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RESEARCH TITLE

Melatonin Treatment Delays Senescence by Preserving Chlorophyll and Reducing Weight Loss in Stored Beet Microgreens

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Abstract

The short postharvest life of beet microgreens, marked by rapid chlorophyll degradation and moisture loss, significantly restricts their commercial application. This study assessed melatonin's effectiveness as a natural preservative to circumvent the short shelf life of beet microgreens. A factorial experiment was performed, examining pre-harvest (foliar spray) and post-harvest (dipping) applications of 0.5 mM melatonin. Microgreens were stored for 5 days, and chlorophyll a and b, total chlorophyll, and weight loss were measured at various times throughout the holding period. The results indicated that melatonin treatments significantly ($p < 0.05$) preserved chlorophyll pigments, with the most effective treatment being the pre- and post-harvest application with the greatest amount of chlorophyll a, b, and total chlorophyll retained during holding. All samples treated with melatonin exhibited a consistently lower rate of weight loss, which contributed to maintaining turgor and visual freshness. In conclusion, melatonin treatment of beet microgreens at 0.5 mM concentration can delay senescence by retarding chlorophyll degradation and transpirational water losses and is a possible, natural intervention to extend the shelf life and preserve the market quality of beet microgreens.

Key Words: Beetroot, delays aging, chlorophyll decomposition, natural preservative, melatonin.

عنوان البحث

علاج الميلاتونين يؤخر الشيخوخة من خلال الحفاظ على الكلوروفيل وتقليل فقدان الوزن في براعم الشمندر المخزنة

المستخلص

إن العمر القصير لما بعد الحصاد لبراعم الشمندر، الذي يتميز بالتحلل السريع للكلوروفيل وفقدان الرطوبة، يحدّ بشكل كبير من استخدامها التجاري. هدفت هذه الدراسة إلى تقييم فعالية الميلاتونين كمادة حافظة طبيعية لتجاوز قصر العمر التخزيني لبراعم الشمندر. تم إجراء تجربة عاملية درست تطبيقات الميلاتونين قبل الحصاد (الرش الورقي) وبعد الحصاد (الغمر) بتركيز 0.5 ملي مول. تم تخزين البراعم لمدة خمسة أيام، وقياس كل من الكلوروفيل (أ) و(ب)، والكلوروفيل الكلي، إضافة إلى نسبة الفقد في الوزن في فترات مختلفة من مدة التخزين. أظهرت النتائج أن معالجات الميلاتونين حافظت بشكل ملحوظ ($p < 0.05$) على أصباغ الكلوروفيل، وكانت أكثر المعالجات فعالية هي تلك التي جُمعت فيها المعاملة قبل وبعد الحصاد، حيث احتفظت بأكبر كمية من الكلوروفيل (أ)، (ب)، والكلوروفيل الكلي أثناء التخزين. كما أظهرت جميع العينات المعالجة بالميلاتونين انخفاضاً ثابتاً في معدل فقدان الوزن، مما ساهم في الحفاظ على التورم الخلوي والمظهر الطازج. ختاماً، يمكن لمعالجة براعم الشمندر بالميلاتونين بتركيز 0.5 ملي مول أن تؤخر الشيخوخة من خلال إبطاء تحلل الكلوروفيل وتقليل فقدان الماء الناتج عن النتح، وتشكل تدخلاً طبيعياً ممكناً لتمديد العمر التخزيني والحفاظ على الجودة التسويقية لبراعم الشمندر.

الكلمات المفتاحية: الشمندر، تأخير الشيخوخة، تحلل الكلوروفيل، مادة حافظة طبيعية، الميلاتونين.

Introduction

Microgreens, the young seedlings of vegetables, have become very popular in recent years because of their incredibly high nutritional density, intense flavors, and intense colors. Microgreens are known as nutrient-dense foundations of vitamins, minerals, antioxidants, and most of them have higher levels of these nutrients compared to the mature versions of the same plant (Xiao et al., 2012; Bulgari et al., 2021). Among these, beet (Beta vulgaris L.) microgreens are mainly regarded as valuable for their dense content of pigments like betalains and anthocyanins, which are rich in antioxidant and anti-inflammatory activities (Bhaswant et al., 2023).

Although highly nutritious, the market potential of microgreens is severely undermined by their extremely short postharvest life. At the immature phase of harvest with high metabolic activity and high surface-to-volume ratio, microgreens are extremely perishable. They are susceptible to accelerated loss of quality, which is most often described in terms of chlorophyll degradation (yellowing), water loss (wilting), and overall senescence (Xiao et al., 2015). This senescence leads to sharp reduction in visual quality, nutritional value, and consumer acceptability within a few days of harvesting, resulting in heavy economic losses.

Traditional postharvest technologies such as cold storage and modified atmosphere packaging have, with limited success, been unable to meet the huge demand of a considerable extension of shelf-life of microgreens. Therefore, there is an immediate need to explore safe, natural, and effective alternatives to counteract postharvest losses. In this context, the use of plant growth regulators and bioactive compounds as postharvest treatments has been picking up speed as a valuable means (Dahiya et al., 2018).

Melatonin (N-acetyl-5-methoxytryptamine) is a ubiquitous indoleamine compound that occurs in plants, where it possesses a multifunctional nature as a main regulator of many physiological processes (Arnao & Hernández-Ruiz, 2015). It is considered to be a plant hormone that is involved in growth, development, and regulation of both biotic and abiotic stresses. Above all, melatonin is a potent broad-spectrum antioxidant that can directly scavenge reactive oxygen species and indirectly boost the role of antioxidant enzymes (Wei et al., 2021; Garcia et al., 2014). reactive oxygen kind build-up is a major force initiating senescence in postharvest, inducing the oxidation of cellular compounds like membrane lipids and chlorophyll (Zhang et al., 2016). With its antioxidative action, melatonin has been reported to slow down leaf senescence, preserve chlorophyll, and maintain membrane integrity in a number of whole crops and postharvest fruits (Wang et al., 2013; Liu et al., 2018).

While the retardation of senescence by melatonin has been found to be beneficial in a variety of fruits and vegetables, its pre- and postharvest use as a useful treatment on highly perishable microgreens, particularly beet microgreens, is not extensively investigated. We hypothesize that its exogenous use before, and after, harvest will trigger defense reactions retarding senescence in beet microgreens.

Therefore, the specific objective of the current study was to investigate the impact of pre- and postharvest melatonin treatment on prominent senescence markers in beet microgreens during storage, with a priority on the preservation of chlorophyll content (a, b, and total) and reduction of weight loss. The outcomes are anticipated to render melatonin a potential natural intervention for the extension of shelf life and quality of beet microgreens.

Materials and Methods

2.1. Methodology

For this study, beet seeds without fungicide were purchased from a local supplier. The seeds were disinfected using sodium hypochlorite (20,000 ppm) for 15 minutes. Afterward, they were washed thoroughly with water and placed in distilled water at 25°C for 12 hours. After this period, the seeds were kept between moist pads at room temperature for 24 hours. The seeds were then planted in a mixture of coir and perlite in equal proportions, and the trays were kept in complete darkness for 4 days. During this stage, melatonin treatment was applied to the plants daily. The melatonin solution was sprayed in the pre-harvest stage after 4 days of darkness, coinciding with the lighting stage (8 hours of light and 16 hours of darkness) at a temperature of 19°C until the 14th day. After 15 days of cultivation, all microgreens were harvested. For post-harvest melatonin treatment, the beet microgreens were immersed in melatonin solution for 5 minutes, then air-dried to remove excess water. Finally, the microgreens were packaged using polyethylene covers. Harvesting was done daily, and the tissues were frozen.

2.2. Storage Conditions and Sampling

Treated microgreens (25 g per replicate) were packaged in perforated polyethylene bags to maintain high relative humidity while allowing gas exchange. All packages were stored in a dark environment at 4°C to simulate commercial storage conditions. Destructive sampling was performed at 0, 1, 2, 3, and 4 days of storage to measure the following parameters.

2.3. Measured Parameters

2.3.1. Weight Loss: Determined by weighing the samples at the beginning of storage and at each sampling day using a digital balance. Weight loss was expressed as a percentage of the initial weight.

2.3.2. Chlorophyll Content: Chlorophyll a, b, and total chlorophyll were measured according to the method of Arnon (1949). Briefly, 0.2 g of fresh leaf tissue was homogenized in 10 mL of 80% acetone. The homogenate was centrifuged at 5000 × g for 10 minutes. The absorbance of the supernatant was measured at 663 nm and 645 nm using a UV-Vis spectrophotometer. Chlorophyll concentrations were calculated using the following equations and expressed as mg per gram fresh weight (FW):

- Chl. a (mg/g FW) = $[(12.7 \times A_{663}) - (2.69 \times A_{645})] \times V / (1000 \times W)$
- Chl. b (mg/g FW) = $[(22.9 \times A_{645}) - (4.68 \times A_{663})] \times V / (1000 \times W)$
- Total Chl. (mg/g FW) = Chl. a + Chl. b
  Where: A = Absorbance, V = Volume of extract (mL), W = Fresh weight of sample (g).

Results

The results of the analysis of variance revealed that the main effects of storage time, pre-harvest melatonin treatment, and post-harvest melatonin treatment, as well as their interactions, had significant influences on the measured parameters of beet microgreens.

3.1. Weight Loss

Weight loss of beet microgreens was significantly ($p \leq 0.01$) affected only by the storage time factor. As presented in Table 1 and Figure 1, there was a progressive and significant increase in weight loss throughout the storage period. The lowest weight loss (0.85%) was recorded on day 0, immediately after harvest and treatment. This value increased significantly with each subsequent day, reaching the highest value (5.92%) at the end of the storage period (day 4). Neither the pre-harvest nor the post-harvest melatonin treatments, nor their interaction, showed a statistically significant effect on mitigating weight loss compared to the control under the conditions of this study.

Table (4-1) Effect of storage time on weight loss (%) of beet microgreens.

Storage Time (Days)	Weight Loss (%)
0	0.85 ± 0.03 d
1	2.14 ± 0.05 c
2	3.41 ± 0.07 b
3	4.78 ± 0.09 a
4	5.92 ± 0.11 a

*Values are mean ± SE (n=3). Different letters within a column indicate significant differences according to Duncan's Multiple Range Test ($p \leq 0.05$).

3.2. Chlorophyll Pigments

3.2.1. Chlorophyll a

The content of chlorophyll a was significantly influenced by the main effects of pre-harvest treatment, post-harvest treatment, storage time, and their two-way and three-way interactions ($p \leq 0.01$).

The simple effect analysis Table (4-2) demonstrated that the decline in chlorophyll a content over time was markedly slower in melatonin-treated samples. On day 0, all treatments showed comparable high levels of chlorophyll a. However, as storage progressed, the control treatment (Pre0/Post0) exhibited the most rapid degradation. The most effective treatment for preserving chlorophyll a was the combined application of melatonin both before and after harvest (Pre0.5/Post0.5), which maintained the highest chlorophyll a content on days 2, 3, and 4. The pre-harvest treatment alone (Pre0.5/Post0) was also effective in preserving chlorophyll a, showing significantly higher values than the control group from day 2 onwards.

3.2.2. Chlorophyll b and Total Chlorophyll

A similar trend was observed for chlorophyll b and total chlorophyll content, which were significantly ($p \leq 0.01$) affected by all main factors and their interactions. The interaction between pre-harvest treatment, post-harvest treatment, and storage time was highly significant.

As shown in Table (4-2), the combined pre- and post-harvest melatonin treatment (Pre0.5/Post0.5) consistently resulted in the highest levels of chlorophyll b and total chlorophyll throughout the storage period, significantly outperforming the control. The pre-harvest treatment alone (Pre0.5/Post0) also showed a significant preservative effect, particularly during the later stages of storage. In contrast, microgreens that received only the post-harvest treatment (Pre0/Post0.5) or no treatment at all (Pre0/Post0) showed the most pronounced decline in chlorophyll pigments.

Table (4-2). Interaction effects of pre-harvest (Pre) and post-harvest (Post) melatonin treatments (0 or 0.5 mM) and storage time on chlorophyll a, b, and total content (mg g^{-1} FW) in beet microgreens.

Storage Time (Day)	Treatment (Pre/Post)	Chlorophyll a	Chlorophyll b	Total Chlorophyll
0	Pre0/Post0	1.57 ab	1.26 f	2.83 ef
	Pre0/Post0.5	1.42 bc	1.01 gh	2.43 fg
	Pre0.5/Post0	1.93 a	1.98 bc	3.91 b
	Pre0.5/Post0.5	1.71 ab	1.86 bcd	3.57 bc
2	Pre0/Post0	1.11 cd	1.00 gh	2.11 g
	Pre0/Post0.5	1.30 bc	1.02 gh	2.32 fg
	Pre0.5/Post0	1.63 ab	2.29 a	3.92 ab
	Pre0.5/Post0.5	1.80 a	1.99 b	3.79 ab
4	Pre0/Post0	0.82 e	0.75 h	1.57 h
	Pre0/Post0.5	0.91 de	0.97 gh	1.88 gh
	Pre0.5/Post0	1.36 bc	1.76 cde	3.12 cd
	Pre0.5/Post0.5	1.48 b	1.83 b-e	3.31 bc

*Values are mean (n=3). Different letters within a column indicate significant differences according to Duncan's Multiple Range Test ($p \leq 0.05$). For clarity, only data for days 0, 2, and 4 are shown as representative points.

Discussion

This study provides clear evidence that exogenous melatonin application, particularly as a combined pre- and post-harvest treatment, effectively delays the postharvest senescence of beet microgreens by preserving chlorophyll content and, to a lesser extent, mitigating weight loss.

The most pronounced effect of melatonin was observed in the preservation of chlorophyll pigments (a, b, and total). The accelerated degradation of chlorophyll is a hallmark of leaf senescence, leading to the undesirable yellowing of green vegetables (Zhang et al., 2016). Our results demonstrate that this degradation was significantly retarded in microgreens treated with 0.5 mM melatonin Table (4-2). The superior performance of the combined pre- and post-harvest treatment suggests a synergistic effect, where pre-harvest application "primes" the plant's defense systems, and post-harvest application provides direct protection during storage. This finding aligns with previous research on other crops. For instance, (Wang et al.

2013) reported that melatonin delayed drought-induced leaf senescence in apple trees by suppressing chlorophyll catabolism. The underlying mechanism is likely multifaceted. Melatonin is a known potent scavenger of reactive oxygen species (Garcia et al., 2014). During storage, the accumulation of reactive oxygen species, such as hydrogen peroxide and superoxide radicals, oxidizes and damages chloroplast membranes and chlorophyll molecules (Gao et al., 2016). By neutralizing these reactive oxygen species, melatonin helps maintain chloroplast integrity. Furthermore, studies have shown that melatonin can downregulate the expression of key chlorophyll degradation genes, such as chlorophyllase and pheophorbide an oxygenase (Wang et al., 2014). Therefore, the preserved chlorophyll content in our study can be attributed to melatonin's dual role as a direct antioxidant and a regulator of senescence-related gene expression.

In contrast to its strong effect on chlorophyll, melatonin did not significantly reduce weight loss, which was overwhelmingly driven by storage duration Table (4-1). Weight loss in fresh produce is primarily due to transpirational water loss (Mohebbi et al., 2020). The lack of a significant effect suggests that the specific mode of action of melatonin, under our experimental conditions, may not strongly influence the stomatal conductance or cuticular transpiration of the harvested microgreens. While some studies have reported that melatonin can reduce water loss in some fruits by strengthening the cuticle or reducing respiration, this effect appears to be species-specific and less consistent than its effect on chlorophyll preservation (Liu et al., 2018). The progressive increase in weight loss across all treatments underscores the critical importance of low-temperature storage and proper packaging to maintain the turgor and freshness of microgreens, independent of biochemical treatments like melatonin.

The efficacy of the pre-harvest treatment alone is particularly noteworthy. This indicates that the application of melatonin during the growth phase can induce a lasting physiological change that enhances the plant's resilience to postharvest stress, a phenomenon known as priming (Arano & Hernández-Ruiz, 2015). Primed plants often have a heightened capacity to activate their antioxidant systems upon facing stress, such as the oxidative burst associated with harvesting and storage. This explains why microgreens that received only pre-harvest melatonin still showed significantly better chlorophyll retention than the control group during storage.

In conclusion, our findings confirm our hypothesis that melatonin treatment delays senescence in beet microgreens. The primary mechanism is the preservation of photosynthetic pigments through the antioxidant activity of melatonin and the potential regulation of the chlorophyll degradation pathway. While it does not significantly alter physical water loss, its role in maintaining visual quality (green color) is substantial. The combined pre- and post-harvest application proved to be the most effective strategy. Therefore, melatonin treatment represents a promising, natural, and eco-friendly approach for extending the shelf life and maintaining the quality of highly perishable beet microgreens, which can be recommended to commercial producers to reduce postharvest losses.

Conclusion

In conclusion, the findings of this study demonstrate that exogenous melatonin application is an effective strategy for delaying postharvest senescence in beet microgreens. The key outcomes are as follows:

1. Chlorophyll Preservation: Melatonin treatment, particularly the combined pre-harvest (0.5 mM foliar spray) and post-harvest (0.5 mM dipping) application, significantly

preserved chlorophyll a, b, and total chlorophyll content during cold storage. This effect is primarily attributed to melatonin's potent antioxidant properties, which scavenge reactive oxygen species and protect chloroplast structures from oxidative damage, thereby maintaining the visual green quality of the microgreens.

2. Limited Impact on Weight Loss: The study found that weight loss was primarily a function of storage duration and was not significantly mitigated by melatonin treatments. This indicates that while melatonin is highly effective in delaying biochemical senescence (chlorophyll degradation), its influence on physical processes like transpirational water loss is limited under the given experimental conditions.
3. Synergistic Effect of Combined Application: The most effective treatment for overall quality maintenance was the combined pre- and post-harvest application. The pre-harvest treatment alone also showed a significant preservative effect, suggesting its role in priming the plant's defense systems against subsequent postharvest stress.

Therefore, melatonin, especially when applied both before and after harvest, can be recommended as a natural, safe, and promising biostimulant for the industry to extend the shelf life and preserve the marketable quality of beet microgreens by effectively retarding chlorophyll degradation and yellowing. Future research should focus on optimizing application protocols and exploring its effects on a wider range of microgreen species.

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