



# Application of Melatonin in Maintaining Post Harvest Quality of Fruits and Vegetables: A Review

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10.18805/ag.R-2092

## ABSTRACT

Melatonin (N-acetyl-5-methoxytryptamine) is a nontoxic biological molecule produced naturally in a pineal gland of animals and different tissues of plants. Melatonin acts as an antioxidant during postharvest storage and augments the shelf life of fruits and vegetables. Our review highlighted the role of pre and post harvest application of melatonin in extending post harvest shelf life and alleviating chilling injury in fruits and vegetables in cold storage. Review also included available information regarding biosynthesis of melatonin in plants and mode of action of melatonin in maintaining post harvest quality.

**Key words:** Fruits, Melatonin, Post-harvest, Quality, Vegetables.

Melatonin was discovered in bovine pineal gland in 1958 (Lerner *et al.*, 1958). After its discovery, for the ensuing four decades it was considered exclusively as an animal hormone, especially a neurohormone (Reiter, 1991). In 1993, melatonin was found in the Japanese morning glory (*Pharbitis nil*) (Van-Tassel *et al.*, 1995). Also in the same year, the existence of melatonin in a number of edible plants was indisputably demonstrated (Dubbels *et al.*, 1995; Hattori *et al.*, 1995). However, even prior to its identification in plants, melatonin had been shown to have effects on endosperm cells of the amaryllidacean *Scadoxus multiflorus* (syn. *Haemanthus katherinae*) and on the epidermal cells of *Allium cepa* (Jackson, 1969; Banerjee and Margulis, 1973). Currently, research on plant melatonin is in an exponential growth phase and its functions in overcoming biotic and abiotic stress and in improving the storage life and quality of fruits and vegetables, have been uncovered in numerous plants; the number of publications related to plant melatonin has rapidly increased within the last decade (Arnao and Hernández-Ruiz, 2015; Reiter *et al.*, 2015).

Melatonin has been reported for its involvement in improving seed germination, photosynthesis, biomass production, circadian rhythm, redox network, membrane integrity, root development, leaf senescence, osmoregulation, abiotic stress (Lee *et al.*, 2014, Shi *et al.*, 2014, Qian *et al.*, 2015, Zhang *et al.*, 2014). Recently melatonin has been explored by various researchers for improving the post harvest life and maintaining quality of fruits and vegetables through pre-harvest and post harvest application (Cao *et al.*, 2016; Gao *et al.*, 2016; Liu *et al.*, 2016; Ma *et al.*, 2016; Meng *et al.*, 2015).

The aim of this review is to emphasize the various aspects of melatonin from the studies available over its role in prolonging freshness of fruits and vegetables, reducing chilling injury and browning in cold storage and protecting them from post harvest deterioration. Additionally, the contribution of melatonin in regulating gene expression has

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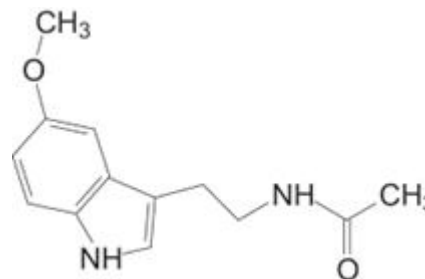
**How to cite this article:** Gurjar, P.S., Killadi, B., Pareek, P.K. and Hada, T.S. (2021). Application of Melatonin in Maintaining Post Harvest Quality of Fruits and Vegetables: A Review. Agricultural Reviews. DOI: 10.18805/ag.R-2092.

**Submitted:** 08-09-2020 **Accepted:** 14-09-2021 **Online:** 09-10-2021

been presented here. The melatonin molecular structure, biosynthesis pathway and mode of action have also been presented in this review with illustration and diagrammatic sketches. Furthermore, we discussed the melatonin concentration in different fruits and vegetables.

## Melatonin structure

Melatonin, a tryptophan-derived natural product, is a central biomolecule that is produced in almost all living organisms, including animals and plants (Reiter and Tan, 2002). Melatonin has a chemical formula of  $C_{13}H_{16}N_2O_2$  and a molecular mass of 232.278 g/mol.



### Melatonin biosynthesis in plants

Mitochondria and chloroplast are referred to as the original site of melatonin synthesis in plants (Tan *et al.*, 2013). Melatonin biosynthesis from tryptophan requires four-step reactions and six genes, *i.e.*, TDC, TPH, T5H, SNAT, ASMT, and COMT, are involved in the synthesis of melatonin in plants, suggesting the presence of multiple pathways (Nawaz *et al.*, 2016). Two major pathways have been proposed based on the enzyme kinetics (Fig 1). One is the tryptophan/tryptamine/serotonin/N-acetylserotonin/melatonin pathway, which may occur under normal growth conditions; the other is the tryptophan/tryptamine/serotonin/5-methoxytryptamine/melatonin pathway, which may occur when plants produce large amounts of serotonin, *e.g.*, upon senescence (Back *et al.*, 2016; Sharif *et al.*, 2018). The melatonin biosynthetic capacity associated with conversion of tryptophan to serotonin is much higher than that associated with conversion of serotonin to melatonin, which yields a low level of melatonin synthesis in plants. Many melatonin intermediates are produced in various sub cellular compartments, such as the cytoplasm, endoplasmic reticulum and chloroplasts, which either facilitate or impede the subsequent enzymatic steps. Depending on the pathways, the final subcellular sites of melatonin synthesis vary at either the cytoplasm or chloroplasts, which may differentially affect the mode of action of melatonin in plants.

### Occurrence of melatonin in fruits and vegetables

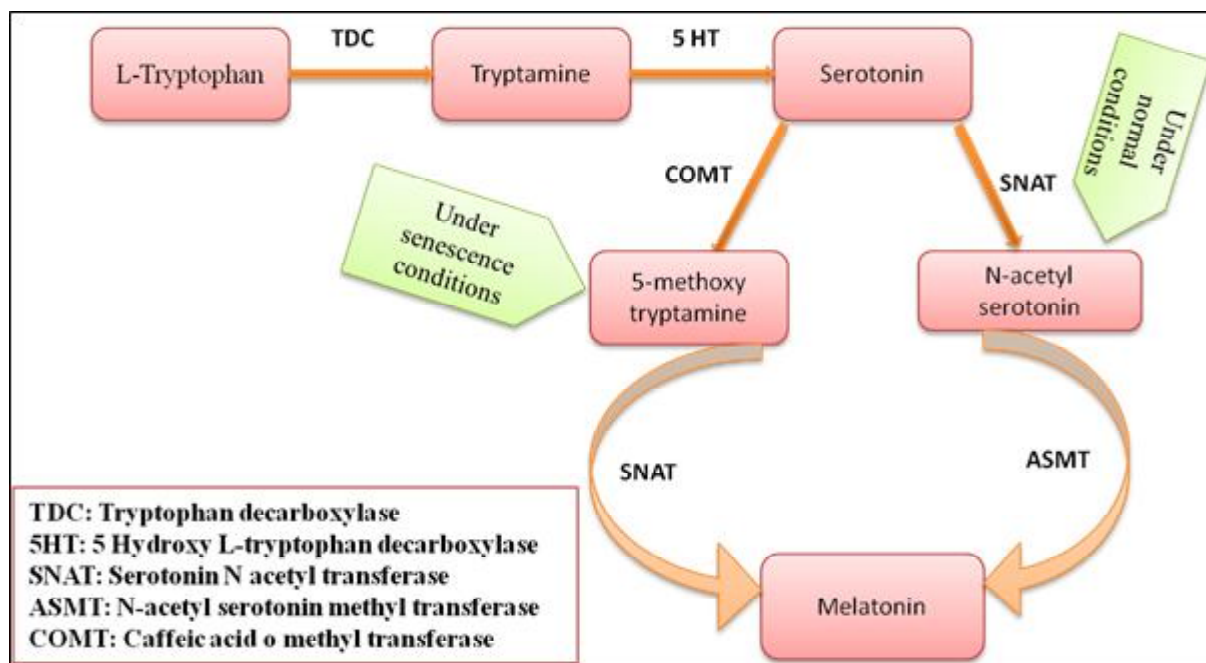
Melatonin concentration in fruits and vegetables have been detected by several methods including radioimmunoassay (RIA), enzyme-linked immune sorbent assay (ELISA), gas chromatography-mass spectrometry (GS-MS) and high-performance liquid chromatography (HPLC) with electrochemical detection (HPLC-ECD), fluorescence detection (HPLC-FD),

or HPLC-MS. The highest melatonin concentrations (227–233 µg/g) have been reported in four different varieties of Pistachio nut (*Pistacia vera* L.) (Oladi *et al.*, 2014). The amount of melatonin detected in fruits and vegetable are listed in Table 1.

### Mode of action of melatonin

The harvested fruits and vegetables produce are the living material, therefore several biochemical and physiological activities such as respiration, dehydration, hormonal and enzymatic changes are undergoing in these products (Velenzuela *et al.*, 2017). The high respiration rate coupled with dehydration and active metabolism lead to a rapid deterioration of produce during marketing and storage. For this reason, many treatments have been implemented to maintain the quality and shelf life of postharvest fruits and vegetables (Nazari *et al.*, 2015; Sharif *et al.*, 2018).

After harvesting of fruits and vegetables, usually the produce is stored in two ways: at room temperature for short period and in cold storage for longer period. In cold storage, cold environment induces oxidative stress by elevating the production of reactive oxygen species (ROS); this is the main drawback of cold storage (Sun *et al.*, 2016). However, treatment with melatonin alleviates the ROS activity and increases the antioxidant enzymes production (Cao *et al.*, 2018). In other cases, the application of exogenous melatonin triggered the endogenous melatonin biosynthetic activity *via* the antagonistic crosstalk with calcium, preventing the product from chilling injury and postharvest deterioration as reflected in Fig 2. At room temperature storage, melatonin treatment reduced relative membrane-leakage rate and inhibited the generation of superoxide radicals ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ), ethylene synthesis and malondialdehyde (MDA) that results in prolonging freshness



Common Name	Scientific name	Family	Concentration (ng/gm)	References
Fruits				
Apple	<i>Malus domestica</i>	Rosaceae	0.47	Dubbels <i>et al.</i> , 1995
Banana	<i>Musa paradisiaca</i>	Musaceae	0.16	Badria, 2002
Cherry	<i>Prunus avium</i>	Rosaceae	0.06	Gonzalez-Gomez <i>et al.</i> , 2009
Kiwi fruit	<i>Actinidia chinensis</i>	Actinidiaceae	0.02	Hattori <i>et al.</i> , 1995
Pomegranate	<i>Punica granatum L</i>	Punicaceae	0.17	Badria, 2002
Grape (skin)	<i>Vitis vinifera L.</i>	Vitaceae	0.03	Iriti <i>et al.</i> , 2006
Strawberry	<i>Fragaria ananasa</i> Duch.	Rosaceae	0.01	Hattori <i>et al.</i> , 1995
Orange	<i>Citrus sinensis</i> Osbeck.	Rutaceae	0.15	Johns <i>et al.</i> , 2013
Mango	<i>Mangifera indica L</i>	Anacardiaceae	0.70	Johns <i>et al.</i> , 2013
Papaya	<i>Carica papaya L</i>	Caricaceae	0.24	Johns <i>et al.</i> , 2013
Walnut	<i>Juglans regia L</i>	Juglandaceae	3.5	Reiter <i>et al.</i> , 2005
Vegetables				
Tomato	<i>Solanum lycopersicum L</i>	Solanaceae	0.30	Badria, 2002
Carrot	<i>Daucus carota</i>	Apiaceae	0.49	Badria, 2002
Radish	<i>Raphanus sativus L</i>	Brassicaceae	0.76	Badria, 2002
Cabbage	<i>Brassica oleracea L var.capitata</i>	Brassicaceae	0.30	Badria, 2002
Broccoli	<i>Brassica oleraceae</i>	Brassicaceae	0.439	Aguilera <i>et al.</i> , 2015
Turnip	<i>Brassica campestris L</i>	Brassicaceae	0.50	Badria, 2002
Onion	<i>Allium cepa L</i>	Amaryllidaceae	0.29	Badria, 2002
Garlic	<i>Allium sativum L</i>	Amaryllidaceae	0.58	Badria, 2002

and reduction of browning and chlorophyll/pigments degradation (Zhang *et al.*, 2018, Hu *et al.*, 2018).

#### Applications of melatonin in fruit and vegetables for improving post harvest life

The fruits and vegetables are perishable commodities therefore the shelf-life and quality of postharvest produce deteriorate after harvesting. Melatonin has been identified in nearly all organs and tissues of plants and is shown to be a signaling molecule involved in numerous physiological processes such as differentiation, growth, ripening and senescence of plant and the protective effect against various forms of environmental stress (Reiter *et al.*, 2015). Recently, few researchers were utilized melatonin for maintaining post harvest quality by applying it at pre and post harvest stages.

#### Pre-harvest application of melatonin

The postharvest quality of horticultural produce is mainly dependent on the pre-harvest factors and intercultural operations carried out during growth and fruiting of the crop as it cannot be augmented after harvesting but can only be maintained (Arah *et al.*, 2015). Two times application of melatonin 100  $\mu\text{mol L}^{-1}$  in grape at veraison stage increased the contents of total phenols, flavonoids and anthocyanins in berry and wine produced. Additionally, melatonin treatment also enhanced the endogenous melatonin in berry and adds

to antioxidant capacity of berry and wine (Meng *et al.*, 2015; Lili *et al.*, 2018). Xu *et al.* (2017) reported that particularly 100  $\mu\text{mol L}^{-1}$  melatonin treatments at pre veraison stage increased the contents of total anthocyanins, phenols, flavonoids and proanthocyanidins in grape berries. In case of individual phenolic compounds, particularly resveratrol was enhanced; in tandem with the up-regulation of STS gene expression. Melatonin treatment on tomato increase yield and enhanced health promoting bioactive compounds such as ascorbic acid, phenols and lycopene in fruits under acid rain stress (Debnath *et al.*, 2018). In another study seeds soaked in melatonin prior to germination influence the quality and yield of tomato. Plants irrigated weekly with melatonin-supplemented nutrient solutions showed significant improvements in their contents of soluble solids, ascorbic acid, lycopene, citric acid and P element when compared with control plants that received only a standard solution (Liu *et al.*, 2016). Melatonin 300 mg  $\text{L}^{-1}$  treatment in blue berry effectively promoted the accumulation of soluble sugar, which is increased by 68.6% comparing with the control fruits (Zhang *et al.*, 2017). The 150  $\mu\text{mol/L}$  exogenous melatonin spraying promoted the growth and biomass accumulation and also enhanced the activities of antioxidant enzymes (POD, CAT and SOD) and the contents of soluble protein and chlorophyll content in leaves of radish (Jiang *et al.*, 2016).

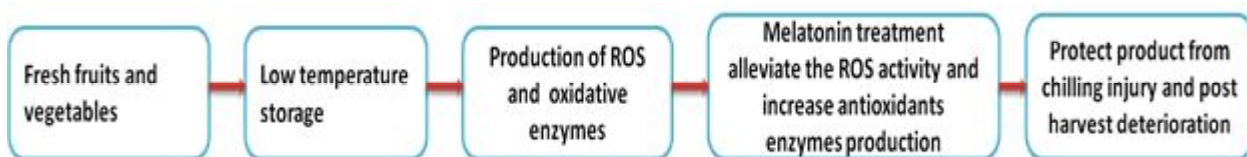


Fig 1: Flowchart of mode of action of melatonin in alleviating chilling injury in cold storage.

### Post harvest application of melatonin in fruit and vegetables

Recently, attention has been partially directed toward the post harvest exogenous melatonin application on fruits and vegetables to augment shelf life and alleviate chilling injury in cold storage. Sun *et al.* (2016) found that melatonin plays a crucial role in the regulation of senescence in tomato fruit. Gao *et al.* (2016) put forward a similar result that melatonin treatment at a concentration of 0.1 mM leads to a clear delay of senescence in peach fruit during ambient storage, through antioxidative mechanism. Gao *et al.*, (2018) proved that melatonin treatment delayed the chilling injury symptoms development in peach fruit stored in cold storage at 1 degree C for 28 days. The impact of 0, 1, 10, 100 and 1000 mol/L melatonin on attenuating fungal decay and maintaining nutritional quality of strawberry fruits was investigated during storage at 4°C for 12 days by Aghdam and Fard (2017). Melatonin treatment at 100 mol/L concentration attenuating postharvest decay in strawberry fruits by mechanisms that trigger the accumulation of hydrogen peroxide and phenolic compounds and induces the activity of  $\gamma$ -aminobutyric acid shunt. Strawberry fruits dipped for 5 minutes in melatonin solution (0.1 mmol L<sup>-1</sup>) enhanced the expression of melatonin biosynthetic genes including FaTDC, FaT5H, FaSNAT and FaASMT and consequently increased the content of endogenous melatonin resulting in delayed senescence during storage at 4°C for 12 days (Liu *et al.*, 2018). Cao *et al.* (2016) proved that melatonin ensures better prevention of chilling injury in peach fruit during low temperature storage and such effect has been attributed in part to melatonin-induced promotion of polyamine,  $\gamma$ -aminobutyric acid and proline. Exogenous melatonin pretreatment induces resistance against *Botrytis cinerea* infection via increasing the activities of defensive enzymes in apple var. 'Fuji'. The activities of defensive enzymes such as peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) were increased 1.86 to 8.73 times in exogenous melatonin treated fruit. Pretreatment with 0.2 mmol L<sup>-1</sup> for 72 h reduced *Botrytis cinerea* load up to 83% (Cao *et al.*, 2018). Ma *et al.* (2016) proved that the melatonin treatment delayed cassava roots postharvest physiological deterioration (PPD), through less H<sub>2</sub>O<sub>2</sub> accumulation as a result of increasing antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) activity and causes higher membrane integrity. Exogenous application of melatonin resulted delayed postharvest banana ripening. However, this effect is concentration-dependent, with 200 and 500  $\mu$ M treatments found more effective. It also led to elevated endogenous melatonin content, reduced ethylene production through regulation of the expression of *MaACO1* and *MaACS1* and delayed sharp changes of quality indices (Hu *et al.*, 2018). Melatonin strongly suppressed pericarp browning and delayed discoloration during storage of litchi at ambient conditions. Melatonin treatment reduced relative membrane-leakage rate and

inhibited the production of superoxide radicals (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and malondialdehyde (MDA) and enhanced the activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) while the activities of browning-related enzymes including polyphenol oxidase (PPO) and peroxidase (POD) were reduced. It also up-regulated the expression of four genes encoding enzymes for repair of oxidized proteins, including *LcMsrA1*, *LcMsrA2*, *LcMsrB1* and *LcMsrB2* (Zhang *et al.*, 2018). Post harvest dip treatment for 12h with 100  $\mu$ M melatonin delayed the ethylene burst in pear var. Starkrimson. In 'AbbéFétel' and 'Red Anjou', normally softening pears, melatonin inhibited ethylene production during the entire senescence process. The limited ethylene production resulted in a lower loss of firmness in melatonin-treated fruit. PcPG, a major cell wall degradation-related gene, was inhibited by melatonin in all three cultivars (Zhai *et al.*, 2018). The exogenous melatonin treatment at 100  $\mu$ M L<sup>-1</sup> slowed down the deterioration of chlorophyll, vitamin C and reduced the active oxygen content, electrolyte leakage, respiration intensity and the ethylene production, also reduced the oxidative damage to cells and maintained a more complete cell structure in cucumber during storage (Xin *et al.*, 2017). Zhu *et al.* (2018) observed that melatonin delayed the senescence process of postharvest broccoli florets through regulating the respiratory metabolism and antioxidant activity. Total chlorophyll in melatonin-treated florets was 24.15% higher, 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<sub>2</sub>O<sub>2</sub> compared to the control. In another study, post harvest application of different concentrations (10, 100 or 1000  $\mu$ M L<sup>-1</sup>) of melatonin were evaluated for delaying in fruit softening and maintaining nutritional quality of mango fruit during storage at 15  $\pm$  1°C and 85 $\pm$ 1% relative humidity. Treatment of 1000  $\mu$ M L<sup>-1</sup>, melatonin has retained the firmness, ascorbic acid, phenolic compound and antioxidant capacity of mango during storage. Melatonin significantly controlled the activity of PPO and increased the activity of the catalase and peroxidase enzymes during storage (Rastegar *et al.*, 2020).

### CONCLUSION AND FUTURE PROSPECTS

The plethora of research available about roles of melatonin in alleviating biotic and abiotic stresses in plants. However, more recently researchers documented melatonin treatment for improving post harvest quality of some fruits and vegetables crops. Till date post harvest application of melatonin is not been used for reducing chilling injury during post harvest low temperature storage of cold sensitive tropical and subtropical fruits like mango, guava, papaya and vegetables. The role of pre and post harvest melatonin application in fruit crops on ethylene biosynthesis, delay in



ripening and senescence need to be investigated. In light of available studies it seems that melatonin may prove to be an important molecule to improve post harvest quality of fruits and vegetables. In addition, melatonin is considered as a nontoxic biodegradable molecule; therefore it could be used for the promotion of organic farming of horticultural crops.

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