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Research Article

Pre-harvest application of proline, methionine, and melatonin improves shelf-life and maintains nutritional quality of *Brassica oleracea* florets during cold storage

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Abstract

The reduction in shelf-life and nutritional value of cauliflower florets are the most vital problems during cold storage. This research was performed to explore the impact of the pre-harvest foliar implementation of proline (PR), methionine (MT), and melatonin (ML), at a rate of 25 mg.L^{-1}, on shelf-life and active composites of cauliflower florets (cv. 'Arasya') stored at 10 °C for 14 d. The obtained results exhibited that florets from treated plants with PR, MT, and ML were lower in water loss, electrolyte leakage, browning index, and titratable acidity (TA) compared with untreated plants, at the end of storage. Furthermore, these pre-harvest treatments significantly slow down the loss of total sugar content, reducing sugar, total soluble solid (TSS), glucosinolates concentration (Gly), total phenols (TP), vitamin C (VC), and antioxidant activity (DPPH) than control samples. After 14 days of storage, the application of PR, MT, and ML significantly improved the activity levels of phenylalanine ammonia-lyase (PAL), and catalase (CAT) as well as declined the polyphenol oxidase (PPO), peroxidase (POD) activates in the florets during the cold storage. Therefore, the pre-harvest application of PR, MT, and ML might be promising substances to keep the nutritional quality of cauliflower forests and reduce the browning index during cold storage.

Keywords: antioxidants; browning index; cauliflower plants; postharvest quality; storability

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Introduction

Cauliflower (*Brassica oleracea* L. *botrytis*) is a popular vegetable plants and is extensively consumed due to its distinctive taste, alimentary value, and bioactive ingredients. Furthermore, cauliflower florets are a nutritious and health – promoting food, due to their richness of ascorbic acid, phenolic compounds, fiber, glucosinolates, and carotenoids (Kapusta-Duch *et al.,* 2019). These metabolic compounds can increase the level activity of antioxidants and reduce human diseases such as cancer, which epidemiological and animal carcinogenesis studies (El-Beltagi *et al*., 2018; Nasrin *et al*., 2022). Nevertheless, the storability of cauliflower floret is quite limited because of its high-water loss, and respiratory rate (Picchi *et al.,* 2020). Therefore, several researchers stated that pre and/or postharvest applications are needed to prolong shelf life and conserve florets' quality and nutritional quality after harvesting (Lai *et al*., 2011; Han *et al*., 2022; Elsayed *et al.,* 2022). Various pre and postharvest materials have been used to increase the storability and delay the damage of *Brassica* floret such as utilization of methyl jasmonate (Darwish *et al.*, 2021), melatonin (Onik *et al.*, 2021), ozone and ultraviolet-C radiation application (Huyskens-Keil *et al.,* 2012), and tapioca-starch edible coating (Owolabi *et al.,* 2021). However, the application of some previous post-harvest treatments couldn't be applicable due to the high cost, low customer acceptance, or difficulty to apply. Therefore, the pre-harvest implementation of environmentally-friendly bio-substances to prolong shelf-life and conserve bioactive constituents of vegetable and fruit products might be proper alternative. Several pre-harvest treatments have been applied to extend the preservation time and conserve the quality of horticultural products, such as the application of salicylic acid (Jiang *et al.,* 2022) and methyl jasmonate (Wang *et al.,* 2021). Furthermore, essential oils and gamma irradiation can be used to extend the shelf life of horticulture products (Afify *et al*., 2012 a, b, c; Aly *et al*., 2023). According to our knowledge, the impact of the pre-harvest treatments such as PR, MT, and ML on nutritional quality and self-life cauliflower florets during refrigerated storage, has not been previously studied.

Proline is a proteinogenic amino acid which biosynthesized in plants through glutamate pathway (Hossain *et al.*, 2019). This amino acid accumulates in plants under environmental stress conditions and acts as antioxidant, and signal molecule as well as an osmotic agent in the plant's tolerance to abiotic stress conditions (Hayat *et al.,* 2012). Recent publications have shown that proline plays vital role not only in improving cellular osmolarity and steadying membrane structures but also in reducing oxidative injury caused by reactive oxygen species (ROS)and protecting cell components under adverse stress conditions (Hayat *et al.,* 2012). Previous studies have been performed to examine the influence of the exogenous application of proline on the storage ability and nutritional value of vegetables and fruits (Gohari *et al.,* 2021). For example, many researchers stated that pre-harvest foliar application of proline during fruits development of peach "Maleiki" resulted in lowering rate of weight loss and fungal deterioration as well as higher TSS, total sugars, ascorbic acids, and firmness, during cold storage (Gohari *et al.,* 2021). In addition, pre-treatment with proline significantly increased antioxidant capacity and phenylalanine ammonialyase (PAL) and conversely reduced the activity level of polyphenol oxidase, hydrogen peroxide, malondialdehyde (MDA), and electrolyte leakage in stored fruit and vegetable products (Liu *et al.,* 2022).

Likewise, methionine (MT), a sulfur-containing amino acid, is found at low levels in plants and contributes to different physiological functions. It effectively modulates the growth of crop plants under normal and abnormal environmental conditions (You *et al.,* 2019). It was shown that postharvest methionine application to vegetable or fruit products significantly conserved quality, decreased browning, and increased the activity level of antioxidant enzymes (Qi *et al.,* 2022). Application of MT to litchi fruit considerably decreased pericarp browning, malondialdehyde content (MDA), hydrogen peroxide (H_2O_2), electrolyte

leakage, POD, and PPO enzyme activities with maintaining anthocyanin content in pericarp fruits during cold storage (Ali *et al.,* 2018).

Melatonin plays a critical role in several physiological processes in different plants, including seed germination, growth development, machinery, ripening and senescence, osmotic regulation, and tolerance to many abiotic stresses (Yang *et al*., 2022). Recent reports have also indicated the impact of pretreatment with melatonin on the post-harvest manner of fruit such as delay in maturation and decay occurrence and induced antioxidant and secondary metabolites (Xu *et al*., 2019). The current study suggested that the pre-harvest treatments of PR, MT, and ML could extend the shelf life and conserve the nutritional quality of cauliflower florets during refrigerated storage. Hence, this study was conducted to examine the influence of pre-harvest application of cauliflower plants with proline PR, ML, and MT on physicochemical properties i.e., weight loss, browning index, total sugar content, titratable, vitamin C, total soluble solids (TSS), acidity, glucosinolates (Gly), total phenols (TP), antioxidant activity (DPPH) and activity levels of antioxidant enzymes of cauliflower florets (cv. 'Arasya').

Materials and Methods

Treatments and florets sampling

Seeds of cauliflower (cv. 'Arasya', obtained from Gaara Seed Company, Egypt) were germinated in foam trays filled with a growing media consisting of peatmoss, vermiculite, and perlite in 1:1:1 volume. After germination, seedlings were grown in a controlled greenhouse in a private farm under 25/15 ± 2 °C for 28 days. Four weeks after seed germination, seedlings were placed in transplanting into plots. Transplanting of cauliflower seedlings was performed on 24th of September 2021 and harvesting was conducted on 1st of January 2022. The experimental plot was 2.1 m^2 and the distance among cauliflower plants was 0.5 m. Likewise, 10-12 plants were grown per plot, and the soil of the experimental field was characterized by clay loam texture (Table 1). The field experiment was organized as a randomized complete block design (RCBD), consisting of four treatments, and each treatment was replicated four times. Treatments were pre-harvest sprayed on entire cauliflower plants. Treatments of this study included: proline, methionine, and melatonin at ratio of 25 mg L^{-1} for each treatment, and the control treatment was treated with tap water. At 15-day intervals, pre-harvest foliar applications of all treatments were done five times (at 30, 45, 60, 75, and 90 days after transplanting) in different stages of cauliflower plant development: seedling, foliage growth, and growth of the head, respectively.

Table 1. Physiochemical properties of experimental soil

Particle size distributions				Chemical properties								
Clay (%)	Silt (%)	Fine sand (%)	Texture	pH	EC (dS/m)	OM* (%)	*Av. N (mg/kg)	Av. P (mg/kg)	K^+ (meq/l)	Ca^{++} (meq/l)	Av. Fe (mg/kg)	Av. Mn (mg/kg)
36	34	30	Clay loam	7.8	2.10	1.57	35.2	22.3	32	7.17	4.1	20.1

*OM= organic matter, *Av= Available

Storage experiment design

Cauliflower heads were harvested, after 90 days from transplanting, at the maturity stage and transported immediately to the postharvest laboratory. Florets free from defects, physical injuries, diseases, or/ and insect damages, with high quality parameters (compact and white color) of each treatment; were selected and packed in polyethylene trays, then conserved at 10 °C and 95% RH for 14 days. Six replicates were utilized for each treatment. The following physicochemical properties were taken every week (7 days).

Weight Loss, electrolyte leakage, and browning index

Cauliflower florets of each treatment were weighed directly after transporting them to the postharvest laboratory and at each sampling time to determine weight loss. According to previous study, the difference between the initial weight and sample weight was utilized to compute the weight loss percentage. Electrolyte leakage (EL) of florets was estimated using the method defined by Cliffe-Byrnes and O'Beirne (2008). To assess the initial EL values, six discs (1 cm) of control and treated florets samples were soaked in mannitol solution (0.3 M) for 35 min and were assessed by pH meter. Then, obtained discs were boiled at 98 °C for 10 min and cooled at 25 °C to determine relative electrolyte leakage rate and the results were reported as a percentage. Browning index was estimated individually on the 7^{th}, and 14^{th}, day of storage and scored using a scale of 0 to 2, where 0 = none, 1 = moderate, and 2 = severe browning.

Activity level of antioxidant enzymes

The extraction technique of catalase (CAT) and peroxidase (POD) activities were defined by Aebi (1984). In brief, one gram of fresh samples was milled in liquid nitrogen and mixed with 10 mM borate buffer (pH 8.5) 5 ml potassium phosphate buffer (100 mM and pH 7). The obtained mixture was then centrifuged at 12,500 ×g for 20 min at 5 °C and the supernatants were used to assess the activity of CAT (EC 1.11.1.6) by Aebi (1984) and POD (EC1.11.1.7) by Nakano and Asada (1981).

The activity level of phenylalanine ammonialyase (PAL, EC 4.3.1.5) and polyphenol oxidase (PPO, EC 1.14.18.1) were assessed utilizing technique defined by Southerton and Deverall (1990) with slight modification. In brief, approximately 0.5 g of frozen florets tissue was mixed with borate buffer (25 mM) containing EDTA, 2-mercaptoethanol, and PVPP. Then, homogenates were centrifuged at 14,000× g for 20 min at 5 °C. For polyphenol oxidase (PPO), the PPO extract, citric buffer (0.1 M) was mixed with one gram of frozen florets sample. The activity level of PPO was assessed at absorbance 420 nm at zero (0) time and after one minute (Bradford, 1976). The activity levels of these enzymes were expressed as U mg^{-1} protein.

Total phenols, glucosinolates and antioxidant activity

Ethanolic extract

Briefly, 5.0 g of the fresh florets samples were grinded with liquid nitrogen after that 25 ml of 80% methanol and shaking for 24 h at room temperature. Whatman filter paper number one was used to filter the extracts, then the extraction was repeated twice (Aly *et al.,* 2022). The resulting methanolic extracts volume were adjusted and used for the analysis of total phenolic content, soluble sugars, reducing sugars, and antioxidant activity (DPPH) analysis. The total phenols concentration (TP) of treated cauliflower florets was estimated using the Folin–Ciocalteu colorimetric procedure described by Gao *et al*. (2011) with slight modification. A spectrophotometer was utilized to assess concentration of total phenols in the filtrated extract at 760 nm. The results were reported as $mg.g^{-1}$ dry weight.

Glucosinolates were measured following the methods of Slominski and Campbell (1987). Briefly, 50 mg of treated and untreated samples were homogenized with 10 ml of ultrapure water and 5 ml of trichloroacetic acid (20%) using a homogenizer. After that, the homogenates were centrifuged at 3000 rpm for ten minutes and 3 ml of supernatant was homogenized with 3 ml of 0.4M Fe $(NO_3)_3$ in 1N HNO_3. The absorbance was measured at 460 nm. The results are reported as potassium thiocyanate equivalents in µg/5 mg of extract.

For determination of 2,20-diphenyl-1-picrylhydrazyl (DPPH), DPPH antioxidant activity was assessed utilizing technique of Ao *et al*. (2008), with minor modification. Briefly, approximately 0.1 ml of ethanol floret extract was taken and homogenized with 3.9 ml of DPPH solution (0.004 percent). The absorbance of control and samples was determined at 517 nm wavelength after incubation in dark at 25 °C for 30 min, using spectrophotometer. The results of DPPH were calculated using the following equation: Antioxidant capacity (%) = [(A control−A sample/A control) × 100].

Where, A control is the absorbance of the DPPH solution and A sample is the absorbance of the treated floret samples.

Titratable acidity, and vitamin C

Titratable acidity (TA) was evaluated using the procedure mentioned by Perdones *et al.* (2016). Briefly, 10 g aliquots of florets were homogenized with 90 ml of distilled water, then filtered; 10 ml of filtrate was titrated with 0.1N sodium hydroxide (NaOH) in the existence of phenolphthalein as an indicator. The finding was presented as a percentage of citric acid.

Vitamin C content was estimated in cauliflower florets using titration method, as reported previously by Shehata *et al.* (2020). Briefly, ten grams of florets were homogenized in oxalic acid (90 ml, 6%) and then filtered. After that, 25 ml of filtrate was titrated by 2,6–dichlorophenol indophenol (Qualikems, Gujarat, India), and the finding was stated as milligram of 100 gram of fresh sample (mg/100 g FW).

Total soluble solid, total sugars, reducing sugars and non-reducing sugars

Twenty grams of cauliflower florets were milled in blinder for obtaining juice extraction. A digital refractometer was utilized to evaluate total soluble solid (TSS%) in the extracted juice of cauliflower florets (Shehata *et al*., 2020). Total sugar content was estimated using the colorimetric method of anthrone and sulfuric acid (Njie *et al.,* 2022). While, reducing sugars was determined using the technique of 3, 5-Dinitrosalicylic acid according to Miller (1959). The difference between total sugar content and reducing sugar content was used to calculate the non-reducing sugar content. The findings of non-reducing sugar content were expressed as percentages.

Statistical analysis

The results of this experiment were reported as mean ± standard error (SE). The experimental data were statistically analyzed using analysis of variance (ANOVA) by Statistical 7 software. Differences between all applied treatments were considered significant at a probability of 5% (95% confidence level) according to the Tukey test. Furthermore, principal component analysis (PCA), correlation heatmap, and Pearson's correlation are multivariate statistical procedures applied to determine relationship between all measurements.

Results

Effect of pre-harvest treatments on weight loss, electrolyte leakage and browning index

The influence of the foliar application of PR, MT, and ML on weight loss rate, dry matter content, electrolyte leakage, and browning index of cauliflower florets (stored at 10 °C for 14 d) is presented in Figures 1 (A, B, C, and D) for both seasons. As shown in Figure 1 A, the values of weight loss of treated and untreated florets increased significantly by increasing the storage time. While, these values were lower in florets of cauliflower plants treated with PR, MT, and ML than untreated ones, at the end of the storage period. After 14 d of storage, the increment rate of weight loss in the untreated control was higher than pre-harvest treatments by 16.47, 15.93, and 17.76% in the first season and 11.82, 17.56, and 8.67% in the second season for PR, MT, and ML treatments, respectively.

Similarly, all treatments showed an increase in floret's electrolyte leakage and browning index during the whole storage period. After 14 days, pre-harvest treatments application with PR, MT, and ML showed a reduction in floret's electrolyte leakage ratio than control treatment (Figure 1B). After 14 d, the reduction rate of floret's electrolyte leakage was reached in plants treated with MT followed by PR, and ML -26.71, -16.51, and -8.87% at the first season and -27.48, -16.59, and -13.018% at the second season, respectively. A linear increase in browning index of cauliflower florets was recorded during the whole storage period for all applied treatments. The maximum values of the browning index were observed in florets of untreated cauliflower plants. In contrast, the minimum values of the browning index were recorded in florets of treated with MT followed by PR and ML, at the end of storage periods. Furthermore, heatmap correlation and principal

component analysis (PCA) showed that water loss rate related positively to electrolyte leakage and browning index (Figure 6 A and B).

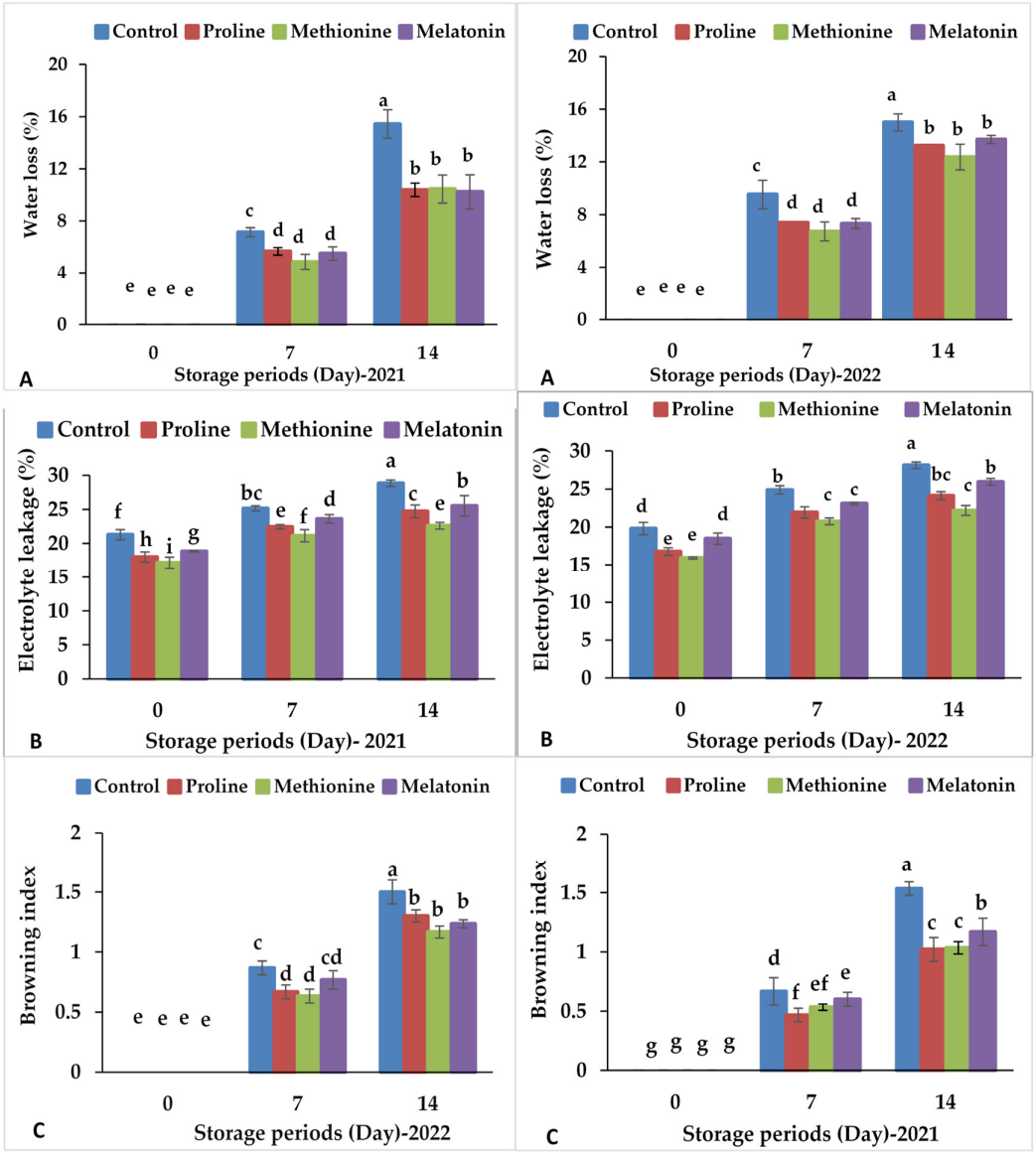

Figure 1. Effect of pre-harvest application of proline, methionine, and melatonin on water loss (A), electrolyte leakage (B) and browning index (C) of cauliflower florets stored for 14 days at 10 °C, at both seasons

The columns followed by different letters show significant differences according to Tukey test ($p < 0.05$). Vertical bar indicates standard error.

Effect of pre-harvest treatments on antioxidant enzymes activity

Regarding the antioxidant enzymes activity, the activity level of antioxidant enzymes (CAT, PAL, PPO, and POD) in cauliflower florets for all treatments exhibited an increase from the beginning until 14 days of storage (Figure 2, A, B, C, and D). At the end of preservation period, the highest activity levels of POD and PPO were observed in florets of control samples, while the PR, MT, and ML treatments displayed the lowest POD and PPO (Figure 2 A and B). On the other hand, after 14 d, the activity levels of PAL and CAT in florets

of cauliflower plants sprayed with pre-harvest treatments were greater than in florets of untreated plants (Figure 2 C and D). Moreover, water loss is associated is positively with POD, and PPO activity, as presented in Figures 6 (A and B)

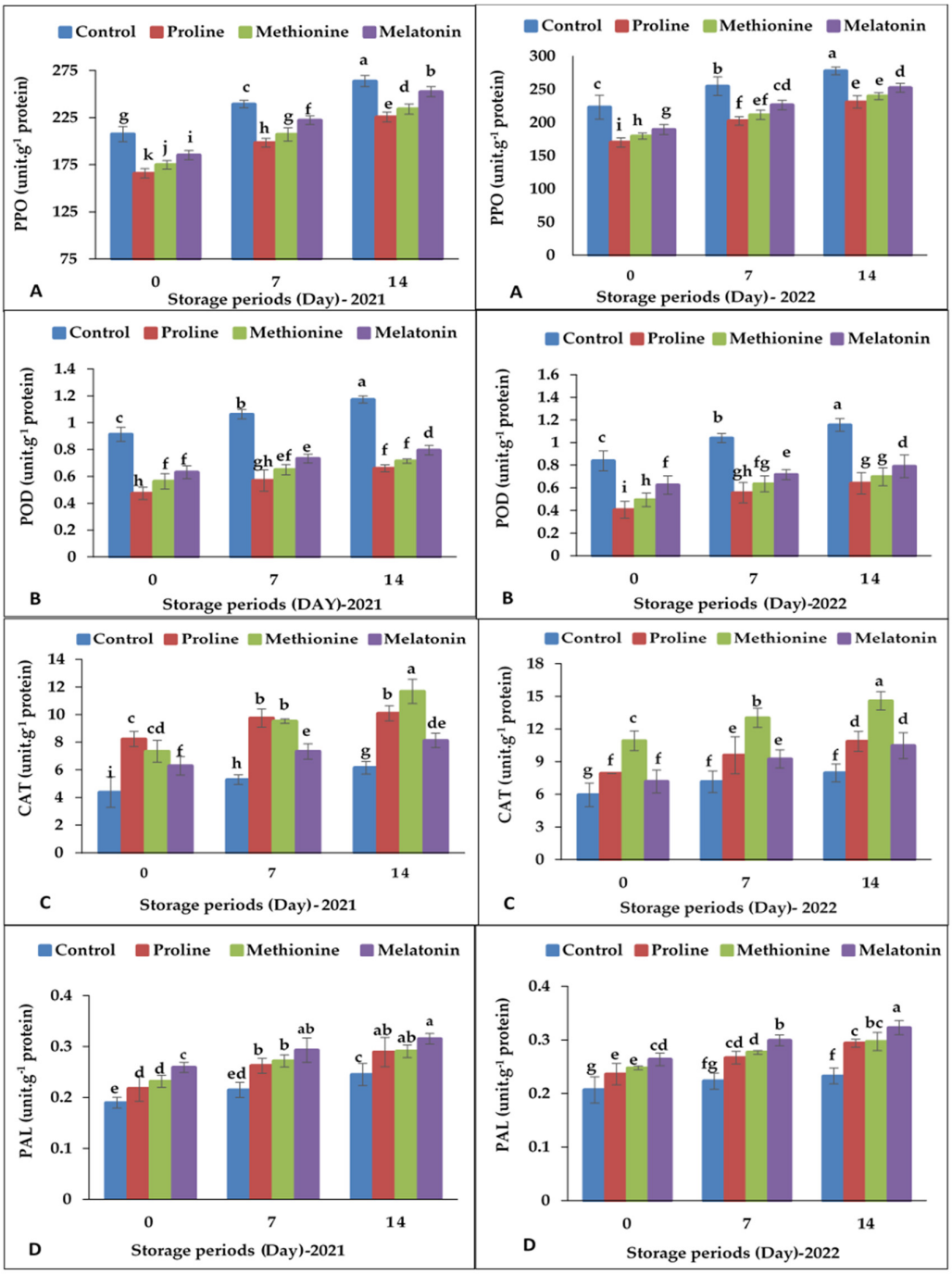

Figure 2. Effect of pre-harvest Effect of pre-harvest application of proline, methionine, and melatonin on activity levels PPO- A, POD- B, CAT-C, and PAL D of cauliflower florets stored for 14 days at 10 °C, at both seasons

Columns followed by different letters show significant differences between treatments according to Tukey test ($p < 0.05$). Vertical bar indicates standard error.

Effect of pre-harvest treatments on total phenols, glycosinolates, and DPPH

As illustrated in Figure 3(A, B, and C), the concentrations of total phenolic compounds (TP), glucosinolates (Gly), and antioxidant activity (DPPH) were rapidly decreased during the refrigerated storage in all applied treatments by increasing the storage time. The TP content in florets of cauliflower plants sprayed with PR, MT, and ML treatments was greater than in the control treatment (Figure 3A). After 14 d of storage, the PR, MT, and ML treatments slow down the loss in TP content by 16.19, 20.26, and 24.30% in the first season and 12.04, 16.10, and 19.69% in the second season, respectively, compared to untreated control. A positive relationship between TP content, water loss rate, and electrolyte leakage was observed (Figure 6 A and B).

Glucosinolates content in cauliflower florets was reduced quickly during cold storage in all treatments (Figure 3B). Before storage (zero days of storage), the levels of Gly were similar in all treatments without any significant difference among them. After 14 days of cold storage, the Gly content in the florets of cauliflower plants sprayed with PR, MT, and ML was greater than control treatment by 24.25, 28.50, and 33.03% in the first year and 23.29, 27.57, and 31.89%, respectively.

As presented in Figure 3 C, Foliar application of PR, MT, and ML significantly decreased the loss in antioxidant activity (DPPH) in florets compared with untreated control from initial until the end of storage time. After 14 day of storage, higher levels of DPPH activity were observed in florets of cauliflower plants sprayed with PR, MT, and ML, as compared with the untreated plants. The highest level of DPPH activity was recorded in florets of cauliflower plants treated with ML followed by PR, and MT compared to the control treatment. Antioxidant activity was related positively with TP content, Gly content, PAL and CAT activity (Figure 6 A, and B).

Effect of pre-harvest treatments on titratable acidity and vitamin C contents

The titratable acidity (TA) and vitamin C (Vit. C) of stored florets are significantly affected by foliar applications of pre-harvest treatments (Figure 4). The titratable acidity content of florets increased by increasing the storage time. From 0 to 14 d of cold storage, the highest value of titratable acidity content was noted in florets of untreated plants compared with the other treatments (Figure 4A). Among the pre-harvest treatments, minimum titratable acidity content was registered in the florets of treated plants with MT followed by PR, and ML in comparison to untreated plants. Whereas, the reduction ratio of total acidy in stored florets was 41.26, 71.19, and 16.76% in the first season and was 40.131, 67.7, and 13.92% in the second season for PR, MT, and ML, respectively, at the end of preservation period.

On the other side, the reduction of vitamin C in cauliflower florets was increased by increasing the storage period in treated and untreated plants (Figure 4B). But the reduction in florets and vitamin C in treated plants was lower than in untreated plants Furthermore, significant differences were found in vitamin C content among all treated florets and the controls. Compared with the all-applied treatments, the lowest content of vitamin C was found in the florets of untreated plants. Among the treated plants, the maximum values of vitamin C were noted in florets of cauliflower plants sprayed with PR, MT, and ML. The improvement ratio of Vitamin C in stored florets was 15.6, 19.3, and 11.7% in the first season and was 16.0, 19.14 and 11.66 % in the second season for PR, MT, and ML, respectively, at the end of preservation period. Likewise, the vitamin C content was associated negatively with water loss rate, titratable acidity, and electrolyte leakage (Figure 6 A, B, and Supplementary Table 1).

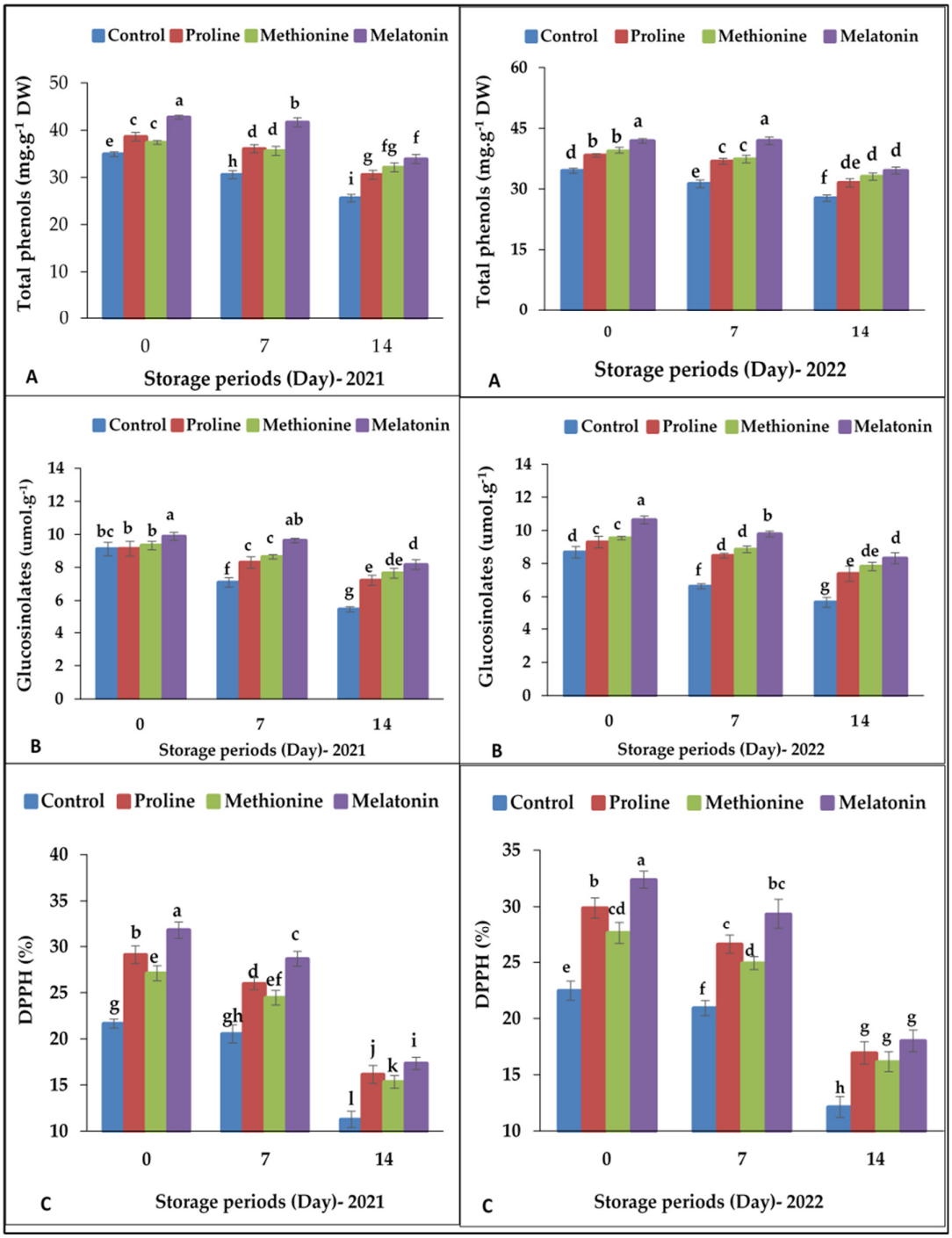

Figure 3. Effect of pre-harvest Effect of pre-harvest application of proline, methionine, and melatonin on total phenols (A), glucosinolates (B), and DPPH- C of cauliflower florets stored for 14 days at 10 °C, at both seasons

Columns followed by different letters show significant differences between treatments according to Tukey test ($p < 0.05$). Vertical bar indicates standard error.

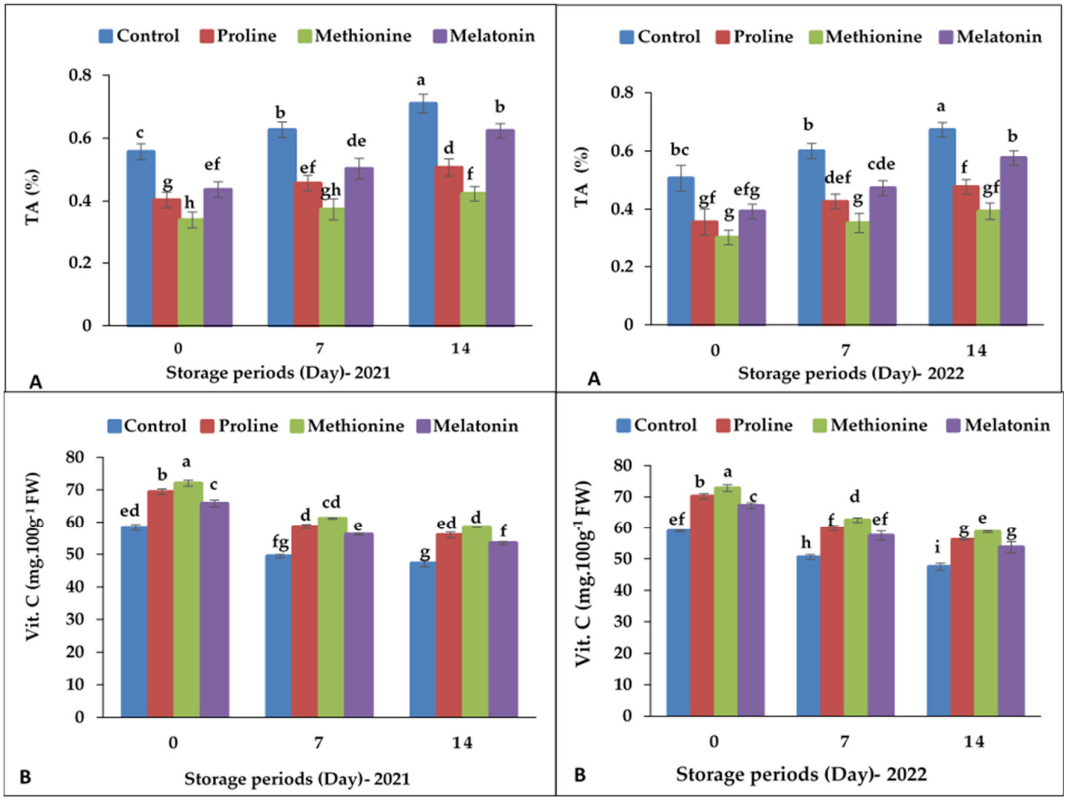

Figure 4. Effect of pre-harvest application of proline, methionine, and melatonin on titratable acidity content (A), and Vitamin C (Vit.C - B) of cauliflower florets stored for 14 days at 10 °C, at both seasons
Columns followed by different letters show significant differences between treatments according to Tukey test ($p < 0.05$). Vertical bar indicates standard error.

Effect of pre-harvest treatments on TSS, total sugars, reducing sugars and non-reducing sugars content

The effect of PR, MT, and ML application on TSS, total sugars, reducing sugars (R.S), and non-reducing sugars (N.R.S) content of cauliflower florets (stored at 10 °C for 14 d) is shown in Figures 5 (A, B, C, and D). Before storage (at 0 d), the pre-harvest treatments significantly increased TSS, total sugars, reducing sugars, and non-reducing sugars values in cauliflower florets ($p<0.05$). The values of the aforementioned parameters (TSS, total sugars, non-reducing sugars, and reducing sugars content) significantly declined with prolonging the storage periods, at both seasons. After 14 d of refrigerated storage, the maximum values of TSS, total sugars, non-reducing sugars, and reducing sugars were recorded in florets of cauliflower plants sprayed with MT followed by PR and ML while the minimum values were observed in florets of untreated plants, at both seasons (Figure 2 A, B, C, D). As presented in Figure 6 (A and B) and supplementary Table 1, TSS content was linked positively to the contents of total sugars, reducing sugars (R.S), and non-reducing sugars (N.R.S) negatively with water loss rate and electrolyte leakage.

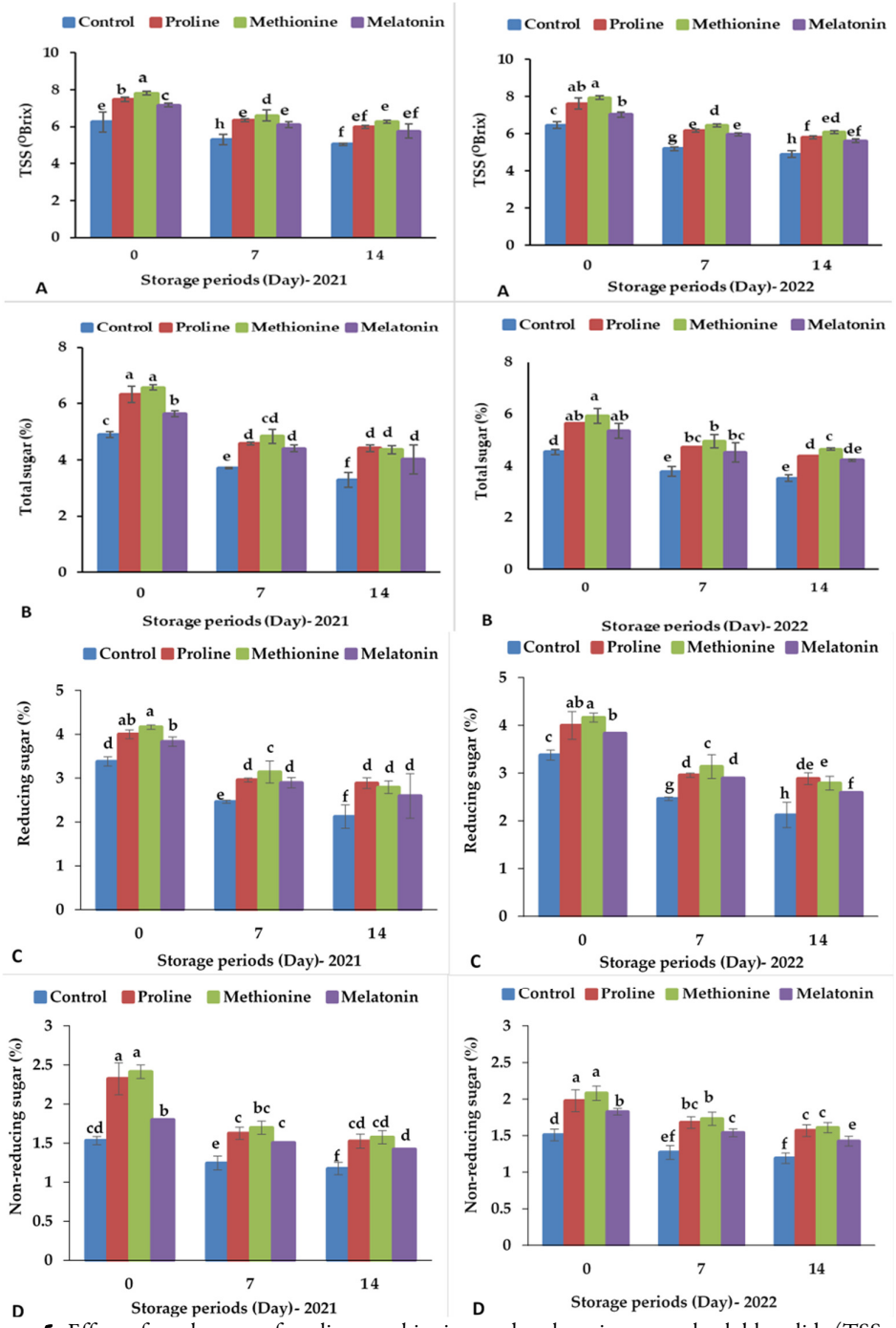

Figure 5. Effect of pre-harvest of proline, methionine, and melatonin on total soluble solids (TSS- A), Total sugars (B), reducing sugars (C) and Non-reducing sugars (D) of cauliflower florets stored for 14 days at 10 °C, at both seasons

Columns followed by the different letter show significant differences according to Tukey test ($p < 0.05$). Vertical bar indicates standard error.

Correlation study

Principal component analysis (PCA), and correlation heatmap of variations in physical and chemical traits of cauliflower florets during the preservation period were shown in Figure 6 (A and B) Regarding variations in the florets quality, stored at 10 °C for 14 days, sprayed their plants with PR, MT, and ML, 15 indexes of florets during cold preservation periods were combined utilizing two-dimensional PCA with Statistical 7 program. The PCA was utilized to additional linked and analyze results of all postharvest parameters of cauliflower florets. Principal components (PCs) gave 90.68% of total variance of data set. The partnership rates of PC1 and PC2 was 59.38% and 31.26% of variance in the group of data, respectively. PC1 shows a high positive loading for total soluble solid (TSS), reducing sugar (R.S), Non-reducing sugar (N.R.S), vitamin C (Vit. C), and high negative correlation for water loss ratio (W.L). PC2 had a high positive correlation for browning index (BI), total phenolic compounds (TP), PAL, POD, PPO, glycosinolate (Gly) and total antioxidant activity (DPPH). In Figure 6 B (heatmap correlation), it can be clearly observed the positive and negative relationships between the physiochemical properties of stored cauliflower florets during storage periods. Heatmap correlation show that water loss ratio (WL) correlated positively with BI, titratable acidity, TPC, CAT, POD, PPO, PAL, Gly, DPPH, EL and correlated negatively with TSS, R.S, N.R.S, and V.C.

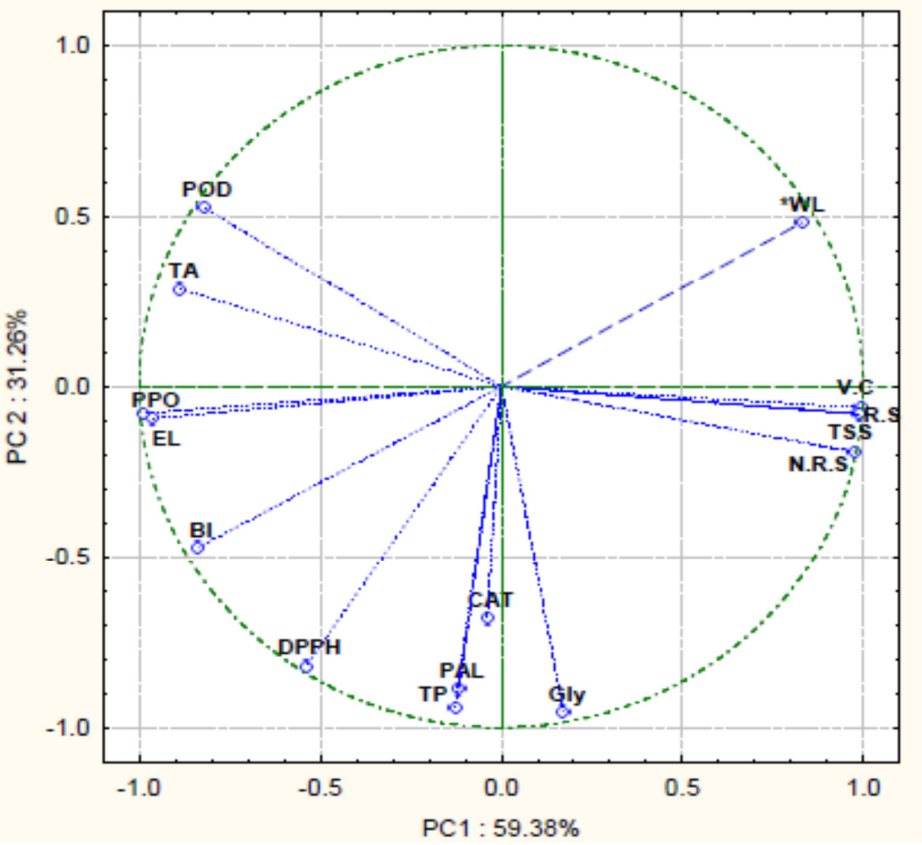

Figure 6A. Principal component analysis of some physical and chemical properties of cauliflower florets. Water loss (WL), titratable acidity (TA), total soluble solid (TSS), reducing sugars (S.R), Non-reducing sugars (N.R.S), Browning index (BI), glucosinolates concentration (Gly), total phenols (TP), vitamin C (Vit. C = V.C), and antioxidant activity (DPPH), PAL, CAT, PPO and POD

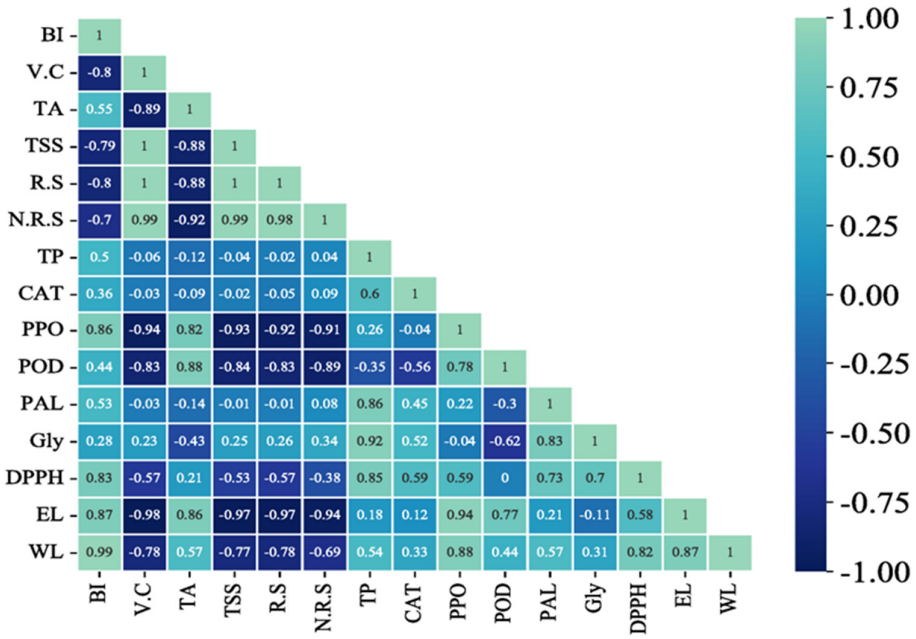

Figure 6B. Correlation heatmap between some physical and chemical properties of cauliflower florets. Water loss (WL), titratable acidity (TA), Browning index (BI), total soluble solid (TSS), reducing sugar (S.R), Non-reducing sugar (N.R.S), glucosinolates concentration (Gly), total phenols (TP), vitamin C (Vit. C = V.C), and antioxidant activity (DPPH), PAL, CAT, PPO and POD

Discussion

Cauliflower florets are seasonal vegetables; one of their main limitations for continuous supply in local market is their climacteric nature. Therefore, an attempt to reduce postharvest florets deterioration, prolong shelf life and keeping quality is essential in supply chain of cauliflower florets. Concerning pre-harvest treatments application and their impact on storage ability and delay floret deterioration, the current results showed that pre-harvest PR, MT, and ML treatments decreased the increasing water loss rate, and electrolyte leakage as well as slowed down the reducing browning index of florets compared with the untreated control in both seasons, during cold storage (Figure 1 A and C). These findings agree with earlier studies (Gohari *et al.,* 2021; Ali *et al.,* 2018; Hernández-Ruiz *et al.,* 2022), confirming that PR, MT, and ML treatment significantly decreased the rate of water loss, and tissue electrolyte leakage of stored vegetable and fruit products. The decline in water loss rate and tissue electrolyte leakage rate by pre-harvest treatments could be resulted from the reduction in the transpiration rate and decreasing activity level of enzymes associated with florets softening and respiration, which was noted by many investigators (Sohail *et al.,* 2021; Hernández-Ruiz *et al.,* 2022). Several studies also have reported that the exogenous application of PR, MT, and ML treatments delayed water loss and reduced electrolyte leakage in vegetables and fruits during storage due to enhancing cuticle rigidity through stimulating wax biosynthesis; inhibition of the aquaporin genes expression of the plasma membrane, thus hindering intracellular water influx; activating NCED1 gene expression accountable for ABA accumulation in plant tissues; decreasing the respiration rate and ethylene production; and keeping cellular redox homeostasis by eliminating excessive reactive oxygen species (EL-Bauome *et al.,* 2022).

Browning is considered one of the most important disorders of cauliflower florets during storage period, which shows post long-term storage at cold temperature (Zhan *et al.,* 2014). Determining the browning index (BI) is consider the most practical techniques to assess browning, which is linked to oxidation of polyphenolic

substances by PPO and POD during storage (Ismail *et al.,* 2023). These enzymes oxidize polyphenol compounds to quinone and quinine-like compounds that finally produce brown complexes. In the present study, the findings revealed that application of PR, MT, and ML treatments displayed lower browning index in treated cauliflower florets compared with the untreated control during cold storage. Furthermore, correlation study showed that the browning index in florets correlated positively with PPO and POD activity (Figure 6 A and B). Numerous studies confirmed the efficiency of PR, MT, and ML in decreasing browning progression. For example, in fresh-cut potatoes (Meng *et al.,* 2021), lettuce (Khan *et al.,* 2019), broccoli (Sohail *et al.,* 2021), and spinach leaves (Sohail *et al.,* 2021), the application of these pre-harvest treatments suppressed tissue browning by decreasing the activity of POD and PPO enzymes (Figure 2 A and B) during storage of vegetable and fruit products. Furthermore, the correlation study confirmed that browning index (BI) correlated positively with POD and PPO, as presented in Figure 6 (A and B).

Accumulating evidence has recently revealed that exogenous application of PR, MT, and ML treatments significantly reduced the decay, slowdown respiration rate, suppressed ethylene production, inhibited tissue browning, minimize the generation of ROS, and increase the shelf-life of stored horticultural products by increasing the production of enzymatic and non-enzymatic antioxidants (Ali *et al.,* 2018; Zhang *et al.,* 2018). In the current study, the foliar application of these pre-harvest treatments revealed a higher activity level of CAT in florets than untreated control (Figure 2 C). Whereas, catalase plays a vital role in scavenging ROS and increases the defense system versus oxidative stress in postharvest fruits by catalyzing the disputations' reaction of hydrogen peroxide molecules into oxygen and water molecules (Nandi *et al.,* 2019). Several scientific reports have mentioned the beneficial impact of methionine and melatonin in increasing CAT, during the storage, as noted in litchi fruit (Ali *et al.,* 2018) and pomegranate (Jannatizadeh, 2019). In addition, comparable responses have been stated in treated peaches (Gohari *et al.,* 2021) with the treated exogenously with proline.

For PAL enzyme, it is accountable for the biosynthesizing of phenols and glucosinolate content in plants. This enzyme catalyzes the conversion of phenylalanine into trans-cinnamic acid, a precursor of phenolic composites (Aly *et al*., 2023). The recent findings displayed a higher activity level of PAL enzyme in florets of cauliflower plants sprayed with PR, MT, and ML treatments than in untreated controls (Figure 2D) in both seasons.

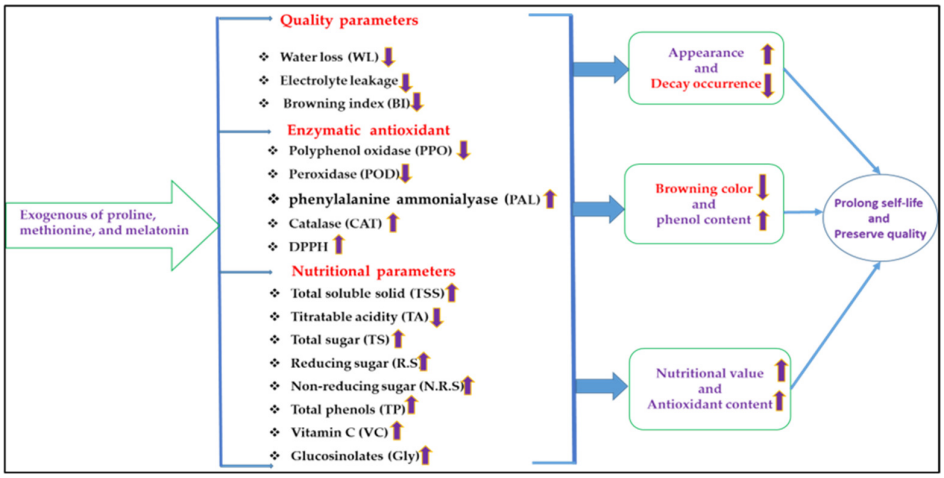

Figure 7. Schematic illustration of proline (PR), methionine (MT), and melatonin (ML) antioxidant system improvement. The pervious treatments declined florets decay through decreasing weight loss (WL), browning index (BI), and electrolyte leakage (E.L). Furthermore, these treatments also maintain the decrease in TSS, total sugars, reducing sugars, and non-reducing sugars and content and increase TA. Application of proline, methionine, and melatonin also improve activity levels of PAL enzyme, enzymatic antioxidant (CAT), and non-enzymatic antioxidants (VC, Gly, TP) and reduce the activity levels of the PPO and POD enzymes. Abbreviations: Water loss (WL), titratable acidity (TA), total soluble solid (TSS), reducing sugar (S.R), Non-reducing sugar (N.R.S), glucosinolates concentration (Gly), total phenols (TP), vitamin C (VC), and total antioxidant activity (DPPH) phenylalanine ammonia-lyase (PAL), and catalase (CAT), polyphenol oxidase (PPO) and peroxidase (POD).

In this study, the improvement in activity level of PAL enzyme, by applied pre-harvest treatments, was associated with an upsurge in total phenol and glucosinolate content which consequently increase the DPPH activity. These results indicate that pre-harvest treatment application efficiently inhibited oxidative stresses, thereby reducing florets decay and increasing DPPH activity during the refrigerated storage of cauliflower florets. Furthermore, PCA and - confirmed that PAL activity correlated positively with CAT, total phenol content (TP), glucosinolate concentration (Gly), and DPPH activity (Figure 6 A andB). In agreement with our findings, the activity level of PAL enzyme was significantly improved by PR, MT, and ML application which stimulates the accumulation of TP, Gly, VC, and increases DPPH activity (Figure 6 A and B), this is favorable in preventing fruit decline and extending shelf-life of postharvest mango, peach, fresh-cut potato, and broccoli (Gohari *et al.,* 2021; Meng *et al.,* 2021; Sohail *et al.,* 2021; Njie *et al.,* 2022).

Vitamin C, also recognized as ascorbic acid, is a natural antioxidant that can decrease of the hazard of cancer by reducing reactive oxygen species (ROS) in human body (Meščić *et al*., 2019). However, it is quickly diminishing in fresh vegetable and fruit products due to several conditions, including refrigerated storage. Hence, it is necessary to keep vitamin C concentration through long-term of storage in low temperatures. These results revealed that foliar application of pre-harvest treatments was highly effective for conserving Vit.C in cauliflower florets during refrigerated storage (Figure 4B). Similar results have been reported in previous studies for treated vegetables and fruits with PR, MT, and ML application (Bahmani *et al*., 2022; EL-Bauome *et al*., 2022). The reduction in vitamin C loss by pre-harvest treatments application might be due to its role in activating ascorbate peroxidase activity, which results in higher content of vitamin C in fresh vegetable and fruit products (Galani *et al*., 2017; Aghdam *et al*., 2020; Sohail *et al*., 2021). Other research also has stated that high vitamin C accumulation by these treatments might be associated with biosynthesis of dehydroascorbate (Galani *et al.,* 2017). In addition, there is a negative correlation was found between vitamin C and water loss as well as EL, this indicating that vitamin C plays a vital role; with CAT, polyphenol, and glucosinolate; in

elevating the total antioxidant activity, reducing ROS production, and extending the shelf-life of stored vegetables in cold conditions (Figure 6 A and B).

Hence, TSS and TA are important parameters correlated to the ripening of horticultural crops. Sugars are essential to fruit's edible quality, mainly granting sweetness and considerably affecting consumer gratification. According to the obtained findings, the TSS, total sugars, non-reducing sugars, and reducing sugars content increased (Figure 5A), in contrast, the TA content (Figure 4 A) declined in florets treated with PR, MT, and ML throughout cold storage period in both studied seasons. However, a continuous reduction in TA in florets of control treatment is evident and is considered a key indicator of faster senescence. Because respiration consumes organic acids as a substrate, this process tends to reduce TA (Al-Dairi *et al.,* 2021). Several investigators reported that application of pre-harvest treatments (PR, MT and ML) could be regulated sugar metabolism, which indicates higher TSS and reduced glucose and fructose loss (Miranda *et al.,* 2020; El-Beltagi *et al.,* 2023a,b; Sati *et al.,* 2023). Moreover, the findings of this study confirmed that PR, MT, and ML application significantly affects TSS, total sugar content, and TA in some fruits and vegetables during cold storage.

To simplify the understanding of the role of PR, MT, and ML in retarding florets decay, decreasing browning index, improving the enzymatic and non-enzymatic antioxidants, and enhancing TSS and sugar content, the findings of postharvest experiment are concluded in schematic diagram (Figure 7) including all measured parameters in this study. The diagram pointed out actions of applied treatments and their contributions to shelf-life prolongation and conservation of postharvest quality in cauliflower florets during refrigerated storage.

Conclusions

In conclusion, it was observed that exogenous application of proline (PR), methionine (MT), and melatonin (ML) extended self-life of stored cauliflower florets at 10 °C for 14 days. All applied treatments maintained postharvest quality of florets during storage by diminishing the weight loss, browning index, electrolyte leakage, and deterioration occurrence more than the untreated control in both seasons. These findings are evident with lessened PPO and POD activity and improved PAL enzyme, which links to preserving a higher content of TP, Gly, vitamin C, and antioxidant activity (DPPH) during cold storage. However, these treatments also cause a reduction in the loss of TSS, non-reducing sugars, reducing sugars, and total sugars and conserved the increase in TA content in stored cauliflower florets. Further molecular experiments are required to understand the influence of pre-harvest application PR, MT, and ML on genes expressions of PPO, POD and PAL enzymes. Further research also is needed to understand relationship between these treatments and sugar biosynthesis.

Authors' Contributions

Conceptualization: HSE-B, EAA, HASF, SMAD, MAMA, SMA, MMEl-M, AM, and HAE; Data curation: HSE-B, EAA, HASF, SMAD, and HAE; Formal analysis: MAMA, SMA, MMEl-M, AM, and HAE; Funding acquisition: HSEB and HASF; Investigation: HSEB and EAA; Methodology: HSE-B, EAA, HASF, SMAD, MAMA, SMA, MMEl-M, AM, and HAE; Project administration: HSEB, MMEl-M and EAA; Resources: EAA, HASF, SMAD, MAMA, SMA and HAE; Software: EAA and HAE; Supervision: HSEB, MMEl-M and EAA; Validation: HSEB, MMEl-M and EAA; Visualization HSE-B, EAA, HASF, SMAD, MAMA, SMA, MMEl-M, AM, and HAE; Writing - original draft: EAA, HASF, SMAD, MAMA, SMA and HAE; Writing - review and editing: HSE-B, EAA, HASF, SMAD, MAMA, SMA, MMEl-M, AM, and HAE. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Aebi H (1984). Catalase *in vitro*. Methods in Enzymology 105:121-126. *https://doi.org/10.1016/S0076-6879(84)05016-3*

Afify AE-MMR, El-Beltagi HS, Aly AA, El-Ansary AE (2012a). Antioxidant enzyme activities and lipid peroxidation as biomarker for potato tuber stored by two essential oils from caraway and clove and its main component carvone and eugenol. Asian Pacific Journal of Tropical Biomedicine 2:S772-S780. *https://doi.org/10.1016/S2221-1691(12)60312-8*

Afify AE-MMR, El-Beltagi HS, Aly AA, El-Ansary AE (2012b). The impact of γ-irradiation, essential oils and iodine on biochemical components and metabolism of potato tubers during storage. Notulae Botanicae Horti Agrobotanici Cluj Napoca 40(2):129-139. *https://doi.org/10.15835/nbha4028268*

Afify AE-MMR, El-Beltagi HS, Aly AA, El-Ansary AE. (2012c). Antioxidant enzyme activities and lipid peroxidation as biomarker compounds for potato tuber stored by gamma radiation. Asian Pacific Journal of Tropical Biomedicine 2(3):S1548-S1555.

Abdel-Hakim SG, Shehata ASA, Moghannem SA, Qadri M, El-Ghany, MFA, Abdeldaym EA, Darwish OS (2023). Nanoparticulate fertilizers increase nutrient absorption efficiency and agro-physiological properties of lettuce plant. Agronomy 13:691. *https://doi.org/10.3390/agronomy13030691*

Aghdam MS, Luo ZS, Li L, Jannatizadeh A, Fard JR, Pirzad F (2020). Melatonin treatment maintains nutraceutical properties of pomegranate fruits during cold storage. Food Chemistry 303:125385. *https://doi.org/10.1016/j.foodchem.2019.125385*.

Al-Dair M, Pathare PB, Al-Yahyai R (2021). Chemical and nutritional quality changes of tomato during postharvest transportation and storage. Journal of the Saudi Society of Agricultural Sciences 20(6):401-408. *https://doi.org/10.1016/j.jssas.2021.05.001*

Ali S, Khan AS, Malik AU, Shaheen T, Shahid M (2018). Pre-storage methionine treatment inhibits postharvest enzymatic browning of cold stored 'Gola'litchi fruit. Postharvest Biology and Technology 1:100-106. *https://doi.org/10.1016/j.postharvbio.2018.02.016*.

Ao C, Li A, Elzaawely AA, Xuan TD, Tawata S (2008). Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. extract. Food Control 19:940-948. *https://doi.org/10.1016/j.foodcont.2007.09.007*.

Aly AA, El-Desouky W, Abou El-Leel O F (2022). Micropropagation, phytochemical content and antioxidant activity of gamma irradiated blackberry (*Rubus fruticosus* L.) plantlets. *In Vitro* Cellular & Developmental Biology-Plant 58:457-46. *https://doi.org/10.1007/s11627-021-10244-7*

Bahmani R, Razavi F, Mortazavi SN, Gohari G, Juárez-Maldonado A (2022). Evaluation of proline-coated chitosan nanoparticles on decay control and quality preservation of strawberry fruit (cv. Camarosa) during cold storage. Horticulturae 8:648. *https://doi.org/10.3390/horticulturae8070648*

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72:248-254. *https://doi.org/10.1006/abio.1976.9999*

Cliffe-Byrnes V, O'Beirne D (2008). Effects of washing treatment on microbial and sensory quality of modified atmosphere (MA) packaged fresh sliced mushroom (*Agaricus bisporus*). Postharvest Biology and Technology 48(2):283-294. *https://doi.org/10.1016/j.postharvbio.2007.10.012*.

Darwish OS, Ali MR, Khojah E, Samra BN, Ramadan KMA, El-Mogy MM (2021). Pre-harvest application of salicylic acid, abscisic acid, and methyl jasmonate conserve bioactive compounds of strawberry fruits during refrigerated storage. Horticulturae 7:568. *https://doi.org/10.3390/horticulturae7120568*.

EL-Bauome HA, Abdeldaym EA, Abd El-Hady MAM, Darwish DBE, Alsubeie MS, El-Mogy MM, ... Doklega SMA (2022). Exogenous proline, methionine, and melatonin stimulate growth, quality, and drought tolerance in cauliflower plants. Agriculture 12:1301. *https://doi.org/10.3390/agriculture12091301*

El-Beltagi HS, Al-Otaibi HH, Ali MR (2023). A new approach for extending shelf-life of pomegranate arils with combined application of salicylic acid and methyl jasmonate. Horticulturae 9:225. *https://doi.org/10.3390/horticulturae9020225*.

El-Beltagi HS, El-Yazied AA, El-Gawad HGA, Kandeel M, Shalaby TA, Mansour AT, ... Ibrahim MFM (2023) Synergistic impact of melatonin and putrescine interaction in mitigating salinity stress in snap bean seedlings: reduction of oxidative damage and inhibition of polyamine catabolism. Horticulturae 9:285. *https://doi.org/10.3390/horticulturae9020285*

El-Beltagi HS, Mohamed HI, Abdelazeem AS, Youssef R, Safwat G (2018). GC-MS analysis, antioxidant, antimicrobial and anticancer activities of extracts from *Ficus sycomorus* fruits and leaves. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 47(2):493-505. *https://doi.org/10.15835/nbha47211405*

Elsayed N, Hassan AA, Abdelaziz SM, Abdeldaym EA, Darwish OS (2020). Effect of whey protein edible coating incorporated with mango peel extract on postharvest quality, bioactive compounds and shelf life of broccoli. Horticulturae 8:770. *https://doi.org/10.3390/horticulturae8090770*

Fatima F, Basit A, Younas M, Shah ST, Sajid M, Aziz I, Mohamed, HI (2023). Trends in potassium permanganate (ethylene absorbent) management strategies: towards mitigating postharvest losses and quality of mango (*Mangifera indica* L) fruit. Food and Bioprocess Technology 1-12. *https://doi.org/10.1007/s11947-023-03047-8*

Galani JHY, Mankad PM, Shah AK, Patel NJ, Acharya RR, Talati JG (2017). Effect of storage temperature on vitamin C, total phenolics, UPLC phenolic acid profile and antioxidant capacity of eleven potatoes (*Solanum tuberosum*) varieties. Horticultural Plant Journal 3(2):73-89. *https://doi.org/10.1016/j.hpj.2017.07.004*.

Gao H, Cheng N, Zhou J, Wang B, Deng J, Cao W (2011). Antioxidant activities and phenolic compounds of date plum persimmon (*Diospyros lotus* L.) fruits. Journal of Food Science and Technology 51:950. *https://doi.org/10.1007/s13197-011-0591-x*

Gohari G, Molaei S, Kheiry A, Ghafouri M, Razavi F, Lorenzo JM, Juárez-Maldonado A (2021). Exogenous application of proline and l-cysteine alleviates internal browning and maintains eating quality of cold stored flat 'Maleki' peach fruits. Horticulturae 7:469. *https://doi.org/10.3390/horticulturae7110469*.

Han X, Wang X, Shen C, Mo Y, Tian R, Mao L, Luo Z, Yang H (2022). Exogenous ABA promotes aroma biosynthesis of postharvest kiwifruit after low-temperature storage. Planta 554:82. *https://doi.org/10.1007/s00425-022-03855-w*

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A (2012). Role of proline under changing environments: a review. Plant Signal Behaviour 7(11):1456-1466. *https://doi.org/10.4161/psb.21949*

Hernández-Ruiz J, Ruiz-Cano D, Giraldo-Acosta M, Cano A, Arnao MB (2022). Melatonin in Brassicaceae: Role in postharvest and interesting phytochemicals. Molecules 27:1523. *https://doi.org/10.3390/molecules27051523*.

Hossain MA, Kumar V, Burritt DJ, Fujita M, Mäkelä P (2019). Osmoprotectant-mediated abiotic stress tolerance in plants. In: Proline Metabolism and Its Functions in Development and Stress Tolerance. Springer Nature: Cham, Switzerland, pp 41-72.

Huyskens-Keil S, Hassenberg K, Herppich WB (2012). Impact of postharvest UV-C and ozone treatment on textural properties of white asparagus (*Asparagus officinalis* L.). Journal of Applied Botany and Food Quality 84:229.

Ismail HA, Richard I, Ramaiya SD, Zakaria MH, Lee SY (2023). Browning in relation to enzymatic activities and phytochemical content in terap peel (*Artocarpus odoratissimus Blanco*) during postharvest ripening. Horticulturae 9:57. *https://doi.org/10.3390/horticulturae9010057*

Jannatizadeh A (2019). Exogenous melatonin applying confers chilling tolerance in pomegranate fruit during cold storage. Scientia Horticulturae 246:544-549. *https://doi.org/10.1016/j.scienta.2018.11.027*.

Jiang B, Fang X, Fu D, Wu W, Han Y, Chen H, Liu R, Gao H (2022). Exogenous salicylic acid regulates organic acids metabolism in postharvest blueberry fruit. Frontiers in Plant Science 13. *https://doi.org/10.3389/fpls.2022.1024909*.

Kapusta-Duch J, Szelag-Sikora A, Sikora J, Niemiec M, Grodek-Szostak Z, Kubon M, Leszczynska T, Borczak B (2019). Health promoting properties of fresh and processed purple cauliflower. Sustainability 11:4008. *https://doi.org/10.3390/su11154008*.

Khan S, Yu H, Li Q, Gao Y, Sallam BN, Wang H, Liu P, Jiang W (2019). Exogenous application of amino acids improves the growth and yield of lettuce by enhancing photosynthetic assimilation and nutrient availability. Agronomy 9:266. *https://doi.org/10.3390/agronomy9050266*

Lai T, Wang Y, Li B, Qin G, Tian S (2011). Defense responses of tomato fruit to exogenous nitric oxide during postharvest storage. Postharvest Biology and Technology 62(2):127-132. *https://doi.org/10.1016/j.postharvbio.2011.05.011*.

Liu P, Xu N, Liu R, Liu J, Peng Y, Wang Q (2022). Exogenous proline treatment inhibiting enzymatic browning of fresh-cut potatoes during cold storage. Postharvest Biology and Technology 184:111754. *https://doi.org/10.1016/j.postharvbio.2021.111754*.

Meng Z, Dong T, Malik AU, Zhang S, Wang Q (2021). Harvest maturity affects the browning of fresh-cut potatoes by influencing contents of amino acids. Postharvest Biology and Technology 173:111404. *https://doi.org/10.1016/J.POSTHARVBIO.2020.111404*

Meščić Macan A, Gazivoda Kraljević T, Raić-Malić S (2019). Therapeutic perspective of vitamin c and its derivatives. Antioxidants 8:247. *https://doi.org/10.3390/antiox8080247*.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31(3):426-8. *https://doi.org/10.1021/ac60147a030*.

Miranda S, Vilches P, Suazo M, Pavez L, García K, Méndez MA, ... Del Pozo T (2020) Melatonin triggers metabolic and gene expression changes leading to improved quality traits of two sweet cherry cultivars during cold storage. Food Chemistry 319:126360. *https://doi.org/10.1016/j.foodchem.2020.126360*.

Mohamed MK, Abouel-Yazeed AM, Abdalla AE, Maraei RW, Aly AA (2023). Quality characteristic of white button mushrooms (*Agaricus bisporus*) affected by gamma irradiation and volatile oils during storage. Radiochimica Acta. *https://doi.org/10.1515/ract-2023-0135*

Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiology 22: 867-880. *https://doi.org/10.1093/oxfordjournals.pcp.a076232*

Nandi A, Yan LJ, Jana CK, Das N (2019). Role of catalase in oxidative stress- and age-associated degenerative diseases. Oxidative Medicine and Cellular Longevity 11:9613090. *https://doi.org/10.1155/2019/9613090*

Nasrin TAA, Yasmin L, Arfin MS, Rahman MA, Molla MM, Sabuz AA, Afroz M (2022). Preservation of postharvest quality of fresh cut cauliflower through simple and easy packaging techniques. Applied Food Research 2:100125. *https://doi.org/10.1016/j.afres.2022.100125*.

Njie A, Zhang W, Dong X, Lu C, Pan X, Liu Q (2022). Effect of melatonin on fruit quality via decay inhibition and enhancement of antioxidative enzyme activities and genes expression of two mango cultivars during cold storage. Foods 11:3209. *https://doi.org/10.3390/foods11203209*

Onik JC, Wai SC, Li A, Lin Q, Sun Q, Wang Z, Duan Y (2021). Melatonin treatment reduces ethylene production and maintains fruit quality in apple during postharvest storage. Food Chemistry 337:127753. *https://doi.org/10.1016/j.foodchem.2020.127753*.

Owolabi IO, Songsamoe S, Khunjan K, Matan N (2021). Effect of tapioca starch coated-rubberwood box incorporated with essential oils on the postharvest ripening and quality control of mangosteen during transportation. Food Control 126:108007. *https://doi.org/10.1016/j.foodcont.2021.108007*.

Perdones Á, Escriche I, Chiralt A, Vargas M (2016) Effect of chitosan-lemon essential oil coatings on volatile profile of strawberries during storage. Food Chemistry 197: 979-986. *https://doi.org/10.1016/j.foodchem.2015.11.054.*

Picchi V, Fibiani M, Scalzo RL (2020). Cauliflower in nutritional composition and antioxidant properties of fruits and vegetables. Academic Press: Cambrige, MA, USA 1:19-32.

Qi X, Zhao J, Jia Z, Cao Z, Liu C, Li J, Su Y, Pan Y, He C, Xu Y, Qin G (2022). Potential metabolic pathways and related processes involved in pericarp browning for postharvest pomegranate fruits. Horticulturae 8(10):924. *https://doi.org/10.3390/horticulturae8100924*.

Sati H, Khandelwal A, Pareek S (2023). Effect of exogenous melatonin in fruit postharvest, crosstalk with hormones, and defense mechanism for oxidative stress management. Food Frontiers. *https://doi.org/10.1002/fft2.180*.

Shehata SA, Abdeldaym EA, Ali MR, Mohamed RM, Bob RI, Abdelgawad KF (2020). Effect of some citrus essential oils on post-harvest shelf life and physicochemical quality of strawberries during cold storage. Agronomy 10:1466. *https://doi.org/10.3390/agronomy10101466*.

Slominski BA, Campbell LD (1987). Gas Chromatographie determination of indole glucosinolates—A re-examination. Journal of the Science of Food and Agriculture 40:131-143. *https://doi.org/10.1002/jsfa.2740400205*.

Sohail M, Wills, RB, Bowyer MC, Pristijono P (2021). Impact of exogenous arginine, cysteine and methionine on the postharvest senescence of six green leafy vegetables. Journal of Horticulture and Postharvest Research 4(3):253-262. *https://doi.org/10.22077/jhpr.2020.3844.1180*.

Southerton SG, Deverall BJ (1990). Changes in phenylalanine ammonia lyase and peroxidase activities in wheat cultivars expressing resistance to the leaf- rust fungus. Plant Pathology 39:223-230. *https://doi.org/10.1111/j.1365-3059.1990.tb02496.x*

Wang SY, Shi XC, Liu FQ, Laborda P (2021.) Effects of exogenous methyl jasmonate on quality and preservation of postharvest fruits: A review. Food Chemistry 353:129482. *https://doi.org/10.1016/j.foodchem.2021.129482*.

Xu T, Chen Y, Kang H (2019). Melatonin is a potential target for improving post-harvest preservation of fruits and vegetables. Frontiers in Plant Science 10:1388. *https://doi.org/10.3389/fpls.2019.01388.*

Yang X, Chen J, Ma Y, Huang M, Qiu T, Bian H, Han N, Wang J (2022). Function, mechanism, and application of plant melatonin: An update with a focus on the cereal crop, barley (*Hordeum vulgare* L.). Antioxidants 11(4):634. *https://doi.org/10.3390/antiox11040634.*

You J, Zhang Y, Liu A, Li D, Wang X, Dossa K, Zhou R, Yu J, Zhang Y, Wang L (2019). Transcriptomic and metabolomic profiling of drought-tolerant and susceptible sesame genotypes in response to drought stress. BMC Plant Biology 19:267. *https://doi.org/10.1186/s12870-019-1880-1*

Zhan L, Hu J, Pang L, Li Y, Shao J (2014). Light exposure reduced browning enzyme activity and accumulated total phenols in cauliflower heads during cool storage. Postharvest Biology and Technology 88:17-20. *https://doi.org/10.1016/j.postharvbio.2013.09.006*.

Zhang Y, Huber DJ, Hu M, Jiang G, Gao Z, Xu X, Jiang Y, Zhang Z (2018). Delay of postharvest browning in litchi fruit by melatonin via the enhancing of antioxidative processes and oxidation repair. Journal of Agricultural and Food Chemistry 66(28):7475-7484. *https://doi.org/10.1021/acs.jafc.8b01922*