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Decreasing postharvest chilling injury of guava fruit by using melatonin treatment

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Guava fruit is a tropical fruit thus sensitive to the chilling injury. In this study the effects of melatonin (known to protect membrane integrity and to help to face abiotic and biotic stress) is evaluated for reduction of chilling injury during postharvest. Guava fruits were dipped into 10, 100 and 1000 µmol L^{-1} melatonin solutions, then kept at cold storage (10±1°C and 90% relative humidity) for 21 days. Several parameters including chilling injury, malondialdehyde content, electrolyte leakage and increased total phenolic compounds and antioxidant activity, phospholipase D and lipoxygenase activity were measured after treatment. Measurements were made every 7 days during the storage. Results showed that melatonin decreased chilling injury, malondialdehyde content, electrolyte leakage and increased total phenolic compounds and antioxidant activity compared to the control. Also, results indicated that chilling injury of guava fruit by using melatonin decreased through increasing integrity of membrane and reducing phospholipase D and lipoxygenase activity. Thus, melatonin can be a useful treatment for decreasing postharvest chilling disorder of guava fruit.

1. Introduction

Guava (*Psidium guajava* L.) is one of the most important fruits of tropical and sub-tropical regions in the world. The fruits are delicious, rich in vitamin C and minerals (Deepthi *et al.,* 2016). There is a great demand of guava fruits in both domestic and international markets for fresh and processing purposes.

Cold storage is one of postharvest technologies for maintaining quality of horticultural crops until human consumption. However, guava is sensitive to chilling disorder of cold storage (temperature of below 12°C). Signs of chilling injury include irregular ripening and surface pitting on the fruit which decreases quality of fruit (Etemadipoor *et al.,* 2020). Resistance to the chilling temperature related to several factors. One of the most important factors is maintaining membrane integrity

(Wongsheree *et al.,* 2009). Membrane integrity can be measured using leakage, malondialdehyde content, lipoxygenase and phospholipase D (Aghdam *et al.,* 2014). Several methods used to decrease chilling injury symptoms of fruit rely on the use of hot water and UV-C (Pongprasert *et al.,* 2011).

Melatonin plays in fruit ripening and senescence and membrane integrity and protection against abiotic and biotic stresses (Rastegar *et al.,* 2020). Melatonin treatment maintained quality of in pear (Liu *et al*., 2019), peach (Gao *et al.,* 2016), and grape (Xu *et al.,* 2018) and tomato (Aghdam *et al.,* 2019) fruits during cold storage.

However, melatonin effects on reducing chilling injury of guava fruit have not been evaluated during cold storage. Therefore, the purpose of this study was to investigate melatonin effects on chilling injury reduction.

2. Materials and Methods

The guava fruits (green stage maturity) were bought from a commercial orchard in Hormozgan province, Iran and the uniform sized fruits were transferred to the laboratory. Twelve fruits per four replications were dipped into 10 (T2), 100 (T3) and 1000 (T4) µmol L^{-1} melatonin solutions for 10 min. Distilled water was used as the control (T1). Fruit were kept at cold storage (10±1°C and 90% relative humidity) for 21 days. Several parameters were measured soon after treatment, and then the measurements were made every 7 days during the storage. Finally, the parameters were checked again one day after exposing to the ambient temperature (25±1°C). The measurements included chilling injury index assessment, percentage of ion leakage, malondialdehyde content, weight loss, titratable acidity, soluble solids concentration (SSC), ascorbic acid, total phenolic content (TPC), antioxidant activity, phospholipase D and lipoxygenase activity.

Chilling injury (CI) index assessment, Percentage of ion leakage (EL) and Malondialdehyde (MDA) content

CI index was assessed subjectively with a scale from 1 to 5, where 5= >50% surface pitting area, 4= 31-50% surface pitting area, 3= 16-30% surface pitting area, 2= 1-15% surface pitting area, 1= 0% no chilling symptoms.

EL was measured by using method described by Madani *et al.* (2016) and results expressed as percentage.

The MDA content was determined based on the method described by Wang *et al.* (2015). The content of MDA was expressed as nmol g^{-1} FW.

Weight loss, Soluble solids concentration (SSC), Titratable acidity (TA), Ascorbic acid, Total phenolic content (TPC) and Total Antioxidant activity

Weight loss was measured based on initial and final experiment at 7-day intervals during storage using a digital balance, and results were expressed as percentage.

Soluble solids concentration (SSC) and TA of pulp tissues were measured by using the method of Ali *et al.* (2011) and the results were expressed as %SSC and %TA, respectively. Ascorbic acid was measured using dye method described by Ranggana (1986) and results were expressed as mg 100 g^{-1} fresh weight (FW).

TPC were assayed using Folin-Ciocalteu procedure (Singleton and Rossi, 1965). Results were expressed as mg of gallic acid equivalents (GAE) per gram of fresh weight (mg GAE g^{-1} FW). The DPPH assay was measured according to the method described by Mirshekari *et al.* (2019). Results were expressed as percentage.

Phospholipase D and Lipoxygenase activity

Phospholipase D and lipoxygenase assay was determined based on the method described by Aghdam and Mohammadkhani (2014). One unit of Phospholipase D was defined as the amount of enzyme that catalyzed the formation of 1 nmol D-nitrophenol h^{-1}. One unit of lipoxygenase was defined as the amount of enzyme which causes an increase in absorption of 0.01 min^{-1} at 234 nm and 25°C when linoleic acid was used as the substrate. Protein content was estimated according to Bradford (1976). Enzymes activities were expressed as units per milligram of protein.

Statistical analysis

Experiments were carried out using completely randomized design. Four replications per treatment used for this study. Data were analyzed using (SAS) version 8.2 (SAS Institute Inc., Cary, NC, USA). Variation Sources were storage days and treatments and means were compared with Duncan's Multiple Range Test (DMRT) at significance level of 0.05.

3. Results and Discussion

Chilling injury (CI), Electrolyte leakage (EL), and Malondialdehyde (MDA)

In the present study, melatonin treatment reduced chilling injury index of guava fruit after 7 days of chilling storage when compared with the control (Table1). Moreover, CI increased with storage time (Table 1). At the end of storage day control (T1) had the severe chilling injury index (4.8) with highest pitting signs, while T4 had the lowest chilling injury index of 3.2. Usually, CI happens at the cell membrane, and maintaining its integrity reduces CI (Mirdehghan *et al.,* 2007; Mirshekari *et al.,* 2020).

Accordingly, Electrolyte leakage has been used as an indicator of membrane damage. In this study, EL increased during storage for control and treated melatonin fruits (Table 1). However, at the end of storage day EL was lower in T3 and T4 compared to the T2 and T1 (Table 1). These results showed the role of melatonin in maintenance of membrane integrity. Comparable results have been stated for sapota fruit by Mirshekari *et al.* (2020). Researches have shown that melatonin treatments affect electron flow acceleration in mitochondria to maintain membrane integrity (Tan *et al.,* 2013).

As shown in Table 1, MDA of control fruit increased during storage. Also, lower MDA content were observed in all melatonin treated fruits compared to the (T1) after 21 days of chilling storage (Table 1). One of the first events in the CI is membrane lipid peroxidation. MDA is the final product of lipid peroxidation (Imahori *et al.,* 2008). Lower temperatures are the main inducers of oxidative damage which produce higher ROS and change the ratio of unsaturated fatty acids to saturated forms (Antunes and Sfakiotakis, 2008). Melatonin treatments lowered MDA accumulation of sapota fruit (Mirshekari *et al.,* 2020).

Weight loss, SSC, TA, Ascorbic acid, TPC and Total Antioxidant activity

Weight loss was at the highest rate (14.3 %) in the control fruits (T1) after 21 days. Treated fruit (T4) showed lower weight loss (6.7%) compared to the T1 and T2 at the end of storage day (Table 2). Weight loss is an index for assessing quality of fruits during storage (Yaman and Bayonidirli, 2002). Skin strength properties of fruit by using melatonin treatment might lower weight loss. Our results are comparable with Rastegar *et al.* (2020) who indicated that the weight loss was decreased by using melatonin treatment in mango.

SSC and TA concentration are main factors for fruit quality judgment. The initial SSC value of this study was 5.2% in control fruits (T1) and increased

Table 1 - Melatonin treatments (0, 10, 100 and 1000 µmol L^{-1}) effects on chilling injury index (CI), electrolyte leakage (EL) and malondialdehyde (MDA) content in guava fruit stored at 10°C for up to 21 days

Treatment (µmol L^{-1})	Storage (day)			
	0	7	14	21
CI				
0 (T1)	0 a D [z]	2.3 a C	3.5 a B	4.8 a A
10 (T2)	0 a D	2.1 a C	3.2 ab B	4.1 b A
100 (T3)	0 a D	1.1 b C	2.5 bc B	3.8 b A
1000 (T4)	0 a D	1.3 b C	2.1 c B	3.2 c A
EL (%)				
0 (T1)	6.5 a D	18.0 a C	32.3 a B	49 a A
10 (T2)	6.5 a D	16.5 a C	30.2 a B	47 a A
100 (T3)	6.5 a D	12.5 b C	22.2 b B	31.7 b A
1000 (T4)	6.4 a D	10.5 b C	20.5 b B	32.3 b A
MDA (nmol g^{-1} FW)				
0 (T1)	5.5 a D	8.3 a C	12.3 a B	15.6 a A
10 (T2)	5.3 a D	7.2 b C	11.6 a B	13.1 b A
100 (T3)	5.0 a B	5.6 c B	7.6 b A	8.5 c A
1000 (T4)	5.6 a B	5.9 c B	8.2 b A	9.1 c A

[z] Small and capital letters show significant differences by DMRT at P= 0.05 between treatments in columns, and storage time for each parameter, respectively.

Table 2 - Melatonin treatments (0, 10, 100 and 1000 µmol L^{-1}) effects on weight loss, soluble solids content (SSC), titratable acidity (TA), ascorbic acid, total phenolic content (TPC) and total antioxidant activity (TAA) in guava fruit stored at 10°C for up to 2

Treatment (µmol L^{-1})	Storage (day)			
	0	7	14	21
Weight loss (%)				
0 (T1)	0 a D [z]	4.5 a C	9.5 a B	14.3 a A
10 (T2)	0 a D	3.2 b C	8.3 b B	13.4 a A
100 (T3)	0 a D	2.6 c C	5.2 c B	8.6 b A
1000 (T4)	0 a D	2.1 c C	4.2 d B	6.7 c A
SSC (%)				
0 (T1)	5.2 a D	8 a C	12.1 a B	13.4 a A
10 (T2)	5..1 a C	7.3 ab B	11.7 a A	12.6 a A
100 (T3)	5.2 a D	6.6 bc C	8.2 b B	9.2 b A
1000 (T4)	5.2 a C	5.8 c BC	6.9 c AB	7.5 c A
TA (%)				
0 (T1)	0.9 a A	0.6 a B	0.4 b C	0.3 bc C
10 (T2)	0.9 a A	0.7 a B	0.4 b C	0.2 c C
100 (T3)	0.9 a A	0.8 a A	0.7 a A	0.5 ab B
1000 (T4)	0.9 a A	0.7 a AB	0.6 a BC	0.5 a C
Ascorbic acid (mg 100 g^{-1} FW)				
0 (T1)	135.5 a B	145.6 b A	121.5 b C	118.3 c C
10 (T2)	136.4 a B	149.2 ab A	126.5 b C	122.4 bc C
100 (T3)	136.2 a B	151.2 a A	132.7 a C	127.2 ab D
1000 (T4)	134.5 a B	150.3 ab A	133.8 a B	129.1 a C
TPC (mg GAE g^{-1} FW)				
0 (T1)	175 a D	210.6 c A	189.7 b B	181.5 d C
10 (T2)	172 a C	201.2 d A	191 b B	188.5 c B
100 (T3)	173.2 a D	233 a A	213.2 a B	200.7 b C
100 (T4)	174 a D	224.2 b A	218.7 a B	214.2 a C
TAA (%)				
0 (T1)	52.3 a C	62..1 b B	70 c A	65.2 b AB
10 (T2)	52 a C	64.2 b B	72.7 c A	67 b B
100 (T3)	53.2 a C	72.4 a B	78.5 a A	74.2 a B
1000 (T4)	52.7 a B	75 a A	77.2 ab A	75.5 a A

[z] Small and capital letters show significant differences by DMRT at P= 0.05 between treatments in columns, and storage time for each parameter, respectively.

during storage (Table 2). However, at the end of storage, T3 and T4 melatonin treatments decreased SSC content compared to untreated (T1) and T2 treatments. Lower SSC content in this research is in agreement with results of Liu *et al.* (2018) who showed that SSC decreased with melatonin treatments during storage. Lower amounts of SSC might be due to the slower respiration rate and a weaker metabolic activity due to the reduced rate of carbohydrate hydrolysis.

Table 2 shows TA content of treated and control fruits. TA content was the highest at harvest day (0.9%). However, TA content decreased during storage. At the end of storage T3 and T4 showed higher TA content compared to the T1 and T2 (Table 2). The higher amounts of TA in melatonin treated fruit can be related to the reduction of respiration rate during storage (Han *et al.,* 2004).

Ascorbic acid decreased during storage. After 21 days of storage, ascorbic acid was higher in T3 and T4 compared to the T1 (Table 2). One of the most significant signs of the nutrient value of fruits is ascorbic acid. The ascorbic acid reduction during storage can be related ascorbic acid oxidase (Choudhary *et al.,* 2016). Melatonin treatment increases oxidative stress resistance by increasing ascorbic acid (Gao *et al.,* 2016). Our results are comparable with Gao *et al.* (2016) who stated that melatonin treatment main-

tained ascorbic acid content of peach.

TPC and antioxidant activity decreased during chilling storage. However, melatonin treated fruit had higher TPC and antioxidant capacity compared with control (Table 2). It has been shown that melatonin treatment increased TPC by regulating gene expression in phenyl propanoid pathway (Zhang *et al.,* 2016). Moreover, Liu *et al.* (2018) stated that melatonin treatment increased TPC and DPPH scavenging capacity of strawberry. This indicated that melatonin showed positive effect on antioxidant activity of guava fruit.

Phospholipase D and Lipoxygenase activity

Phospholipase D and lipoxygenase activity increased during chilling storage. However, melatonin treatment decreased their activities during storage (Table 3). Similarly, melatonin treatment decreased CI signs and inhibited Phospholipase D and lipoxygenase activity of sapota fruit (Mirshekari *et al.,* 2020). Studies indicated that CI was achieved by the activities of membranous lipolytic enzymes like Phospholipase D and lipoxygenase which catalyse peroxidation of polyunsaturated fatty acids and are believed to be major contributors to CI in plant tissue (Aghdam and Mohammadkhani, 2014).

4. Conclusions

Results of this study showed that melatonin treatments during cold storage of guava fruit decreased chilling injury, soluble solids concentration, malondialdehyde content, electrolyte leakage, phospholipase D and lipoxygenase activity and increased titratable acidity, ascorbic acid, total phenolic compounds and antioxidant activity compared to the control. Accordingly, we found that melatonin application reduced CI of guava fruit with enhancing membrane integrity and decreasing phospholipase D and lipoxygenase activity.

Concerning the different treatments, we found out that while 10 µmol L^{-1} produced results which were not significantly different from control treatment, 100 µmol L^{-1} and 1000 µmol L^{-1} were significantly different for CI, weight loss, soluble solids concentration, total phenolic compounds and phospholipase D activity at the end of storage. In conclusion, melatonin application at 1000 µmol L^{-1} can be recommended to be used to decrease CI in guava fruit under cold storage.

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Table 3 - Melatonin treatments (0, 10, 100 and 1000 µmol L^{-1}) effects on phospholipase D (PLD) and lipoxygenase (LOX) in guava fruit during storage at 10°C for up to 21 days

Treatment (µmol L^{-1})	Storage (day)			
	0	7	14	21
PLD (U mg^{-1} protein)				
0 (T1)	24 a D [z]	31.5 b C	46.7 a B	51 a A
10 (T2)	22.5 a D	26.7 b C	43.5 a B	51.7 a A
100 (T3)	24.2 a C	24.5 b C	31.2 b B	44 b A
1000 (T4)	22.7 a C	25.5 b BC	28.2 b B	37 c A
LOX (U mg^{-1} protein)				
0 (T1)	1.45 a D	2.95 a C	5.12 a B	8.9 a A
10 (T2)	1.65 a D	2.85 a C	4.47 a B	8.52 a A
100 (T3)	1.72 a C	2.1 b C	2.95 b B	8.25 b A
1000 (T4)	1.47 a C	1.85 b C	3.27 b B	5.65 b A

[z] Small and capital letters show significant differences by DMRT at P= 0.05 between treatments in columns, and storage time for each parameter, respectively.

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