



Research Article

The Effect of Melatonin Applications on Quality Characteristics of Brussels Sprouts During Storage Period

Emircan DİNÇER, Necdettin SAĞLAM, Osman Nuri ÖCALAN,
 Onur SARAÇOĞLU, Ala Asi Mohammed Al-Salihi, Fatmanur ÇEZİK*

Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Horticulture, 60100, Tokat, Türkiye

*Corresponding author e-mail: fatmanurczk00@gmail.com

Abstract: The edible portion of Brussels sprouts, their sprouts, is recognized for its high nutritional value. These sprouts are rich in glucosinolate compounds, which play a crucial role in enzyme regulation, DNA protection against degradation, and cancer prevention. However, the storage life of Brussels sprouts is relatively short. Thus, this study targeted to investigate the impacts of different melatonin concentrations (50 µM, 100 µM, and 150 µM) on the postharvest quality of Brussels sprouts. Brussels sprouts were immersed in melatonin solutions at concentrations of 50 µM, 100 µM, and 150 µM for 5 minutes, with pure water serving as the control. Following immersion, the sprouts were stored at 1 ± 0.5 °C and $90 \pm 5\%$ relative humidity (RH) for 28 days. Key quality criteria, including weight loss, titratable acidity, soluble solids content, vitamin C, chlorophyll, total phenolic content, and total antioxidant capacity, were analyzed at every seven days (on days 7, 14, 21, and 28). Melatonin treatments significantly influenced the postharvest quality of Brussels sprouts. The highest chlorophyll content was noted in the 50 µM melatonin treatment, whereas the maximum vitamin C levels were recorded in both the control and 50 µM melatonin applications. The greatest total antioxidant capacity was achieved with the 100 µM melatonin treatment. Conversely, weight loss and total phenolic content were not significantly affected by melatonin applications. In conclusion, the application of 50 µM melatonin was found to effectively preserve the quality of Brussels sprouts, allowing for commercial storage of up to 28 days.

Keywords: Antioxidant, Chlorophyll, Melatonin, Post-harvest, Vegetable

Melatonin Uygulamalarının Depolama Süresince Brüksel Lahanalarının Kalite Özellikleri Üzerindeki Etkisi

Öz: Brüksel lahanasının yenilebilir kısmı olan filizleri, yüksek besin değeriyle tanınır. Bu filizler, enzim düzenlenmesinde, DNA'nın bozulmaya karşı korunmasında ve kanser önlenmesinde önemli rol oynayan glukozinolat bileşikler açısından zengindir. Ancak, Brüksel lahanasının depolama ömrü nispeten kısadır. Bu nedenle, bu çalışma farklı melatonin konsantrasyonlarının (50 µM, 100 µM ve 150 µM) Brüksel lahanasının hasat sonrası kalitesi üzerindeki etkilerini araştırmayı hedeflemiştir. Brüksel lahanaları, kontrol olarak saf su kullanılarak 50 µM, 100 µM ve 150 µM konsantrasyonlarındaki melatonin solüsyonlarına 5 dakika daldırıldı. Daldırmanın ardından filizler $1 \pm 0,5$ °C ve 90 ± 5 bağıl nemde (RH) 28 gün boyunca saklandı. Ağırlık kaybı, titre edilebilir asitlik, çözünür katı madde içeriği, C vitamini, klorofil, toplam fenolik içerik ve toplam antioksidan kapasitesi dahil olmak üzere temel kalite kriterleri her yedi günde bir (7., 14., 21. ve 28. günlerde) analiz edildi. Melatonin uygulamaları Brüksel lahanalarının hasat sonrası kalitesini önemli ölçüde etkiledi. En yüksek klorofil içeriği 50 µM melatonin uygulamasında kaydedildi, maksimum C vitamini seviyeleri ise hem kontrol hem de 50 µM melatonin uygulamalarında kaydedildi. En büyük toplam antioksidan kapasiteye 100 µM melatonin uygulamasıyla ulaşıldı. Öte yandan, ağırlık kaybı ve toplam fenolik içerik melatonin uygulamalarından önemli ölçüde etkilenmedi. Sonuç olarak, 50 µM melatonin uygulamasının Brüksel lahanalarının kalitesini etkili bir şekilde koruduğu ve 28 güne kadar ticari depolamaya olanak sağladığı bulundu.

Anahtar Kelimeler: Antioxidan, Hasat sonrası, Klorofil, Melatonin, Sebze

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1. Introduction

Brussels sprouts (*Brassica oleracea* L. var. *gemmifera*), a Brassicaceae member, have been widely studied regarding sowing date, fertilization, plant density, planting time, cessation, and temperature-growth relationships (Kurtar, 2006). Brussels sprouts are a biennial vegetable that reproduces by seed and needs low temperatures for flowering. However, if it is exposed to temperatures below 10 °C for a few weeks, it blooms in the same year (Guvenc, 2016). The sprouts, which are the consumed part of Brussels sprouts, are rich in nutrients. The key nutritional components include fiber, vitamin C, niacin, and minerals. It also contains substantial amounts of glucosinolate compounds (glukorafanin, glukobrassisin), which regulate enzymes to protect against DNA degradation and may help prevent cancer (Verkerk et al., 2009; Yilmaz & Demirel, 2012; Sarvan et al., 2014; Kraśniewska et al., 2016). However, Brussels sprouts do not have a long shelf life. Beneficial compounds have been decreased at inappropriate conditions. Brussels sprouts can be preserved for up to one month at a temperature of 1-2°C and a relative humidity (RH) of 90-95% (Guvenc, 2016).

Discoloration is the biggest problem during storage (Kraśniewska et al., 2016). Loss of nutritional and commercial value are the problems (Hasperué et al., 2016; Bilgin & Aslantaş 2022). Tissue senescence is often accompanied by accumulation of reactive oxygen species. Researchers have recently begun to examine the impact of melatonin on green vegetables to alleviate this problem (Xin et al., 2017; Miao et al., 2020; Wu et al., 2021; Hu et al., 2022). Melatonin is an animal hormone that plays a role in many regulatory processes, especially sleep. In 1958, the hormone melatonin was first named by Lerner et al. (1960) (Demirsoy, 2018). It is called phyto-melatonin after discovered in plants, in 1995 (Arnao & Hernández-Ruiz, 2020). Studies indicate that melatonin plays a significant role in various physiological processes, such as supporting root regeneration by stimulating the formation of the root system in plants, acting as an antioxidant to increase plant tolerance to high and low temperatures, chemical pollutants, and other environmental stressors, protecting chlorophyll from oxidative stress, and accelerating the rate of photosynthesis in plants (Ahmad et al., 2023; Sharma et al., 2024; Khan et al., 2024; Cai et al., 2025). One of the most attractive properties of melatonin, distinguishing it from most antioxidants, is its potential to eliminate both reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Hu et al., 2022). The benefits of melatonin in plants include scavenging free radicals and helping plants resist biotic and abiotic stresses (Arnao & Hernández-Ruiz, 2015). Post-harvest melatonin applications can improve the quality of tomatoes, strawberries, and apples (Sun et al., 2016; Aghdam & Fard, 2017; Liu et al., 2018; Onik et al., 2021). In addition, it has been reported that melatonin application in peaches delays senescence and reduces cold damage (Cao et al., 2016; Fu et al., 2017; Gao et al., 2018). There is also information in the literature that passion fruit preserves its physicochemical properties and delays senescence (Cai et al., 2024).

There are several studies investigating the effect of exogenous melatonin applications on broccoli (Miao et al., 2020; Wei et al., 2020a; Wei et al., 2020b; Hu et al., 2022). However, there is no study on the application of melatonin in Brussels sprouts. Thus, the objective of this study is to explore the impact of different doses of melatonin on the postharvest quality of Brussels sprouts.

2. Material and Methods

2.1. Plant material

Divino F1 Brussels sprouts cultivar was used as a material. Same size sprouts were harvested and transferred to the bioactive laboratory. Brussels sprouts, free from mechanical damage, stains, and disease, were first subjected to pre-cooling at a temperature of 1±0.5 °C and a relative humidity (RH) of 95±5% for 24 hours. (Pre-Cooling: It is called bringing the harvested products to the transportation or storage temperature as soon as possible. It is done to maintain post-harvest quality and reduce quality losses). Each treatment used plastic boxes containing approximately 400 g of sprouts. Before storage, pre-storage analyses were conducted. The plastic boxes were then stored at a temperature of 1±0.5 °C and a relative humidity of 95±5%. For treatments, melatonin was applied in doses of 50 µM, 100 µM, and 150 µM. Brussels sprouts were soaked in melatonin solutions for 5 minutes, followed by drying at room temperature (23 °C) for 2 hours. Pure water was used as a control. Analyses were performed on

days 7, 14, 21, and 28 of cold storage. The experiment was conducted using a randomized plot design with three replications (Figure 1).

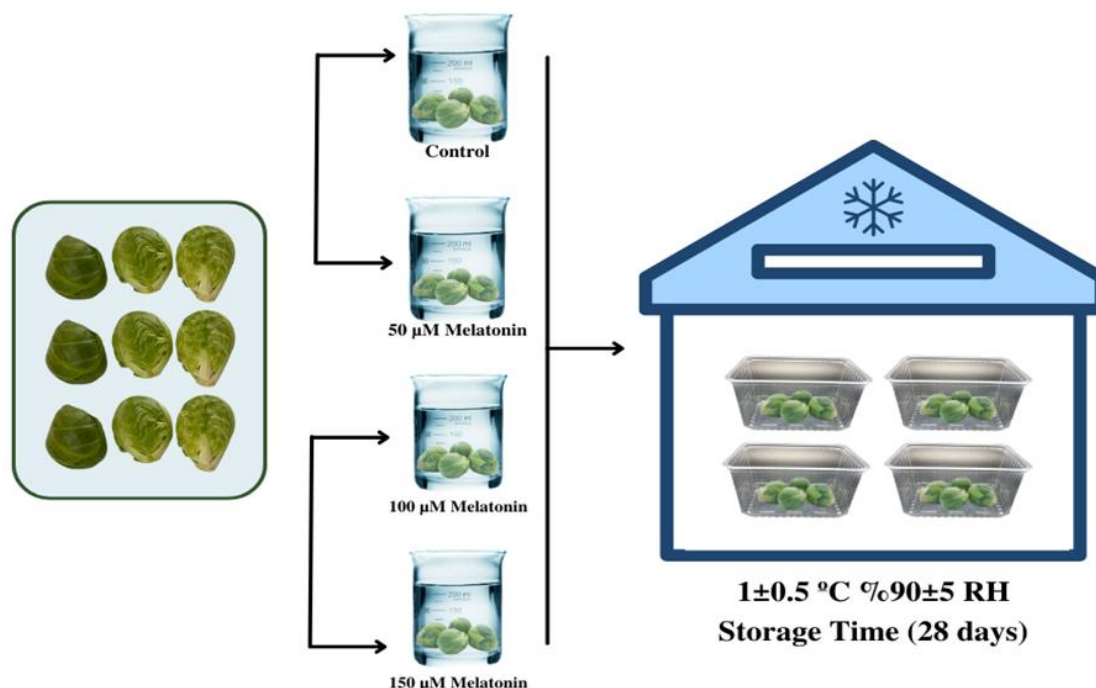


Figure 1. Schematic representation of harvested Brussels sprouts, postharvest melatonin treatments (50 µM, 100 µM, and 150 µM), and storage conditions.

2.2. Weight loss (WL)

At the beginning of storage, the initial weight of the sprouts was measured using a digital balance (Radwag, Poland) with an accuracy of 0.01 g. Subsequent measurements of the final weights were taken on the 7, 14, 21, and 28th days of storage. The weight loss was calculated as a percentage by taking the difference between the initial and final weights and dividing it by the initial weight.

2.3. Soluble solids content (SSC), titratable acidity (TA) and vitamin C

In every repetition, five sprouts were homogenized using a blender (Promix HR2653, Philips, Turkey), and the water content was subsequently removed. Soluble solids content (SSC) was assessed using a digital refractometer (Atago PAL-1, Japan, Brix = 0–53%) and recorded as a percentage (%). Titratable acidity (TA) was calculated as the percentage of citric acid, according to the volume of NaOH consumed during titration. For vitamin C content, ascorbic acid test strips (Catalog no: 116981, Merck, Germany) were used, and the resulting value was presented as mg L⁻¹ (Aglar et al., 2017).

2.4. Chlorophyll content (Total, a and b)

A 0.1 g sample taken from the outer leaves of Brussels sprouts was weighed and crushed by adding 2-3 mL of 80% acetone. The samples were homogenized with 80% acetone until the total volume reached 10 mL, and then filtered through filter paper. The chlorophyll a, chlorophyll b, and total chlorophyll contents of the samples were then recorded at wavelengths of 645, 663, and 652 nm, respectively, with a UV-vis spectrophotometer (Model T60U, PG Instruments). The results were calculated using the formulas provided by Lichtenthaler & Wellburn (1983) and Sarac et al. (2022).

2.5. Total phenolic content (TPC)

Total phenolic content was determined using the method described by Singleton & Rossi (1965). 2 g of pureed samples were weighed and acetone buffer was added and the extraction process was applied for 24 hours. The next day, 0.5 mL of the samples were taken and 0.5 mL of Folin-Ciocalteu's chemical was added, followed by 9 mL of pure water, and 2.5 mL of 7% sodium carbonate was added after 8 min. After two hours, the spectrophotometer was read at 750 nm wavelength. The results are expressed as micrograms (μg) of gallic acid equivalent (GAE) per gram fresh weight (fw).

2.6. Total antioxidant activity (TAA)

Total antioxidant activity was determined using the TEAC (Trolox equivalent antioxidant capacity) method as described by Ozgen et al. (2006). According to the method, 2.97 mL of the prepared TEAC solution was added to 30 μL of sample and the spectrophotometer was read at a wavelength of 734 nm after 10 minutes. The results were presented as Trolox equivalent (TE) micromoles (μmol) per gram fresh weight (fw).

2.7. Statistical analysis

Statistical analyses were carried out using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). The means were compared using Tukey's range test, with the significance level set at 1% and 5%.

3. Results

3.1. Weight loss (%)

The weight loss was significantly influenced by storage time ($p < 0.01$), but was not influenced by melatonin applications ($p > 0.05$). Weight losses ranged from 2.89% to 2.99% on the 7th day, and from 11.27% to 11.90% on the final day of storage (28th day) (Figure 2).



Figure 2. The impact of melatonin treatments on Brussels sprout weight loss throughout storage at 1 ± 0.5 °C and a RH of $95\pm5\%$.

3.2. Soluble solids content (%), titratable acidity (%) and vitamin C (mg L⁻¹)

Soluble solid content (SSC) was affected by the main factors (storage time ($p < 0.01$) and applications ($p < 0.05$)). It was observed that the SSC increased during storage, while the application of 50 μ M melatonin throughout the storage period caused in a reduction in SSC compared to the control. SSC showed no notable effect from the treatments on day 7; however, significant changes were observed on the 14th, 21st, and 28th days. On the last day of the storage period, SSC was higher in the control and 50 μ M melatonin treatments, while it was lower in the 150 μ M melatonin treatment (Figure 3).

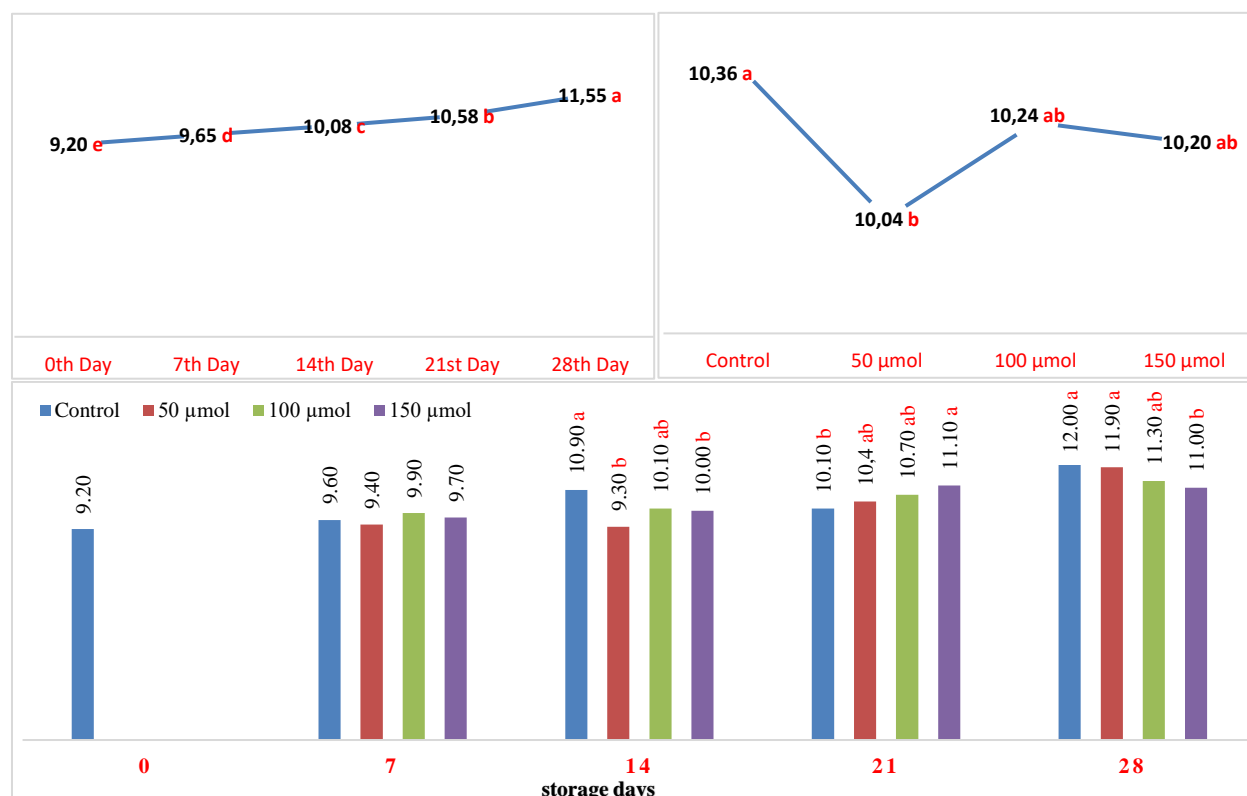


Figure 3. The impact of melatonin treatments on the soluble solid content of Brussels sprouts throughout storage at 1 ± 0.5 °C and a RH of $95 \pm 5\%$.

Titratable acidity (TA) was influenced by both main factors: storage time ($p < 0.01$) and treatments ($p < 0.05$). TA was found to increase throughout the storage period, with the most significant increase occurring on the 14th day. The applications of 50 and 150 μ M melatonin during storage resulted in higher TA compared to the control. TA content of Brussels sprouts was significantly affected by the treatments at all storage periods, except on the 14th day. Melatonin applications on the 7th and 21st days significantly increased TA levels compared to the control. However, by the 28th day, the control treatment exhibited a higher TA content than the melatonin treatments (Figure 4).

Vitamin C content was influenced by the main factors (storage time and treatments, $p < 0.05$). It was noted that vitamin C increased during storage, but the applications of 100 and 150 μ M melatonin throughout storage reduced vitamin C compared to the control. The treatments had a significant effect on vitamin C content on the 7th day of storage, but no notable effects were detected in the subsequent periods. On day 7, vitamin C content decreased with 100 μ M and 150 μ M melatonin treatments in comparison to the control, while the 50 μ M melatonin treatment had a similar effect as the control (Figure 5).

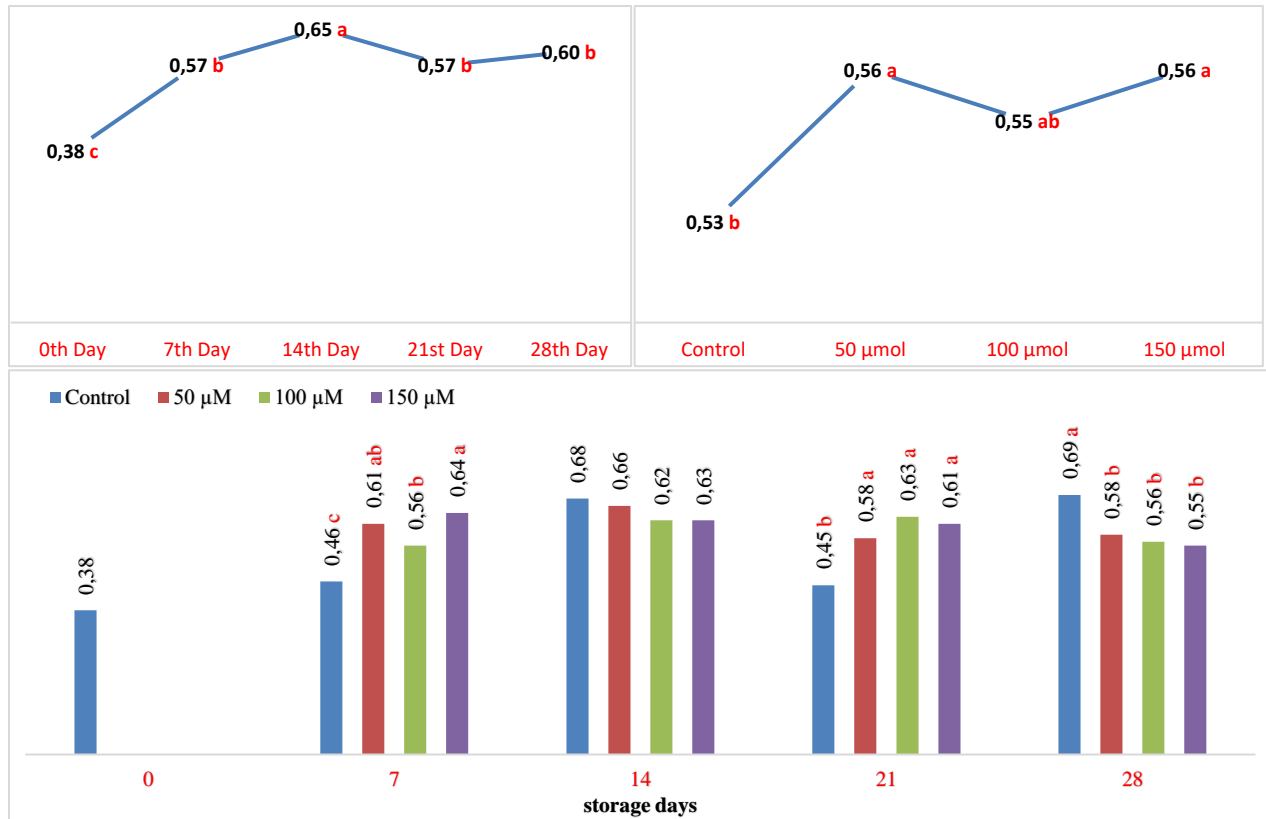


Figure 4. The impact of melatonin treatments on the titratable acidity of Brussels sprouts throughout storage at 1 ± 0.5 °C and a RH of $95\pm5\%$.

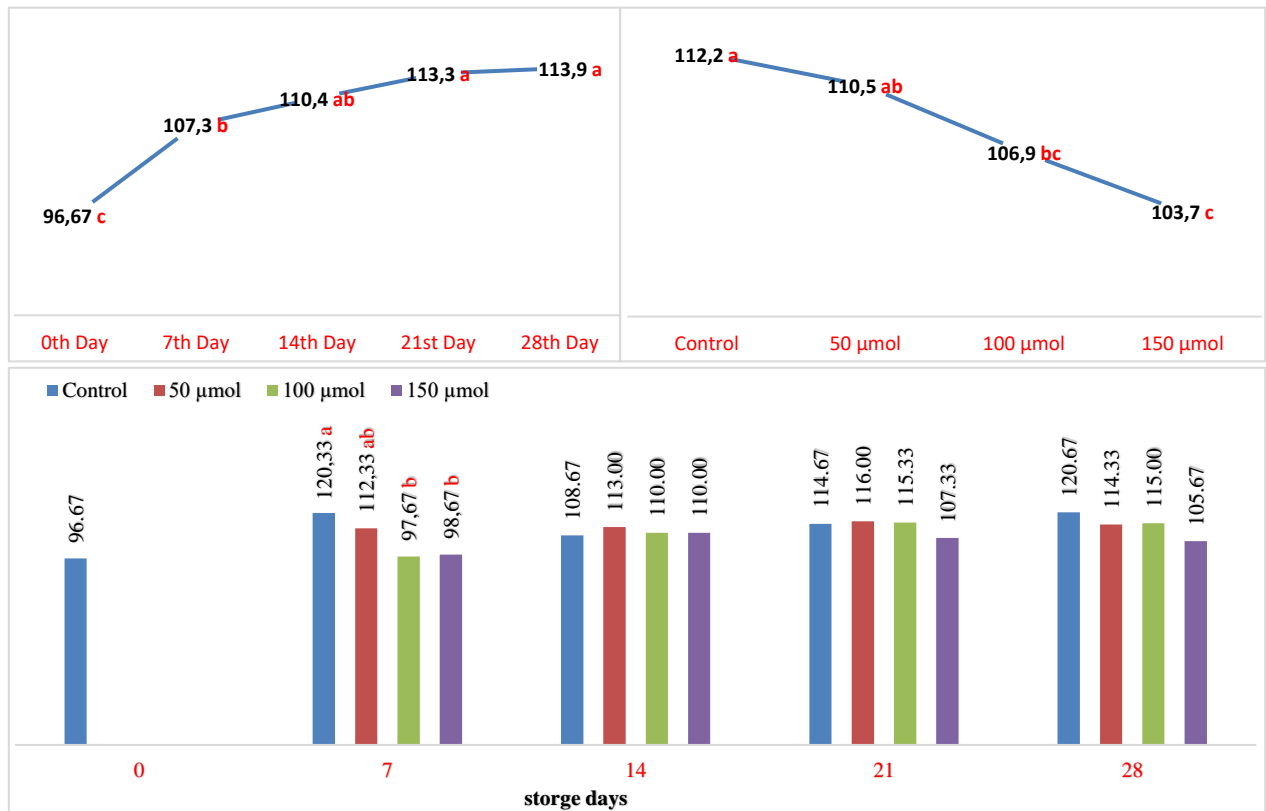


Figure 5. The impact of melatonin treatments on vitamin C of Brussels sprouts throughout storage at 1 ± 0.5 °C and a RH of $95\pm5\%$.

3.3. Chlorophyll content (mg g⁻¹)

The chlorophyll content (total, a, and b) was influenced by the main factors (storage time and treatments, $p < 0.05$). It was determined that chlorophyll content (total, a, and b) increased during the storage period, with the most significant increase occurring on the 14th day. The applications of melatonin throughout storage resulted in higher chlorophyll content in comparison to the control, with the greatest rise monitored in the 50 μ M melatonin treatment (Figures 6, 7, and 8).

Total chlorophyll content of Brussels sprouts was significantly affected by the treatments only during the final period of storage (Figure 6). Analysis conducted on the 28th day revealed that all melatonin applications (50, 100, and 150 μ M) concluded in an increase in total chlorophyll content in comparison with the control.

When chlorophyll-a content was examined, it was found to be affected by the treatments on days 21 and 28 of storage (Figure 7). On the 21st day, the effect of melatonin applications was similar to the control, with the highest chlorophyll-a value monitored in the 50 μ M melatonin treatment and the lowest in the 100 μ M melatonin treatment. By the 28th day, chlorophyll-a values were higher in all melatonin treatments (50, 100, and 150 μ M) compared to the control.

Chlorophyll-b values were affected by the treatments only on the 28th day of storage (Figure 8). On this day, the applications of 50 and 150 μ M melatonin increased chlorophyll-b values compared with the control, while the 100 μ M melatonin application showed a similar effect to the control.

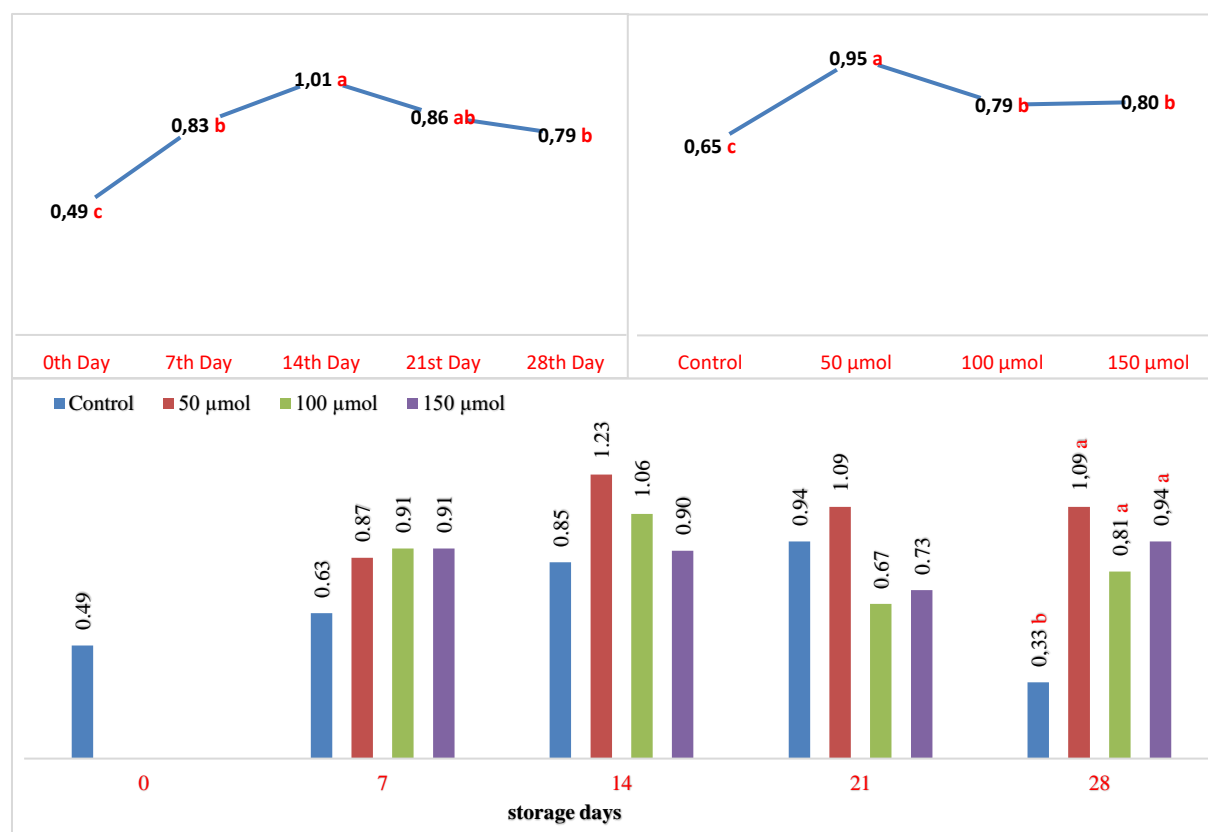


Figure 6. The impact of melatonin treatments on the total chlorophyll of Brussels sprouts throughout storage at 1 ± 0.5 °C and a RH of $95 \pm 5\%$.

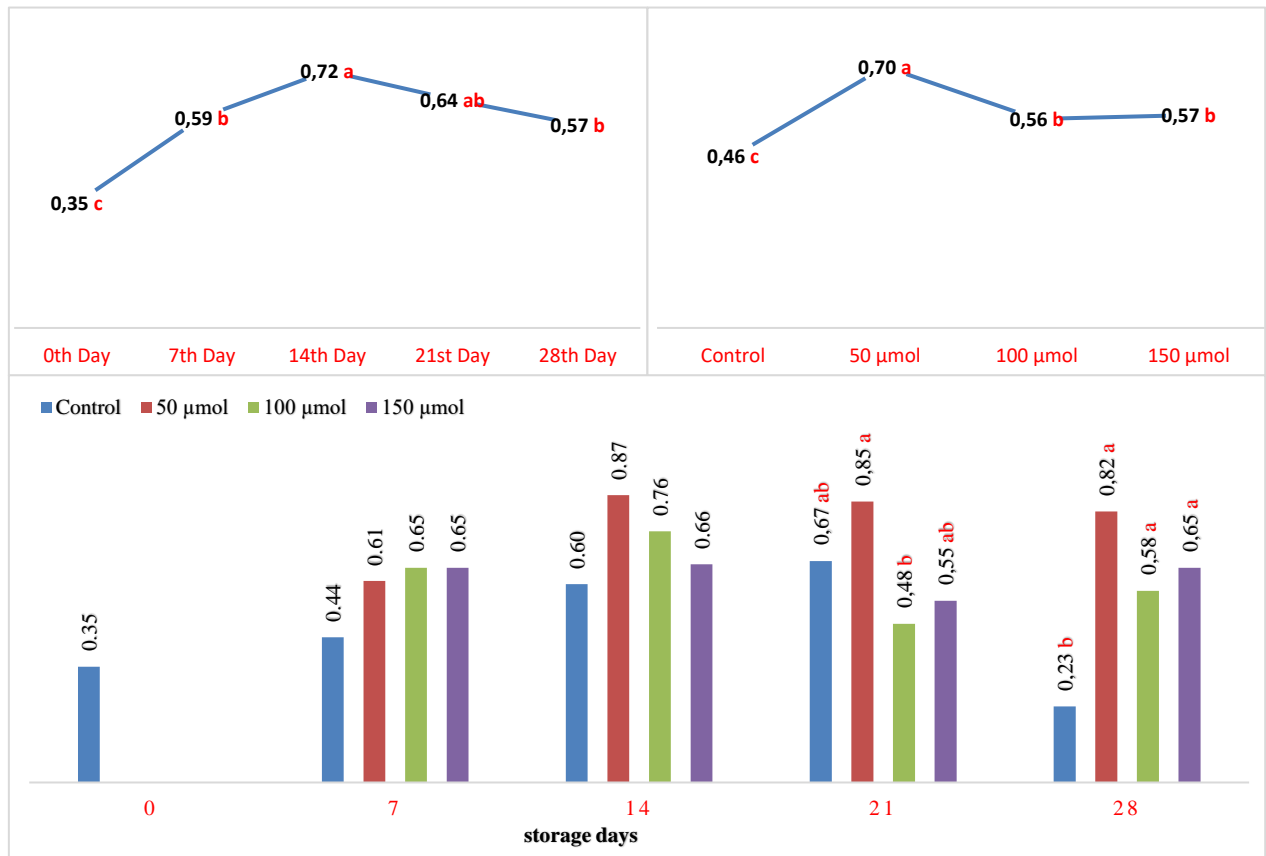


Figure 7. The impact of melatonin treatments on the chlorophyll-a content of Brussels sprouts throughout storage at 1 ± 0.5 °C and a RH of $95\pm5\%$.

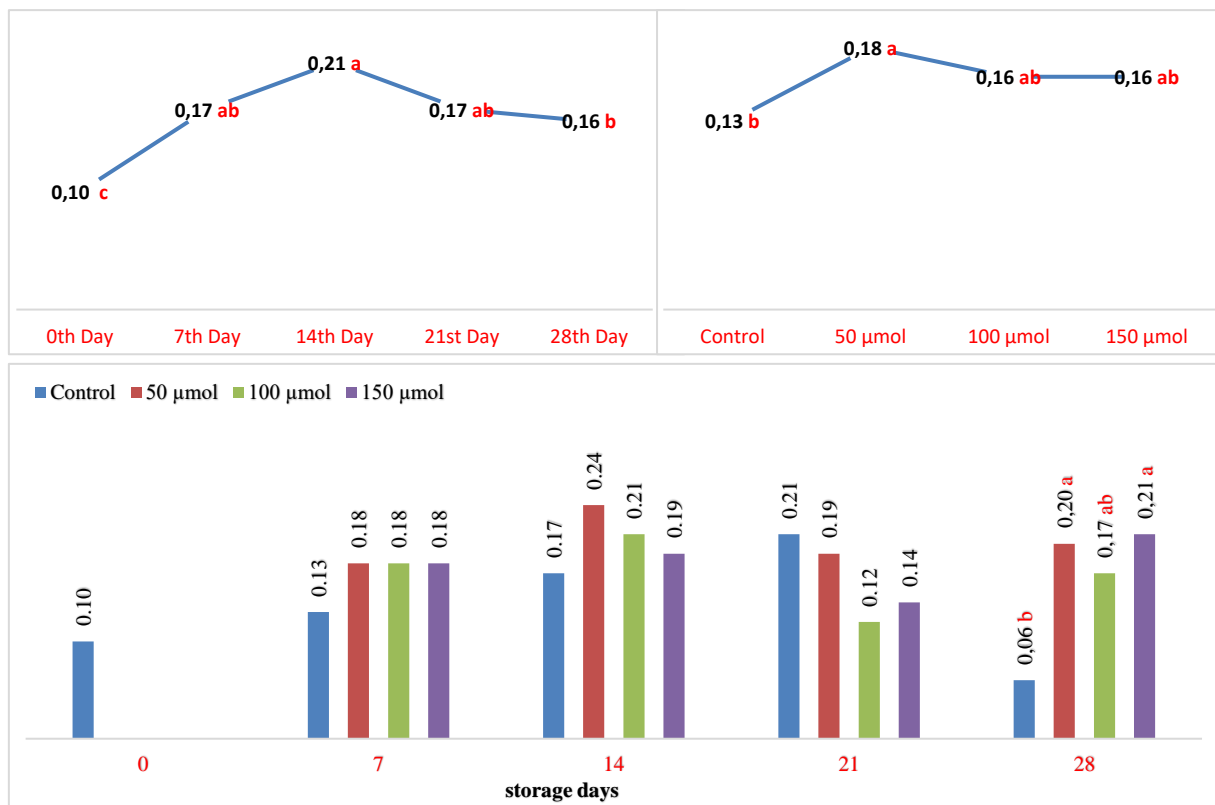


Figure 8. The impact of melatonin treatments on the chlorophyll-b of Brussels sprouts throughout storage at 1 ± 0.5 °C and a RH of $95\pm5\%$.

3.4. Total phenolic content ($\mu\text{g GAE g}^{-1}\text{ fw}$)

The total phenolic content (TPC) was significantly influenced by storage time ($p < 0.05$) but was not influenced by melatonin applications ($p > 0.05$). It was observed that TPC increased during the storage period, with the most notable increase occurring on the 28th day. Analysis performed on the 28th day revealed that TPC was $2853.34 \mu\text{g GAE g}^{-1}$ in the control and $3215.84 \mu\text{g GAE g}^{-1}$ in the $150 \mu\text{M}$ melatonin treatment. However, this difference was found to be statistically insignificant (Figure 9).

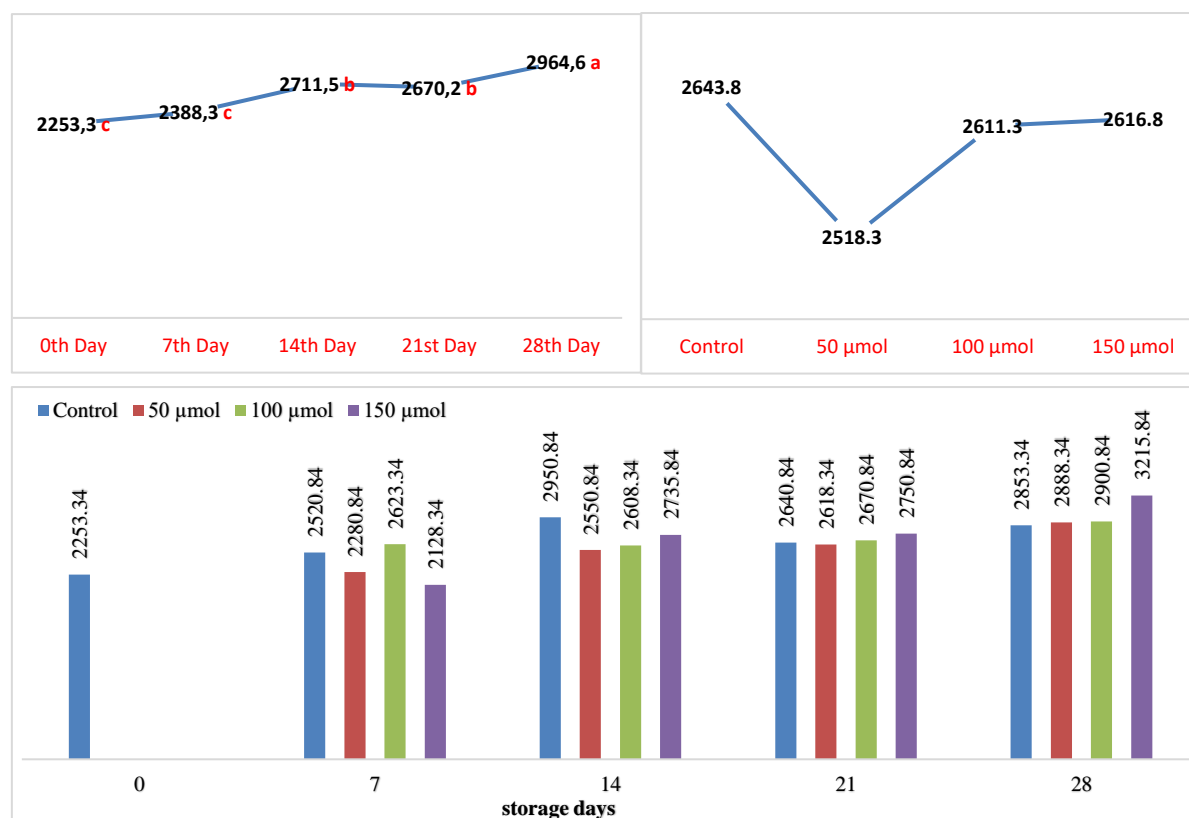


Figure 9. The impact of melatonin treatments on the total phenolic content of Brussels sprouts throughout storage at $1 \pm 0.5^\circ\text{C}$ and a RH of $95 \pm 5\%$.

3.5. Total antioxidant activity ($\mu\text{mol TE g}^{-1}\text{ fw}$)

Total antioxidant activity (TAA) was influenced by the main factors (storage time, $p < 0.01$, and treatments, $p < 0.05$). It was observed that TAA increased during the storage period, with the most significant increase occurring on the 28th day, similar to the trend seen in TPC. The applications of 100 and $150 \mu\text{M}$ melatonin throughout storage resulted in higher TAA compared to the control. TAA was significantly affected by the treatments only on the 7th day. The results from this period showed that all melatonin doses led to higher TAA values compared with the control. Among the melatonin treatments, the $100 \mu\text{M}$ dose exhibited the highest antioxidant activity (Figure 10).

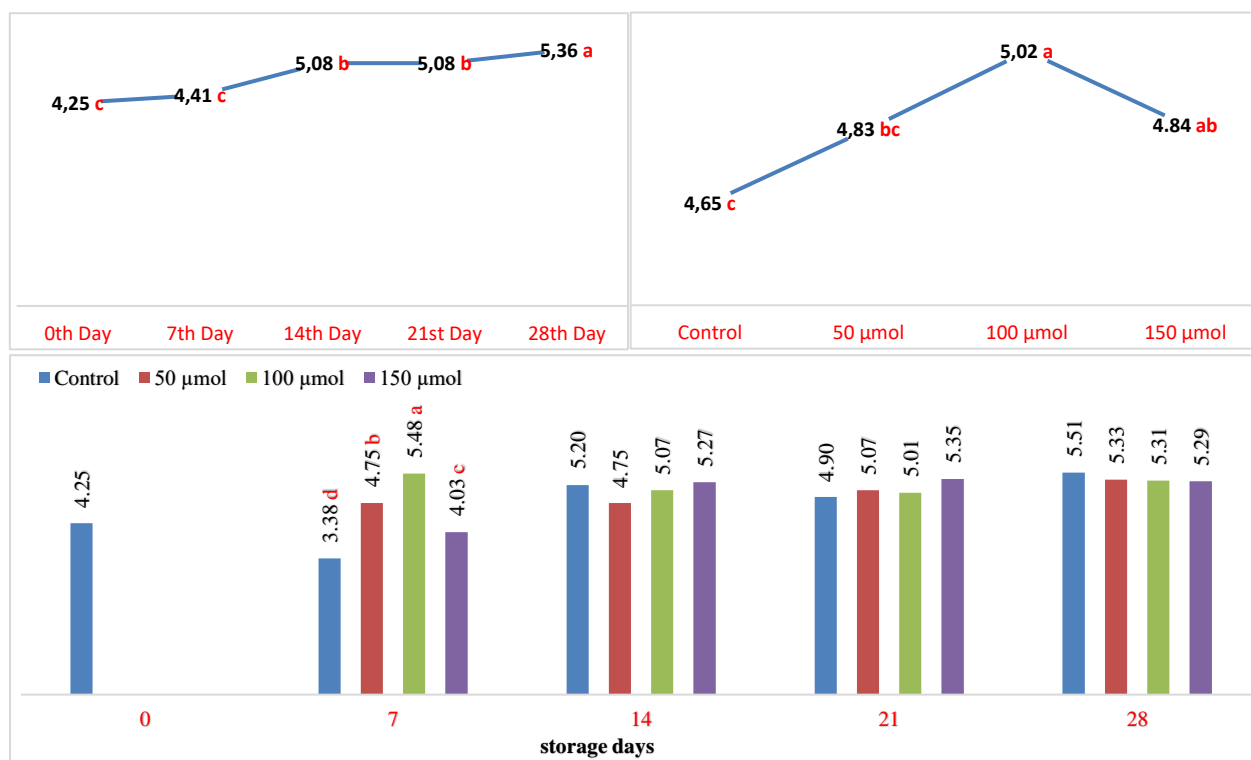


Figure 10. The impact of melatonin treatments on the total antioxidant activity of Brussels sprouts throughout storage at 1 ± 0.5 °C and a RH of $95\pm5\%$.

4. Discussion

The quality of freshly consumed fruits and vegetables, influenced by pre-harvest factors, does not improve after harvesting. In this context, all post-harvest treatments are aimed at maintaining the existing quality. Minimizing water loss (wilting), loss of green color (yellowing), and senescence (accelerated metabolic processes) is crucial for preserving the quality of green vegetables after harvest. Water loss not only results in weight loss in quantitative terms but also leads to the softening of the product, the development of a spongy texture, fading, and wrinkling of the skin. Generally, the acceptable rate of water loss during storage ranges between 5-10%, depending on the species and cultivar of the product (Turk et al., 2017). In a study by Kasim & Kasim (2007), it was found that Brussels sprouts lost up to 10% of their weight after 21 days of storage. In this study, consistent with the literature, the weight loss of Brussels sprouts ranged from 11.27% to 11.90% after 4 weeks of storage (at 1 ± 0.5 °C and a RH of $95\pm5\%$). Additionally, melatonin applications did not significantly impact weight loss (Figure 2).

An increase in the amount of soluble solids content (SSC) and titratable acidity (TA) was observed at the end of the storage period. However, melatonin applications limited the increase in SSC compared to the control group. In general, melatonin applications also preserved the TA content better. When both parameters were considered, the 50 µM dose of melatonin applications was the most effective. Similarly, in the study by Bilgin & Aslantaş (2022), it was observed that TA and SSC values of Brussels sprouts rose after a 28-day of storage. Kowalczyk et al. (2019) also reported an increase in TA content over time in their study with Brussels sprouts. However, in another study, contrary to our findings, it was reported that SSC decreased after 10-day storage of Brussels sprouts at 22°C (Hasperué et al. (2016)). This discrepancy may be attributed to differences in storage conditions.

The most abundant and common vitamin in fruits and vegetables is ascorbic acid, commonly referred to as vitamin C. Besides its role as a cofactor in the synthesis of violaxanthin in chloroplasts within plant cells, ascorbic acid is an important antioxidant. It is particularly abundant in citrus fruits and horticultural products rich in citric acid. During storage, ascorbic acid content decreases due to degradation, which is influenced by the ambient temperature (Kasim & Kasim, 2007; Karaçalı, 2016; Turk et al., 2017; Kowalczyk et al., 2019). No reduction in vitamin C content was noted after cold

storage in this study (Figure 5). Consistent with our findings, [Viña et al. \(2007\)](#) stated that the vitamin C content of Brussels sprouts remained stable throughout storage, with no significant decrease. However, studies on broccoli have shown a decline in vitamin C levels, with melatonin helping to limit this decrease compared to the control ([Lerner et al., 1960](#); [Demirsoy, 2018](#); [Arnao & Hernández-Ruiz, 2020](#); [Miao et al., 2020](#); [Wei et al., 2020a](#); [Wu et al., 2021](#); [Hu et al., 2022](#)). In our study, no such effect of melatonin was observed. This discrepancy may be attributed to the fact that the vegetables used in the two studies belong to different species.

Chlorophylls are green pigments with a lipid-protein structure located in the chloroplasts of plant cells. Chlorophyll molecules are classified into four types: chlorophyll a, b, c, and d. In all highly organized plants, algae, and cyanobacteria, chlorophyll serves as the primary photosynthetic pigment. All highly organized plants and green algae contain chlorophyll b, except for mutant forms. Diatoms, dinoflagellates, and brown algae contain chlorophyll c. Red algae are the only organisms where chlorophyll d is found ([Demirsoy, 2021](#)). In fresh vegetables and fruits, chlorophyll a constitutes the majority of the chlorophyll composition, with chlorophyll b making up the remainder. Chlorophyll is rapidly broken down into small, colorless particles through enzymatic reactions, which are influenced by environmental factors and senescence. During this process, changes in pH, the action of chlorophyllase, and oxidation in the vacuole play key roles in the degradation of chlorophyll ([Karaçalı, 2016](#)). [Tan et al. \(2019\)](#) stated that abscisic acid (ABA) and melatonin play opposing roles in the regulation of leaf senescence in plants. Exogenous melatonin application was reported to reduce endogenous ABA levels by downregulating the expression of genes (BrABF1, BrABF4, BrABI5) associated with chlorophyll degradation and ABA biosynthesis. Consequently, melatonin postponed the senescence of Chinese flowering cabbage. Subsequent studies have documented that melatonin stimulates chlorophyll production in tomato seedlings ([Siddiqui et al., 2020](#)), pepper ([Kaya et al., 2020](#)), and corn leaves ([Ahmad et al., 2020](#)). In post-harvest studies, [Hu et al. \(2022\)](#) and [Miao et al. \(2020\)](#) stated that chlorophyll content in broccoli deteriorated throughout the storage, but these declines were largely prevented by the application of melatonin. Similarly, in our study, it was found that melatonin applications increased the chlorophyll levels in Brussels sprouts compared with the control.

Phenolic compounds and vitamin C, which are abundant in *Brassica* vegetables, are particularly notable for their high content and strong antioxidant activity. When consumed, these compounds can contribute to preventing chronic diseases such as cancer, heart disease, and diabetes by protecting cells from oxidative damage ([Podsędek, 2007](#)). The conservation of these compounds after harvest is crucial for maintaining their health benefits. Our study showed a trend of increasing both total phenolic content and antioxidant activity during storage. However, no effect of melatonin on phenolic content was observed. The effect of the applications on antioxidant activity was found to be significant only on the 7th day. On this day, melatonin applications increased antioxidant capacity compared with the control group. The highest antioxidant activity was detected in the 100 µM melatonin treatment, followed by melatonin doses of 50 µM and 150 µM, respectively. Consistent with our findings, [Miao et al. \(2020\)](#) and [Wei et al. \(2020a\)](#) also stated that total phenolic content and antioxidant capacity in broccoli flowers increased by the deadline of the storage period. [Miao et al. \(2020\)](#) indicated that there was no notable difference between the control group and the 1 µM melatonin treatment after storage, whereas [Wei et al. \(2020a\)](#) observed that 100 µM melatonin treatment led to an increase in TPC and TAA contents in comparison to the control. In our study, melatonin did not exhibit such an effect. This discrepancy could be due to the varying responses of Brussels sprouts and broccoli flowers to melatonin treatment.

5. Conclusion

As a result, postharvest quality of Brussels sprouts was significantly affected by melatonin applications. Application of 50 µM melatonin limited the increase in SSC while promoting the increase in TA levels. All tested melatonin concentrations (50, 100 and 150 µM) resulted in an increase in total chlorophyll and chlorophyll a and b levels compared to the control group. During the first seven days of cold storage, 100, 50 and 150 µM melatonin applications showed the highest antioxidant activity, respectively. However, 50 µM melatonin application provided the highest vitamin C content. Melatonin application had no significant effect on weight loss or phenolic content. In general, melatonin did not adversely affect the quality of Brussels sprouts and preserved the external color and sugar/acid ratio of the product. The best quality results were obtained with the application of 50 µM melatonin. These

results indicate that melatonin can be effectively used in postharvest applications. Brussels sprouts can be stored commercially for up to 28 days, after which time weight loss exceeds 10%.

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