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Modified Atmospheric Packaging of Fresh-Cut Amaranth (*Amaranthus tricolor* L.) for Extending Shelf Life

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Abstract: Fresh-cut vegetables are prone to microbiological contamination and oxygenation during handling and storage. In this study, fresh-cut amaranth was subjected to various gas ratios (5–15% O_2, 5–15% CO_2, 80% N_2) for 12 days. Chlorophyll content, ascorbic acid content, antioxidant enzyme activity, microbial population, and physiological and biochemical indicators were measured to evaluate the impact of atmospheric packaging. Suitable atmospheric packaging could slow the respiration of amaranth, delay the decline in physiological and biochemical characteristics, maintain the antioxidant enzyme activity, promote the sensorics, and prolong the shelf life by 2 days. According to the analysis of the results, modified atmospheric packaging (10% O_2, 10% CO_2, 80% N_2) retarded the decline in fresh-cut amaranth quality, provided effective antioxidative browning, and inhibited *Pseudomonas fluorescens* development.

Keywords: fresh-cut amaranth; modified atmospheric packaging; antioxidation ability; antibacterial ability

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1. Introduction

Amaranth has high nutritional value, containing high levels of iron, calcium, and other minerals, as well as carotene and vitamin C [1]. Amaranth is simple, suitable for all kinds of climatic conditions, and contains lots of high-quality protein and minerals. Selling amaranth is mainly in the form of fresh-cut goods. Fresh-cut vegetables not only preserve their original fresh state but also guarantee their hygiene and safety through processing. The stomata in amaranth leaves are distributed widely and are susceptible to infection by spoilage bacteria [2]. Once microorganisms enter the stomata, they are difficult to remove; furthermore, fresh-cut amaranth undergoes juice loss [3] and intense respiration.

At present, the atmospheric packaging process for storage and preservation is frequently used for vegetables and fruits. Atmospheric packaging is an elegant way of preserving vegetables by controlling the composition of gas in the storage environment, thus reducing the rate of vegetable respiration and metabolism, inhibiting the growth and reproduction of spoilage bacteria and extending the storage period of vegetables [4]. Mudau et al. [5] found that the total antioxidant activity and the flavonoid content of fresh-cut spinach were well maintained at 4 °C in an aerated package (5% O_2, 15% CO_2, 80% N_2). Francisco et al. [6] indicated that a high O_2 atmosphere inhibited microbial growth and effectively suppressed the browning of fresh-cut lettuce. Sajid et al. [7] demonstrated that the free-radical-scavenging activity and antioxidant enzyme activity in litchi were visibly ameliorated during storage in modified atmospheric packaging. Artes et al. [8] found that fresh-cut vegetables with lower O_2 and moderately enriched CO_2 in the atmosphere were shown to delay chlorophyll degradation. Some studies have shown that MAP (20/% O_2,

3% CO_2) can limit the growth of *E. coli* O157:H7 on spinach [9] and MAP (3% O_2, 7% CO_2) reduced the number of *Pseudomonas* on cucumber [10].

These reactions can aggravate the degradation process of the metabolic enzymes associated with fresh-cut amaranth and its infestation by organisms. At this stage, fewer studies were conducted to conserve fresh-cut amaranth by MAP technology. Therefore, it is important to focus on maintaining the shelf-life quality of fresh-cut amaranth in response to the consumer demand for fresh-cut amaranth by finding suitable preservation methods.

2. Materials and Methods

2.1. Treatment Method of Amaranth and Experimental Setup

Amaranth plants were purchased from the Shanghai Dolly Farm fruit and vegetable industrial park (Pudong New District, Shanghai, China). The plantation increased the added value of products and widened the industrial chain of the botanical garden by experiencing vegetable planting methods. The freshly picked amaranth plants were washed and sent to the laboratory for processing within 3 h. Residual pesticides and other impurities were removed using ultrapure water, followed by a secondary washing with 1.8 mg/L ozonated water. Amaranth samples that were not yellowed and free of spots were selected. A 1 cm scalpel was used to cut fresh amaranth at a distance of 8 cm from the stem. The specimens were weighed on sterilized trays (80 g/tray), dried, and placed in BOPP/PE (high-pressure polyethylene plastic) bags (30 × 40 cm), divided into four groups of CK (control), MA1, MA2, and MA3, according to the proportion of gas filled, as detailed in Table 1, before storage at 4 °C for 12 days.

Table 1. Sample grouping according to gas ratio (%).

Group	O_2 (%)	CO_2 (%)	N_2 (%)
CK (air)	21	1	78
MA1	5	15	80
MA2	10	10	80
MA3	15	5	80

Four experimental groups were formed, with eight fresh-cut amaranth samples in each group. While in storage, the gas concentration was checked during periodic checks using the FBI-Dansensor CheckPoint O_2/CO_2 (MR-07825-00, FBI-Dansensor America Inc., Glen Rock, NJ, USA). The samples were packed and stored in 4 °C refrigerated storage conditions featuring different gas ratios, as detailed in Figure 1. Physical and chemical indices such as weight loss, ascorbic acid content, chlorophyll content, bacterial colony count, and antioxidant enzyme activity were measured by sampling in triplicate every 2 days; the average value was recorded.

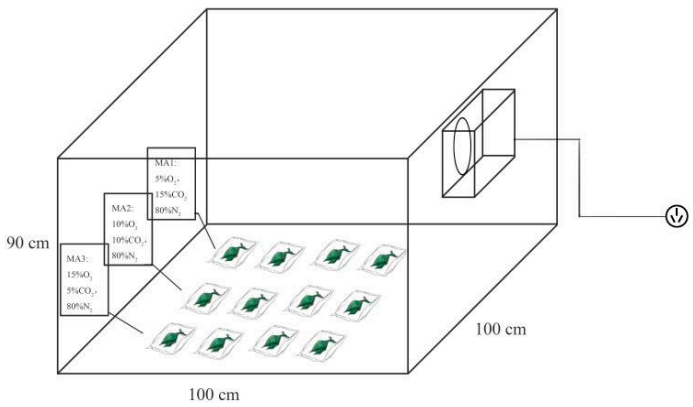

Figure 1. Schematic diagram of experimental setup.

2.2. Sensory Properties

The sensory evaluation panel included 12 experts with sensory experience. Fair and impartial scores were given for color, shape, and odor of fresh-cut amaranth samples. The indices were scored from 0 to 10 (10-point scale), repeating three times, as detailed in Jin et al. [11].

2.3. Weight Loss Rate

The differential method was used as a reference for measurement, and the weight of each treatment at 0 days was recorded, with the corresponding sample mass measured at 2-day intervals throughout storage.

$$\text{Weight loss rate\%} = \frac{X_0 - X_t}{X_0} \times 100\%, \quad (1)$$

where X_0 is the sample mass after pretreatment, and X_t is the sample mass on storage day t.

2.4. Water Distribution and Migration

The water distribution and migration were evaluated according to the method of Zhang et al. [12].

2.5. Soluble Solids

The soluble solid content was determined using a PR-101α digital display whole sugar meter (Shanghai Yuyi Instruments Company). The samples were fully ground and centrifuged at 5000× *g* for 5 min; the supernatant was then extracted and placed in the instrument for evaluation, then the scale reading was taken (%) and the reading was recorded and repeated three times.

2.6. Chlorophyll Content

Chlorophyll content was recorded according to Gutiérrez et al. [13] using a spectrophotometric method with some modifications. First, Leaf tissue of amaranth (5 g) was homogenized in 20 mL of 80% acetone with a tissue homogenizer at 2000× *g* for 30 s. The homogenate was then filtered through a filter paper and centrifuged at 3500× *g* for 10 min. The absorbance of the filtered homogenate was measured in triplicate using a UV-1102 instrument (Tianmei Instrument Co., Ltd., Shanghai, China) at 645 and 663 nm, and the results were expressed as $mg \cdot kg^{-1}$ of chlorophyll on a fresh weight basis.

2.7. Aerobic Plate Count and Specific Spoilage Organism (SSO) Count

This study applied the aerobic plate counting method of Sothornvit et al. [14]. Samples (5 g) were mixed with 45 mL of 0.85% sterile saline, shaken well, and diluted with 0.85% sterile saline in a gradient manner to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} concentrations. Then, the diluted samples were added dropwise onto nutrient agar plates for colony counting under aseptic conditions and cultured at 37 °C for 24 h.

The specific spoilage organism (SSO) count was determined according to the method of Paillart et al. [15].

2.8. Ascorbic Acid (AsA) Content

Ascorbic acid content was measured using 2,6-dichlorophenol indophenol titration according to the method of Bottino et al. [16] with some modifications. First, 1.0 g of sample was ground with 5 mL of oxalic acid (0.05 mol/L) until the tissue fluid was exuded; it was then centrifuged at 5000× *g* for 8 min, and the supernatant was abstracted for analysis.

2.9. Malondialdehyde (MDA) Content

MDA content was determined according to the method of Liu et al. [17] with some modifications. First, 1.0 g of sample was extracted with 2 mL of trichloroacetic acid (TCA)

(0.61 mol/L), before being ground and centrifuged at low temperature for 20 min (5000× *g*, 4 °C). The supernatant's absorbance was then measured at 450 nm, 532 nm, and 600 nm.

2.10. Peroxidase (POD) Activity

POD activity was determined according to the method of Qiao et al. [18].

2.11. Superoxide Dismutase (SOD) Activity

SOD activity was determined according to the method of Guner et al. [19] using nitro blue tetrazolium (NBT) reduction.

2.12. Ascorbate Peroxidase (APX) Activity

APX activity was determined according to the method of Li et al. [20].

2.13. NBT Staining

Amaranth leaves were washed thrice with phosphate-buffered saline (PBS), soaked in DAB staining solution, and placed in dark conditions for decolorization over 4–8 h. 3,3′-Diaminobenzidine (DAB) staining was applied to determine the production of hydrogen peroxide according to the method of Tan et al. [21]. Leaves were immersed in NBT staining solution, excess stain was rinsed with industrial alcohol; they were left in the dark for 6 h. Decolorization was recorded as a function of DAB staining. ROS was confirmed by observing the degree of purple dyeing.

2.14. Data Analysis

All experiments were conducted in triplicate. The results were expressed as the mean ± standard deviation and subjected to one-way analysis of variance (ANOVA), with significant differences between averages considered at $p < 0.05$ and verified by Duncan's multiple range test using the SPSS 17.0 statistical program (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Changes in Sensory Properties

Experts are able to directly detect the quality and status of fresh-cut goods using their senses, representing the main factor controlling their purchase behavior. The sensory evaluation provided an impression of the changes in quality of fresh-cut amaranth samples [22]. As shown in Figure 2, the scores for smell (A), form (B), and color (C) decreased with storage time. CK samples underwent rapid quality deterioration, approaching the sensory threshold on day 8, before losing commercial value. With the exception of MA3 samples, which reached the critical acceptance point on day 10, the other experimental groups maintained commercial value with sensory scores above 5. The results of this experiment showed that modified atmospheric packaging allowed retaining more freshness in comparison to the CK group during the storage period.

3.2. Changes in Weight Loss and Water Migration

The dissipation of moisture is a major factor affecting fresh-cut vegetable quality. As shown in Figure 3A, the weight loss of fresh-cut amaranth increased at a steady rate for all sample groups. The weight loss rate (WLR) is an essential indicator of the wilting, spoilage, and rotting of fruits. This occurred due to the mechanical injury of amaranth leaves following cutting, which accelerated aging and led to material depletion [23]. The MA2 group exhibited the lowest WLR (9.03% on day 10) among all samples, best maintaining the quality of fresh-cut amaranth. Evapotranspiration represents the primary pathway of water loss in fresh-cut vegetables, whereby water is dispersed as water vapor and coalesces inside the bags. The O_2 consumption rate was lower than the CO_2 consumption rate in low-temperature storage, where the CO_2 load dominated. At a CO_2 load of 10%, the physiological metabolism of fresh-cut amaranth was suppressed during storage, thereby curbing the increase in WLR and maintaining the amaranth sample quality. Dite et al. [24]

reached similar conclusions. A 15% higher O_2 content in MA3 might lead to intense leaf physiological activity, resulting in severe water loss.

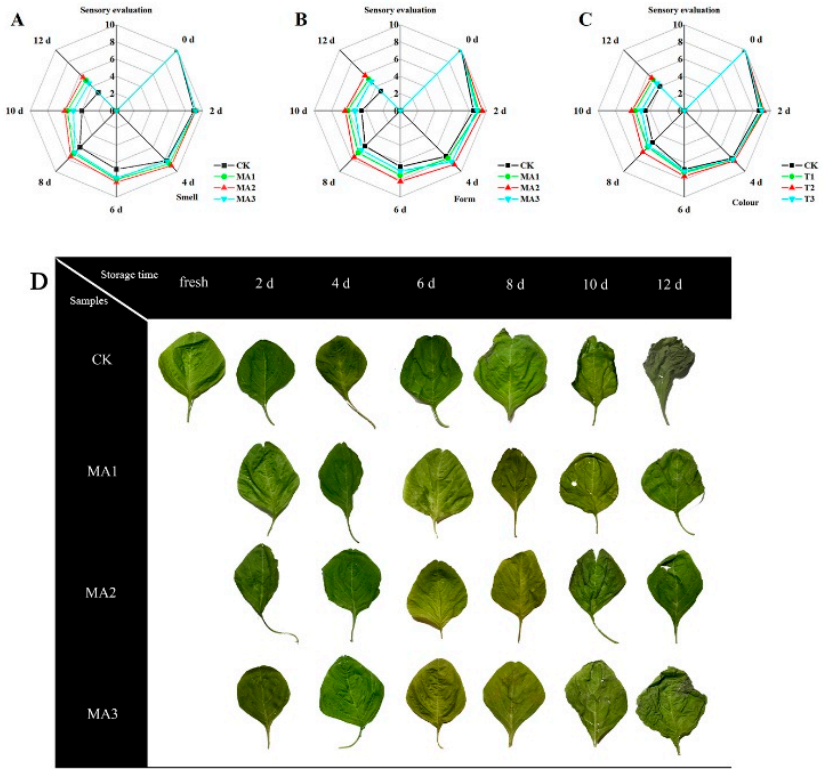

Figure 2. Changes in sensory properties: (**A**) smell; (**B**) form; (**C**) color; (**D**) shelf life. (CK:air, MA1: 5% CO_2 15% O_2, 80% N_2, MA2: 10% CO_2 10% O_2, 80% N_2, MA3: 15% CO_2 5% O_2, 80% N_2).

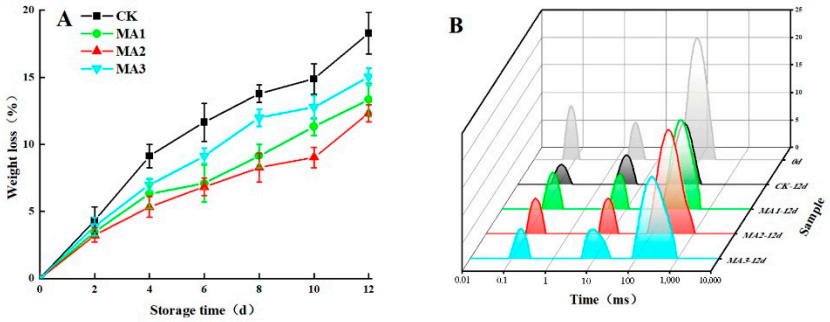

Figure 3. Changes in (**A**) weight loss and (**B**) water migration. (CK:air, MA1: 5% CO_2 15% O_2, 80% N_2, MA2: 10% CO_2 10% O_2, 80% N_2, MA3: 15% CO_2 5% O_2, 80% N_2).

As shown in Figure 3B, the free water content was reduced in all groups by day 12, with the CK group exhibiting the most dramatic decrease in peak area (51.55%), in contrast to the MA2 group (36.25%). This finding was in accordance with the WLR results, confirming the better water retention of fresh-cut amaranth when subjected to atmospheric packaging. The bound water was maintained at a higher level in the treatment groups, indicating that the physiological and metabolic activities in amaranth were still ongoing. The relaxation time was significantly delayed on day 12, which might have been due to the atmospheric packaging effectively extending plant respiration. In summary, the MA2 group performed best in terms of WLR and moisture retention.

3.3. Changes in Soluble Solid Content

The soluble solid content is an indicator of vegetable maturity and metabolic rate. As shown in Figure 4, the soluble solid content of the four groups declined to different degrees as a function of amaranth respiration. The CK group exhibited the most marked drop in levels ($p < 0.05$). The soluble solid content of MA1 samples decreased the most among experimental groups during the early storage period due to the higher CO_2 volume fraction boosting the aerobic respiration of fresh-cut amaranth, while consuming organic matter [25]. On day 12, the MA2 group yielded the finest outcome (3.2%), representing a 40.62% higher level than the CK group. Song et al. [26] confirmed that MAP (80% N_2, 10% O_2, 10% CO_2) treatment effectively delayed the respiration rate of blueberry, decreased its loss of soluble solid content, and prolonged its shelf life. Zhang et al. [27] demonstrated that MAP (85% N_2, 10% O_2, 5% CO_2) treatment successfully alleviated the reduction in soluble solid content in pak choi.

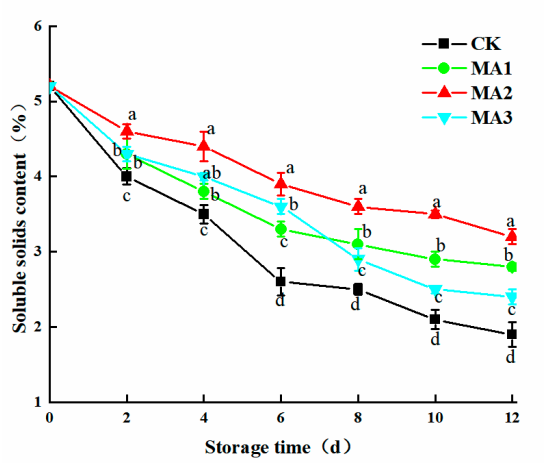

Figure 4. Changes in soluble solid content. (CK:air, MA1: 5% CO_2 15% O_2, 80% N_2, MA2: 10% CO_2 10% O_2, 80% N_2, MA3: 15% CO_2 5% O_2, 80% N_2) Points are means with different lowercase letters (a–d) in figure significantly differ ($p < 0.05$) according to Duncan's multiple range test.

3.4. Changes in Chlorophyll Content

Chlorophyll content serves as a key indicator of color change in vegetables, as it degrades during storage, thus representing a reduction in quality and commercial value. As shown in Figure 5, all groups lost chlorophyll content, with MA2 samples performing best. On day 12, the modified atmospheric packaging groups all exhibited a higher chlorophyll content than the CK group ($p < 0.05$). The 5–10% CO_2 conditions were able to control amaranth respiration to variable degrees, stunting the pre-storage decline in chlorophyll content [28]. Low-oxygen (5% O_2) packaging led to the sample tissues undergoing anaerobic respiration, thereby accelerating senescence during the late storage period [29]. Darani et al. [30] confirmed that chlorophyll content was optimally maintained in spinach under packaging treatment (10% O_2, 10% CO_2), with a further increase in CO_2 level accelerating spinach deterioration. Amin et al. [31] also confirmed this result in their study.

3.5. Aerobic Plate Count and SSO Count

Fresh-cut vegetables suffer tissue damage, thus leading to rapid microbial growth [32]. As shown in Figure 6A, the total bacterial count increased with storage time, with the highest level recorded in the CK group. Atmospheric packaging was able to slow this increase (aerobic plate counts on day 12: MA1, 6.2 log CFU/g < MA2, 6.8 log CFU/g < MA3, 7.6 log CFU/g << CK, 8.5 log CFU/g). Microbial proliferation might depend on a sample's resistance, in addition to the atmospheric environment. A lower O_2 and higher CO_2 level can lead to an imbalance in tissue respiration, changes in anaerobic enzymes, accelerated tissue aging, weakened resilience, and a stimulation of bacterial growth [33]. Chandra

et al. [34] demonstrated that strawberries stored in 15–20% CO_2 lower microbial counts toward the end of storage, in agreement with this experiment. CO_2 is able to reduce inner microbial pH and withdraw phospholipid and hydrophilic complexes, thereby leading to microbial inactivation [35]. Irazoqui et al. [36] found that MAP technology applied to fresh-cut lettuce reduced microbial proliferation.

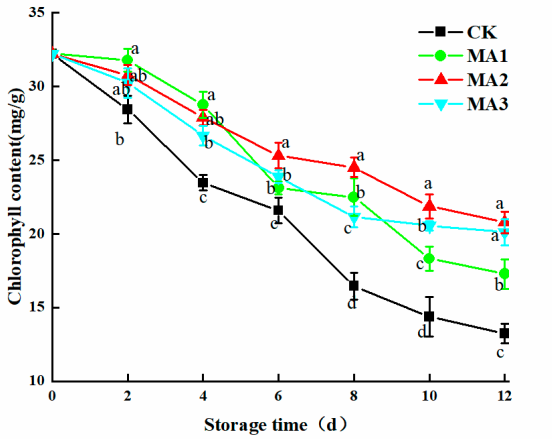

Figure 5. Changes in chlorophyll content. (CK:air, MA1: 5% CO_2 15% O_2, 80% N_2, MA2: 10% CO_2 10% O_2, 80% N_2, MA3: 15% CO_2 5% O_2, 80% N_2) Points are means with different lowercase letters (a–d) in figure significantly differ ($p < 0.05$) according to Duncan's multiple range test.

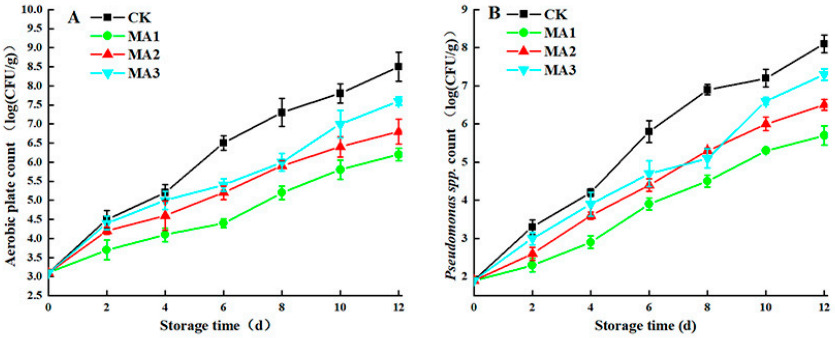

Figure 6. Changes in (**A**) aerobic plate count and (**B**) *Pseudomonas* spp. count. (CK:air, MA1: 5% CO_2 15% O_2, 80% N_2, MA2: 10% CO_2 10% O_2, 80% N_2, MA3: 15% CO_2 5% O_2, 80% N_2).

Pseudomonas fluorescens [37] is a dominant spoilage bacterium and a primary driver of leaf rot and poor quality in vegetables. As shown in Figure 6B, the MA1 group only recorded 6.2 log CFU/g on day 12, representing a decrease of 37.10%, 22.58%, and 9.2%, compared to the CK, MA3, and MA2 groups. Thus, an appropriate level of CO_2 in atmospheric packing was effective in curbing *Pseudomonas* spp. growth. *Pseudomonas* spp. is an aerobic bacterium; thus, storage in low-oxygen conditions would prevent rapid proliferation [38]. Furthermore, carbonic acid, which is formed following the dissolution of CO_2 in water, can actively suppress *Pseudomonas* spp. growth, with a better antibacterial effect observed at 4 °C [39]. On the other hand, the presence of N_2 can not only avoid package collapse during storage but also postpone the development of aerobic spoilage bacteria, thereby indirectly limiting an increase in microbial count [40]. Ioannidis et al. [41] presented concordant results.

3.6. Changes in AsA Content and MDA Content

Ascorbic acid possesses antioxidant and antifungal abilities; however, it is unstable and susceptible to oxidation. As shown in Figure 7A, the MA1 group showed the slowest reduction in AsA content over the first 4 days of storage, due to the lower O_2 level retarding

sample respiration [42]. The gradual O_2 depletion in MA1 samples may indicate anaerobic enzymolysis with intense activity, which resulted in a marked decrease on day 6, following the trend seen in Figure 5. Thus, MA1 packaging was only suitable for slowing fresh-cut amaranth oxidation within the first 6 days of storage. Zhang et al. [27] drew similar conclusions in their study.

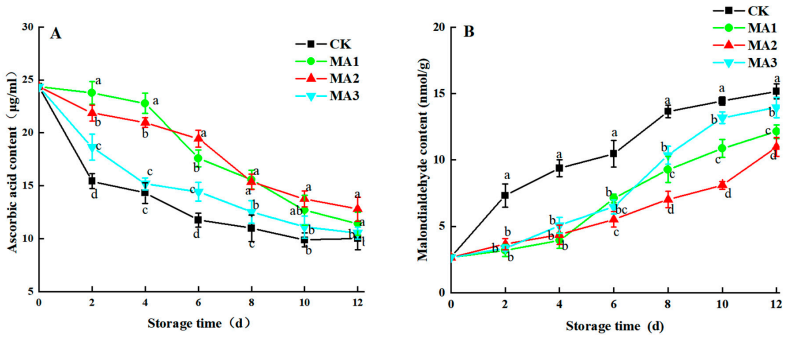

Figure 7. Changes in (**A**) AsA content and (**B**) MDA content. (CK:air, MA1: 5% CO_2 15% O_2, 80% N_2, MA2: 10% CO_2 10% O_2, 80% N_2, MA3: 15% CO_2 5% O_2, 80% N_2) Points are means with different lowercase letters (a–d) in figure significantly differ ($p < 0.05$) according to Duncan's multiple range test.

MDA content reflects the degree of lipid membrane peroxidation, serving as an indicator of quality. It is an intermediate component of plant senescence and is closely associated with plant tissue decay. Plant cells produce O^{2-} and OH^-, thereby inducing unsaturated fatty-acid anion peroxidation, stimulating lipid membrane peroxidation and degrading plant cells [43]. As shown in Figure 7B, the MAP groups successfully curtailed the rapid increase in MDA content with respect to the CK group ($p < 0.05$). Chen et al. [44] also found that MAP inhibited chlorophyll degradation and chloroplast destruction, thereby maintaining the green color of lettuce, by blocking MDA production. ROS induction plays a major role in enhancing MDA content [45]. In line with our results, Wang et al. [46] discovered that MAP (high O_2 atmosphere) caused ROS accumulation in vegetables during the late storage period, leading to an increase in MDA content.

3.7. Changes in Antioxidant Enzyme Activity

POD activity strictly corresponds to vegetable decomposition, reflecting the storage quality of fresh-cut fruits and vegetables. A higher POD activity induces more acute browning and hastens decay [47]. SOD enzymes and APX enzymes play roles in the ASA–GSH cycle [48], and their activity levels denote a plant's antioxidant capacity. SOD enzymes catalyze O^{2-} into O_2 and H_2O_2 via disproportionation reactions, cooperating with APX enzymes to scavenge H_2O_2, thereby lowering the O^{2-} generation rate, removing ROS, and slowing lipid peroxidation [49]. As shown in Figure 8A, all treatment groups showed dramatically lower activity than the CK group, with atmospheric packaging treatment delaying the peak appearance, in agreement with Lu et al. [50]. As shown in Figure 8B,C, the antioxidant enzyme activity was clearly higher in the treatment groups during storage. The SOD and APX activities were notably improved by 41.36% and 48.65% in the MA2 group compared to CK samples on day 12 ($p < 0.05$). The MA1 group exhibited the highest activity during the first 4 days of storage, which was attributed to the high oxidative stress in vegetables in relatively high-CO_2 conditions, thus stimulating SOD enzyme expression [51]. Khan et al. [52] studied the changes in antioxidant enzyme activity in longan during MAP (85% N_2, 5% O_2, 10% CO_2) storage, finding similar results to this paper. Serrano et al. [53] found that MAP (<5% O_2, >10%CO_2) inhibited the decline in total antioxidant activity in broccoli during storage.

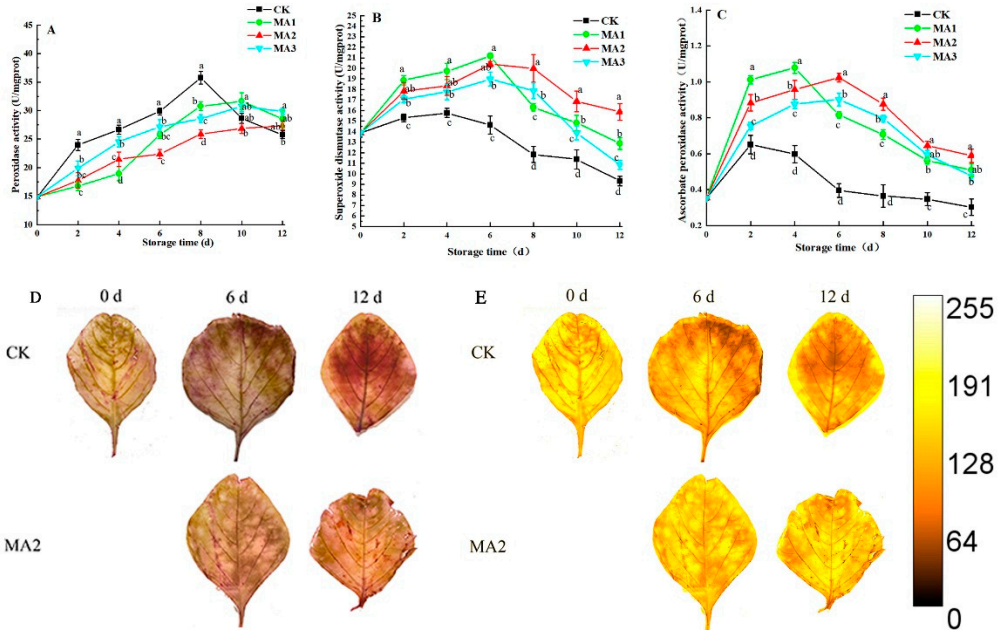

Figure 8. Changes in antioxidant enzyme activity: (**A**) POD activity; (**B**) SOD activity; (**C**) APX activity; (**D**) NBT staining, (**E**) NBT dyeing result. (CK:air, MA1: 5% CO_2 15% O_2, 80% N_2, MA2: 10% CO_2 10% O_2, 80% N_2, MA3: 15% CO_2 5% O_2, 80% N_2) Points are means with different lowercase letters (a–d) in figure significantly differ ($p < 0.05$) according to Duncan's multiple range test.

To verify whether atmospheric packaging could impact ROS presence, ROS staining experiments were conducted. As shown in Figure 8D,E, senescent leaves showed a sharp increase in ROS with storage time, with particularly substantial H_2O_2 and O^{2-} levels recorded during the late storage period [21]. On the other hand, the MA2 group was less affected than the CK group. Combined with the antioxidant enzyme results, this indicated that atmospheric packaging successfully blocked the increase in O^{2-}, thus prolonging the shelf life, as shown previously.

3.8. Correlation Analysis

As shown in Figure 9, POD enzyme activity, MDA content, and total bacterial count were positively correlated, whereas POD enzyme activity, chlorophyll content, and soluble solid content were negatively correlated. These findings demonstrate that fresh-cut amaranth quality decreased with an increase in storage time due to redox senescence and an increase in bacterial colony count, thereby affecting its shelf life. Furthermore, SOD enzyme activity and APX enzyme activity presented a substantial positive correlation, as both enzymes act simultaneously via the ASA–GSH cycle to protect fresh-cut amaranth against oxidative stress and to maintain quality. As confirmed in Figure 8D, MAP technology could strongly curb the oxidative process in amaranth leaves. Overall, the MA2 treatment (10% O_2, 10% CO_2, 80% N_2) demonstrated the best antioxidant and decay-reducing effects in fresh-cut amaranth during storage.

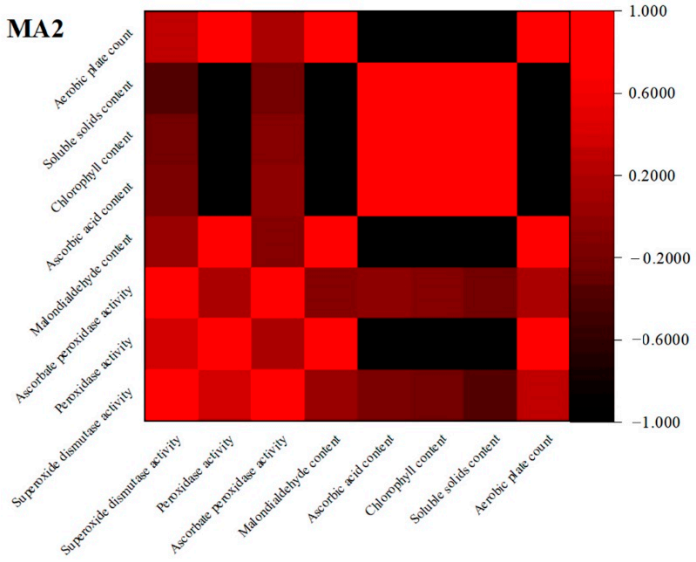

Figure 9. Correlation analysis of antioxidant enzymes and physiological and biochemical indices.

4. Conclusions

The results of this study showed that an appropriate atmospheric ratio (10% O_2, 10% CO_2, 80% N_2) is optimal for maintaining the quality of fresh-cut amaranth. An environment of 5% O_2, 15% CO_2, 80% N_2 exhibited favorable effects within 4 days, followed by accelerated decay. According to the physical and chemical indices measured, MA2 packaging (10% O_2, 10% CO_2, 80% N_2) was able to maintain fresh-cut amaranth sensory properties during storage, delay the reduction in chlorophyll and ascorbic acid content, improve water retention, control malondialdehyde accumulation, support antioxidant enzyme activity, reduce cell membrane peroxidation, maintain cell membrane integrity, and postpone tissue aging. Furthermore, it could effectively inhibit the growth and reproduction of *Pseudomonas fluorescens*, thereby prolonging the shelf life of fresh-cut amaranth by 2 days. MAP technology in general is a mature technology, but industrialization still needs more intensive research. In the subsequent research, it will be further developed in combination with other preservation technologies.

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