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Hydroxyl radical is involved in cell wall disassembly and aril breakdown in mulberry fruit during storage

Hangjun Chen [a], Shifeng Cao [b], Haiyan Gao [a,*], Xiangjun Fang [a], Jiaojiao Li [a]

[a] Institute of Food Science, Zhejiang Academy of Agricultural Sciences, Key Laboratory of Fruits and Vegetables Postharvest and Processing Technology Research of Zhejiang Province, Hangzhou, 310021, China
[b] Nanjing Research Institute for Agricultural Mechanization, Ministry of Agriculture, Liuying 100, Nanjing 210014, China

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ABSTRACT

The effect on hydroxyl radical (•OH) on cellular wall disassembly and aril breakdown in mulberry fruit was investigated *in vitro* and *in vivo*. The *in vitro* experiment showed •OH induced total sugar and uronic acid released from the cell wall material of mulberries in a dose-dependent manner. Then the harvested mulberries were treated with 5 mM •OH to find out its effect in vivo. The results suggested that the application of exogenous •OH induced degradation of cellular wall polysaccharides, especially water soluble pectin (WSP) and hemicellulose, which was associated with the higher incidence of aril breakdown in •OH-treated mulberries. Meanwhile, the results of gel permeation chromatography analysis further demonstrated that •OH enhanced depolymerization and structural modifications of polysaccharides of mulberries during storage as experienced by the downshift in molecular mass of WSP and hemicellulose fractions. These results indicated that as a consequence of the enhanced degradation of cell wall polysaccharides, •OH could increase the incidence of aril breakdown in harvested mulberry fruit during storage.

1. Introduction

Mulberry is a Chinese traditional edible fruit and well known as a good source of natural antioxidants. Due to its various health benefits, unique taste, and nutritional value, the production and consumption of mulberries have increased rapidly in recent years (Baea and Suh, 2007). However, the fruit quality deteriorates rapidly after harvest due to aril breakdown and decay development. Aril breakdown is an extremely important postharvest event in mulberries which determines fruit quality, shelf life and consumer acceptance (Li et al., 2016). Therefore, delaying or reducing aril breakdown should be an important approach to extend storage life or maintain quality of mulberry fruit. However, since the exact cause of this physiological disorder is not understood well in mulberry, there is no corresponding measure to resolve the problem.

It has been well established that the aril breakdown in longan fruit is a result of cell wall modification caused by enzymatic and non-enzymatic factors (Duan et al., 2011; Lin et al., 2007; Wang et al., 2013). The enzymatic depolymerisation and structural modifications of polysaccharides caused by polygalacturonase, pectin methyl esterase and β-galactosidase were reported to be responsible for aril breakdown of harvested longan fruit (Duan et al., 2011). In addition, non-enzymatic degradation of cell wall polysaccharides may also account for this disorder (Wang et al., 2013). Duan et al. (2011) suggest that hydroxyl radical could initiate the disassembly of cellular wall polysaccharides in longan. However, little attention has been paid to the same phenomenon in harvested mulberry fruit.

Research has shown that the modification of polysaccharide in aril breakdown of postharvest fruit was mediated by non-enzymatic events partly (Duan et al., 2011). The objective of this study was to evaluate the effect of ROS, especially OH, on the depolymerisation and solubilisation of cellular wall polysaccharides *in vitro*. In addition, the effects of exogenous OH on disassembly of cell wall polysaccharides and aril breakdown *in vivo* were also investigated. This work could be helpful for understanding the biochemical and physiological mechanism of aril breakdown in mulberry fruit during storage.

2. Materials and methods

2.1. Plant materials

Mulberry fruit (*Morus indica* L. Dashi) were hand-harvested at the commercial mature stage from an orchard in Huzhou, Zhejiang

* Corresponding author.
E-mail address: spsghy@163.com (H. Gao).

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Province. They were transported to our laboratory within 2 h. The fruits were selected for uniform size, colour and absence of visual defects.

2.2. Experiment in vitro

2.2.1. Cell wall material (CWM) preparation

CWM was prepared according to the method of Cao et al. (2010) with some modifications. Two hundred grams of aril tissues were homogenized in 80 mL of cold 95% (v/v) alcohol over ice for 3 min, which was then boiled for 25 min. The suspension was filtered under vacuum through glass fiber filters (GF/C, Whatman) and washed with 100 mL of 95% (v/v) ethanol. The residue was transferred to 100 mL of chloroform-methanol (1:1, v/v) and stirred for 30 min at room temperature. Then the suspension was filtered under vacuum through glass fiber filters (GF/C, Whatman) and washed with 100 mL of 100% acetone. Starch was removed by re-extracting overnight in 90% DMSO and no starch was detected using the KI-I_2 method. The extract suspension was then centrifuged at 4000*g* for 15 min. The precipitate was washed twice with 70% ethanol, then filtered and finally dried in the vacuum oven at 50 °C for 48 h. The dried powder was considered as CWM. Results were expressed as mg g^{-1} fresh weight (FW).

2.2.2. Effect of ROS on levels of total sugar and uronic acid released from CWM in vitro

The CWM from harvested mulberry aril was washed with 100 mM acetate buffer (pH 5.0) overnight at 25 °C by stirring to remove soluble polysaccharides and the mixture solution was filtered. The insoluble residue was then re-suspended in 40 mM acetate buffer (pH 5.0) to obtain CWM suspension (1%, w/v). Effects of ROS on contents of the total sugars released and uronic acid from CWM suspension were investigated by the method of Duan et al. (2011). The reaction mixture contained 0.2 ml of 1% CWM suspension, 0.7 ml of 40 mM sodium acetate buffer (pH 5.0) and 0.1 ml of ROS solutions. In this study, the final concentration of ROS solutions was 1 mM paraquat (O_2^- production) (Takizawa et al., 2007), 1 mM H_2O_2 or 1 mM $FeSO_4$ and 1 mM H_2O_2 (•OH production) (Halliwell, 1965), respectively. Distilled water was instead of ROS solutions as the control. The mixtures were shaken for 12 h at 120 rpm and 25 °C, and then filtered. The filtrate was used for measurements of total sugar and uronic acid contents. Total sugar content were quantified using a phenol-sulfuric acid method (Dubois et al., 1956). Glucose was used as a standard. The uronic acid content was determined by the m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973). Galacturonic acid was used as a standard.

2.2.3. Effect of Fe^{2+}/H_2O_2 concentration on levels of total sugar and uronic acid released from CWM in vitro

The effect of different Fe^{2+}/H_2O_2 concentrations on total sugar and uronic acid released from CWM was measured in a mixture of 0.84 ml of 50 mM sodium acetate buffer (pH 5.0) and 0.24 ml of 1% CWM suspension were incubated with 0.1 ml of •OH reaction solutions ($FeSO_4/H_2O_2$). The final concentrations of Fe^{2+}/H_2O_2 in the reaction mixture were 0, 0.1, 0.5, 1 and 5 mM, respectively. The total sugar and uronic acid were measured as described above.

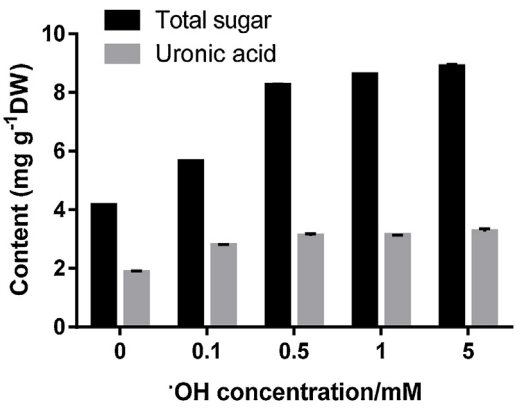

Fig. 1. Effect of Fe^{2+}/H_2O_2 concentration on levels of total sugar and uronic acid released from CWM *in vitro*. Values are the means ± SE. Each value is the mean of three replicates. Vertical bars represent the standard errors of the means.

2.3. Experiment in vivo

The mulberries were divided into two groups randomly. Fe^{2+}/H_2O_2 at 0.5 mM was chosen as the optimal concentration based on our previous study. For Fe^{2+}/H_2O_2 treatment, the first group of fruit was immersed into a solution of 0.5 mM Fe^{2+}/H_2O_2 for 3 min; the second group of fruit was considered as the control and was soaked in sterile deionized water for 3 min. All fruit were then air-dried for approx. 30 min and stored at 0 °C for 12 d (at 80%–90% RH). There were three replicates of 3 kg of fruit each per treatment. Samples were taken initially and at 2-d intervals during storage.

2.3.1. Fruit firmness

Fruit firmness was measured on 20 fruit from each replicate with a TA-XT2i texture analyser (StableMicro SystemLtd., U.K.) with a 5 mm diameter probe at a speed of 1 mms^{-1}.

2.3.2. Aril breakdown index

Aril breakdown index manifested as the breakdown area on each berries was evaluated visually using 30 fruit from each replicate. For each fruit, aril breakdown index was scored according to a 5-grad scale, where 0 = none; 1 = slight; 2 = moderate; 3 = moderately severe; 4 = severe. Results were expressed as an aril breakdown index calculated using the following formula: Aril breakdown index (between 0 and 4) = [Σ (aril breakdown level) × (number of fruit at the aril breakdown level)]/(total number of fruits in the treatment).

2.3.3. Extraction and analysis for cell wall polysaccharides

CWM was prepared as described above. Then, the CWM (50 mg) was fractionated using the methods described previously (Deng et al., 2005). The uronic acid content in the water (WSP), CDTA (CSP) and Na_2CO_3 (NSP) fractions were determined by the m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973). Results were expressed as mg of galacturonic acid per g of CWM. The cellulose and hemicelluloses contents were quantified

Table 1
Effect of ROS on contents of total sugars released and soluble uronic acid from CWM of pulp tissues of harvested mulberry fruit *in vitro*. •OH was produced by Fenton reaction with 1 mM Fe^{2+} and 1 mM H_2O_2. The means within a column followed by the same letter were not significantly different at 5% level.

	Relative percentage of total sugars released	Relative percentage of uronic acid
Control	100.00a	100.00b
Paraquat	104.16a	109.47b
H_2O_2	109.01a	54.67a
•OH	546.88b	489.04c