3 [2.5% (w/v) Na_2CO_3] until effervescence ceased. The hydrolysate was filtered, and the filtrate was brought to a final volume of 100 ml with distilled water. For the colorimetric assay, 1 ml of 5% (w/v) phenol solution and 5 ml of concentrated sulfuric acid (98%) were added to 1 ml of the filtrate and incubated at ambient temperature in the dark for 10 minutes, followed by a secondary incubation at 30°C for 20 minutes. Absorbance was measured at 490 nm using a UH5300 spectrophotometer. A standard calibration curve was constructed using D-glucose concentrations ranging from 0 to 100 µg/ml, and results were expressed as a percentage (%) on a FW basis [27].

2.6. 2,2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Assay

Fresh mesocarp tissue was macerated in absolute methanol at a 1:10 (w/v) ratio and filtered. Methanolic extract (5 ml) was combined with 1 ml of 0.3 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution prepared in methanol. The reaction mixture was incubated in the dark at ambient temperature for 30 minutes, and absorbance was measured at 518 nm using a UH5300 spectrophotometer. The percentage of DPPH radical scavenging activity (SA%) was calculated using the following equation:

$$SA\ (\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

Where $A_{control}$ = absorbance values of the control (DPPH plus solvent without sample extract) and A_{sample} = absorbance values of the samples [26].

2.7. Estimation of Hydrogen Peroxide

Fresh tissue (0.5 g) was homogenized in 5 ml of ice-cold 0.1% (w/v) trichloroacetic acid using a pre-chilled mortar and pestle. The homogenate was centrifuged at 10,000 × g (4°C) for 10 minutes to isolate the clarified supernatant. For the analytical reaction, 0.5 ml of the supernatant was combined with 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI. Following a 10-min incubation in darkness, the absorbance was recorded at 390 nm using a UH5300 spectrophotometer. Concentrations were quantified using a standard calibration curve of reagent-grade hydrogen peroxide (H_2O_2) and expressed as µmol/g FW [28].

2.8. Ascorbate Peroxidase Activity Assay

Fresh mesocarp tissue (0.5 g) was homogenized in 5 ml of ice-cold 50 mM potassium phosphate buffer (pH 7.0) supplemented with 0.1 mM Ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 12,000 × g (4°C) for 15 minutes. The assay was performed using 0.1 mM H_2O_2 in a 3 ml reaction volume consisting of 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM L-ascorbate, 0.1 mM EDTA, and 0.1 ml of the protein extract. The enzymatic rate was quantified by measuring the decrease in absorbance at 290 nm for 1 minute, which corresponds to the stoichiometric consumption of the ascorbate substrate (ε = 2.8 mM/cm). One unit of ascorbate peroxidase (APX) activity was defined as the amount of enzyme required to oxidize 1 µmol of ascorbate per min (expressed as µmol ascorbate oxidized min/g FW) [29].

2.9. pH

Following the standard protocol of the Food Safety and Standards Authority of India [30], 10 g of fruit pulp was accurately weighed and dispersed in 100 ml of distilled water. The mixture was then pulverized and filtered to obtain a clear extract and maintained at 28˚C while measuring pH using a calibrated digital pH meter (Adwa AD1000 pH/mV).

2.10. Moisture Analysis

The moisture content of the fruit samples was determined using a Radwag MA 50/1. R.WH halogen moisture analyser. For each analysis, ~3 g of the sample was distributed evenly on the sample pan to ensure uniform heat exposure. The determination was conducted at a drying temperature of 99°C until a consistent weight was achieved.

2.11. Fourier Transform Infrared Analysis

Samples were macerated at a 1:10 ratio (w/v) for 2 hours at room temperature, and then filtered to prepare clear extracts for analysis. The spectral data were recorded using a Bruker Alpha II ATR-FT-IR spectrometer equipped with a zinc selenide Attenuated Total Reflectance crystal. Spectra were collected over the range of 4,000–500 cm^{-1}, at a resolution of 4 cm^{-1} by averaging 32 scans for each sample.

2.12. Commission Internationale de l'Éclairage (CIELAB) Colour Analysis

Fruit was sliced into uniform sections (~5 mm thick) to ensure a flat surface for measurement. Colour coordinates were recorded using a Hunterlab ColorFlex EZ colorimeter, calibrated and operated under standard illuminant D65 and a 10° standard observer. Measurements were taken at three randomly selected points on each slice, and the mean values were calculated for further analysis. The total colour difference (ΔE_{ab}) between the control and eCO_2-treated samples was calculated using the following equation:

$$\Delta E_{ab} = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2}$$

here, L_1^*, a_1^* and b_1^* indicate the colour values of the control, while L_2^*, a_2^* and b_2^* indicate the colour values of the CO_2-treated samples.

2.13. Multivariate Statistical Analysis

The experiment consisted of four treatments with three independent biological replicates per treatment. Each biological replicate comprised three fruits processed together under identical treatment conditions. Data are presented as the mean ± SD. Statistical analyses were performed using XLSTAT (Addinsoft, Paris, France), integrated with Microsoft Excel (MS Office 2021). Differences among treatments were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons. Statistical significance was established at $p < 0.05$.

For multivariate analysis, for each treatment, individual replicate values of all measured physicochemical and biochemical variables, including ascorbic acid content, TPC, DPPH radical scavenging activity, H_2O_2 content, APX activity, moisture content, pH, colour parameters, and other measured quality attributes, were subjected to *Z*-score standardization. Standardization centred the data to a mean of zero and scaled them to unit variance to ensure equal weighting of all variables.

The standardized dataset consisting of four observations (control, 5, 10, and 15 minutes CO_2 treatments) and the corresponding measured variables was used for heat-map visualization and Pearson correlation analysis. Principal component analysis (PCA) was performed using the same standardized data matrix in XLSTAT to reduce dimensionality and visualize relationships among treatments and variables. PCA score plots were used to assess patterns among treatments, while loading plots were used to evaluate the contribution of individual variables to the principal components.

3. RESULTS AND DISCUSSION

3.1. Microscopic Structural Response of Amla Tissue to Short-term CO_2 Exposure

Microscopic analysis was performed to evaluate early cellular alterations induced by short-term CO_2 exposure. Microscopic

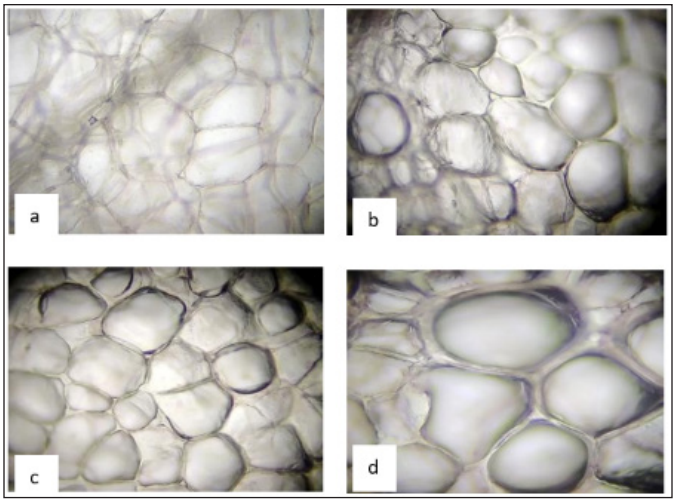


Figure 1. Microscope images of amla tissue at 40 × magnification under a light microscope. Representative light micrographs of transverse sections of amla (*Phyllanthus emblica* L.) fruit following short-term CO_2 exposure (control, 5, 10, and 15 minutes). Images were acquired using a light microscope at 40× magnification. The micrographs illustrate general tissue organization. a, Control; b, 5 minutes exposure; c. 10 minutes exposure; d, 15 minutes exposure.

examination of control samples revealed normal parenchymal cell morphology with well-defined cell boundaries and uniform cytoplasmic distribution (Fig. 1). In contrast, CO_2-treated samples exhibited noticeable cellular swelling, which may be associated with rapid gas diffusion and transient osmotic adjustments within the tissue matrix. Unlike oxygen, CO_2 can diffuse efficiently through both intercellular spaces and the intracellular liquid phase, enabling rapid interaction with the cellular environment [31]. eCO_2 treatments have been reported in other horticultural commodities to help maintain cell wall–plasma membrane association, preserve membrane integrity, and reduce structural deterioration during postharvest storage, thereby contributing to improved tissue quality [32].

3.2. Effect of CO_2 Exposure on Ascorbic Acid Content

Short-duration exposure to eCO_2 significantly increased the ascorbic acid content of amla samples (Fig. 2a). Mean vitamin C content increased progressively from 370.83 ± 1.54 mg/100 g in the control to 427.23 ± 1.52, 439.02 ± 1.01, and 458.00 ± 2.04 mg/100 g following 5, 10, and 15 minutes of CO_2 exposure, respectively. One-way ANOVA showed a highly significant effect of CO_2 treatment on ascorbic acid levels ($F = 1693.42$, $p = 1.5 \times 10^{-11}$), while Tukey's HSD test confirmed significant differences among treatment groups, demonstrating a positive exposure-time-dependent increase in ascorbic acid content within the investigated range. The progressive reduction in the rate of increase at longer exposure durations indicates a transient metabolic response that may reflect an equilibrium between biosynthesis, utilization, and antioxidant regulation under short-term CO_2 stress [23,33–35].

3.3. Effect of CO_2 Exposure on TPC

The TPC of amla exhibited a transient increase following short-term CO_2 exposure (Fig. 2b). Control samples showed a baseline TPC of ~6.01 mg GAE/g. After 5 minutes of exposure, TPC increased to 6.28 mg GAE/g. However, with longer exposure durations, phenolic levels gradually declined toward baseline values, reaching 6.07 mg GAE/g at 10 minutes and 6.03 mg GAE/g at 15 minutes. The initial increase in TPC suggests activation of stress-responsive phenolic metabolism under short-term CO_2 exposure [36,37]. Similar increases in phenolic accumulation under eCO_2 conditions have been reported in several fruit systems [38,39]. The gradual decline observed at longer exposure durations may indicate partial metabolic adjustment or utilization of phenolic compounds during the maintenance of cellular stability under continued gas exposure [39,40].

3.4. Effect of CO_2 Exposure on Total Carbohydrate Content

Total carbohydrate content (TCC) in amla exhibited a transient decline followed by partial recovery during short-term CO_2 exposure (Fig. 2c). Control samples showed a baseline carbohydrate content of 6.577%. After 5 minutes of exposure, carbohydrate levels decreased significantly to 6.389%. With increasing exposure duration, carbohydrate content partially recovered to ~6.54% at 10 minutes and stabilized at 6.41% after 15 minutes. Similar transient alterations in sugar metabolism under eCO_2 conditions have been reported in other horticultural products, including strawberries, where short-term CO_2 exposure influenced glucose, fructose, and sucrose accumulation [23,35].

3.5. Effect of CO_2 exposure on antioxidant scavenging activity

Amla fruit possesses a strong natural antioxidant system comprising ascorbic acid, phenolic compounds, and other antioxidant metabolites that contribute to its radical scavenging capacity [4,41]. In the present study, the radical scavenging activity of the control sample was 37.89%. Following 5 minutes of CO_2 exposure, scavenging activity increased markedly to 74.35% (Fig. 2d). With increasing exposure duration, the activity gradually declined to 70.17% at 10 minutes and 67.88% at 15 minutes; however, the values remained substantially higher than those of the control samples. This response corresponded with the heightened ascorbic acid and TPC observed in Sections 3.1. and 3.3., indicating coordinated enhancement of antioxidant metabolism [23]. Similar increases in antioxidant capacity under eCO_2 conditions have been reported in several plant systems [1]. Antioxidant enzymes such as APX may contribute to the regulation of reactive oxygen species generated during CO_2 exposure [42].

3.6. Effect of CO_2 Exposure on H_2O_2 Levels

Short-term CO_2 exposure induced a transient increase in H_2O_2 levels in amla tissue (Fig. 2e). Control samples exhibited comparatively low baseline H_2O_2 levels, whereas exposure for 5 minutes resulted in a marked increase in H_2O_2 accumulation. At longer exposure durations (10 and 15 minutes), H_2O_2 levels showed a tendency toward stabilization. In plant stress physiology, H_2O_2 functions not only as a reactive oxygen species but also as an important signalling molecule involved in the activation of defence-related pathways [37,43]. The transient increase observed after 5 minutes of exposure may therefore contribute to the induction of antioxidant responses, including the enhanced ascorbic acid accumulation and antioxidant scavenging activity observed in Sections 3.1. and 3.5. [23,37]. The subsequent stabilization of H_2O_2 levels at longer exposure durations suggests partial restoration of redox balance [42–44]. These findings indicate that short-term CO_2 exposure induces a controlled oxidative response rather than severe oxidative damage, supporting the role of CO_2 treatment as a mild abiotic stress stimulus in amla fruit.

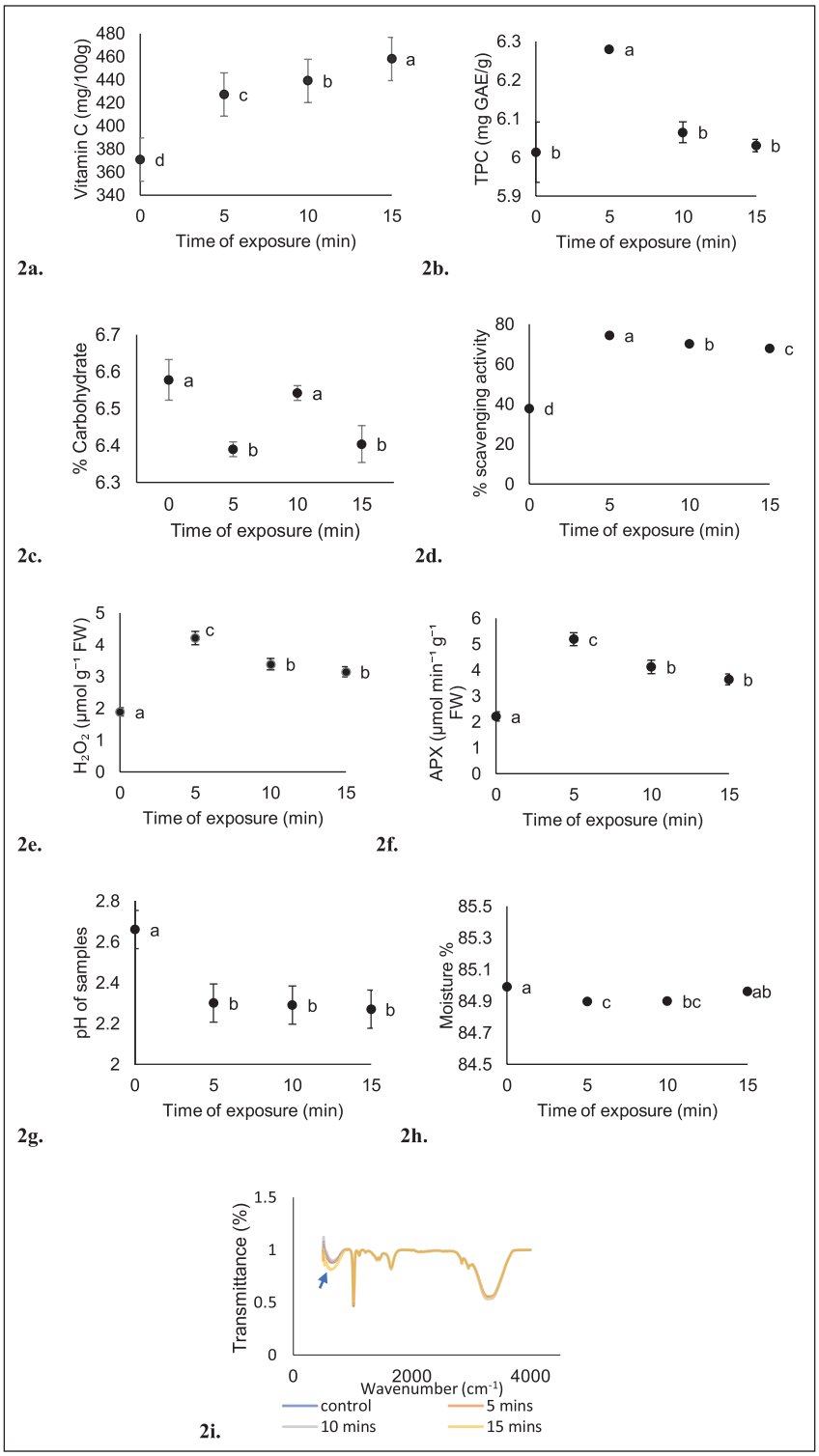


Figure 2. Measures of physicochemical parameters to quantify the effect of CO_2 exposure. Effect of short-term CO_2 exposure (control, 5, 10, and 15 minutes) on various physicochemical and biochemical parameters of amla (Phyllanthus emblica L.) fruits. Data are presented as the mean (n = 3), and error bars represent the SD. Different lowercase letters indicate significant differences among treatments according to one-way ANOVA followed by Tukey's HSD test at $p < 0.05$. a, Ascorbic acid content; b, TPC; c, TCC; d, scavenging activity; e, H_2O_2 content; f, APX activity; g, pH values; h, moisture content; 2i, FT-IR spectra (4,000–500 cm-1) of control (0 minutes) and CO_2-treated samples following 5, 10, and 15 minutes exposure.

3.7. Effect of CO_2 Exposure on APX Activity

Modulation of antioxidant metabolism under eCO_2 exposure in several horticultural commodities is well reported [41,45]. In the present study, APX activity increased significantly following CO_2 exposure, reaching a maximum at 5 minutes of treatment that corresponded with the observed increase in H_2O_2 levels (Fig. 2f). With longer exposure durations, APX activity showed a tendency toward stabilization while remaining higher than the control

Table 1. CIELAB colour analysis.

Attributes	Control	5 minutes	10 minutes	15 minutes
L*	49.03 ± 0.01	47.77 ± 0.03	48.45 ± 0.008	48.38 ± 0.04
a*	−1.24 ± 0.03	−1.46 ± 0.011	−1.39 ± 0.002	−1.04 ± 0.023
b*	3.53 ± 0.06	4.68 ± 0.02	3.37 ± 0.01	4.23 ± 0.07
ΔE_{ab}	-	1.72	0.62	0.98

Values are expressed as the mean ± SD for L* (lightness), a* (red–green axis), and b* (yellow–blue axis).

samples. APX plays an important role in the detoxification of H_2O_2 by utilizing ascorbate as an electron donor [42,46,47]. Similar increases in antioxidant enzyme activity under eCO_2 conditions have been reported in other plant systems, where APX contributes to the maintenance of redox homeostasis and protection against oxidative damage [32,48].

3.8. Effect of CO_2 Exposure on pH

The untreated amla samples exhibited a pH of 2.66, which is consistent with the naturally high acidity reported for the fruit [49,50]. Following CO_2 exposure, a gradual decrease in pH was observed across all treatment durations, indicating a mild increase in acidity (Fig. 2g). The dissolution of CO_2 within the aqueous cellular environment to form carbonic acid (H_2CO_3), which subsequently dissociates into bicarbonate ions and protons, could have contributed to the reduction in pH ($CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$) [50]. Similar reductions in pH under eCO_2 conditions have been reported in plant tissues and post-harvest systems [51,52].

3.9. Effect of CO_2 Exposure on Moisture Content

The moisture content of the control sample was 84.99%, while CO_2-treated samples treated for 5, 10, and 15 minutes showed values of 84.897%, 84.900%, and 84.963%, respectively (Fig. 2h). The moisture content remained within a narrow range across all exposure durations, indicating that short-term CO_2 treatment did not induce significant water loss or net water gain within the fruit tissues. Similar observations have been reported in previous studies, where eCO_2 treatments were able to maintain moisture stability and reduce dehydration-related deterioration in horticultural products [53,54]. The stability of moisture levels observed in the present study further indicates that the biochemical and antioxidant responses induced by CO_2 exposure were unlikely to be attributable to concentration effects arising from tissue dehydration.

3.10. Effect of CO_2 Exposure Using FT-IR Spectral Analysis

FT-IR spectroscopy was performed to evaluate whether short-term CO_2 exposure induced structural or chemical alterations in amla tissue. Spectra recorded within the range of 3,500–500 cm^{-1} showed highly similar peak patterns and absorption profiles for both control and treated samples (Fig. 2i). No major peak shifts or formation of new absorption bands were observed following treatment, indicating that the overall chemical composition of the fruit matrix remained largely unaffected by short-term CO_2 exposure. The spectra exhibited characteristic absorption bands corresponding to major biomolecular constituents of amla fruit. The broad band between 3,500 and 3,200 cm^{-1} was associated with O–H stretching vibrations of water, sugars, and phenolic compounds [55]. Peaks near 2,924 and 2,853 cm^{-1} corresponded to asymmetric and symmetric C–H stretching vibrations of aliphatic groups commonly present in carbohydrates and lipids [56]. The absorption band around 1,730 cm^{-1} was attributed to C=O stretching vibrations of organic acids, esters, and pectic substances [55,57]. In addition, the fingerprint region between 1,200 and 900 cm^{-1} contained intense bands related to C–O and C–C stretching vibrations associated with mono- and polysaccharides [58]. However, the absence of major spectral shifts across the remaining regions suggests that no substantial alterations occurred in the major biochemical constituents of the fruit under the conditions evaluated. These findings indicate that the CO_2 treatment induced only transient biochemical responses.

3.11. Effect of CO_2 Exposure Using CIELAB Colour Analysis

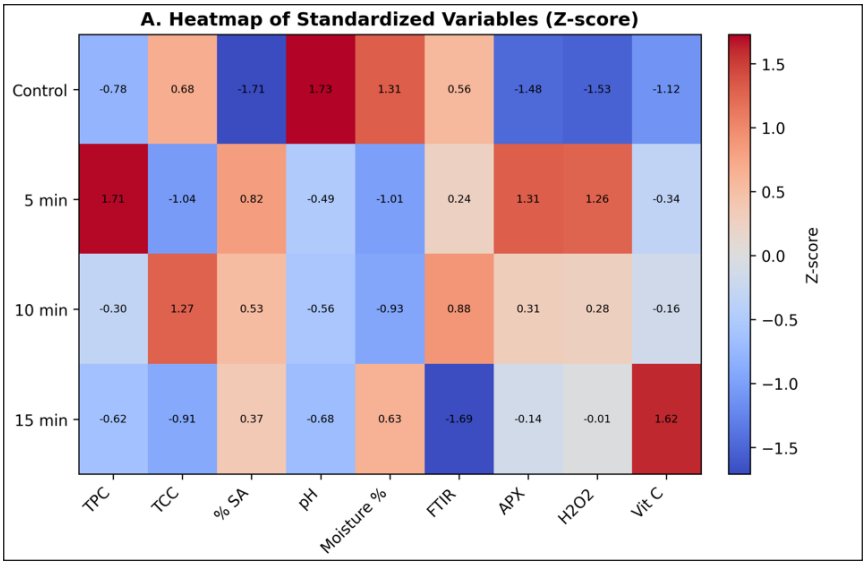
The CIELAB colour parameters of amla fruits following CO_2 exposure are presented in Table 1. The ΔE_{ab} relative to the control ranged from 0.62 to 1.72 across the different exposure durations. According to established perceptibility thresholds, ΔE_{ab} values below 1.0 are generally considered visually imperceptible, whereas values between 1.0 and 2.0 are only slightly perceptible under close observation, although these thresholds can vary depending on the food matrix and viewing conditions [59]. Thus, the colour changes are unlikely to adversely affect the visual appearance or consumer acceptability of the fruit. Similar findings have been reported in post-harvest studies where CA treatments effectively maintained colour quality and limited oxidative deterioration in fresh produce [60,61].

3.12. Multivariate Heat Map Analysis of Treatment-Induced Characteristics

Multivariate analysis was performed to evaluate the overall relationship between CO_2 exposure duration and the biochemical responses of amla fruit. The heat map of *Z*-score standardized variables (Fig. 3a) showed that the 5 minutes treatment exhibited relatively higher values for TPC, scavenging activity, APX activity, and H_2O_2 levels compared with the other treatments. The pattern is consistent with the enhanced antioxidant response observed in the individual biochemical analyses [23,37]. In contrast, the 15 minutes treatment was associated with increased ascorbic acid content [33,35].

The Pearson correlation matrix (Fig. 3b) is suggestive of a positive association among scavenging activity, APX activity, and H_2O_2 levels. These relationships propose the role of APX in the detoxification of H_2O_2 using ascorbate as an electron donor [42,46]. TPC also showed positive correlation with antioxidant-related parameters, suggestive of the contribution of phenolic compounds to the overall antioxidant capacity of the fruit [36]. In contrast, vitamin C showed a negative correlation with pH and Fourier Transform Infrared Spectroscopy (FT-IR) transmittance, indicating an inverse association among these variables under the experimental conditions evaluated.

The PCA biplot (Fig. 3c) provided an exploratory visualization of the relationships among the treatment means and the measured variables, with the first two principal components accounting for 88.9% of the total variance. The 5 minutes treatment was positioned in proximity to antioxidant-related variables, including APX, H_2O_2, TPC, and scavenging activity, whereas the 15 minutes treatment was positioned closer to vitamin C. The control treatment was associated with pH and moisture content. These observations are consistent with the trends obtained from the individual biochemical analyses under the present experimental conditions. Similar transient oxidative adjustments have been reported in horticultural commodities treated with modified atmospheric conditions, where moderate reactive oxygen species accumulation functions as a signalling mechanism that stimulates antioxidant metabolism and stress acclimation [46,48].



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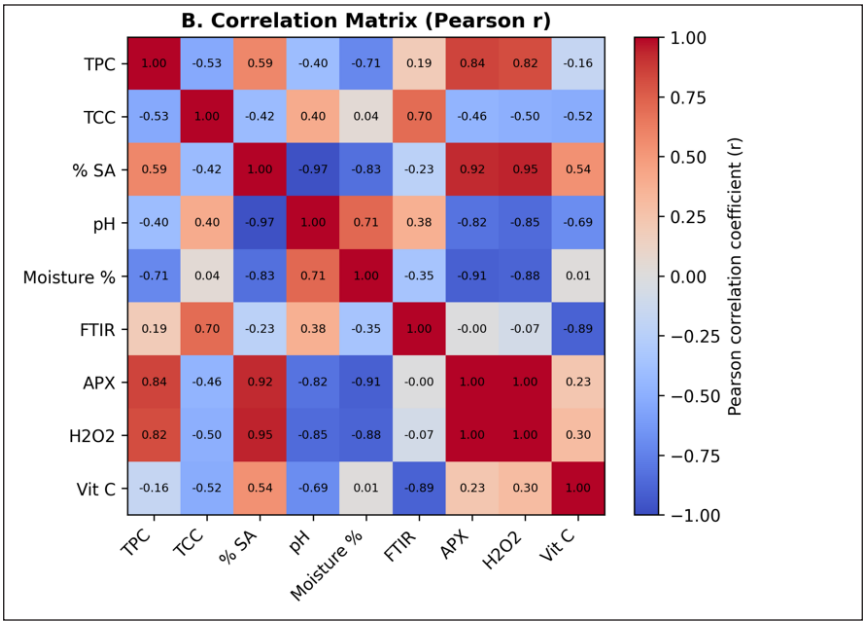

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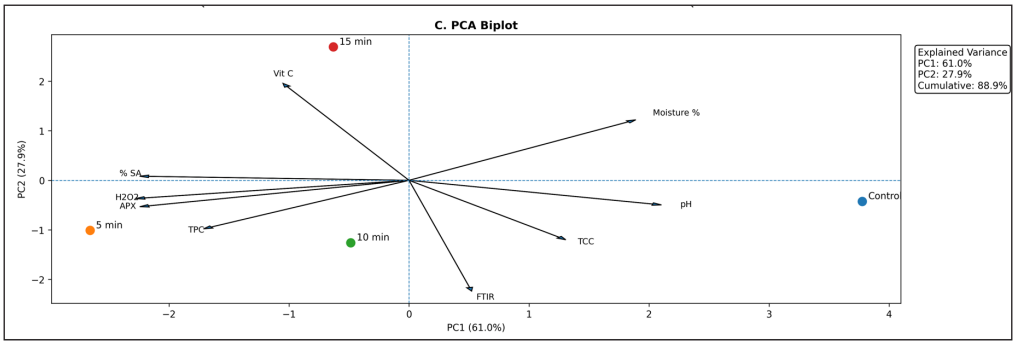

3c.

Figure 3. Multivariate analysis of physicochemical and antioxidant parameters of CO_2-treated amla fruit. a, Heat map of *Z*-score-standardized physicochemical and antioxidant variables. Darker shades indicate relatively higher values, whereas lighter shades indicate lower values relative to the mean. b, Pearson correlation matrix of physicochemical and antioxidant parameters. Positive correlations are represented by darker shades, and negative correlations by lighter shades. c, The PC1 and PC2 of the PCA biplot explained 61.0% and 27.9% of the total variance, respectively, with a cumulative explained variance of 88.9%.

4. CONCLUSION

Short-term CO_2 exposure enhanced antioxidant-related biochemical responses in amla fruit while producing only minor changes in the measured physicochemical and structural characteristics. A 5 minutes treatment produced the strongest overall antioxidant response, characterized by increased radical scavenging activity, increased APX activity, transient H_2O_2 accumulation, and higher phenolic content. In contrast, a 15 minutes exposure favoured greater ascorbic acid accumulation, accompanied by only minor changes in colour and molecular characteristics. The limited changes observed in moisture content, FT-IR spectral profiles, and colour parameters suggest that short-term CO_2 exposure produced only minor alterations in the measured characteristics during the treatment period. Furthermore, studies focusing on molecular and transcriptomic responses could help clarify the regulatory mechanisms underlying these antioxidant responses.

5. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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7. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

8. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

9. PUBLISHER'S NOTE

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10. CONFLICTS OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

11. DECLARATION OF GENERATIVE AI

During the preparation of this work, ChatGPT (OpenAI Version 4.0) was used to paraphrase draft content and perform general English language corrections to improve readability.

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