INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 3, p. 213-217, 2014

RESEARCH PAPER OPEN ACCESS

Changes in the chemical composition, superoxide dismutase and catalase activities during ripening of raspberry fruit

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Key words: *Rubus astarae*, antioxidant enzymes, fruit ripening, chemical composition.

http://dx.doi.org/10.12692/ijb/4.3.213-217 Article published on February 14, 2014

Abstract

Raspberry (*Rubus astarae Gili*) is a naturally growing fruit in Gilan. Consumption of fresh raspberry has increased in the past few years in Iran. In this study, changes in the activities of superoxide dismutase (SOD) and catalase (CAT) as well as in the levels of soluble solids, Titratable acidity, anthocyanins, total phenols and ascorbic acid during ripening stages of raspberry fruits were investigated. The highest SOD and CAT activates were at the unripe stage, as well as the highest contents of total phenols. Enhanced fruit ripening was reflected by decreased values for titratable acidity and increased concentration of total anthocyanins, soluble solids and ascorbic acid. Analysis of variance revealed ($P<0.01$) differences in these parameters based on ripeness stages.

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Introduction

Raspberry fruits (Rubus sp) are highly appreciated by consumers for their aromatic taste. They provide nutrients and micronutrients essential for health, particular vitamin C and a significant dietary source of numerous phytochemicals with health benefits, mainly anthocyanins and phenolics (de Ancos *et al*., 1990; Wang and Lin., 2000; Mullen *et al*., 2002). Rubus astara Gili is a Rubus hybrid between R. anatolicus and R. persicus (Sabeti., 1976). It is a deciduous crop that belongs to complex of small fruit species called brambles which have perennial root system and biennial canes. The raspberry is an aggregate fruit, developed from an individual flower with several ovaries from which originate clusters of fleshy drupelets surrounding a hard-coated achene (Gabriel, 2006).

Oxidative damage is thought to be one of the major mechanisms involved in chronic human diseases such as cancer and heart disease (Zhang *et al*., 2008). Lot of studies suggests 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 (Kris-Eeherton *et al*., 2002; Ruxton *et al*., 2006). Antioxidants mainly include a group of oxidative enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) etc. and phenolic compounds of various chemical structures (e.g. catechins, flavonoids, anthocyanins) and vitamin (C,E and A) (Ferreyra *et al*., 2007). 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 (Meyskens and Szabo., 2005). The contents of antioxidants and antioxidant capacity vary strongly during growth and maturation (Wang and Lin., 2000; Huseyin *et al*., 2008). Antioxidant enzymes also belong to antioxidants, scientific information regarding the changes in antioxidative enzymes is scare to our knowledge in raspberry during ripening.

The study was conducted to examine the changes in the contents of total phenolic, soluble solids, total sugar, ascorbic acid, superoxide dismutase and catalase activities during the development and ripening of the raspberry fruits.

Materials and methods

Fruit samples

Raspberries that were evaluated in this study (*R. astarae*) were collected from the north (Gilan province) region of Iran. Raspberries were picked at different development stages: unripe, semi-ripe, ripe and slightly over-ripe. Fruits were handled carefully to prevent damage or wounds, and placed in 6 cm-deep shallow containers bearing only three layers of berries to prevent crushing of fruit in the bottom layer. Raspberries free from decay and defects were immediately sent to the laboratory. All biochemical assays were performed on drupelets separated from the receptacle.

Soluble solids content (SSC), and titratable acid (TA)

Frozen fruit samples were thawed at room temperature and homogenized in a mixer. Following seeds removal, 10 g of pulp from each sample were poured into a 150 ml vial and 80 ml of distilled water was added. The vials were placed in a water bath at 80˚C for 2 hours. The extract was transferred to 100 ml volumetric flasks and filtered through a cheese cloth. Titratable acidity was determined by titration with 0.1 N NaOH to a pink color using 1% phenolphthalein as indicator and expressed as g.100g^{-1} citric acid. The pH of the diluted pulp of each sample was determined using a pH-meter and soluble solids (˚Brix) were determined using a handheld refractometer.

Determination of ascorbic acid content

Total ascorbic acid content was determined using the dinitrophenylhydrazine (DNPH) method (Terada *et al*., 1978). Five grams of homogenized fruit tissue was added to 100 ml of a mixture of 6% metaphosphoric acid in 2 moll^{-1} acetic acid. The mixture was centrifuged at 17,000 × g for 15 min at 4^{o}C and supernatant was filtered through Whatman filter

paper. One milliliter aliquot of the supernatant was mixed with 0.05 ml of 0.2% 2, 6-dichlorophenolindolphenol (DCIP) and the solution was incubated at room temperature for 1 h. After that, 1 ml of 2% thiourea in 5% metaphosphoric acid and 0.5 ml of 2% DNPH in 4.5 moll^{-1} sulfuric acid were added to the solution, and then incubated at 60^{o}C for 3 h. The reaction was stopped by placing the tubes in an ice bath and slowly adding 2.5 ml of cold 90% sulfuric acid. Total ascorbic acid was measured by absorbance at 540 nm using a standard curve. The concentrations were expressed as ascorbic acid on a fresh weight basis, mg per 100 g of fruit.

Determination of total phenolic content

Total phenol in the methanol extracts was determined with Folin-Ciocalteu reagent by the method of Slinkard and Singleton (1972). Gallic acid (GAE) was used as a standard and results were expressed as mg Gallic acid equivalents per 100 g fresh weight.

Extraction and measurement of SOD and CAT 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 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.

Extraction and measurement of total anthocyanins

Some of frozen tissue was ground to a fine powder under liquid nitrogen by cold mortar and pestle and 1g of the resultant powder was added to 10 ml of methanol containing HCl (1%, v/v) and held at 0^{o}C for 10 min (Cordenunsi *et al.*, 2003). The slurry was centrifuged at 17,000× g for 15 min at 4 ^{o}C and then the supernatant was used. Total anthocyanins content was measured with the pH differential absorbance method, as described by Cheng and Breen (1991). Briefly, absorbance of the extracts were measured at 510 and 700 nm in buffers at pH 1.0 (hydrochloric acid-potassium chloride, 0.2 M) and 4.5 (acetate acid-sodium acetate, 1 M). Anthocyanin content was calculated using a molar extinction coefficient of 29,600 (cyaniding-3- glucoside).

Absorbance (A) = $(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$

Results were expressed as mg cyaniding 3-glucoside equivalent per 100g of fresh weight.

Statistical analysis

Statistical analyses were performed using the SPSS for Windows version 16.0 (SPSS Inc.,USA). Differences among the means were compared between species using one-way analysis of variance. Multiple-comparison was done using either Tukeys or Dunnett·s T3 test. Differences at $P< 0.05$ were considered to be significant.

Results and discussion

Soluble solids content (SSC) and titratable acid (TA)

Flavor is derived from the interactive taste and aroma of many chemical constituents. SSC and TA contribute to fruit flavor. High sugars and high acids are required for good berry flavor (Kader., 1991). High acid with low sugar results in a tart berry, while high sugar and low acid results in a bland taste. When both are low, the fruit is tasteless (Kader, 1991). Raspberries SSC and TA values varied among different maturity levels at harvest. The SSC were in the range of 7.02-9.85 % and the TA was 5.18-1.1 % in berries from unripe to slightly over-ripe stage. At harvest, the slightly over-ripe stage fruit had the greatest SSC, but lower TA values (Fig 1). Ripening stage as a main effect reduced titratable acidity significantly as expected, but there was only a tendency for enhanced soluble solids.

Total anthocyanins and total phenolics

The different ripening stages significantly affected the total anthocyanin concentration only, with slightly over-ripe berries showing the highest concentration. The increase of total anthocyanins during raspberry fruit development and ripening is well known and described in the literature, for both raspberry, Wang

and Lin (2000), Beekwilder *et al.* (2005) and for boysenberry (Rubus hybrid) Vincente *et al.* (2006). Total phenol contents of each ripening stage were statically different and showed a declining trend with advancing maturity (Fig 2). According to Wang and Lin (2000), the content of total phenolics increased in black and red raspberry from the pink to the ripe stage. Also Gabriel. (2006) reported high levels of phenolics is a common feature in green stage and reduction in phenolic contents during development has been described for other soft fruit species (Cheng and Breen, 1991). Shin *et al.* (2008) reported that total phenol and flavonoid contents were higher at the white tip than at the red ripe stage of ripening strawberry.

Ascorbic acid

In the present study, ascorbic acid content of the berries was affected by the enhanced ripening (Fig 3). To our Knowledge, there is no information available about the ascorbic acid content of raspberries at different maturity stages. However, for strawberry the situation seems to be controversial. Shin *et al.* (2008) found an increase of ascorbic acid from the white-tip to the red ripe stage and also Cordenunsi *et al.* (2002) have reported an increase in the ascorbic acid level during fruit development.

SOD and CAT activities

SOD activity gradually decreased during the development of the fruits followed then by an increase during the ripening period, whereas the activity of SOD in the unripe stage was higher than ripe stage. The lowest activity was found at the semi-ripe stage. To our knowledge this is the first report of SOD activity during raspberry ripening. Ya Luo *et al.* (2011) also found a similar behavior during strawberry ripening. CAT activity gradually decreased from unripe to semi-ripe stage, from semi-ripe to ripe stage CAT activity increased slightly and decreased again at slightly over-ripe stage (Fig 3).

Conclusions

The results presented in this work showed that there were important changes in the activities of SOD and CAT as well as in the contents of TSS, TA, ascorbic acid, phenols and anthocyanins. Significant variation is found in total phenols, ascorbic acid, anthocyanins, SOD and CAT activites. Unripe stage has the highest antioxidant activites of SOD and CAT, which should be valued and explored in specific details of this trend as source of antioxidant compounds, not discarded during fruit-thinning.

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