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Phenolic compounds and antioxidant activity in red-fleshed apples

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ABSTRACT

Red-fleshed apples are receiving increased attention because of their remarkable anthocyanin contents. To provide sufficient experimental evidence about their phenolic composition and strong antioxidant capacity, three red-fleshed varieties from Xinjiang, P.R. China and one from the USA were evaluated. For comparison, two commercial white-fleshed varieties were also included. In samples of the flesh, contents of phenolics (total phenolics, flavonoids, flavanols, and anthocyanins) and antioxidant activity were significantly higher for red-fleshed varieties than for white-fleshed varieties. Flavonoid profiles also differed between the red- and white-fleshed varieties. Among the red-fleshed varieties, 'Roberts Crab' had the highest amounts of total phenolics, flavonoids, and anthocyanins, as well as the strongest antioxidant activity, while 'Xiahongrou' had the lowest levels of total phenolics and flavonoids and the weakest activity. HPLC analysis revealed that individual phenolic compounds varied significantly among red-fleshed varieties. These results suggest that red-fleshed apples are a promising source of antioxidants for human nutrition.

1. Introduction

In a highly oxidative environment, many processes involved in metabolism may lead to oxidative stress. In particular, cardiovascular disease and cancer can arise from oxidative stress, damaging biomolecules such as DNA, lipids, and proteins (Li et al., 2014). Antioxidant molecules can inhibit oxidation, thereby retarding the progress of many chronic diseases and lipid peroxidation (Gülçin, 2012). Fruits and vegetables contain various phytochemicals with antioxidant activity that can protect the human body against cellular oxidation reactions (Giovanelli & Buratti, 2009). A major class of phytochemicals commonly found in such products is the phenolics. These compounds have strong antioxidant properties that enable them to scavenge free radicals, donate hydrogen atom, chelate metal ions, break radical chain reactions, and quench singlet oxygen *in vitro* and *in vivo* (Meng, Fang, Qin, Zhuang, & Zhang, 2012).

The apple is one of the most important dietary sources of phenolic compounds (Tsao, Yang, Xie, Sockovie, & Khanizadeh, 2005). Fruits contain five major groups of phenolic compounds, namely, hydroxycinnamic acids, dihydrochalcones, flavanols, flavonols, and anthocyanins (Łata, Trampczynska, & Paczesna, 2009). Distribution of these compounds differs among varieties and tissue types.

Although the pigments associated with colour can vary, the amount and composition of anthocyanins within the phenolics classification are the major determinants of apple fruit skin reddening (Ubi et al., 2006). In the typical red apple,

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anthocyanin accumulations are limited to only a few epidermal and hypodermal cell layers, whereas the internal tissues generally lack anthocyanin and are non-pigmented. However, the fruits of several genotypes also have red flesh because of the presence of anthocyanin pigments. Therefore, this germplasm is an attractive starting point in both traditional breeding and biotechnology to develop novel varieties for consumption and nutraceutical purposes (van Nocker et al., 2012).

Red-fleshed apples, in particular, are deduced to demonstrate strong antioxidant capacity, mainly as a result of their high levels of anthocyanins and other phenolic compounds. Research with those types has focused on their anthocyanin contents, antioxidant activity, and processing techniques (Balázs et al., 2012; Joshi, Rupasinghe, & Khanizadeh, 2009; Mazza & Vefioglu, 1992; Rupasinghe, Huber, Embree, & Forsline, 2010; Sadilova, Stintzing, & Carle, 2006; Sun-Waterhouse et al., 2013). However, no detailed quantitative analysis of individual phenolic compounds has been conducted with these particular varieties. Nearly all red-fleshed apple varieties can be traced back to wild *Malus niedzwetzkyana* (van Nocker et al., 2012). This presumed natural form is thought to have originated from central Asia (e.g., Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan, Turkmenistan, and China's Xinjiang Province). To the best of our knowledge, however, no study has previously been reported on the varieties found in Xinjiang, P.R. China, nor have they been investigated for their phenolic compounds and antioxidant activity.

The objective of the present study was to evaluate the phenolic profiles and antioxidant activities in fruits of three red-fleshed Xinjiang varieties and one from the USA. For comparison, two commercially grown white-fleshed varieties were also included. This study was carried out to obtain good experimental evidence that could support future efforts toward the development and utilization of their fruits.

2. Materials and methods

2.1. Chemicals and instruments

DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-tripyridyl-S-triazine), ABTS (2,2′-azino-bis-(3-ethylben zothiazoline-6-sulphonic acid) diammonium salt), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), *p*-DMACA (*p*-dimethylaminocinnamaldehyde), and Folin-Ciocalteu's phenol reagent were purchased from Sigma–Aldrich Chemical Company (Shanghai, China). All phenolic standards were obtained from Sigma–Aldrich (St. Louis, MO, USA), Extrasynthese (Genay Cedex, France) and AApin Chemicals (Abingdon, Oxon, UK). Other chemicals were of analytical grade whereas the solvents used for chromatography were HPLC-grade.

Absorbance was measured with a UV–visible spectrophotometer (Shimadzu, UV-1700, Kyoto, Japan). HPLC analysis was conducted on an Agilent 1200 Liquid Chromatograph equipped with a diode array detector (Agilent Technology, Palo Alto, CA, USA) and an Inertsil ODS-3 column (5.0 μm particle size, 4.6 mm × 250 mm; GL Sciences Inc., Tokyo, Japan). The resulting data were processed using the Agilent Chemstation for LC systems.

2.2. Apple samples

Red-fleshed apples 'Xiahongrou', 'No.1 Hongxun', and 'Hongrouguo' (three Xinjiang varieties) and 'Roberts Crab' (a variety from the USA) were studied. All are within *Malus niedzwetzkyana*. In addition, two commercially grown white-fleshed varieties of *M. domestica*, 'Gale Gala' and 'Golden Delicious', were used for comparison of phenolic compositions and antioxidant capacities. All varieties differed in peel and flesh colours (Fig. 1). The 6~8-year-old trees were grafted and

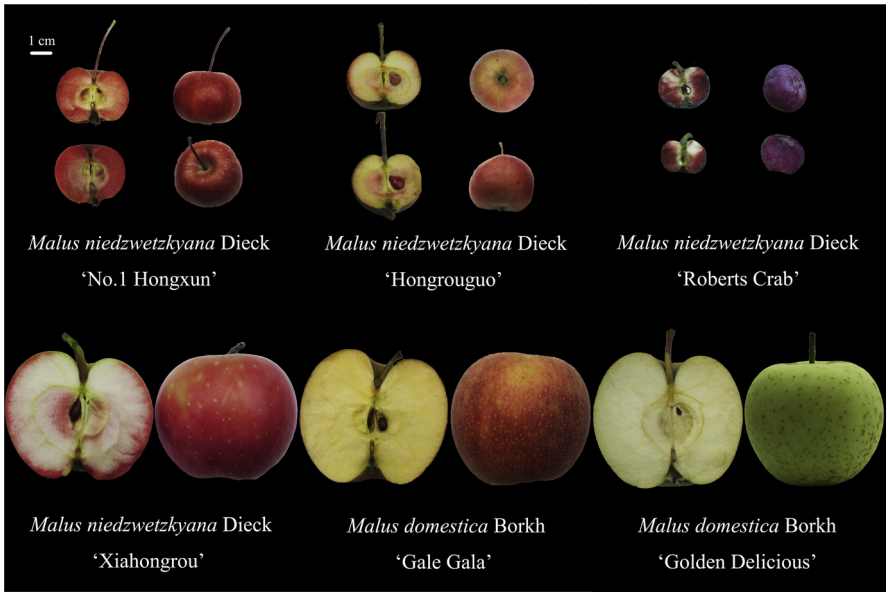

Fig. 1 – Varieties of red- and white-fleshed apple fruits examined here.