Food Chemistry 152 (2014) 205–209

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Anthocyanin contributes more to hydrogen peroxide scavenging than other phenolics in apple peel

Xiuli Bi [1], Jiangli Zhang [1], Changsheng Chen, Di Zhang, Pengmin Li *, Fengwang Ma

State Key Laboratory of Crop Stress Biology for Arid Areas, College of Horticulture, Northwest A&F University, Yangling, Shaanxi 712100, China

ARTICLE INFO

Article history:
Received 5 June 2013
Received in revised form 13 November 2013
Accepted 15 November 2013
Available online 25 November 2013

Keywords:
Anthocyanin
Apple peel
H_2O_2
Phenolic compounds

ABSTRACT

The phenolic compounds in apple peel extracts were quantified in the presence of hydrogen peroxide (H_2O_2) to identify which phenolic compound contributed more to H_2O_2 scavenging. The results showed that the phenolics extracted from 'Golden Delicious' apple peel had a strong ability for scavenging H_2O_2. After incubating with H_2O_2 for 30 min, cyanidin-3-galactoside concentrations in the phenolic extract decreased as H_2O_2 concentrations increased. In contrast, the concentrations of other phenolic compounds remained unchanged. Exogenous application of H_2O_2 enhanced the synthesis of phenolics, especially anthocyanin, in 'Golden Delicious' apple peel under sunlight. After incubating the peel extract of H_2O_2-treated apples in the dark for 30 min, the concentration of cyanidin-3-galactoside significantly decreased to a greater extent than that of other phenolic compounds. Based on these data, anthocyanin is more sensitive to H_2O_2 and contributes more to H_2O_2 scavenging than other phenolic compounds.

1. Introduction

Phenolic compounds are low molecular weight secondary metabolites and are widely distributed in fruits and vegetables. Phenolic compounds attract more attention than other bioactive compounds since they are thought to have positive effects on human health as a result of their high antioxidant capacity (Chen & Chen, 2013; Williams, Spencer, & Rice-Evans, 2004). The antioxidant capacity of phenolics has been well-demonstrated (Rhodes, 1996; Shahidi, Janitha, & Wanasundara, 1992), and epidemiological studies have shown that they can protect humans from some age-related diseases (Commenges et al., 2000), cancer (Chen & Chen, 2013; Knekt et al., 1997), and cardiovascular disease (Jia et al., 2012; Ju et al., 2012).

For humans, one good source of phenolic compounds is apples which are considered an important source of dietary antioxidants (Wolfe & Liu, 2003; Wolfe, Wu, & Liu, 2003). The main phenolics found in apples are flavonols, anthocyanins, flavanols, phenolic acids, and dihydrochalcones (Chen, Zhang, Wang, Li, & Ma, 2012; Treutter, 2001). In apples, flavonols mainly occur as quercetin conjugated glycosides such as galactoside, glucoside, xyloside, arabinoside, rutinoside, and rhamnoside whereas the major anthocyanin is cyanidin-3-galactoside. Compared to flavonols and anthocyanins, flavanols such as catechin, epicatechin, procyanidin B1, and procyanidin B2 are not glycosylated and are either monomeric or oligomeric. The phenolic acids in apples are divided into two subgroups: hydroxycinnamic acids (particularly chlorogenic acid) and benzoic acids (e.g., gallic acid). Lastly, apples also contain dihydrochalcones in the form of phloridzin and its derivatives.

The capacities of phenolic compounds to scavenge 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals vary. Consequently, Rice-Evans, Miller, and Paganga (1997) have suggested that a hierarchy of phenolic antioxidant activities exist. In particular, they reported that the antioxidant capacities of cyanidin and quercetin were similar and remarkably higher than that of other phenolic compounds (Rice-Evans et al., 1997). Lee, Kim, Kim, Lee, and Lee (2003) have also reported that the antioxidant capacity of quercetin is higher than other phenolics. In apples, since cyanidin and quercetin are unstable, they are usually present in their glycosylated forms. Do the antioxidant capacities of glycosylated cyanidin and quercetin remain similar and also higher than that of other phenolic compounds, however?

Previous studies have shown that the red leaves of plants have a higher antioxidant capacity than their green counterparts for α,α-diphenyl-β-picrylhydrazyl (DPPH). This may be attributed to the higher anthocyanin content in these tissues (Hughes, Neufeld, & Burkey, 2005; Neill & Gould, 2003; Neill, Gould, Kilmartin, Mitchell, & Markham, 2002a, 2002b; Shao et al., 2007). However, besides anthocyanin, phenolic concentrations in red plant tissues are also higher than that of green tissues. Therefore, whether the higher antioxidant capacity of red tissues can be mainly attributed to their anthocyanin content is debatable. Chen, Nagao, Itani, and Irifune (2012) found that the anthocyanins in red and black rice cultivars

* Corresponding author. Tel./fax: +86 29 87082648.
E-mail address: Lipm@nwsuaf.edu.cn (P. Li).
[1] These two authors contributed equally to this work.

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.11.088

contributed little to the total antioxidant capacity of rice. Moreover, antioxidant activity was measured by the ability to scavenge free radicals such as ABTS or DPPH radical. Because ABTS and DPPH are artificial free radicals, a higher capacity for scavenging ABTS or DPPH might not represent actual antioxidant activity of scavenging reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) or superoxide anions, the most commonly produced ROS in plants, animals, or human beings.

The most active free radical is H_2O_2 which can diffuse across membranes (Yamasaki, Sakihama, & Ikehara, 1997) and cause serious health problems (Jia et al., 2012; Ju et al., 2012; Wang, Peng, Zhu, & Ren, 2007). In this study, we examined the degradation of phenolic compounds in apple peel in the presence of H_2O_2 to determine which compound was more sensitive to H_2O_2 and contributes more to total phenolic antioxidant capacity.

2. Materials and methods

2.1. Plant materials and treatments

The 'Golden Delicious' apple (*Malus domestica* Borkh.) fruit was used in this study. The trees were 19 years-old on M111 rootstock, planted at a spacing of 3.0 m × 4.0 m, in Luochuan, Shaanxi, China (35.765°N, 109.442°E; elevation: 1033 m). The tree received standard horticultural treatment for diseases and pest control. In the middle of May 2012, fruits were placed in light-impermeable double-layer paper bags (outer layer was yellow on the outside and black on the inside, and the inner layer was red).

Thirty mature fruits per tree (5 replications) were harvested without removing the bags at the end of August. The collected apples were taken from their bags in weak light and divided into two groups. One group (totally 50 fruits, 10 per replicate) was immersed in 150 mM H_2O_2 overnight while the other group (totally 100 fruits, 5 replicates) was immersed in water. Before sunrise the next morning, all apples were removed from either the H_2O_2 solution or water and placed on four layers of wet cheesecloth in an open space for exposure to sunlight. After 3 days of light treatment, the peels from each apple immersed in H_2O_2 solution and from half the apples immersed in water were collected. Peels from the remaining water-immersed apples were collected 7 days after light treatment. Sun-exposed peels (about 1 mm thick) were collected using a peeler and immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

2.2. Phenolic compounds analysis

Phenolic compounds were extracted using 70% methanol containing 2% formic acid at 0–4 °C. After centrifugation (10,000*g* for 10 min), the supernatant was passed through a 0.45 μm syringe filter. Extracts from the samples taken 3 days after sunlight exposure were analysed immediately or after a 30 min incubation in the dark at room temperature. Extracts from the samples taken 7 days after sunlight exposure were incubated in the dark for 30 min after adding 0 mM, 2.5 mM, 25 mM, or 150 mM H_2O_2. Reaction mixtures were analysed by high-performance liquid chromatography.

Phenolic compounds were analysed using an HP1200 Liquid Choromatograph equipped with a diode array detector (Agilent Technology, Palo Alto, CA, USA) as previously described by Chen et al. (2012). The Inertsil ODS-3 column (5.0 μm particle size, 4.6 mm × 250 mm; GL Sciences Inc., Tokyo, Japan) preceded by an Inertsil ODS-3 Guard Column (5.0 μm, 4.0 mm × 10 mm) was used for the separation. Solvent A consisted of 10% formic acid (11.36% 88% formic acid) dissolved in water and solvent B was 10% formic acid (11.36%, 88% formic acid) and 1.36% water in acetonitrile (HPLC grade, purity: 99.9%). The gradient was 95% A (0 min), 85% A (25 min), 78% A (42 min), 64% A (60 min), and 95% A (65 min). The post-run time was 10 min, and the flow rate was 1.0 ml/min at 30 °C. Simultaneous monitoring was performed at 280 nm for catechin, epicatechin, procyanidin B1, procyanidin B2, phloridzin, and gallic acid; 320 nm for chlorogenic acid, caffeic acid, ρ-coumaric acid, and ferulic acid; 365 nm for quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside, quercetin-3-rutinoside, quercetin-3-xyloside, and quercetin-3-arabinoside; and 520 nm for cyanidin-3-galactoside, respectively. Peaks were identified by comparing retention times and UV spectra with authentic standards. The concentration of individual phenolic compounds was determined based on peak area and calibration curves derived from corresponding authentic phenolic compounds. All phenolic standards were obtained from Sigma–Aldrich (St. Louis, MO, USA), Extrasynthese (Genay Cedex, France), and AApin Chemicals (Abingdon, Oxon, UK).

2.3. H_2O_2 analysis

H_2O_2 concentration was assayed using an Oxytherm liquid-phase oxygen electrode system (Hansatech Instruments, Norfolk, UK). After mixing 100 μl 250 mM H_2O_2 solution with 900 μl of the phenolic extract (anthocyanin concentrations: 0, 1.5, 3, and 6 mM), O_2 evolution was analysed after incubating for 30 min in the dark. In the O_2 evolution assay, 20 μl reaction mixture was immediately added to the Oxytherm reaction chamber filled with 2.0 ml 100 mM phosphate buffer, pH 7.0. Next, 20 units of catalase were added, and O_2 evolution was continuously monitored until the signal remained constant. The difference in O_2 evolution between before adding the reaction mixture and after adding catalase reflected the H_2O_2 concentration of the mixture.

Phenolic extracts were obtained from the apples exposed to sunlight for 7 days. Briefly, phenolic compounds were extracted using 90% methanol containing 2% formic acid. The extract solution was then evaporated to dryness under a stream of nitrogen gas, and the resultant was finally dissolved in 70% methanol containing 2% formic acid to enrich the concentration of the phenolic compounds, with that of anthocyanin being 1.5, 3, and 6 mM, respectively.

2.4. Statistical analysis

All data were statistically analysed by *t*-test using SPSS 16.0 software, $P < 0.05$.

3. Results and discussion

We used an Oxytherm liquid-phase oxygen electrode system to investigate the H_2O_2 scavenging capacity of phenolics extracted from apple peel. After a 30 min incubation of the mixture of H_2O_2 and phenolic extracts, H_2O_2 concentrations were analysed. It was found that with increasing concentrations of phenolics, H_2O_2 concentrations decreased linearly, indicating that the phenolic extract could effectively scavenge H_2O_2 (Figs. 1 and 2). This was consistent with previous studies where phenolics exhibited strong antioxidant abilities (Rhodes, 1996; Shahidi et al., 1992).

'Golden Delicious' is a green/yellow apple cultivar, however, the sun-exposed peel may turn red when fruits are bagged for long time with light-impermeable bags and then suddenly re-exposed to sunlight (Chen, Li, Zhang, Li, & Ma, 2013; Fig. 3A and B). Besides anthocyanin, eighteen phenolic compounds in the extract were identified and quantified after incubating with different concentrations of H_2O_2 (Table 1, Fig. 3A) for 30 min. Cyanidin-3-galactoside concentrations decreased with increasing H_2O_2 concentration (Fig. 3A). In contrast, the concentrations of other phenolics