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RESEARCH ARTICLE

Melatonin application increases accumulation of phenol substances in kiwifruit during storage

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ABSTRACT

To investigate the effect of melatonin on the fruit quality during storage, fruits of 'Jingshi No 1', a new yellow-fleshed kiwifruit variety, were used as materials by soaking in 0.1 mmol L^{-1} melatonin (MT) solution. The results showed that MT treatment significantly delayed the decline of fruit hardness, reduced the loss of soluble protein, and significantly lowered the accumulation of malondialdehyde when compared with the control. Moreover, MT application improved content of antioxandants, such as total phenolic, total flavonoid and flavanol content, and the antioxidant capacity measured by ABTS and FRAP. These results indicate that MT application can delay fruit softening and improve antioxidant ability by enhancing the accumulation of phenolic substances during storage.

Keywords: Melatonin; Kiwifruit; Antioxidant; Antioxidant capacity

INTRODUCTION

In recently years, kiwifruit becomes more popular around the world due to its bright flesh color, enjoyable taste and high value nutrients. Kiwifruit is rich in organic acids, minerals and 17 amino acids that humans need (He et al., 2017). In addition, it is known for its high content of Vc and antioxidants as its functional properties being confirmed as highly correlated with the antioxidant capacity in kiwifruit (Krupa et al., 2011). Phenolic substances acts as antioxidants (Wolfe et al., 2003, Fattouch et al., 2005) and can influence the sensory properties of fruit (Lesschaeve and Noble, 2005).

Kiwifruit is a kind of typical respiration climacteric fruit and have less postharvest life (Crisosto et al., 2001). When harvested, fruit is firm and starchy; in the process of storage and post-ripening, fruit hardness decreases rapidly, accompanied with water losing and texture softening, which direct affected fruit commodity characters (Nishiyama et al., 2004). At the same time, the contents of antioxidant substances, such as phenolic substances, flavonoids and flavanols, and soluble solids, soluble protein and titratable acid also changed to alter the flavor and nutritive components in fruit (Possingham, 1991).

Up to now, the storage and preservation techniques of kiwifruit mainly include low temperature and chemical treatments, such as 1-methylcyclopropene (Lim et al., 2016), polyamines (Jhalegar et al., 2012), nitric oxide (Zhu et al., 2008; Zheng et al., 2017) and hydrogen-rich water (Hu et al., 2014). These preservation techniques can harm the consumers by chemical residues. Melatonin (MT) is an indole derivative of tryptophan and is defined as an antioxidant due to its high radical scavenging activity (Kolář and Macháčková, 2010). MT was shown to protect organisms against reactive oxygen and nitrogen species by two manners: (a) direct free radical scavenging by providing electronic; (b) stimulation of activities of antioxidant enzymes, such as POD, CAT, APX (Arnao and Hernandez, 2015), as well as other antioxidants, including AsA, GSH, *etc.* (Reiter and Tan, 2002). Furthermore, MT has the function in regulating fruit development. For example, application of MT promoted the fruit maturity and enhanced the fruit quality in tomato (Liu et al., 2016), and delayed the fruits senescence after harvest and regulated the active oxygen metabolism in peach (Gao et al., 2016) and strawberries (Aghdam and Fard, 2017).

At present, few studies focused on the effect of MT on the quality of kiwifruit during storage. In this study, fruits of a

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new yellow-fleshed kiwifruit variety 'Jinshi No 1' were used as material, treated by 0.1 mmol L^{-1} melatonin solution and stored at 4°C. Fruit quality indicators, content of phenolic substances, and antioxidant capacity were determined to investigate the effect of melatonin on kiwifruit during storage.

MATERIALS AND METHOD

Materials and treatment

Fruits of 'Jingshi No.1' were harvested from the Shifang Kiwifruit Resources Base of Sichuan Province Natural Resources Science Academy, China in October, 2016. Total 200 uniform fruits with no disease and mechanical damage were selected and picked when reached to physiological mature with over 7% TSS content, and then quickly transported to the laboratory for treatment.

Selected fruits were dipped in 5% hypochlorite solution for 10 minutes for sterilizing, and washed three times with distilled water. Then the fruits were randomly divided into two groups of 50 each. The first group was soaked in distilled water for 30 min as control (CK). The second group was soaked in 0.1 mmol L^{-1} melatonin solution for 30 min as MT treatment. After drying, the fruits were stored in the 4°C refrigerator, one bag for every 10 fruits. 10 samples were randomly taken for every 7 days during storage at 0, 7, 14, 21 and 28d to immediately measure the fruit weight, hardness, soluble solids, titrable acid. The remaining pulp was diced and stored at -80°C for the determination of other indicators, such as malondialdehyde (MDA) content, etc.

Measurements of physical properties

Fruit weigh was measured with an electronic scale. Fruit hardness was measured by GY-3 hardness tester. Soluble solids (TSS) were measured with PAL saccharimeter of ATAGO.

MDA content assay

MDA content was measured according to the thiobarbituric acid method (Hodges et al., 1999). Briefly, 0.3 g samples was ground as homogenate using 5 ml cold 5% (w/v) trichloroacetic acid solution (TCA) and centrifuged at 10000 g for 10 min at 4°C. Then, 2 ml supernatant and 0.67% (w/v) thiobarbituric acid (TBA) were mixed, sealed and heated for 10 min in boiling water bath. After cooling, the absorbance of supernatant was measured at 450, 532, 600 nm.

Soluble protein content measurement

The determination of soluble protein content was performed by coomassie brilliant blue G-250 staining. 0.3 g sample was ground as homogenate with 50 mM cold potassium phosphate buffer (PBS) (pH 7.8). The homogenate was centrifuged at 10000 g for 10 min at 4°C. The supernatant was added to coomassie brilliant blue G250 solution (dissolved in 90% ethanol and 85% (w/v) phosphoric acid) and mixed. The absorbance was determined at 595 nm.

Determination of total phenolic, flavonoid and flavanol contents

The methods of determining total phenolics (TPC), flavonoids (TFC) and flavanols (TFAC) were described by Wang (2015). In brief, 0.2 g of samples was ground as homogenate with cold 70% (v/v) methanol containing 2% (v/v) formic acid and 28% (v/v) ethanol. The homogenate was ultrasonically extracted for 30 min and shake at 250 rpm for 2 h at 30°C. Then the homogenate was centrifuged at 10000 g for 10 min at 4°C and the supernatant was filtrated by 0.45 μm filter membrane for further analysis. Folin-Ciocalteu method was used for TPC measurement with some adaptations (Singleton and Rossi, 1965; Tang et al., 2015). Results were expressed as mg gallic acid equivalents per gram of freeze-dried sample (mg GAE/g FDW). TFC was determined according to the method of Tang et al. (2015) with modifications. Results were expressed as mg catechin equivalents per gram of freeze-dried sample (mg CE/g FDW). The slightly modified DMACA-HCl method was used to evaluate TFAC (Li et al., 1996; Ma et al., 2017). Results were also expressed as mg CE/g FDW. The above indexes were repeated for more than 3 times, and the average value was taken as the measured value of each treatment.

Determinations of antioxidant capacity

Measurements of free radical scavenging ability, include DPPH, ABTS, and FRAP methods. ABTS was measured following the method of Re's group with modifications (Xu and Chen, 2011; Re et al., 1999). The ability to scavenge DPPH free radicals was assessed using modified DPPH method established by Brandwilliams's group (Brandwilliams et al., 1995; Tang et al., 2015). The ferric reducing ability of plasma (FRAP) assay was carried out based on the method of Benzie's group with a few adaptations (Benzie and Strain, 1996; Tang et al., 2015). All results were expressed as micromole trolox equivalents per gram of freeze-dried sample (μmol TE/g FDW). The above indexes were repeated for more than 3 times, and the average value was taken as the measured value of each treatment.

Statistic analysis

Excel 2010 was used for data processing and graphing. Analysis of variance was performed by the statistical program SPSS 22.0. Significant differences were detected by Duncan's multiple range tests at the 0.05 level.

RESULTS

Fruit weight loss rate and hardness

In the process of storage at 4°C, the fruit weight loss rate continued to rise, as shown in Fig. 1A. At 7 d, the weight loss rate in CK was higher than that in MT group by 0.81%. After then (14d and 21 d), weightlessness rate in CK were lower than that in MT, reached to 0.68% and 0.44%, respectively. The results indicated that melatonin treatment delayed the weight loss of kiwifruit in the early stage of storage.

Fruit hardness continued to decline during the storage (Fig. 1B). At 0-7 days, fruits in CK and MT group maintained high hardness with no differences. While after then, the hardness decreased sharply, and the hardness of CK group fall largely than MT group by 20.1% at 14d. At 28d, there was no significant difference between CK and MT. The results showed that MT treatment delayed the decrease of the hardness of kiwifruit.

Soluble solids and soluble protein content

During storage at 4°C, the content of TSS continued to increase, and reached edible degree at 7d (TSS>10%), and reached the maximum at 21d with TSS>12%. While the soluble protein content decreased. There was no significant differences between CK and MT group whether TSS or soluble protein content, thus indicated MT treatment had little effect on the TSS and soluble protein content in kiwifruit.

MDA content

The content of MDA exhibited a declined trend during whole storage except at 28d whether in CK or MT group (Fig. 3). But the MDA content in MT was significantly lower than that in CK by 17.24% at 7d, 32.51% at 14d, 47.38% at 21d, except at 28d, with no significant difference. It is proved that MT treatment can significantly reduce the content of MDA in kiwifruit to maintain fruit quality at certain extent.

Content of total phenols, total flavonoids and total flavanol

During the storage at 4°C, TPC in fruit was showed a slowly rising trend in both MT and CK group (Fig. 4A). TPC in MT group was slightly higher than CK, but not significant, except for 21 d, by 16.01% higher than CK. The results indicated that MT treatment can increase the TPC but had no apparent effect.

TFC in both MT and CK group fall slightly down in initial stage (7d), but increased quickly from 7d to 21d (Fig. 4B).

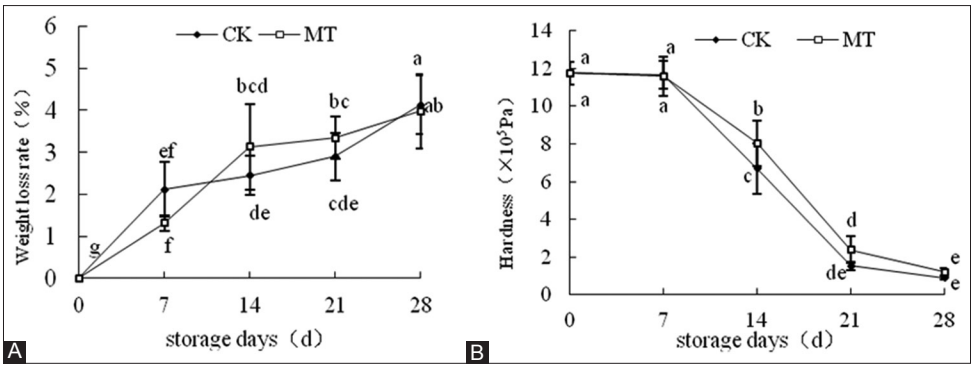

Fig 1. Changes of fruit weight loss rate and hardness during fruit storage under MT treatment and control (CK). Data are show as means±SE (n=6), different letters indicate significant differences at p=0.05 level.

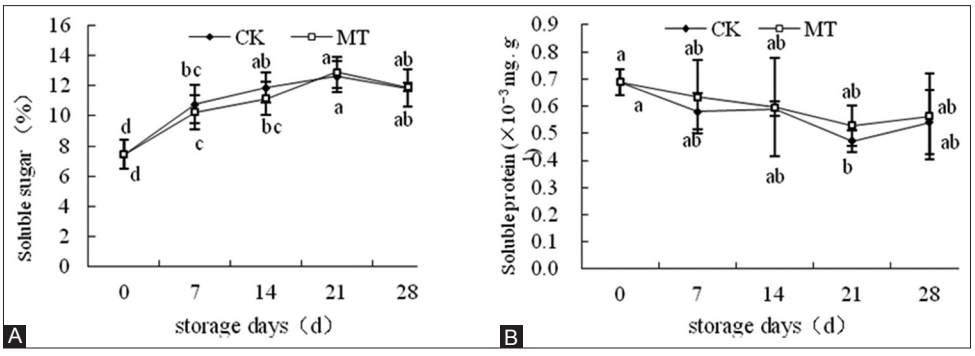

Fig 2. Changes of soluble solids and soluble protein content during fruit storage under MT treatment and control (CK). Data are show as means±SE (n=6), different letters indicate significant differences at p=0.05 level.

During the whole storage period, the TFC in MT was significantly higher than that in CK, by 36.31% at 7d, 62.95% at14d, 23.20% at 21d and 7.59% at 28d higher, respectively. It is proved that MT treatment can increase the TFC of kiwifruit significantly.

TFAC in CK and MT group fluctuated during the whole storage (Fig. 4C). CK reached the lowest point of $2.5x10^{-2}$ mg·g^{-1} at 14 d, reached the highest value at 28d. TFAC in MT treatment initially increased significantly and reached a maximum of 9.2×10^{-2} mg·g^{-1} at 7 d. During the whole storage process, TFAC in MT was significantly higher than that in CK, by99.55% at 7d, 87.44% at14d, 35.62% at 21d and 2.80% at 28d, respectively, indicating that MT can significantly increase the total flavanol content of kiwi fruit during storage.

Antioxidant ability

During storage at 4°C, ABTS$^+$ free radical scavenging capacity value in MT increases rapidly at the beginning of the storage (0 to 7 days), and then remain the level for the rest of time. While the value of ABTS$^+$ value in CK increased significantly after 7d and then fluctuated (Fig. 5A). During the whole storage period, the value of ABTS$^+$ in MT was significantly higher than that in CK by 14.87% at 7d, 0.64% at 14d, 4.54% at 21dand 1.70% at 28d higher respectively. It was proved that the ABTS$^+$ free radical scavenging ability in kiwifruit was significantly enhanced by MT treatment.

The total FRAP in MT showed an increasing trend and reached the maximum value at 21d and then slightly decreased at 28 d, while FRAP in CK has changed not much as in MT group (Fig. 5B). During the whole storage period, the FRAP of MT was significantly higher than CK, by 68.64% at 7d, 85.96% at 14d, 117.64% at 21d and 76.27% at 28d, respectively. It was proved that MT treatment of kiwi fruit can significantly increase the FRAP in kiwifruit and improved the antioxidant capacity of fruit to a certain extent.

During the process of storage at 4°C, DPPH value at the beginning of the storage (0 to 7 days) increased rapidly both in MT and CK group, after then remain basically unchanged (Fig. 5C). Except at 21d, the DPPH in MT was not significantly different from that in CK during the whole storage period. It is preliminarily believed that MT treatment has little effect on DPPH free radical scavenging ability of kiwifruit.

DISCUSSION

In this study, the effects of MT treatment on internal and external quality of kiwifruit 'Jinshi No.1' were investigated.

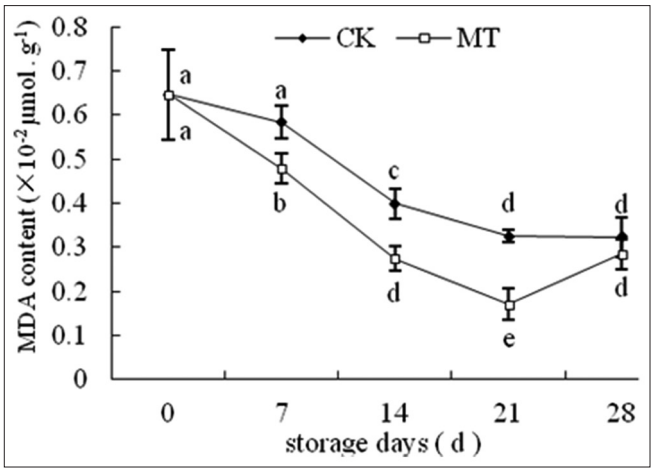

Fig 3. Changes of MDA content during fruit storage under MT treatment and control (CK). Data are show as means±SE (n=6), different letters indicate significant differences at *p*=0.05 level.

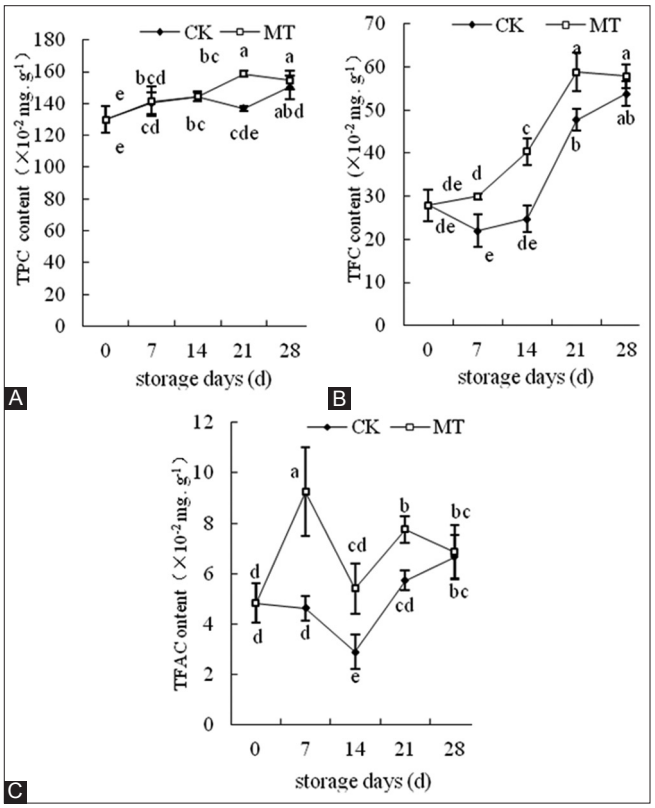

Fig 4. Changes of TPC (A), TFC (B) and TFAC (C) during fruit storage under MT treatment and control (CK). Data are show as means±SE (n=6), different letters indicate significant differences at *p*=0.05 level.

In the post-ripening stage, fruit weight loss rate increased accompanied by hardness decrease and soluble solids increase (Matsumoto et al., 1983). The results showed that the treatment with melatonin significantly delayed the decrease of the hardness of kiwifruit and had little effect on the content of soluble solids, but enhanced the rate of weight loss. At the same time, MT treatment soluble protein content in kiwifruit were slightly higher than CK,

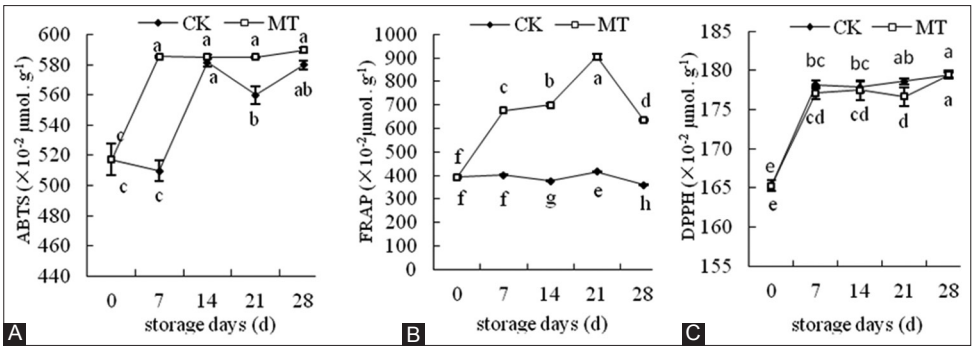

Fig 5. Effect of exogenous melatonin on ABTS.⁺, FRAP and DPPH of kiwifruit during storage. Data are show as means±SE (n=6), different letters indicate significant differences at p=0.05 level.

proving MT processing delay the soften, but maintain the nutritional quality of kiwifruit.

Under adversity stress or aging process, the accumulation of free radicals, reactive oxygen species, lipid free radicals induced membrane lipid peroxide, eventually lead to cell damage or death. MDA is the final product of membrane lipid peroxidation, through the determination of its content may know the extent of the membrane lipid oxidative damage, so as to determine antioxidant capacity of plant (Liang et al., 2018). The results of this experiment showed that the content of MDA in kiwifruit decreased significantly with the treatment of melatonin, which improved the antioxidant ability of fruit to a certain extent.

Phenolic substances are important secondary metabolites in plants. They are abundant in fruits, vegetables and grains and are the main source of antioxidant substances obtained by people (İnan et al, 2017). Phenolics has many biological activities, such as flavonoids polyphenols can inhibit carcinogenic activity of carcinogens, treat various inflammation, and tranquilize, allay excitement, lower blood pressure (Lu et al., 2016). Flavanol polyphenols can remove reactive oxygen free radicals and play an antioxidant role (Bhullar and Rupasinghe, 2015). Therefore, the phenol function to human health has been widely concerned. The results showed that the content of total flavonoids and total flavanols in kiwifruit was significantly increased with MT treatment, but the content of total phenol was not significantly increased. In general, MT treatment increased the content of phenolic substances in kiwifruit.

The method to determine the antioxidant capacity based on scavenging free radicals has become the most popular. DPPH (1,1'-diphenyl phenylhydrazine) is a stable method to measure nitrogen-centered free radical, which changes the color of the solution when it reacts with antioxidants such as phenols (Da, et al.,2000; Standley,et al., 2001). The principle of lipid peroxidation inhibition (ABTS method) is that the blue-green cationic $ABTS^{+-}$ reacts with antioxidants (such as phenols) to become colorless ABTS (Miller,et al.,1993). Iron ion reduction antioxidant capacity (FRAP method) is an ability to reflect the antioxidant capacity of Fe^{3+} by measuring the ability of the subject to reduce Fe^{3+} to Fe^{2+} (Bub, et al.,2000). In this study, results showed that antioxidant capacity in MT measured by ABTS, FRAP were significantly higher than that in control, although the results of the DPPH method (MT) there was no evident difference compared with CK. In overall, MT improved the antioxidant capacity of kiwifruit during storage.

CONCLUSIONS

In conclusion, melatonin treatment delayed significantly the fruit hardness soften, reduced the membrane lipid peroxidation level and MDA accumulation, alleviated the degradation of soluble protein, increased content of antioxidants, thus improving its oxidation resistance. However, the mechanism how MT regulates metabolism of these physiological indicators was remained to be further studied.

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Author contributions

XW carried out the experimental part of this study. XW and DL interpreted the experimental data and drafted the manuscript. HX and DL were the project director who designed the experimental plan. All other authors contributed towards the final shape of the manuscript. In addition, all authors were involved in the review and editing of the final manuscript.

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