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Chemical and physical changes during ripening raspberry (*Rubus caesius* L.) fruits

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Abstract

Raspberries (*Rubus caesius L.*) were harvested a three developmental stages (unripe, semi-ripe, ripe) to study changes associated with (dry mater, soluble solids, titratable acidity, pH, total phenolics and total anthocyanin) in order to understand this behavior during the ripening process. The determination of fruit maturity was based on fruit surface color. Enhanced fruit ripening was reflected by decreased values for titratable acidity, total phenolics and increased concentration of total anthocyanins and soluble solids. Total phenolics displayed their highest values in green fruit. In the early fruit ripening stages, pH decreased, titratable acidity increased, while in the later stage, pH increased, titratable acidity decreased considerably. Analysis of variance (ANOVA) revealed that the effect of ripening stage was significant ($P<0.05$) on chemical and physical characteristics.

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Introduction

For over 2,000 years several species of the genus Rubus have been grown for their edible fruit, for medicinal purpose and as hedges to keep out intruders. Raspberry is deciduous crops that belong to a complex of small fruit species called brambles which have perennial root systems and biennial canes. The raspberry is an aggregate fruit, developed from on individual flower with several ovaries from which originate clusters of fleshy drupelets surrounding a hard-coated achene. The drupelets undergo a sequence of color changes during development and ripening (Gabriel., 2006).

For most fruit ripening process corresponds a number of coordinated biochemical and physiological processes (Ilkay *et al.*, 2008). The evolution of some components during the development of fruit have been studied previously (Ackermann *et al.*, 1992; Perkins-Veazie *et al.*, 1996; Hernandez *et al.*, 1999; Wang & Lin 2000; Raffo *et al.*, 2004). Erika *et al.* (2011) evaluated changes in titratable acidity and soluble solids of raspberry cultivar at three maturity stages. Studied related to changes of phytochemicals with fruit maturity have often been carried out over a wide range of development stages (Beekwilder *et al.*, 2005; Vincente *et al.*, 2006; Ferryra *et al.*, 2007).

The aim of this work was to evaluate and quantify some physical and chemical changes during the maturation of *Rubus caesius L.*fruits.

Materials and methods

Raspberries that were evaluated in this study (R.caesius) were collected from the northwest (Ardebil province) region of Iran. Raspberries were picked at different development stages: unripe, semi-ripe and 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.

Dray matter was determined by drying fruits at 70˚C under vacuum (AOAC, 1984). The soluble solids contents of samples were at 20˚C on an Abb refractometer (Japan), pH was determined at 20˚C. Titratable acidity was determined by titration to pH8.1 with 0.1 M NaOH solution and calculated as grams of citric acid per 100g of sample (AOAC, 1984). 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. 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 0ºC for 10 min (Cordenunsi *et al.*, 2003). The slurry was centrifuged at 17,000× g for 15 min at 4 ºC 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).

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

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

Statistical analysis

The experimental design was randomized with three treatments and four replications. Data were submitted to variance analysis (ANOVA) and averages compared by the Duncan‘s multiple range test at $P<0.05$.

Results and discussion

Dry matter changes in the R. caesius fruits decreased as the fruit ripened. Standard internal quality of fruit can be described by the content of soluble solids content (SSC) and titratable acidity (TA). Titratable acidity increased during development, but was less in ripe fruits. Acidity was inversely correlated to pH. The ripe sample which had a low acid content had a corresponding high pH.

Raspberry SSC values varied among different maturity stages. The SSC was in the range of 5.72 _ 8.95% in berries from unripe to ripe stage. At harvest, the ripe stage fruit had the greatest SSC, but lower TA values. The effects of different stage (unripe, semi-ripe and ripe) on SSC and TA values are presented in Table 1. Berries harvest at unripe stage never developed the levels of SSC value observed in red berries at harvest. The increases in SSC are probably not due to the conversion of starch to soluble sugars since raspberries, similar to strawberry fruit, accumulate very little starch during development (Souleyre *et al.*, 2004).

Table 1. Some physical and chemical properties of the *R. Caesius* in different maturation stages.

properties	unripe	Semi-ripe	ripe
Dry matter (%)	19.65a	15.99b	12.01c
Soluble solids (%)	5.72c	6.03b	8.95a
pH	2.79a	2.23c	2.50b
Titratable acidity (%)	5.93b	12.10a	5.09c

Fruit pH showed higher values at the unripe stage, while lower pH values were recorded in intermediate stage of fruit development (Table 1), in line with previous reports on raspberry and blackberry (Perkins- Veazie and Nonnecke., 1993; Perkins-Veazie *et al.*, 2000). Pectin fragments released from the cell walls may bind to polyphenols as berry fruits mature, bringing about reduced acidity and astringency and an increased homogenate pH (Ozawa *et al.*, 1987).

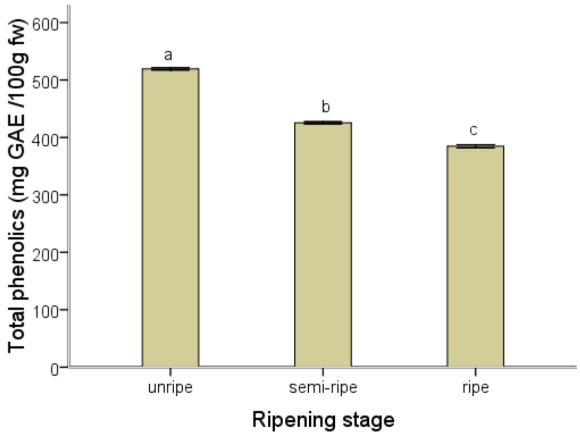

Fig. 1. Total phenolic contents during ripening stage of *R. caesius*. The bars represent the mean of 4 replicates with standard deviation. Means followed by the same letters are not significantly different for P=0.05.

As depicted in Fig 1, phenolic compounds displayed high values at the unripe stage (519.5 mg GAE/100gFW). Relatively high levels of phenolics is a common feature in young fruit (Hyodo., 1971), and reduction in phenol contents during developments has been described for other soft fruit species (Cheng and Breen., 1991). According to Wang and Lin (2000), the content of total phenolics increased in black and red raspberry from the pink to the ripe stage whereas for other berry species like strawberry and blackberry, less ripe fruit have higher contents of total phenolics than fully ripe berries. Also Shin *et al.* (2008) reported decreasing total phenol contents in strawberries with enhanced ripening.

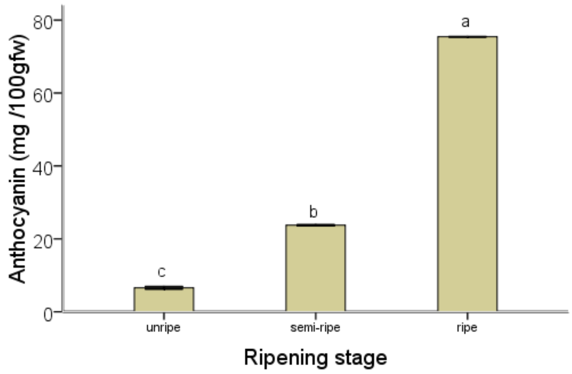

Fig. 2. Total anthocyanincontents during ripening stage of *R. caesius*. The bars represent the mean of 4

replicates with standard deviation. Means followed by the same letters are not significantly different for P=0.05.

The different ripening stages of R. caesius significantly affected the total anthocyanin concentration only, with ripe berries showing the highest concentration (Fig 2). 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). Anthocyanins are the primary pigments in ripe berries and are present in fruit as metal-ion complexes. Anthocyanin level was low at the unripe stage, but increased over 3-fold from the semi-ripe to ripe stage. Ripe fruit displayed 10-fold higher anthocyanin content mg^{-1} FW fruit than unripe fruit. The increase in anthocyanin concentration in raspberry may be related to the rise in sugar synthesis. Glucose, fructose and sucrose positively modulate anthocyanin accumulation in other soft fruit species (Mori and Sakurai., 1994; Perkins-Veazie *et al.*, 2000).

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