



## Effect of Melatonin and Ascorbic Acid Application on Delay of Senescence and Keeping Quality of Fresh Broccoli Florets.

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### ABSTRACT

Broccoli (*Brassica oleracea* L.) is rapidly perishable because of the loss of green color and the consequent yellowing of florets after harvest at ambient temperature. Therefore, the purpose of the study was to investigate the effects of melatonin (MT) solutions at 75, 100 and 125  $\mu$ M and ascorbic acid (AA) solution at 0.5, 1 and 1.5% in addition to control on delay of senescence and quality attributes, and shelf life extending of fresh broccoli florets after harvest and during cold storage at 5°C and 90-95% relative humidity for 16 days, during two successive seasons of 2024 and 2025. The obtained results revealed that the application of melatonin at 100  $\mu$ M was notably effective in reducing weight loss. Moreover, giving a good general appearance (score) and retention of green color (high hue angle), maintained lightness after 16 days at 5°C and as well as maintained the total content of chlorophyll, photonic content and vitamin C. Whereas, ascorbic acid treatments were less effective in this concern in two tested seasons.

**Keywords:** Broccoli- Melatonin- Ascorbic Acid- Total Chlorophyll- Total Phenolic.

### INTRODUCTION

Broccoli (*Brassica oleracea* var. *italica*) is a Brassica genus vegetable used worldwide vegetable with high nutritional value and health benefits, due to its richness in vitamins, antioxidants, anti-carcinogenic substances and health-promoting phytochemicals such as glucosinolates (Yuan et al., 2010.) Epidemiological studies have shown an inverse association between the consumption of Brassica vegetables and the risk of cancer (Day et al., 1994).

Broccoli is a highly perishable vegetable that senesces quickly after harvest and thus its postharvest life is quite short due to yellowing, softening, water loss, decay and off-odor incidences a storage life of 3 to 4 weeks in 0 °C and 95% RH and 2 to 3 days at 20 °C (Vasconcelos and Almeida, 2003). Yellowing caused by either chlorophyll loss or blooming of the buds is a common problem intimately related to ethylene concentration and temperature of storage (Zhuang et al., 1995). During the postharvest period, inflorescences lose their green color, turn yellow and decrease their nutritional and quality, diminishing the

concentration of proteins, sugars, ascorbic acid and glucosinolates (Jia et al., 2009).

Melatonin (N-acetyl-5-methoxytryptamine) is an endogenously produced indoleamine in all plant species, since fruits and vegetables are perishable goods, their shelf life and quality decline after they are harvested. It has been determined that melatonin is a signaling molecule that is involved in several physiological processes, including plant development, differentiation, ripening, and senescence, as well as protecting against different types of environmental stress (Reiter et al., 2015). Melatonin is also recognized as an abiotic and biotic anti-stress and has been found to function in delaying senescence and improving quality in plants (Arnao and Hernández, 2019). Exogenous melatonin (MT) improves the storage life and quality of broccoli, (Cano et al., 2022). Zhu et al. (2018) observed that melatonin delayed the senescence process of postharvest broccoli florets by regulating the respiratory metabolism and antioxidant activity. Total chlorophyll in melatonin-treated florets was 24.15% higher and the



total respiration rate of postharvest broccoli florets by reducing the operating proportion of embden-meyerhof-parnas (EMP) tricarboxylic acid cycle (TCA) and phosphopentose pathway (PPP). It also ameliorates the buildup of reactive oxygen species and  $H_2O_2$  compared to the control.

Ascorbic acid (AA) treatments in the preservation of horticultural crops are a biologically safe molecule that can be used to maintain post-harvest quality (Balouchi et al., 2012). Broccoli heads contain high levels of (AA), but it has been observed that this compound decreases rapidly in the postharvest period, especially during the shelf life (Nishikawa et al., 2003). During

the senescence trend of broccoli, it was observed that the accumulation of active oxygen species increases and thus the antioxidant content, especially ascorbic acid, decreases. AA 1.5 % treatments lead to a delay in chlorophyll degradation and florets senescence in broccoli at 0°C (Balouchi et al., 2012).

Therefore, the main objectives of the present study were to investigate the effect of melatonin and ascorbic acid treatments as postharvest treatments to maintain the quality, delay yellowing and extend the shelf life of broccoli florets during refrigerated storage at 5°C and 95% RH.

## MATERIALS AND METHODS

Broccoli (*Brassica oleracea* var. *italic Imperial F1 hybrids*), was harvested at the proper stage of marketing on the 1<sup>st</sup> and 2<sup>nd</sup> of January in the 2024 and 2025 winter seasons respectively, from Experimental Farm for Central Lab. for Agric. Climate in Giza Governorate, to study the effect of melatonin and ascorbic acid treatments on delay senescence, maintenance quality and extend the shelf life of fresh broccoli florets during cold storage. Broccoli Heads were placed in plastic boxes, with heads pointing upwards and no stacking and transported immediately within 30 minutes to the laboratory of the Department of Vegetable Handling and Postharvest Research Section, Horticultural Research Institute, Agriculture Research Center, Giza Governorate, Egypt. Fresh broccoli heads were selected for uniformity of size, color and being free from any visual defects and absence of physical damage and pests. Then, broccoli heads are separated into florets and stems. The florets were disinfected with sodium hypochlorite at the rate of 150 ppm for 10 min, followed by repeated washing with distilled water. The broccoli florets were divided into 7 groups and dipped for 10 min. in melatonin (MT), a solution of 75, 100 and 125  $\mu$ M,

ascorbic acid (AA) solution at 0.5, 1 and 1.5 % and distilled water represented as control. All treatments of broccoli florets were dried in a well-ventilated room under sterilized conditions and packed in trays sealed with a polyethylene film of 15 $\mu$ m thicknesses and each packed was approximately 200 g represented as an experimental unit (EU). Twelve EU were prepared for each treatment. Samples were arranged in a completely randomized design. All treatments were placed inside carton boxes (40 × 30 × 12.5 cm) and stored at 5°C and 95% RH, for 16 days. Samples of 3 replicates (EU) were randomly taken and examined immediately after treatment and at four days intervals (0- 4-8-12-16) days for the following properties:

### Physical analysis:

- **Weight loss (%):** It was determined to harvest and every four days of storage, the weight of each sample from each of the three replications of each experimental unit was recorded. The percentage loss of the initial weight was used to describe cumulative weight losses (Lemoine et al., 2009)
- **General appearance:** It was determined according to the scale of the scoring



system 9: Excellent, 7: good, 5: fair, 3: poor and 1: unsalable, depending on morphological defects such as flower bud yellowing, texture (crisp to very soft) and pathological defects, (Gorny et al., 2002).

- **External surface color:** Color was evaluated by a color meter (Minolta model CR400, Konica Minolta, Tokyo, Japan), to measure the L\* (lightness), and hue angle, (McGuire, 1992).

#### **Chemical composition:**

- **Total chlorophyll content:** The content of chlorophylls was measured according to (Moran, 1982).

**5. The total phenolic content (mg/100F.W.):** was determined according to Aaby et al. (2005).

**6. Vitamin C content (mg/100g FW):** was determined by the titration method using 2, 6, di phenol indophenols as described in A.O.A.C. (2000).

- **Statistical Analysis:** The experiment was factorial with 2 factors in a complete randomized design (CRD) with 3 replicates. Comparison between means was evaluated by Duncan's Multiple Range Test at 5% level of significance. The statistical analysis was performed according to Snedecor and Cochran (1982).

## **RESULTS AND DISCUSSION**

### **1. Weight loss percentage: -**

As shown in **Table (1)**, the Data exhibited significant increases in weight loss percentage with the extension of the storage period during storage at 5°C the two seasons. The loss in weight may be attributed to respiration and other senescence-related metabolic processes Finger et al., (1999). Similar results were obtained by Jia et al., (2009), Pedro et al., (2013), Bilgin, (2021) on broccoli florets. The Weight loss percentage is one of the most important postharvest issues which have a direct impact on the marketability and quality of broccoli (Nath et al., 2011). Also, water loss is one of the main issues during broccoli storage, causing stalk hardening and bud-cluster turgidity loss (Serrano et al., 2006).

Concerning the effect of dipping of melatonin (MT) and AA treatments on weight loss % of fresh broccoli florets, results demonstrate that in **Table (1)**, there were significant differences among treatments in weight loss percentage during storage. All treatments reduced weight loss % during storage as compared with untreated control. Furthermore, broccoli florets dipped in MT solution at 100 µM was

the most effective treatment in reducing weight loss % during storage at 5 °C in both seasons, followed by dipped in MT solution at 125, 75 µM and AA 1.5%. Meanwhile, untreated control gave the highest values of weight loss in the two seasons. These results agreed with those reported by Cano et al., (2022). The MT treatments are related to its participation in the closing and opening of the stomata, (Wei et al., 2018). Accumulation of ROS and ethylene production during storage caused membrane degradation (Hodges, 2003). Furthermore, accompanied by changes in membrane permeability, changes in ability of cell soluble material preservation, increased leakage in cells and finally loss of weight, therefore possibly ascorbic acid (AA) treatments led to inhibition of ethylene production (Ieamtim et al., 2008) and cellular membrane destruction and reduce ROS level, had led to decreasing weight loss (Shalata and Neumann, 2001).

Regarding, the interactions between various postharvest treatments and storage periods in **Table (1)**. Data conclusively show that significant effect on weight loss percentage after 14 days of storage at 5°C and 95% RH, the lowest value of weight loss



was recorded from the broccoli florets dipped in MT solution at 100  $\mu$ M, while the highest ones were obtained from untreated control in both seasons. Melatonin treatments delayed the senescence process of

postharvest broccoli florets by regulating respiratory metabolism and antioxidant activity. Also, ameliorates the buildup of reactive oxygen species and  $H_2O_2$  compared to the control (Zhu et al., 2018).

**Table (1). Effect of melatonin, ascorbic acid and their interactions on weight loss percentage of fresh broccoli florets during cold storage at 5°C and RH 90-95% during 2024 and 2025 seasons.**

Treatments	Storage periods (per day)					Mean
	0	4	8	12	16	
	2024					
Melatonin (75 $\mu$ M)	0.00q	1.53ko	1.63jn	1.83jk	2.20hi	<b>1.44E</b>
Melatonin (100 $\mu$ M)	0.00q	1.00p	1.26op	1.47lo	1.80jl	<b>1.11F</b>
Melatonin (125 $\mu$ M)	0.00q	1.30np	1.38mo	1.77jl	2.37fh	<b>1.36E</b>
Ascorbic Acid (0.5%)	0.00q	1.90ij	2.30gh	2.60eg	3.53c	<b>2.07B</b>
Ascorbic Acid (1%)	0.00q	1.70jm	1.93ij	2.37fh	2.93de	<b>1.79C</b>
Ascorbic Acid (1.5 %)	0.00q	1.60jn	1.80jl	2.20hi	2.67df	<b>1.65D</b>
Control	0.00q	2.67df	3.00d	4.00b	4.83a	<b>2.90A</b>
Mean	<b>0.00 E</b>	<b>1.67D</b>	<b>1.90C</b>	<b>2.32B</b>	<b>2.90A</b>	
2025						
Melatonin (75 $\mu$ M)	0.00t	1.60oq	1.77mp	2.07jm	2.60fh	<b>1.61E</b>
Melatonin (100 $\mu$ M)	0.00t	1.08s	1.19rs	1.30qs	1.70np	<b>1.05G</b>
Melatonin (125 $\mu$ M)	0.00t	1.47pr	1.63op	1.87lo	2.33hj	<b>1.46F</b>
Ascorbic Acid (0.5%)	0.00t	2.10jl	2.67eg	2.97de	3.40c	<b>2.23B</b>
Ascorbic Acid (1%)	0.00t	1.90ko	2.20ik	2.43gi	2.90ef	<b>1.89C</b>
Ascorbic Acid (1.5 %)	0.00t	1.73np	2.00kn	2.16il	2.93de	<b>1.77D</b>
Control	0.00t	2.57gh	3.23cd	4.10b	4.70a	<b>2.92A</b>
Mean	<b>0.00E</b>	<b>1.78D</b>	<b>2.10C</b>	<b>2.41B</b>	<b>2.94A</b>	

Values with the same letters in the same column and capital letters the same row are not statistically different, at 0.05 level according to Duncan's. Small letters for interactions.

## 2. General Appearance (Score): -

As depicted in **Table. (2)** general appearance (score) of broccoli florets was decreased with the prolongation of the storage period. These results were in agreement with those obtained by Feng et al. (2018) and Liyang et al. (2020). The decreases in general appearance during storage might be due to the degradation of chlorophyll (Page et al., 2001). Branchless and florets lose turgor and become flaccid if water loss is excessive, (Brennan and Shewfelt, 1989). Decay commonly occurs on florets, primarily during advanced stages of senescence.

The broccoli florets treated with MT and AA had significantly the highest score of appearance as compared with untreated control. The broccoli florets dipped in MT solution at 100  $\mu$ M, were the most effective

treatment for maintaining general appearance during storage at 5 °C and 95% RH, followed by those dipped in MT solution at 125, 75  $\mu$ M and AA 1.5%. Whereas untreated control recorded the lowest value of general appearance. These results agree with Liyang et al. (2020) and on broccoli florets. Bilgin et al. (2021) indicated that MT is important for the physiological regulation of cellular homeostasis. In addition, MT helps maintain the morphology of the chloroplast and thylakoid membranes. Thus, the yellowing process of the MT-treated broccoli samples was remarkably delayed, ( Feng et al., 2018). Also, Liyang et al. (2020) reported that melatonin treatment was effective in retaining the green color and fresh weight of fresh-cut broccoli during cold storage.



The effect of interaction between postharvest treatments and storage periods at 5°C and 95% RH on general appearance (score). The broccoli florets dipped in MT solution at 100 µM, gave a good appearance at the end of the storage period of 16 days at 5° C and 95% RH. On the other hand, untreated control had an unsalable

appearance at the end of the storage period in both seasons. The MT treatments lead to maintaining the visual quality and health-promoting properties of broccoli florets during cold storage, by substantially inhibiting the protection against chlorophyll degradation of chlorophyll which is important in the retention of green color in broccoli.

**Table (2). Effect of melatonin, ascorbic acid and their interactions on general appearance (score) of fresh broccoli florets during cold storage at 5°C and RH 90-95% during 2024 and 2025 seasons.**

Treatments	Storage periods (per day)					
	0	4	8	12	16	Mean
<b>2024</b>						
Melatonin (75 µM)	9.00a	9.00a	7.00	6.33be	4.33eg	<b>7.13BC</b>
Melatonin (100 µM)	9.00a	9.00a	9.00a	9.00a	7.00ad	8.60A
Melatonin (125 µM)	9.00a	9.00a	8.33ab	6.33be	5.00df	7.53B
Ascorbic Acid (0.5%)	9.00a	7.67ad	5.00df	4.33eg	1.67h	5.53E
Ascorbic Acid (1%)	9.00a	8.33ab	6.33be	5.67ce	2.33gh	6.33D
Ascorbic Acid (1.5 %)	9.00a	9.00a	6.33be	5.67ce	3.00fh	6.60CD
Control	9.00a	7.00ad	5.00df	3.00fh	1.00h	5.00E
<b>Mean</b>	<b>9.00A</b>	<b>8.43A</b>	<b>6.71B</b>	<b>5.76C</b>	<b>3.48D</b>	
<b>2025</b>						
Melatonin (75 µM)	9.00a	9.00a	7.00	7.67ac	4.33eg	<b>7.40B</b>
Melatonin (100 µM)	9.00a	9.00a	9.00a	8.33ab	7.00ad	<b>8.47A</b>
Melatonin (125 µM)	9.00a	9.00a	8.33ab	7.00ad	5.00df	<b>7.67B</b>
Ascorbic Acid (0.5%)	9.00a	7.67ac	5.00df	5.00df	1.67	<b>5.67D</b>
Ascorbic Acid (1%)	9.00a	8.33ab	6.33be	5.00df	2.33gh	<b>6.20CD</b>
Ascorbic Acid (1.5 %)	9.00a	9.00a	6.33be	5.67ce	3.00fh	<b>6.60C</b>
Control	9.00a	7.00ad	4.33eg	3.00fh	1.00h	<b>4.87E</b>
<b>Mean</b>	<b>9.00A</b>	<b>8.43A</b>	<b>6.62B</b>	<b>5.95C</b>	<b>3.48D</b>	

Values with the same letters in the same column and capital letters the same row are not statistically different, at 0.05 level according to Duncan's. Small letters for interactions.

### 3. Color (hue angle h° value): -

As shown in **Table (3)**. There's a significant hue angle value of broccoli florets with the prolongation of the storage period at 5°C and 95% RH during both seasons. The loss of green color in broccoli florets occurs due to chlorophyll breakdown and this is stimulated by exogenously applied and endogenously produced ethylene (Tain et al., 1996). These results agree with those obtained by Perini et al. (2017) and Liyang et al. (2020). Yellowing is a reflection of to decline in the hue angle

of the flower buds. As well the increase in yellowing rates and the decrease in chlorophyll concentrations during storage are very important factors to the storage life of broccoli (Dong et al., 2004). Hue angel decreased significantly during storage at 4 °C reflecting the transition from a green color to yellow (Fadhel et al., 2018). Yellowness is generally related to senescence which is characterized by chlorophyll degradation during storage (Vasconcelos and Almeida, 2003).





Concerning the effect of melatonin and ascorbic acid treatments on hue angle value as shown in **Table (3)** there was a significant difference between treatments during storage periods at 5°C and 95% RH in two seasons. The broccoli florets which dipped in MT solution at 100 µM were the most effective in retention of green color (high hue angle) which is an indicator of greenness during storage, followed by dipping in MT solution at 125, 75 µM and AA 1.5% were less effective in this concern. While the untreated control recorded the lowest value in both seasons. These results agreed with Jiajun et al. (2023). 100 µM MT treatment was most effective in keeping the green color of florets among all treatments. Also, 100 µM MT treatment showed significantly higher H values and slowed down the reduction rate of chlorophyll content when compared with other treatments and control. Moreover, MT

treatment significantly delayed the degradation of chlorophyll, while POD activity was decreased during storage, (Liyang et al., (2020). MT treatment can keep the chloroplasts intact as well as inhibit the expression of genes and the activity of enzymes involved in chlorophyll degradation (Wu et al., 2021). Ascorbic acid treatments delayed chlorophyll degradation. This delay might be attributed to efficient scavenging of ROS by antioxidants compounds like AA, that would have destroyed the lipid membrane and chlorophyll pigments (Farouk, 2011).

As for the interaction between the interaction between postharvest treatments and storage periods at 5°C and 95% RH on hue angle value, demonstrated in **Table (3)**, the broccoli florets dipped in melatonin solution at 100 µM were the most effective in retention of green color (high hue angle) after 16 days of storage in the two seasons.

**Table (3). Effect of melatonin, ascorbic acid and their interactions on the huge angle (h° value) of fresh broccoli florets during cold storage at 5°C and RH 90-95% during 2024 and 2025 seasons.**

Treatments	Storage periods (per day)					Mean
	0	4	8	12	16	
2024						
Melatonin (75 µM)	130.20a	127.67cd	126.33df	121.00kl	119.07mn	124.85C
Melatonin (100 µM)	130.20a	129.90ab	129.12ac	126.33df	123.50hi	127.81A
Melatonin (125 µM)	130.20a	128.67bc	127.67cd	124.60gh	121.83jl	126.59B
Ascorbic Acid (0.5%)	130.20a	125.10fg	123.33hj	118.33no	116.33p	122.66F
Ascorbic Acid (1%)	130.20a	126.00eg	124.77gh	119.37mn	117.33op	123.53E
Ascorbic Acid (1.5 %)	130.20a	126.80de	125.40eg	120.40lm	118.20q	124.20D
Control	130.20a	122.00ik	116.33p	112.33q	109.50r	118.07G
Mean	130.20A	126.59B	124.71C	120.34D	117.97E	
2025						
Melatonin (75 µM)	131.10ab	129.67cd	128.00ef	124.17ij	120.30no	126.65C
Melatonin (100 µM)	131.10ab	132.33a	130.93bc	127.60eg	123.83jk	129.16A
Melatonin (125 µM)	131.10ab	130.83bc	129.53d	126.40gh	122.77kl	128.13B
Ascorbic Acid (0.5%)	131.10ab	127.17fg	125.17hi	121.03mn	117.10q	124.31F
Ascorbic Acid (1%)	131.10ab	128.00ef	126.40gh	122.07lm	118.17pq	125.15E
Ascorbic Acid (1.5 %)	131.10ab	128.80de	127.40fg	123.13jl	119.13op	125.91D
Control	131.10ab	123.37jk	119.50o	115.63r	113.00s	120.52G
Mean	131.10A	128.60B	126.70C	122.86D	119.19E	

Values with the same letters in the same column and capital letters the same row are not statistically different, at 0.05 level according to Duncan's. Small letters for interactions.

#### 4. Color (lightness L. Value): -

As shown in **Table (4)**. There's a significant increase in lightness loss of



broccoli florets with the prolongation of the storage period at 5°C and 95% RH during the two seasons. These results agree with those obtained by Liyang et al. (2020) and Jiajun et al. (2023) in broccoli. The deterioration of color during the postharvest storage of broccoli is a result of losses in total chlorophyll (Serrano et al., 2006).

Regarding, the effect of dipping heads in MT and AA treatments on lightness value, there were significant differences between treatments during storage periods at 5°C and 95% RH in the two tested seasons. The broccoli florets which dipped in MT solution at 100 µM was the most effective in reducing the increases of lightness loss during storage, Meanwhile the untreated control recorded the highest mean value. These results were achieved in the two seasons and were in agreement with those

reported by Jiajun et al. (2023) and Liyang et al. (2020). The L\* parameter indicates the brightness or luminosity of the broccoli surface. Generally, it is desirable that it remains constant (Cano et al., 2022). 100 µM MT treatment obviously inhibited the increase of L\* value. Furthermore, 100 Mm MT treatment significantly reduced chlorophyll content when compared with control and other treatments and confirmed those of Wei et al. (2018).

Concerning the interaction between postharvest treatments and storage periods on color (lightness L\* Value) of fresh broccoli florets (**Table 4**). The broccoli florets dipped in MT solution at 100 µM was the most effective treatment in reducing the increases of lightness loss after 16 days of storage.

**Table (4). Effect of melatonin, ascorbic acid and their interactions on Lightness (L\* Value) of fresh broccoli florets during cold storage at 5°C and RH 90-95% during 2024 and 2025 seasons.**

Treatments	Storage periods (per day)					Mean
	0	4	8	12	16	
	2024					
Melatonin (75 µM)	35.87n	39.43k	40.10j	42.47g	44.23e	40.42E
Melatonin (100 µM)	35.87n	36.33n	37.07m	38.17l	39.00k	37.29G
Melatonin (125 µM)	35.87n	38.33l	39.00k	41.83h	42.60fg	39.53F
Ascorbic Acid (0.5%)	35.87n	42.10gh	43.80e	45.10d	47.63b	42.90B
Ascorbic Acid (1%)	35.87n	41.07i	42.13gh	44.10e	46.17c	41.87C
Ascorbic Acid (1.5 %)	35.87n	40.27j	41.17i	43.00f	45.03d	41.07D
Control	35.87n	44.07e	45.67c	47.17b	48.67a	44.29A
Mean	35.87E	40.23D	41.28C	43.12B	44.76A	
2025						
Melatonin (75 µM)	37.10a	39.93m	41.13l	42.43j	43.60h	40.84E
Melatonin (100 µM)	37.10a	37.50a	38.17o	39.30n	40.00m	38.41G
Melatonin (125 µM)	37.10a	38.40o	39.99m	40.73l	41.70k	39.58F
Ascorbic Acid (0.5%)	37.10a	43.10i	44.77f	46.03d	47.47c	43.69B
Ascorbic Acid (1%)	37.10a	42.03jk	43.10i	45.10ef	46.33d	42.73C
Ascorbic Acid (1.5 %)	37.10a	41.10l	42.07jk	43.43hi	44.10g	41.56D
Control	37.10a	45.23e	47.07c	48.13b	49.40a	45.39A
Mean	37.10E	41.04D	42.33C	43.60B	44.66A	

Values with the same letters in the same column and capital letters the same row are not statistically different, at 0.05 level according to Duncan's. Small letters for interactions.

### 5. Total chlorophyll content: -

As shown in **Table (5)**, the total chlorophyll content of broccoli florets

decreased and gradually deteriorated during storage at 5°C and 95% RH with the extension of the storage period in both



seasons. These results agree with those obtained by Liyang et al. (2020) on broccoli florets. Chlorophyll degradation is the most obvious visual change, and this is accompanied by losses in membrane lipids and proteins eventually resulting in cell death, (Page et al., 2001). The decrease and

gradual deterioration could be attributed to the gradual increase of destruction by chlorophyll degrading peroxidase POD activity, which is the transformation of chloroplasts to chromoplasts, (Dong et al., 2004).

**Table (5).Effect of melatonin, ascorbic acid and their interactions on total chlorophyll content (mg/100g F.W) of Fresh broccoli florets during cold storage at 5°C and RH 90-95% during 2024 and 2025 seasons.**

Treatments	Storage periods (per day)					Mean
	0	4	8	12	16	
	2024					
Melatonin (75 µM)	99.40a	97.03cd	95.27hi	94.30km	93.00n	96.58C
Melatonin (100 µM)	99.40a	99.10a	97.97b	97.50bc	96.97ce	98.19A
Melatonin (125 µM)	99.40a	98.10b	96.77de	96.03fg	94.98ik	97.06B
Ascorbic Acid (0.5%)	99.40a	95.87gh	94.53jl	93.13n	92.27o	95.04F
Ascorbic Acid (1%)	99.40a	96.60df	95.73gh	95.03ij	93.63mn	96.08D
Ascorbic Acid (1.5 %)	99.40a	97.53bc	96.30eg	95.67gi	94.00lm	95.80E
Control	99.40a	95.00ij	93.17n	92.13o	90.00p	93.94G
Mean	99.40A	97.03B	95.68C	94.83D	93.55E	
2025						
Melatonin (75 µM)	100.10a	97.90cd	96.83fg	95.17j	93.00n	97.52C
Melatonin (100 µM)	100.10a	99.93a	99.63a	99.10b	97.28ef	99.21A
Melatonin (125 µM)	100.10a	99.00b	98.13c	97.57de	96.53gi	98.27B
Ascorbic Acid (0.5%)	100.10a	96.57gh	95.30j	93.60lm	92.13o	95.54F
Ascorbic Acid (1%)	100.10a	97.47de	96.07i	94.20k	93.97kl	96.36E
Ascorbic Acid (1.5 %)	100.10a	98.67b	97.50de	96.27hi	95.07j	96.60D
Control	100.10a	95.43j	93.33mn	92.10o	90.40p	94.27G
Mean	100.10A	97.85B	96.69C	95.43D	94.05E	

Values with the same letters in the same column and capital letters the same row are not statistically different, at 0.05 level according to Duncan's. Small letters for interactions.

Regarding the effect of dipping of melatonin and ascorbic acid treatments on the total chlorophyll content (mg/100g F.W) of fresh broccoli florets, there were significant differences between all used treatments. The broccoli florets dipped in MT solution at 100 µM, exhibited the lowest deterioration and retained more total chlorophyll content during storage periods, followed by dipping in MT solution at 125, 75 µM and ascorbic acid (AA) 1.5% which were less effective in this concern. Whereas the untreated control recorded the lowest mean value in both seasons. These results agreed with Cano et al. (2022) and Balouchi

et al. (2012). MT treatment may keep chloroplasts intact as well as inhibit the expression of genes and the activity of enzymes involved in chlorophyll degradation (Wu et al., 2021). In addition, MT may prevent chlorophyll from degrading (Di et al., 2022). AA treatments delayed chlorophyll degradation. This delay might be attributed to the efficient scavenging of ROS by antioxidant compounds like AA, which would have destroyed the lipid membrane and chlorophyll pigments (Farouk, 2011). Moreover, it has been proposed that AA may retard senescence





either by inhibiting ethylene production or decreasing respiration (Ieamtim et al., 2008).

As for the interaction between postharvest treatments and storage periods on total chlorophyll content of fresh broccoli florets during cold storage, results showed that the broccoli florets dipped in MT solution at 100  $\mu\text{M}$  was more retained and reduced the decline of total chlorophyll content after 16 days of storage at 5°C in two seasons. Zhu et al. (2018) observed that MT delayed the senescence process of postharvest broccoli Florets by regulating

respiratory metabolism and antioxidant activity.

#### 6. Vitamin C content:

Vitamin C content of broccoli florets decreased with extending storage period in both seasons as shown in **Table (6)**. These results agree with those obtained by Rybarczyk-Plonska et al. (2014) and Liyang et al. (2020). This decrease in vitamin C could be due to oxidation or enzymatic degradation, which occur during storage (Kibar et al., 2023). It was previously reported that this decrease may be attributed to respiration and other senescence-related metabolic processes, (Finger et al., 1999).

**Table (6).** Effect of melatonin, ascorbic acid and their interactions on vitamin C content (mg/100g F.W.) of fresh broccoli florets during cold storage at 5°C and RH 90-95% during 2024 and 2025 seasons.

Treatments	Storage periods (per day)					Mean
	0	4	8	12	16	
	2024					
Melatonin (75 μM)	48.73a	46.63df	46.13dg	44.30hi	42.10ln	45.58C
Melatonin (100 μM)	48.73a	48.57a	48.10ab	47.87ac	46.87ce	48.03A
Melatonin (125 μM)	48.73a	47.83ac	47.17bd	45.90eg	43.70ik	46.67B
Ascorbic Acid (0.5%)	48.73a	44.33hi	43.50ik	40.37o	38.23p	43.03F
Ascorbic Acid (1%)	48.73a	45.60fg	44.17hj	41.70mn	39.87o	44.01E
Ascorbic Acid (1.5 %)	48.73a	46.60df	45.03gh	42.63km	41.03no	44.81D
Control	48.73a	43.10jl	40.10o	38.10p	36.60q	41.33G
Mean	48.73A	46.10B	44.89C	42.98D	41.20E	
2025						
Melatonin (75 μM)	47.70a	45.20ce	43.43gh	41.60k	40.13mn	43.61C
Melatonin (100 μM)	47.70a	47.50a	47.07ab	46.20bc	45.70cd	46.83A
Melatonin (125 μM)	47.70a	46.17bc	44.70df	43.37hi	42.20jk	44.83B
Ascorbic Acid (0.5%)	47.70a	43.07hj	40.23m	38.27o	37.10pq	41.27F
Ascorbic Acid (1%)	47.70a	43.83fh	41.43kl	39.73mn	38.03op	42.15E
Ascorbic Acid (1.5 %)	47.70a	44.47eg	42.13jk	40.40lm	39.10no	42.76D
Control	47.70a	42.30ik	38.50o	36.67q	34.27r	39.89G
Mean	47.70A	44.65B	42.50C	40.89D	39.50F	

Values with the same letters in the same column and capital letters the same row are not statistically different, at 0.05 level according to Duncan's. Small letters for interactions.

Concerning the effect of dipping melatonin and ascorbic acid treatments on vitamin C content of fresh broccoli florets, (**Table 6**). There were significant differences between treatments in vitamin C content. All treatments inhibited clear decrease in vitamin C content during storage as compared with untreated control.

Furthermore, broccoli florets dipped in MT solution at 100  $\mu\text{M}$  was the most effective treatment in deterioration inhibitor and more retention of vitamin C content during storage at 5 °C in both seasons. These results agreed with those obtained by Liyang et al. (2020). MT treatments inhibited the decrease in vitamin C content due to the



effective inhibition of oxidation and scavenging of free radicals by MT. Besides, the effect of MT was indirectly demonstrated by the increase in DPPH in broccoli (Feng et al., 2018). The exogenous MT treatment slowed a clear deterioration of vitamin C and reduced the active oxygen content, respiration intensity and ethylene production, also reduced the oxidative damage to cells and maintained more complete cell structure during storage (Xin et al., 2017).

The interaction between postharvest treatments and storage periods on vitamin C content of fresh broccoli florets during cold storage, was shown in **Table (6)**. The broccoli florets dipped in MT solution at 100  $\mu\text{M}$  reduced the decline of vitamin C content after 16 days of storage at 5°C in two seasons.

#### **7. Total phenolic content:**

The total phenolic content of broccoli florets decreased significantly throughout cold storage at 5°C for 16 days in both seasons, (**Table. 7**). These findings were in line with those of Liu et al. (2018) and Di et al. (2022). Vegetables have a lot of enzymes that can oxidize and degradation of phenolic compounds and are caused by the loss of integrity of the cell membrane. PPO and POD were the main agents responsible for the degradation of phenols in plants (Baltacig et al., 2011). Loss of the integrity of the membrane results in the barriers and allowing enzymes to act on their substrates, the decrease in phenolic content on broccoli florets is probably due to the oxidation of the PPO enzyme to give the colored quinones and quinone was oxidized directly by PPO (Zhang et al., 2015).

As shown in **Table (7)**. The phenolic compounds were significantly higher in all treatments. The broccoli florets dipped in MT solution at 100  $\mu\text{M}$ , were the most effectively treated on maintained total phenols during storage as compared with untreated control in both seasons. Following by those dipped in MT solution at 125, 75  $\mu\text{M}$  and AA 1.5%, which were less effective in this concern. While the untreated control recorded the lowest value in both seasons. Similar results were previously reported in broccoli, Melatonin significantly increased the total phenols and total flavonoid contents (Liyang et al., 2020). The increase in total phenols might be explained by a higher phenylalanine ammonia-lyase (PAL) enzyme activity induced by the MT treatment leading to an accumulation of phenols (Aghdam and Fard, 2017). During the long period of storage, cells gradually lose the integrity of the membrane system, which allows enzymes to act on their substrates (Zhang et al., 2015). AA treatment causes increased total phenolic content (Balouchi et al., 2012). AA is extensively used to avoid enzymatic browning of fruit which caused by the reduction of the o-quinones, generated by the action of the PPO, back to the phenolic substrates (Lamikanra and Watson, 2001).

The interaction between postharvest treatments and storage periods on Total phenolic content of fresh broccoli florets during cold storage at 5°C. The broccoli florets dipped in MT solution at 100  $\mu\text{M}$  were retained with the highest value of total phenolic content compared by other treatments. While untreated florets (control) gave the lowest value in this concern in both seasons.



**Table (7). Effect of melatonin, ascorbic acid and their interactions on Total phenols content (mg/100g F.W.) of fresh broccoli florets during cold storage at 5°C and RH 90-95% during 2024 and 2025 seasons**

Treatments	Storage periods (per day)					Mean
	0	4	8	12	16	
	2024					
Melatonin (75 µM)	26.40a	25.03c	23.60e	22.50f	20.70i	23.65C
Melatonin (100 µM)	26.40a	26.20a	25.70b	25.10c	24.30e	25.54A
Melatonin (125 µM)	26.40a	25.57b	24.40d	23.77d	22.70f	24.57B
Ascorbic Acid (0.5%)	26.40a	22.50f	20.23j	19.03l	17.40o	21.11F
Ascorbic Acid (1%)	26.40a	23.50e	21.90g	20.10j	18.63m	22.11E
Ascorbic Acid (1.5 %)	26.40a	24.60d	22.50f	21.40h	19.70k	22.92D
Control	26.40a	21.97g	19.23l	18.07n	15.17p	20.17G
Mean	26.40A	24.20B	22.51C	21.42D	19.80E	
2025						
Melatonin (75 µM)	25.73a	23.10e	20.77h	19.63i	17.97l	21.44C
Melatonin (100 µM)	25.73a	25.40a	24.83b	24.13c	23.63d	24.75A
Melatonin (125 µM)	25.73a	24.54b	23.40de	22.70f	21.67g	23.61B
Ascorbic Acid (0.5%)	25.73a	20.47h	17.60l	16.63m	14.60p	19.01F
Ascorbic Acid (1%)	25.73a	21.40g	18.90j	17.87l	15.76o	19.93E
Ascorbic Acid (1.5 %)	25.73a	22.43f	19.60i	18.50k	16.87m	20.63D
Control	25.73a	19.17j	16.17n	14.63p	12.90q	17.72G
Mean	25.73A	22.36B	20.18C	19.16D	17.63E	

Values with the same letters in the same column and capital letters the same row are not statistically different, at 0.05 level according to Duncan's. Small letters for interactions.

## CONCLUSION

From the previous results, it could be concluded that MT treatments at 100 µM was the most effective treatment in reducing weight loss, giving a good general appearance (score) and retention of green color (high hue angle) and lightness,

Moreover, maintained the total content of chlorophyll, photonic content and vitamin C. during storage at 5°C for 16 Days. Therefore, 100 µM melatonin recommended selected as the best and used to further maintain postharvest attributes.

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### الملخص العربي

تأثير معاملات الملاتونين وحامض الاسكوربيك علي تأخير الشيخوخة والمحافظة علي جودة ما بعد

الحصاد في البركولي الطازج المجزأ

صالح محمد أبو الوفا و صفاء زكريا

قسم بحوث تداول الخضار- معهد بحوث البساتين- مركز البحوث الزراعيه

البروكلي (*Brassica oleracea* L) من النباتات سريعة التلف بعد الحصاد بسبب فقدان اللون الأخضر و اصفرار البراعم لذلك كان الهدف من البحث هو التحقق من معرفة تأثير المعاملات من الميلاتونين (MT) عند 75 و 100 و 125 ميكرومول وحامض الاسكوربيك AA عند 0.5 و 1 و 1.5٪ علي تأخير الشيخوخة و المحافظة علي خصائص الجودة، وإطالة العمر التسويقي للبروكلي الطازجة المقطع جزئياً خلال التخزين المبرد عند 5 ° م ورطوبة نسبية 90-95٪ لمدة 16 يوماً، خلال موسمي 2024 و 2025. أظهرت النتائج المتحصل عليها أن المعاملة بالميلاتونين عند تركيز 100 ميكرومول كان الأكثر فاعلية في تقليل فقد في الوزن و إعطاء مظهر عام جيد والاحتفاظ باللون الأخضر والبريق بعد 16 يوماً من التخزين علي درجة 5 ° م وكذلك الحفاظ على المحتوى الكلي من الكلوروفيل والفينولات وفيتامين سي بينما كانت معاملات حمض الاسكوربيك أقل فعالية في هذا الشأن في كلا موسمين.