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Hydrogen-rich water delays postharvest ripening and senescence of kiwifruit

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ABSTRACT

The effect of hydrogen-rich water (HRW) on prolonging the shelf life of kiwifruit and possible underlying mechanisms were assessed. Our results revealed that HRW (30%, 80%, and 100%) displayed different effects in inhibiting the rot of kiwifruit. Among these treatments, 80% HRW had the most significant effect by decreasing the rot incidence and preserving the firmness of kiwifruit. This conclusion was supported by the fact that 80% HRW treatment could effectively alleviate pectin solubilization and reduce the activities of cell wall-degrading enzymes. On the other hand, HRW treatment was able to reduce the respiration intensity, increase the activity of superoxide dismutase, decrease lipid peroxidation level, and maintain the radical (DPPH$^{\cdot}$, $O_2^{\cdot-}$, and $^{\cdot}OH$)-scavenging activity of kiwifruit. Moreover, the inner membrane of mitochondria exhibited higher integrity. Thus, our results demonstrate that HRW treatment could delay fruit ripening and senescence during storage by regulating the antioxidant defence.

1. Introduction

Kiwifruit is an economically important subtropical fruit crop. There are presently more than 70 species of kiwifruit in the genus *Actinidia*, which are widespread in Asia. It is also grown commercially in other countries or continents, such as the United States, Canada, Chile, New Zealand, and parts of Europe (Garcia, Stevenson, Atkinson, Winz, & Quek, 2013). In China, kiwifruit is one of the most common fruits, widely grown in the northwest region. It contains a wealth of phytonutrients, including ascorbic acid, phenolics, flavonoids, vitamin E, carotenoids and minerals, and serves as the best source of lutein and *myo*-inositol among daily consumed fruits (Zhang, Li, Liu, Song, & Liu, 2012). Thus, consumption of kiwifruit can be beneficial for specific health conditions. For example, regular kiwifruit consumption could reduce DNA fragility (Rush et al., 2006) and exert beneficial effects on the antioxidative status and the risk factors for cardiovascular disease (Chang & Liu, 2009).

Despite its wide popularity, the kiwifruit has a short shelf life (approximately 3–4 days) because of ripening and rapid deterioration (Jhalegar, Sharma, Pal, Arora, & Dahuja, 2011), which affects its economic performance. The decay of kiwifruit is closely related to structural changes in the cell wall. Associated processes, e.g., pectin solubilization and depolymerization (Fischer, Wegryzn, Hallett, & Redgwell, 1996), primarily cause kiwifruit softening by hydrolyzing the cell wall. The key enzymes involved in this process are cellulase, pectinmethylesterases (PME), and polygalacturonase (PG) (Ramana-Rao, Gol, & Shah, 2011).

To extend the storage life and maintain the quality of kiwifruit, various methods have been developed, among which cold storage is the most common. However, the kiwifruit is susceptible to relatively low temperature (e.g., 0, −0.8 °C), resulting in the development of physiological disorders, along with rapid softening after cold storage (Mworia et al., 2012). Although 1-methylcyclopropene can reduce ethylene production and prevent flesh softening of kiwifruit more effectively at 20 °C than at 0 °C (Jhalegar et al., 2011), it has no significant effect on the firmness of kiwifruit during the mid-to-late period of storage (Kim, Hewett, & Lallu, 2001). Therefore, alternative postharvest handling strategy is needed for delaying kiwifruit softening and thus extending its shelf life.

In recent years, accumulating evidence has implicated gases of small molecules such as nitric oxide (NO) and hydrogen sulphide (H_2S) in various developmental processes, including adventitious rooting (Xuan et al., 2012), alleviation of cadmium toxicity (Li, Wang, & Shen, 2012), seed germination (Wang et al., 2012), and preservation of fruits and vegetables (Hu et al., 2012; Lai, Wang, Li, Qin, & Tian, 2011). Despite certain delaying effects of low-dose NO and H_2S on the senescence of plants, high doses of NO and H_2S, especially the latter, can be poisonous to fruits and vegetables (Perna et al., 2011). Owing to stringent safety requirements, the application of NO and H_2S for preservation of fruits and vegetables

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is still largely limited. A previous study has demonstrated hydrogen gas (H_2) evolution and uptake by illuminated leaves (Sanadze, 1961), while other *in vitro* studies have demonstrated H_2 evolution by isolated chloroplasts and postulated the existence of hydrogenase in specific higher plants (Esquivel, Amaro, Pinto, Fevereiro, & Malcata, 2011). Recently, H_2 is proven to have the ability of alleviating various abiotic stresses, including high salinity (Xie, Mao, Lai, Zhang, & Shen, 2012; Xu et al., 2013), and low temperature (Jin, Zhu, Cui et al., 2013) by regulating the antioxidant defence system. It is well known that the senescence of fruits and vegetables is generally accompanied by excessive production of reactive oxygen species (Singh & Singh, 2013). Thus, we speculated that H_2 might also be involved in the regulation of postharvest physiological and biochemical behaviour of kiwifruit during storage. Most important, H_2 is non-toxic and does not react with most compounds, including oxygen gas (O_2) at room temperature (Ohsawa et al., 2007).

In this study, to confirm above deduction and provide new evidence for the function of H_2, we investigated the effects of hydrogen-rich water (HRW) on the parameters of physical quality and antioxidant capacity of kiwifruit during storage. Our results support the idea that H_2 delays the ripening process, reduces lipid peroxidation and maintains the free radical ($DPPH^{\cdot}$, $O_2^{\cdot-}$, and $\cdot OH$)-scavenging activity in kiwifruit. These results suggest that HRW treatment may be a useful technique for maintaining kiwifruit quality and extending its postharvest life.

2. Materials and methods

2.1. Kiwifruit

Kiwifruit (*Actinidia chinesis* cv. Huayou) is selectively bred from the cv. 'Zhonghua' and 'Meiwei' seedlings at an orchard in Yangling District (Shaanxi Province, China). The fruit ripen from the end of September to the middle of October. Fruit were harvested at commercial maturity (Brix $\geqslant$ 6.5) and obtained from the Zhongcai Market in Nanjing (Jiangsu Province, China) on October 15. The fruits of uniform size with no physical injuries or infections were chosen for subsequent treatments.

2.2. Treatment with hydrogen-rich water (HRW)

Purified hydrogen gas (H_2) (99.99%, v/v) was generated from a H_2 generator (AYH-300, Beijing Keshi Xingye Technology Co., Ltd., China) and bubbled into 10 L of distilled water at a rate of 200 ml/min. The bubbling was continued for 3 h to allow the saturation of water with H_2 (Jin, Zhu, Cui et al., 2013). Subsequently, an aliquot of the saturated hydrogen-rich water (HRW; 100% concentration) was immediately diluted to the indicated concentrations for the further experiments. In the preliminary test, we chose a series of HRW concentrations and found that the concentrations of 30–80% HRW obviously maintained a better quality of kiwifruit and reduced the rot incidence during storage, in comparison with distilled water treatment, whereas 100% HRW treatment accelerated the rot incidence (Supplementary Fig. 1). Therefore, HRW at 30%, 80%, and 100% was used in the following experiments.

Afterwards, the fruits were immersed in plastic boxes with 10 L of distilled water (reagent control, CK_1), 30% HRW, 80% HRW, or 100% HRW for 5 min, followed by air-drying at 20 °C for 1 h. Then, the fruits were placed in 21-L Lock & Lock boxes (two holes with a diameter of 1 cm in the diagonal position, which kept the gas composition in the box similar to that in ambient air and prevented an escape of water), then stored at 20 ± 0.2 °C in an MIR-254 culture incubator (Sanyo, Japan) for 16 days and 90–95% relative humidity (RH). Fruit without distilled water and HRW treatment were also used as the blank control (CK_0). In view of the fact that the time of HRW treatment was only about 5 min, the effect of hypoxia occurring in headspace (about 10 L) of the plastic box [for example, upon 80% HRW treatment, 0.78% hydrogen and 16.13% oxygen were detected by gas chromatography (GC 7890, Agilent) in the headspace of plastic boxes, in comparison with about 0% hydrogen and 21% oxygen in the outside air] on fruit was almost negligible. During storage, flesh samples were taken from the fruit at 4 d intervals for physicochemical assays. Each treatment was done in triplicate, with 50 fruits in each replicate group.

2.3. Determination of H_2 content

For analyzing the H_2 content, we took 10 ml of freshly prepared HRW into a vial (20 ml). Afterwards, the vials were immediately capped and kept for 2 h, then 0.5 ml of sample was withdrawn from the headspace with a microsyringe and measured by gas chromatography (GC).

The chromatographic system (GC 7890, Agilent) was composed of a gas chromatograph equipped with a thermal conductivity detector (TCD) and a column containing the Molecular Sieve 5 Å stationary phase (MSA). The column was held isothermally at 70 °C. The injection and detector temperatures were adjusted to 200 and 220 °C, respectively. Nitrogen was used as carrier gas. In our experimental conditions, the H_2 concentration in freshly prepared HRW (100% concentration) analysed by GC was about 0.66 mM.

2.4. Rot incidence evaluation

The external appearance of each fruit and the presence of macroscopic fungal growth were visually evaluated. Fruits with visible decay were defined as 'rot', and no visible changes in the tissues were defined as 'good'. At each sampling day, the number of rotted kiwifruits was recorded. Rot incidence for the treatment unit was calculated as follows:

$$\text{Rot incidence} = \left(\frac{\text{the number of rotted fruit}}{\text{the total number of investigated fruit}}\right) \times 100.$$

2.5. Electronic tongue measurements

The original data were acquired through the αASTREE electronic tongue system (Alpha M. O. S. Co., Toulouse, France), which includes 7 different liquid cross-selective sensors (ZZ, BA, BB, CA, GA, HA, and JB). Each sensor is made from silicon transistors with a specific organic membrane, which interacts with ionic, neutral, and chemical compounds present in the liquid sample in a specific manner. Any interaction at the membrane interface is detected by the sensor and converted into an electronic signal to be analysed. The principle of the method is to detect the potentiometric difference between each individually coated sensor and the Ag/AgCl reference electrode. Therefore, an integral signal of each sample comprises a vector with 7 individual sensor determinations.

All the samples were detected at the room temperature of 25 °C. Before data acquisition from the electronic tongue, a sequence of sample preprocessing was implemented: an 8 ml kiwifruit juice sample was diluted to 80 ml with 72 ml distilled water. Then, the 80 ml dilution of each sample was analysed by the electronic tongue. The measurement time was set at 120 s for each sample, and then the sensors were rinsed for 10 s with distilled water before detecting the next sample. Five samples were tested at one measurement sequence. According to the experience of pre-experiment and the requirement of system stability, each sample was measured 7 times.