DPPH·, ABTS⁺ and FRAP activity of kiwifruit during post-ripening process at ambient temperature

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Abstract. Kiwifruit is well-known for an excellent source of antioxidants. In this study, antioxidant capacity, evaluated by DPPH·, ABTS⁺ and FRAP assays, was investigated in outer/inner pericarp and core during post-ripening process at ambient temperature. The results explored that the changes patterns of free radical scavenging rate of DPPH and ABTS remained relatively steady in whole post-ripening process, while FRAP increased in earlier stage and then declined remarkably.

1. Introduction
Kiwifruit is one of the most popular and widely cultivated temperate fruits around the world. According to the FAO statistical database (FAO, 2013), the world production of kiwifruit is 3,261,474 t. China, with about 54% of the world production in 2013 (1,765,874t), followed by the Italy (447,560t), New Zealand (382,337t), Chile (255,758t) and Greece (162,800t). Most of kiwifruits were consumed in the form of fresh fruit.

As one of the most popular fruits today, kiwifruit is also well-known for an excellent source of antioxidants, such as vitamin C, vitamin E, carotenoids, flavonoids, polyphenol, pigments and others [5,6,8].

The antioxidant activities and its capacities in kiwifruit can be altered by genotypes, cultivation techniques, climatic conditions, even post-harvest storage. The bioactive compounds and the level of antioxidant activity of four kiwifruit cultivars were determined and compared. The results show that the contents of polyphenols, ascorbic acids, protocatechuic and vanillic acids had significant difference among these cultivars [5]. Total soluble phenolics, flavonoids, and total antioxidant capacities were much higher than in 1-MCP treatments. The bioactive compounds and total antioxidant status of fruits increased during the treatment [4].

Here, antioxidant capacities were investigated using three different assays (DPPH·, ABTS⁺ and FRAP) in outer pericarp, inner pericarp and core of ‘Hongyang’ (Actinidia chinensis) kiwifruit, a popular cultivar planted in China with red-flesh endocarp and emerald green or golden outer layer pulp. Our object was to provide references for consumer purchase behavior and theoretical basis for further research on its medicinal value and antioxidant mechanism. This study supplied new information on the antioxidant function of these fruits for consumers, nutritionists and food policy makers.
2. Materials and methods

2.1 Materials

‘Hongyang’ kiwifruits used in this study were harvested from Kiwifruit Resource Orchard in Shifang (104°16′N, 31°13′E), Chengdu, China. Fruits were selected according to the uniformity of the shape on September 20th (all fruits samples have reached physiological maturity) and stored at ambient conditions (25±2 °C and 62±6 % RH) without any treatment. 8 fruits were sampled randomly every 3 days, 3 replicates, and stored at -72°C for further studies.

2.2 Preparation of kiwifruit extracts

At least 8 fruits were combined for each of the three replicated samples. The fruits samples was divided into three part, including outer pericarp, inner pericarp and core, each part (0.1g) was homogenised and extracted three times respectively in 1mL of ethanol: methanol: Formic acid (14:35:1, v/v) at 0°C. The homogenate was transferred to a centrifuge tube; another 1.0 mL of extraction solution was used to wash the mortar and pestle before being added to the first homogenate. After being shaken in a thermomixer at 30 °C for 3h at 400 rpm, this combined homogenate was centrifuged at 10,000 g for 10 min. The supernatant was then filtered through a 0.45μm syringe filter prior to analysis of the total phenolics, total flavonoids, total flavanols, vitamin C and antioxidant activities.

2.3 Antioxidant capacity determined by DPPH·, ABTS·+ and FRAP

The DPPH scavenging activity was determined based on an assay modified by Brandwilliams and others [2]. Absorbance at 517 nm was measured.

The ABTS+ assay was based on the method from Re and others [7]. Absorbance at 734 nm was measured.

The ferric reducing antioxidant power (FRAP) was measured following a procedure derived from Benzie and Strain [1] with some modifications. The absorbance of the reaction mixture was measured using a spectrophotometer at 593 nm.

Results of DPPH, ABTS+ and FRAP were expressed as (mmol TE/kg FW) and Trolox standard solutions were prepared as concentrations.

2.4 Soluble solids contents and firmness of fruit

Soluble solids contents (SSC) were estimated using Fisher Hand-hold Refractometer (scale 0-50) and firmness indexes were determined with hardness tester. At least 8 fruits were estimated for each replicate. Measurements were carried out at the same sites of fruit.

3. Results and discussion

3.1 Antioxidant capacity

Different assays have been used to measure the antioxidant activities of foods and biological samples, so it is difficult to use one antioxidant capacity of the unified standard to evaluate the plants [3]. Therefore, it is necessary to use at least two complementary methods to evaluate the antioxidant capacity. In our study, three antioxidant assays (DPPH, ABTS, and FRAP) were applied to obtain more accurate evaluations of antioxidant activities (Fig.3). The changes patterns of free radical scavenging rate of DPPH (Fig.1A) and ABTS (Fig.1B) were similarly. They all remained relatively steady in whole post-ripening process. In different part of fruit, free radical scavenging rate of DPPH and ABTS had not significant difference.

Different from the capable of free radical scavenging of DPPH and ABTS, the ferric reducing antioxidant power (FRAP) decreased significantly after 3 days of room temperature storage (Fig.1C). However, a observation on storage has been made by Tavarini et al. [8], which exhibited concomitant increase in FRAP and the author concluded that may be attribute to increase in phenolic content or
vitamin C content.

3.2 SSC and firmness of kiwifruit fruits
The SSC and firmness of fruits are important indicators of kiwifruit maturity. SSC and firmness of kiwifruit during post-ripening process in room temperature have been reported in Fig.2. An increase SSC corresponds to a conversion of starch to soluble sugars, and fruit firmness is the parameter of greater concern in kiwifruit storage and marketing with its senescence, rotting, and fruit injuries (Sharma et al. 2015).

Fig. 1 Changes of antioxidant capacity determined by DPPH (A), ABTS$^+$ (B), FRAP (C) of ‘Hongyang’ kiwifruit during post-ripening process at ambient temperature.

Here, at the beginning, the fruits just were reached physiology maturity, with 12.7 kg/cm$^2$ hardness and 6.8% SSC (Fig.2), not suitable to eat. During storage at ambient temperature, fruit firmness consistently decreased, meanwhile with SSC constantly increasing, finally reached to 2
kg/cm² and 17.1% at 12d respectively. From 3d to 6d, there is a dramatically decrease in hardness, followed by a rapid increase in SSC between 6d to 9d, indicating a respiratory climactic process.

Fig. 2 Changes of soluble solid content and firmness of ‘Hongyang’ kiwifruit during post-ripening process at ambient temperature.

4. Conclusions
Here, we investigated the change of antioxidant capacities (described by DPPH·, ABTS⁺ and FRAP) of different kiwifruit tissues (outer pericarp, inner pericarp and core) during post-ripening process. In a summary, the changes patterns of free radical scavenging rate of DPPH and ABTS⁺ remained relatively steady in whole post-ripening process, while FRAP increased in earlier stage and then declined remarkably.

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Reference
[1] Benzie IFF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. Anal Biochem 239(1): 70-76
[2] Brandwilliams W, Cuvelier ME, Berset C. 1995. Use of a free-radical method to evaluate antioxidant activity. Food Science and Technology- LWT, 28(1), 25-30
[3] Du GR, Li MJ, Ma FW, Liang D. 2009. Antioxidant capacity and the relationship with polyphenol and Vitamin C in Actinidia fruits. Food Chem 113:557-562
[4] Park YS, Im MH, Gorinstein S. 2015. Shelf-life extension and antioxidant activity of ‘Hayward’ kiwi fruit as a result of prestorage conditioning and 1-methylcyclopropene treatment. J Food Sci Technol 52(5):2711-2720
[5] Park YS, Leontowicz H, Leontowicz M, Namiesnik M, Suhaj M, Cvikrova M, Martincová O, Weisz M, Gorinstein S. 2011. Comparison of the contents of bioactive compounds and the level of antioxidant activity in different kiwifruit cultivars. J Food Compos and Anal 24(7): 963-970
[6] Park YS, Namiesnik J, Vearasilp K, Leontowicz H, Leontowicz M, Barasch D, Nemirovski A, Trakhtenberg S, Gorinstein S. 2014. Bioactive compounds and the antioxidant capacity in new kiwi fruit cultivars. Food Chem 165:354-361
[7] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio Med 26(9-10): 1231-1237
[8] Tavarini S, Degl’Innocenti E, Remorini D, Massai R, Guidi L. 2008. Antioxidant capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after storage of Hayward kiwifruit. Food Chem 107(1):282-288