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Antioxidant properties and involved antioxidant compounds of strawberry fruit at different maturity stages

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Abstract

The contents of antioxidant compounds superoxide dismutase (SOD), catalase (CAT), ascorbate peroxide (APX), total phenols, flavonoids, ascorbic acid, anthocyanins and vitamin E along seven growth stages of strawberry fruit were determined in this work. Three complementary assays, DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity, FRAP (ferric reducing antioxidant power) assay and superoxide anion radical scavenging ability were used to screen the antioxidant properties of extracts. Significant variations in antioxidant properties and involved compounds were observed at seven different growth stages. The highest antioxidant capacity was at the small green stage accompanying the highest activities of SOD, CAT and APX, as well as the highest contents of total phenols, flavonoids and vitamin E. The concentration of vitamin E, total phenols, ascorbic acid and flavonoids correlated with the antioxidant capacity. Ours results support the use of small green fruit of strawberry as sources of antioxidant compounds.

Key words: Strawberry, antioxidant, antioxidant capacity, antioxidant enzymes.

Introduction

Oxidative damage is thought to be one of the major mechanisms involved in chronic human diseases such as cancer and heart disease [1]. Lot of studies suggest that fruits and vegetables strongly contribute in reducing the risks of chronic human diseases lately, especially the colorful fruits and vegetables. This fact is attributed to various natural antioxidants contained in them [2, 3]. Antioxidants mainly include a group of oxidative enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxide (APX), glutathione reductase (GR) etc. and phenolic compounds of various chemical structures (e.g. catechins, flavonoids, anthocyanins) and vitamins (C, E and A) [4]. They can neutralize harmful free radicals to protect the cell against the attack from the free radicals and reduce oxidative damage, thus preventing a critical step in the onset of carcinogenesis [5].

Strawberries (*Fragaria×ananassa* Duch.) are popular fruits with high visual appeal, desirable taste and flavor. They are also good sources of ascorbic acid and polyphenolics. Studies have demonstrated that strawberries have high antioxidant activity which is two to eleven fold compared with apple, peach, pear, grape, tomato, orange or kiwifruit and antiproliferation activity [6-8]. In addition, current research suggests that strawberry extracts can inhibit $HepG_2$ human liver cancer cell proliferation [9]. This beneficial effect is believed to be due to the action of antioxidants partially. The contents of antioxidants and antioxidant capacity vary strongly during growth and maturation [10-12]. However, there is no consistence about the effects of maturity stages on antioxidant activity or antioxidant compounds. Wang and Lin found that greener stages had the highest antioxidant capacity in blackberries and strawberries, whereas red raspberries had the highest antioxidant capacity at the ripe stage [10]. Fu *et al.* [13] concluded that daylily flowers had the highest antioxidant activity at stage III (flower opening). Moreover, antioxidative enzymes also belong to antioxidants, scientific information regarding the changes in antioxidative enzymes is scare to our knowledge in strawberry during ripening.

'Toyonaka' is a very important strawberry cultivar in China, few works have described its antioxidant compounds and properties during ripening. Considering the importance of antioxidant and antioxidant capacity for functional benefit of strawberry, the aim of this work was to evaluate the influence of seven development stages on changes in antioxidant properties and antioxidant capacity of strawberry cultivar 'Toyonaka' in order to have a clear understanding of antioxidant metabolic changes, phytochemical accumulation and make the best use of the different botanical stages of fruits to extract for dietary supplements.

Materials and Methods

Plant materials and treatments: Strawberry cultivar 'Toyonaka' was grown in greenhouses in Sichuan Agricultural University. Fruits were collected in seven different development stages on April 2009, selected by color: small green (SG), large green (LG), white (W), 25% red, 50% red, 75% red and 100% red. All fruits were transported to lab within 30 min after harvest and damage-free were selected, immediately treated with liquid nitrogen and stored at -80ºC until extraction.

Determination of total phenols (TP), flavonoids, anthocyanins, ascorbic acid (AsA), dehydroascorbic acid (DHA) and vitamin E: TP was extracted from the crushed material using ethanol 80% (1:6, w/v) and determined at 765 nm, using the Folin-Ciocalteau reagent [14]. TP concentration was expressed as mg of gallic acid/ 100 g FW. A gallic acid curve was utilized with gallic acid standard prepared in ethanol (0.01-0.06 $mg \cdot mL^{-1}$). Flavonoids were extracted from the crushed material using ethanol 80% (1:6, w/v) and

determined at 510 nm [15]. Flavonoid concentration was expressed as mg of rutin/100 g FW. A rutin curve was utilized with rutin standard prepared in ethanol (0.0158-0.0948 mmol·L^{-1}). The anthocyanin content of strawberry was measured using a spectrophotometric pH differential protocol [16]. From 1.0 g of crushed material, anthocyanins were extracted by employing 5 mL of HCl (1%) and determined spectrophotometrically at 515 nm and 700 nm. Results were expressed as mg of cyanidin-3-glucoside, where MW= 449.2 and ε = 26900. The AsA and DHA measurements were based on the method of Sun [17]. Samples of 1.0 g of crushed material were extracted using 6 mL metaphosphoric acid 5% (w/v), the extracts were centrifuged at 22,000×g for 15 min and determined at 525 nm. Results were expressed as mg of AsA or DHA/100 g FW. An ascorbic acid curve was utilized with ascorbate standard prepared in metaphosphoric acid (0-40 nmol·mL^{-1}). The concentrations of vitamin E were assayed by the determination kit (Nanjing Jiancheng Bioengineering Institute).

Determination of SOD, CAT and APX activities: SOD (EC 1.15.1.1) and CAT (EC 1.11.1.6) activity were assayed by the determination kit (Nanjing Jiancheng Bioengineering Institute). One unit of SOD activity was defined as the amount of enzyme required for 1 g tissue in 1 mL of a reaction mixture SOD inhibition rate to 50% as monitored at 550 nm. One unit of CAT activity defined as the amount of enzyme required for 1 mg tissue protein decomposed 1 µmol H_2O_2 in 1 min. APX (EC 1.11.1.11) activity was determined spectrophoto-metrically by monitoring the decrease in ascorbate at 290 nm as described by Nakano and AsAda [18].

Determination of DPPH radical scavenging activity: Scavenging activity of the extracts against DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical was measured based on Yang *et al.* [14]. Antioxidants were extracted from the crushed material by using ethanol 80% (1:6, w/v), 200 µL of extract was added to 2.8 mL of DPPH-ethanol solution (60 µmmol·L^{-1}), vigorously shaken and maintained for 30 min at room temperature. Ethanol (80%) was used instead of strawberry extract as a control. The absorbance was measured at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation: DPPH scavenging effect (%) = $[(A_0-A_1)/A_0] \times 100$, where A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample.

Determination of ferric reducing antioxidant power (FRAP): The FRAP assay was performed according to Yang *et al.* [14]. Antioxidants were extracted from the crushed material using ethanol 80% (1:6, w/v), 20 µL of extract was added to 1.8 mL fresh TPTZ solution containing 25 mL acetate buffer (0.3 mol·L^{-1}), 2.5 mL TPTZ solution (10 mmol·L^{-1} in 40 mmol·L^{-1} HCl) and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution (20 mmol·L^{-1}). The reagent was warmed to 37ºC and maintained for 30 min, and then the absorbance was measured at 593 nm. The calibration curve was utilized with $FeSO_4 \cdot 7H_2O$ standard in the range of 0.02- 0.1 mol·L^{-1}.

Determination of superoxide anion scavenging activity: The superoxide anion scavenging activity was measured as described by determination kit (Nanjing Jiancheng Bioengineering Institute).

Statistical analysis: All extracts and determinations were carried out at least in triplicate. Data were expressed as mean ± standard deviation. Differences between means were first analyzed by ANOVA test and then least significant difference (LSD) test (p <0.05).

Results and Discussion

Total phenols and flavonoids: Total phenol and flavonoid contents of each development stage were statically different and showed a declining trend with advancing maturity (Fig. 1). Total phenol and flavonoid contents decreased during strawberry development. Total phenol content in fruits SG was 423.63 mg gallic acid/100 g FW. This value decreased by 57% in the 25% R stage, to remain almost constant until 100% R stage. Flavonoid contents also decreased from 9.78 to 0.96 mg rutin/100 g FW from SG to 100% R stage. Less ripe berries contain higher total phenols and flavonoids [4, 10, 12, 19]. Ferreyra *et al.* [4] observed total phenols content decreased by 80% from the SG to W stage. Shin *et al.* [9] reported that total phenol and flavonoid contents were higher at the white tip than at the red ripe stage of ripening.

Anthocyanins, ascorbic acid, dehydroascorbic acid and vitamin E: Anthocyanin content increased gradually during maturation (Fig. 2A) which was a typical maturation process. Along the ripening stages anthocyanins were detected only from 25% R stage, the content was 0.05 mg cyanidin-3-glucoside/100 g FW and values were very low up to the 50% R stage. From the stage on, the content of anthocyanins increased sharply and reached 1.51 mg cyanidin-3-glucoside/100 g FW at 100% R stage. Ferreyra *et al.* [4] and Wang and Lin [10] also obtained that anthocyanin content increased during maturation, however, it could be detected from W stages. Kalt *et al.* [20] suggest that anthocyanin content increases due to the shift in the pool of total phenols toward anthocyanin synthesis, and an overall decline in the content of other phenolic components during the ripening of highbush blueberry fruits.

AsA could react with singlet oxygen and other free radicals, and suppress peroxidation, thus reducing the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer[13]. In this experiment, the AsA content showed an increasing trend with advancing maturity (Fig. 2B). The contents were 33.24 mg AA/100 g FW at 100% R stage which were 2.80 fold of SG stage and very close to the values reported by Xie *et al.* [21] (37.33

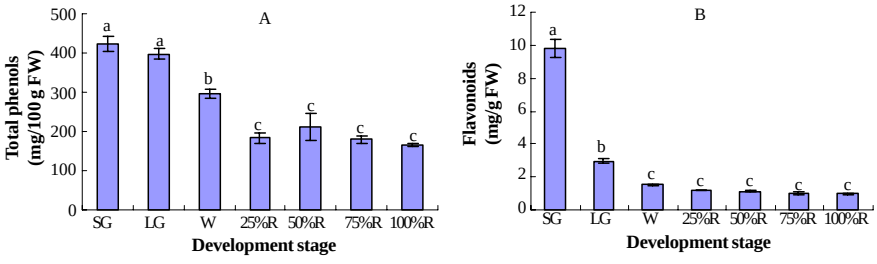

Figure 1. Variation of total phenol (A) and flavonoid (B) contents during strawberry development.
The bars represent the mean of 3 replicates with standard deviation. Means followed by the same letters are not significantly different for p = 0.05.

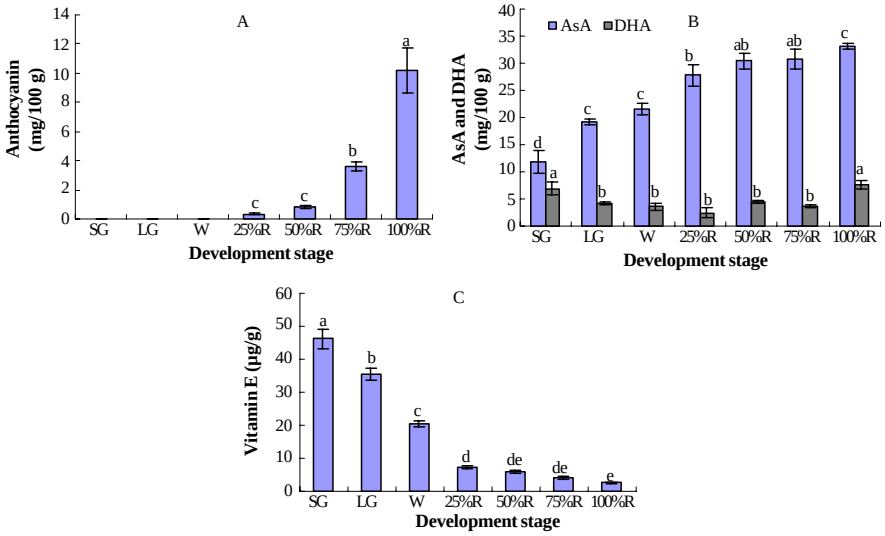

Figure 2. Variation of anthocyanin (A), ascorbic acid, dehydroascorbic acid (B) and vitamin E (C) contents during strawberry development.

The bars represent the mean of 3 replicates with standard deviation. Means followed by the same letters are not significantly different for $p = 0.05$.

mg AA/100 g). AsA content was higher than DHA content in all stages. A similar trend was also reported in daylily flowers, kiwifruit and cauliflower [6, 13, 22] where the highest amount of AsA was present in fully ripe maturity stage. However, Ferreyra *et al.* [4] had different results that the highest AsA content was found in SG stage of strawberry fruit because of a higher proportion of interfering substance.

Fig. 2C shows a strong decrease in the vitamin E content of strawberry cv. Toyonaka during ripening. Vitamin E content in fruit SG stage was 46.15 $\mu g \cdot g^{-1}$ FW and 2.58 $\mu g \cdot g^{-1}$ FW in the 100% R stage which was decreased by 94%. In olive cultivar, Beltran found a similar behavior that the tocopherols decreased during olive ripening [23].

SOD, CAT and APX activities and superoxide anion scavenging activity: Scavenging of superoxide anion radicals is of importance for protection against early events in oxidative damage [24]. The scavenging effect of extracts from seven development stages of strawberry on superoxide anion radical followed the order: W stage > 100% R stage >25% R stage >50% R stage>75% R stage> LG stage > SG stage and were 1.36, 1.30, 1.29, 1.28, 1.26, 1.05 and 0.64 U/g protein. In this experiment, the variation trend of scavenging effect was similar with the variation of ascorbic acid (Fig. 3A, 2B). Greener stages of strawberry fruit had lower ascorbic acid content and poorer capacity of scavenging effect. Fu *et al.* [13] suggested that high superoxide anion radical scavenging activity might be due to synergistic effects of relatively high concentration of ascorbic acid.

SOD is an important enzyme to scavenge superoxide anion radicals and converts superoxide anion radicals into H_2O_2 and O_2. CAT dismutases H_2O_2 into water and O_2. APX utilizes AsA as an electron donor in the neutralization of H_2O_2 [25, 26]. In this experiment, SOD activities decreased sharply from SG to W stage, from W to 75% R stage SOD activities increased slightly and decreased again at 100% R stage. Comparing SG and 100% R stage, SOD activities decreased from 9.55 to 9.04 $U \cdot g^{-1}$ FW (Fig. 3B). Change of SOD activities didn't accord with that of scavenging of superoxide anion radicals. Yuan *et al.* [27] also found a similar behavior during tomato ripening. The variation trend of SOD activities during ripening depends on type of fruit. SOD activities increased with ripening in apple and peach but decreased in tomato and pummelo [28-31].

The activities of CAT showed a rapid decrease and then increased a little (Fig. 3C). The activities of APX also decreased rapidly from SG to W stage by 79%, and after that the activities of APX increased (Fig. 3D). At 100% R stage,activities were 0.0025 $U \cdot min^{-1}$ which was 0.5 fold compared with the SG stage. Both of APX and CAT activities decreased during ripening due to superoxide radical scavenging activity increase and low H_2O_2 accumulation.

DPPH and FRAP: In this experiment, antioxidant capacities were determined by DPPH and FRAP methods during strawberry ripening. The determination methods significantly correlated ($r = 0.947$, $p = 0.01$). Similar trends were present in both methods where the stages had significant difference. The antioxidant capacities at seven stages followed the order: SG stage >LG stage >W stage > 25% R stage > 50% R stage >75% R stage > 100% R stage (Fig. 4). The results showed that greener stages of strawberry fruit had higher antioxidant capacities than mature stages. Similar results have also been found in strawberry, hot pepper, raspberry and cranberry [4, 12, 32, 33]. However, the antioxidant capacities showed an increasing trend along maturation in papaya fruit and daylily flowers [13, 34].

In strawberry cultivar Toyonaka, total antioxidant capacities determined by DPPH and FRAP assay, respectively, were highly correlated with vitamin E ($r = 0.968$ and 0.924), total phenols ($r =$ 0.962 and 0.906), ascorbic acid ($r = -0.946$ and -0.966) and flavonoid concentration ($r = 0.791$ and 0.802) but not with the concentration of anthocyanins. This means that vitamin E, total phenols, ascorbic acid and flavonoids may play a major role for the antioxidant capacity in the extract of strawberry fruit, but not the anthocyanins. At present, there is overwhelming evidence to indicate that the antioxidant capacity was highly correlated with total phenols and flavonoids in various vegetables and fruits [4, 12, 35]. However, there were some different opinions in the literature about the influence of AsA and anthocyanins on the antioxidant capacity. Olsson *et al.* [36] reported that AsA may be a minor contributor to total antioxidant activity. Proteggente *et al.* [37] reported that the highest antioxidant capacity attributed to the highest anthocyanin content in strawberry, raspberry and red plum. Ferreyra *et al.* [4] reported that the variation of antioxidant capacity followed the variations of phenolics and AsA but not anthocyanins in strawberry. Explanations for differences among results may reflect differences of ripening stages and therefore the range of compound concentrations obtained and used for statistical analysis [9]. The author suggested that the differences of various antioxidant compound contents in different species or cultivars may be the cause of different results. Vitamin C contributed to antioxidant capacity much more than phenols or carotenoids in kiwifruits, which is characterised by a high content of vitamin C and a low amount of phenolics [6].

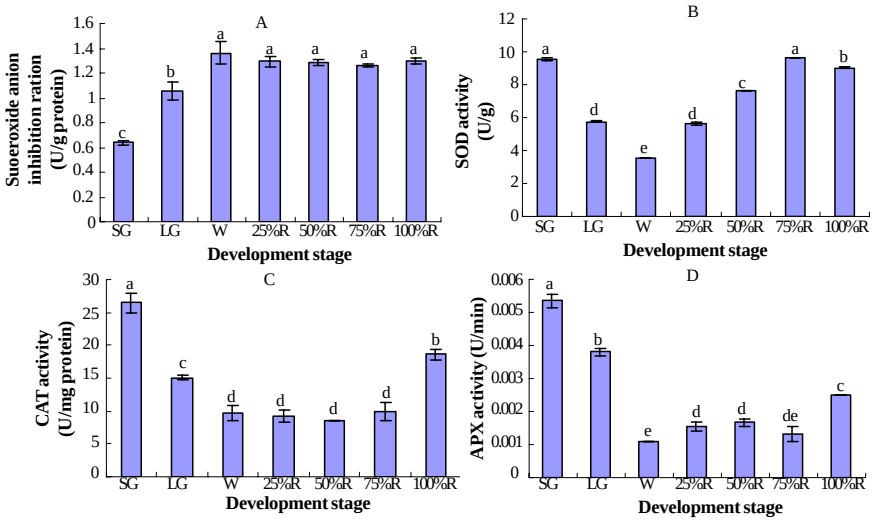

Figure 3. Variation of superoxide anion scavenging activity (A) and SOD (B), CAT (C) and APX (D) activities during strawberry development.

The bars represent the mean of 3 replicates with standard deviation. Means followed by the same letters are not significantly different for $p = 0.05$.

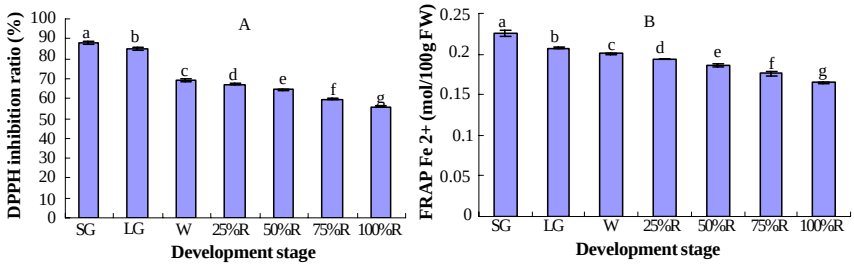

Figure 4. Variation of DPPH inhibition ratio (A) and FRAP values (B) during strawberry development.

The bars represent the mean of 3 replicates with standard deviation. Means followed by the same letters are not significantly different for $p = 0.05$.

Conclusions

The results clearly demonstrated that antioxidant properties and antioxidant compounds of strawberry fruits are affected by maturation stages. Significant variation is found in total phenols, flavonoids, ascorbic acid, anthocyanins, vitamin E, SOD, APX, CAT and antioxidant capacity. Decrease of APX and CAT activities attributed to superoxide radical scavenging activity increase and low H_2O_2 accumulation during ripening. Vitamin E, total phenols, ascorbic acid and flavonoid contents affect the antioxidant capacity. SG stage has the highest antioxidant activities of SOD, CAT and APX and antioxidant capacity, which should be valued and explored in specific details of this trend as sources of antioxidant compounds, not discarded during fruit-thinning.

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