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Original Research Paper

Post-harvest melatonin application reduced browning in minimally processed lettuce (*Lactuca sativa* L.) during low temperature storage

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ABSTRACT

The investigation was carried out to assess the effect of post-harvest dipping of minimally processed fresh cut lettuce with various concentrations (10, 100 and 1000 μmolL^{-1}) of melatonin on shelf-life and sensory quality of lettuce stored at 6±2ºC for 8 days. Melatonin treatment was found effective in maintaining freshness and sensory quality of lettuce during storage. Browning was reduced by 45% and visual quality index increased by 44.10% compared to control in 100 μmol L^{-1} melatonin treated samples on the 6th day of storage. Maximum total chlorophyll, total phenol and total antioxidants and least activity of browning related enzyme *i.e.,* peroxidase (POD) was observed in 100 μmol L^{-1} melatonin treated samples during storage. No significant variation was observed between 10 μmol L^{-1} melatonin treated and control samples. Browning index value had significant negative correlation with total chlorophyll, total phenol and total antioxidants whereas POD activity had significant positive correlation. It can be inferred from the present investigation that post-harvest treatment of 100 μmol L^{-1} melatonin extended shelf-life of minimally processed lettuce for 6 days by preserving phenols, chlorophyll, antioxidants and inhibiting POD activity.

Keywords : Browning, lettuce, melatonin, minimal processing, peroxidase, phenols

INTRODUCTION

Minimally processed vegetables, popularly known as ready-to-use or ready to eat or fresh-cut, are raw vegetables that have been sanitized, peeled, sliced, chopped or shredded and packaged to make them readily usable without decline in freshness and quality (Siddique *et al*., 2011). Since lettuce is having meager number of calories (10 kcal100^{-1} g FW), it is often advised for reducing obesity and also minimizing risk of cataracts, heart ailments, cancers and paralysis due to presence of ample amount of β-carotene and lutein contents (Mampholo *et al*., 2016). In recent years, demand for minimally-processed (MP) vegetables is increasing in India and projected to record 6.5% compound annual growth rate (CAGR) by 2026 due to their minimal processing, ready to consumption form and high dietary value. Lettuce is an important leafy vegetable usually consumed as salads. Currently, share of salads has enhanced in diet and, hotels, restaurants and catering services are demanding lettuce in ready to eat form. Lettuce is highly delicate and prone to surface browning through enzyme action. Minimally processed produce deteriorates more rapidly than whole produce because internal and outer tissues are exposed to external environment. Physical damage during the minimal processing elevate metabolic activities, respiration, biochemical conversion and microbial growth, that often result in dilapidation of texture, color, ûavour and affect visual quality as well as marketability of the product. Several chemical and physical treatments have been widely tried out to manage fresh-cut lettuce browning. However, most of the methods are commonly constrained by toxic nature, cost and potentially spoiling sensory properties and reduction in nutrient content of the produce.

Melatonin is a harmless biological molecule synthesized naturally in mitochondria and chloroplast of the plants (Tan *et al*., 2013). Melatonin works as an antioxidant and augments the post-harvest life of horticultural produce. Earlier, post-harvest treatment of melatonin had found effective in mitigating browning and extending shelf-life in strawberry (Aghdam and Fard, 2017), litchi (Zhang *et al*., 2018), peach (Gao *et al*., 2018), broccoli (Zhu *et al*., 2018) and cut anthurium flowers (Aghdam *et al*., 2019). The objective of this investigation was to assess the impact of post-harvest melatonin treatment on tissue browning and storage quality of fresh-cut lettuce.

MATERIALS AND METHODS

Lettuce var. 'Grishma' grown under aeroponic conditions at vegetable hydroponic centre of ICAR-CISH, Lucknow was procured. Uniform size healthy heads were selected and wrapper foliage was removed and heads were sanitized with 100 ppm chlorine water. Then heads were cut into two halves with sanitized sharp stainless-steel knife. Thereafter, cut pieces were treated with aqueous solution of melatonin (CDH, New Delhi) (10 µmol, 100 µmol and 1000 µmolL^{-1}) by immersing them for 5 min and treated heads were air-dried to evaporate surface water. Dried cut pieces with sample size of 200 g were packed in zip-n-lock polypropylene bags. Packaged samples were stored at 6±2°C temperature for period of 8 days and observation was recorded on 0, 2, 4, 6 and 8 days of storage. Physical and biochemical observations were recorded in four samples for each treatment.

The appearance and browning index of lettuce were recorded as suggested by Tian *et al.* (2014). Five lettuce pieces evaluated for each treatment by 8 panelists. Overall visual quality (OVQ) was measured on a scale from 9 to 1, where 8- 9: excellent (completely devoid of brown spots) 6-8: good (minor defects; not objectionable) and less than 6: poor (moderately to excessive defects) quality. Salability limit was restricted to 6 OVQ rating. The browning index (BI) was calculated by using following formula: BI="(browning scale) × (number of lettuce pieces with that browning level)/ (total number of lettuce pieces). Sample rated BI more than 2.0 was considered unsuitable for marketing. Electrolyte leakage (EL) was measured by using the method of Aghdam *et al.* (2015). The total phenols were determined by the Folic-Ceocalteu method using tannic acid as standard. The total chlorophyll was determined using the equation: Total chlorophyll (µg/ml) = (20.2 x O.D. at 645 nm) + (8.02 x O.D. at 663 nm) as given by Arnon (1949). The antioxidants activity in lettuce was measured as ferric reducing antioxidant potential (FRAP) value (Bhattacherjee *et al.*, 2014). The peroxidase enzyme activity was estimated as number of absorbance units per gram fresh weight of leaf. Experiment was designed in complete randomized block design (CRD) and data was analyzed by using Web Agri Stat Package 2.0 (WASP 2.0) statistical software.

RESULTS AND DISCUSSION

The emergence of brown spots on minimally processed (MP) lettuce leaves increased progressively in all samples during storage period irrespective of post-harvest treatment. During first 2 days of storage, browning index (BI) remained below the threshold limit (less than 2) in all samples. However, BI in control and 10 µmolL^{-1} melatonin treated samples was significantly higher compared to 100 and 1000 µmolL^{-1} melatonin treated samples (Fig. 1). After 2 days of storage, sharp elevation in BI was observed in control and 10 µmolL^{-1} melatonin treated lettuce and it exceeded threshold BI limit (less than 2) on 4th day during storage and with values 3.50 and 3.16, respectively. However, BI in 100 and 1000 µmolL^{-1} melatonin treated leaves was lesser than the threshold limit (below 2) *i.e.*, 1.91 and 1.95, respectively on 6th day of storage. At 6th day of storage, 45% lower BI value was observed in 100 µmolL^{-1} melatonin treated samples compared to control. At 8th day of storage, the lowest BI (2.45) was noticed in 100 µmol melatonin treated samples followed by 1000 µmolL^{-1} melatonin treatment (2.51) whereas, significantly higher BI (3.82) was observed in control and 10 µmol melatonin treated lettuce. Similar outcomes were reported by Aghadam *et al.* (2015) in cut anthurium where 51% lower browning was noticed in 100 µmol melatonin treated flowers. Zhang *et al.* (2018) observed strong suppression of pericarp browning in litchi through post harvest melatonin treatment. Membrane damaged during minimal processing

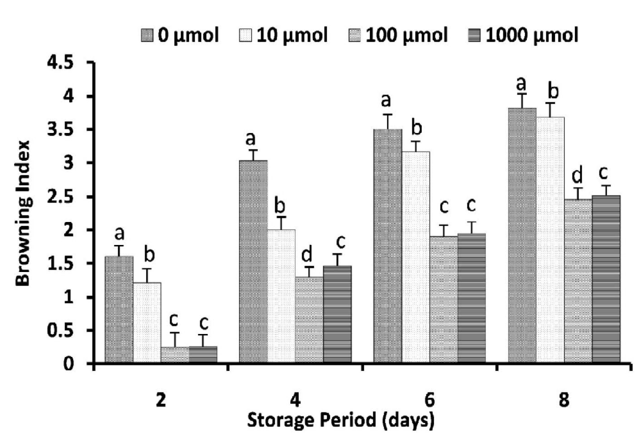

Fig. 1. Browning index score in minimally processed lettuce treated with exogenous application of melatonin during 8 days storage at 6±2°C temperature.

operations caused loss of sub-cellular compartmentalization, leading to contact between browning inducing-enzymes (PPO and POD) and phenolic substrates, further leading to enzymatic browning in fruits and vegetable produce. In the current investigation, less tissue browning in treated samples compared to control might be due to suppression of phenol oxidizing enzymes by melatonin. This is supported by the significant positive correlation (r=0.915) between BI and POD activity at the end of storage period (Table 2).

Visual quality of MP lettuce was considerably retained by exogenous post-harvest dip treatment of 100 and 1000 µmolL^{-1} melatonin. On the 6th day of storage, Visual Quality Index (VQI) was significantly higher *i.e.,* 7.12 (more than threshold limit 6) in 100 µmolL^{-1} melatonin treated samples whereas, in control and 10 µmol melatonin treated lettuce VQI was calculated as 4.78 and 5.02, respectively. On the 8th day of storage, maximum VQI (5.26) was recorded for 100 µmol melatonin treated heads and minimum VQI (3.65) was observed in control (Fig. 2). In 100 µmol melatonin treated samples, VQI value was 44.10% higher than the control samples. No considerable visual quality difference was observed in 10 µmol melatonin treated lettuce and untreated samples. Similarly, 100 µmolL^{-1} melatonin treatments-maintained freshness in broccoli florets for 7 days storage period (Zhu *et al*., 2018). VQI demonstrated significant negative association with browning index (r= -0.945) and POD activity (r= -0.986) whereas, it displayed significant strong positive correlation with total chlorophyll (r=0.0.963), total phenol (r=0.794) and total antioxidants (r=0.961) (Table 2).

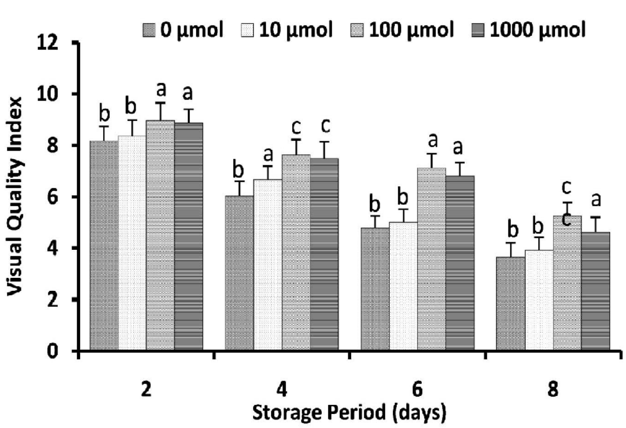

Fig. 2. Visual Quality Index (VQI) score in minimally processed lettuce treated with exogenous application of melatonin during 8 days storage at 6±2°C temperature.

Electrolyte leakage (EL) is correlated with maintenance of membrane integrity during cold storage of fresh produce. An enhancement in EL has been used as an indicator of physiological damage in cell membrane during storage. EL of MP lettuce leaves increased in control as well as melatonin treated samples during storage. During initial two days of storage, non-significant difference was observed in EL among the treatments. However, on 4th, 6th and 8th day of storage considerably lower EL was noticed in lettuce dipped in 100 and 1000 µmolL^{-1} melatonin compared to control (Fig. 3). At the end of storage period, 55.77% enhancement in EL was noticed in 100 µmolL^{-1} melatonin treated samples whereas 97.92% elevation was noticed in control samples. Melatonin treatment slowed down the production of superoxide radicals (O_2^{-}) and hydrogen peroxide (H_2O_2) during post-harvest storage which resulted in protection of membrane structure and lower electrolyte leakage (Aghdam *et al*., 2015). Our results were in concomitant with findings of Aghdam *et al*. (2019) in melatonin treated anthurium cut flowers during cold storage.

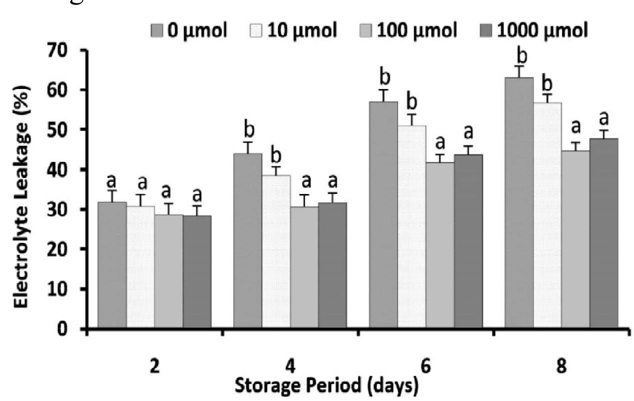

Fig. 3. Electrolyte leakage (%) in minimally processed lettuce treated with exogenous application of melatonin during 8 days storage at 6±2°C temperature.

Chlorophyll in lettuce leaves is a crucial quality parameter with respect to salability and consumer acceptance. Total chlorophyll content decreased in MP lettuce during storage irrespective of post-harvest treatment. The rate of chlorophyll degradation was considerably delayed in 100 and 1000 µmolL^{-1} melatonin solution dipped samples whereas rapid chlorophyll loss was recorded in untreated and 10 µmolL^{-1} melatonin treated samples (Table 1). On the 8th day of storage, 71.61% and 68.16% chlorophyll loss was noticed in control and 10 µmolL^{-1} melatonin treated samples, respectively

Table 1 : Post harvest melatonin treatment effect on total chlorophyll, total phenols and total antioxidants of minimally processed lettuce during low temperature (6±2°C) storage for 8 (days)

Biochemical parameter	Post-harvest treatment	Storage period (D)					
		D_0	D_2	D_4	D_6	D_8	Mean
Total chlorophyll (mg/g FW)	Control	3.18^{a}	2.90^{b}	2.48^{b}	1.34^{c}	0.91^{b}	2.16
	10	3.11^{ab}	2.91^{b}	2.67^{b}	1.59^{c}	0.99^{b}	2.25
	100	3.15^{ab}	3.11^{a}	3.05^{a}	2.45^{b}	1.39^{a}	2.67
	1000	3.12^{b}	3.10^{ab}	2.98^{c}	2.89^{a}	1.32^{a}	2.68
	Mean	3.14	3.05	2.81	2.06	1.15	
Total phenols (TAE mg/g FW)	Control	0.117^{a}	0.168^{a}	0.211^{a}	0.227^{c}	0.236^{b}	0.191
	10	0.118^{a}	0.155^{a}	0.206^{b}	0.220^{c}	0.250^{b}	0.189
	100	0.116^{a}	0.160^{a}	0.237^{ab}	0.253^{b}	0.268^{a}	0.206
	1000	0.117^{a}	0.166^{a}	0.251^{c}	0.237^{a}	0.263^{a}	0.206
	Mean	0.117	0.162	0.226	0.234	0.254	
Total antioxidants (mmol/g FW)	Control	12.91^{a}	15.73^{b}	19.17^{c}	22.39^{b}	24.90^{b}	19.02
	10	12.91^{a}	16.12^{b}	18.15^{c}	23.37^{b}	26.10^{b}	19.33
	100	12.91^{a}	18.31^{a}	23.59^{b}	26.25^{ac}	29.29^{a}	22.11
	1000	12.91^{a}	19.54^{a}	22.34^{a}	27.12^{a}	28.56^{a}	22.09
	Mean	12.91	17.42	20.81	24.78	27.19	

*Means with same superscript are non-significant. TAE=Tannic acid equivalent

whereas in 100 and 1000 µmolL^{-1} melatonin treated samples 44.12% and 42.30% chlorophyll loss was observed. Similar, observation were reported by Zhu *et al.* (2018) who recorded 24.15% higher chlorophyll in 100 µmolL^{-1} melatonin treated broccoli compared to water treated samples on the 6th day of storage. During post-harvest storage of fresh produce chlorophyll, dilapidation occurs through the continuous reduction of chlorophyll binding proteins due to elevated action of chlorophylase enzyme (Arnao and Ruiz, 2008). It may be possible here that melatonin plays a role as antioxidant, prevents the accumulation of free radicals such as ROS and lipid radicals and thus delaying the chlorophyll degradation.

Changes in total phenol content occurred in MP lettuce throughout the storage period. Both treated and untreated samples displayed an enhancing trend with respect to total phenols. Lettuce dipped in 100 and 1000 µmolL^{-1} melatonin demonstrated considerably increased levels of total phenol compared to untreated samples from day 4 to 8 days of storage, whereas no considerable variation was noticed between untreated and 10 µmol L^{-1} melatonin treated lettuce (Table 1). On the 8th day of storage maximum total phenols (0.268 TAE mgg^{-1} FW) was estimated in 100 µmolL^{-1} melatonin treated samples followed by 1000 µmolL^{-1} melatonin treatment (0.263 TAE mgg^{-1} FW) with non-significant difference. Consistent with our findings, high total phenols retained in melatonin treated samples of litchi (Zhang *et al.*, 2018), strawberry (Liu *et al.*, 2018), peaches (Gao *et al.*, 2018) and anthurium (Aghdam *et al.*, 2019) during low temperature storage has been reported earlier. Damage occurs during minimal processing due to induced accumulation of phenols in both untreated and melatonin treated lettuce leaves. Phenolics are converted into quinone through oxidation by PPO and POD in the presence of oxygen which is responsible for browning in fresh produce (Pardossi and Tognoni, 2005). Significantly higher level of total phenols in samples treated with melatonin might be ascribed to the fact that melatonin is mitigating the action of phenol oxidizing agents such as peroxidase (POD) and polyphenol oxidase (PPO) enzyme.

Melatonin treatment significantly influenced the antioxidant activity of MP lettuce leaves during storage. Gradual enhancement in antioxidant activity was noticed during storage irrespective of post-harvest treatment. At the end of storage period, untreated lettuce leaves exhibited lowest (24.90) antioxidant activity as compared to melatonin pre-treated samples.

Among melatonin treated samples, maximum antioxidant activity (19.29) was observed in minimally processed lettuce leaves which were treated with 100 µmolL^{-1} melatonin followed by 1000 µmolL^{-1} melatonin (Table 1). However, non-significant difference was noticed in both the treatments. The findings are in concurrence with the previous reports that melatonin treatment preserved antioxidant level in strawberry (Liu *et al*., 2018), litchi (Zhang *et al*., 2018) and anthurium (Aghdam *et al*., 2019). Phenolics are well recognized for their antioxidant properties. In lettuce strong positive correlation (r=0.884, Table 2) was noticed among total phenols and total antioxidants during storage. Higher antioxidant activity of melatonin treated lettuce might be attributed to higher presence of phenol content accompanied by lower activity of peroxidase (POD) and polyphenol oxidase (PPO).

During storage of MP lettuce POD activity was enhanced in the tissues irrespective of post harvest treatments. The augmenting tendency of POD activity was considerably inhibited by melatonin pre-treatment (Fig. 4). During initial 2 days of storage non-significant variation was observed in POD activity in all treatments. However, it was rapidly enhanced afterwards but remains significantly low throughout the storage period in 100 and 1000 µmolL^{-1} melatonin treated lettuce compared to control (Fig. 4). On the 8th day of storage, POD activity in untreated and 10 µmolL^{-1} melatonin treated samples was 6.60, 6.42 times of the initial value, respectively whereas 100 and 1000 µmolL^{-1} melatonin treated samples had 5.05- and 5.43-fold POD activity compared to initial value. Previous researchers also reported lower POD activity in post-harvest melatonin treated broccoli florets

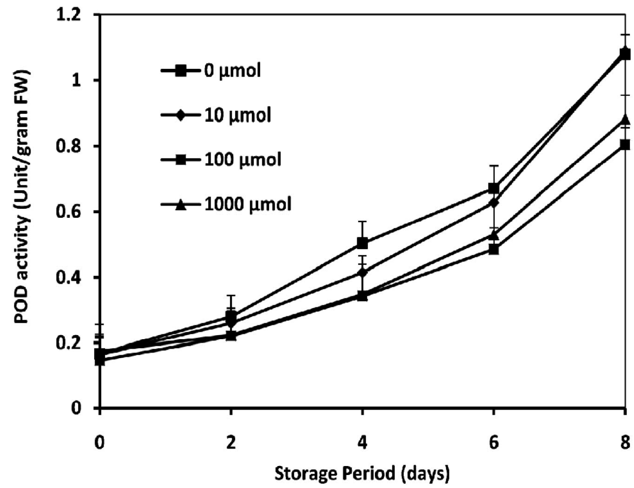

Fig. 4. Peroxidase (POD) activity in minimally processed lettuce treated with exogenous application of melatonin during 8 days storage at 6±2°C temperature.

(Zhu *et al*., 2018), litchi (Zhang *et al*., 2018) and peach (Gao *et al*., 2018). POD takes part in the oxidation of polyphenols into quinones that contributes in development of the brown pigments in minimally processed fresh produce. Slow increases in POD concentration coupled with elevated levels of total phenolics were noticed in treated lettuce signaled that melatonin slowed down the enzyme induced phenolic oxidation and inhibit brown color development in minimally processed lettuce. In present study, POD activity showed strong positive correlation (r=0.915, Table 2) with browning index.

The results of the present study concluded that post-harvest melatonin treatment proved effective in reducing browning, maintaining freshness and quality of minimally processed lettuce for 6 days during storage at 6±2 °C. Comparing to the higher dose (1000

Table 2 : Correlation between browning index and various physical and biochemical parameters recorded during storage of lettuce

Trait	Browning Index	Visual Quality Index	Electrolyte leakage	Total phenol	Total chloro-phyll	Total antioxi-dants	POD activity
Browning Index	1.000						
Visual Quality Index	-0.945	1.000					
Electrolyte Leakage	0.935	-0.891	1.000				
Total Phenol	-0.884	0.794	0.667	1.000			
Total chlorophyll	-0.886	0.961	-0.931	-0.615	1.000		
Total antioxidants	-0.997	0.963	0.921	0.889	-0.898	1.000	
POD activity	0.915	-0.986	0.918	-0.693	-0.993	0.931	1.000

μmolL^{-1}) and control, lower dose of melatonin at100 μmolL^{-1} was found highly beneficial for minimizing browning, quality retention and enhancing shelf life of minimally processed lettuce for 6 days.

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