



## Cultivar, storage conditions and ripening effects on physical and chemical qualities of red raspberry fruit

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### ARTICLE INFO

#### Article history:

Received 9 March 2010

Accepted 4 December 2010

#### Keywords:

*Rubus idaeus*

Cold storage

Fruit ripening

Fruit colour

Antioxidant activity

Anthocyanin profile

Polyphenolics

### ABSTRACT

Ascorbic acid, total polyphenols, anthocyanins, antioxidant capacity (TEAC), soluble solids, titratable acidity and fruit colour ( $L^*$ , chroma and hue angle) were quantified (a) in ripe fruit of four raspberry cultivars and (b) in fruit of different commercial ripening stages (semi-ripe, ripe and slightly over-ripe defined by CIE  $L^*a^*b^*$  measurements) of cv. Tulameen. Fruit were also stored 1 d at 20 °C room temperature or 3 d at 2–4 °C followed by 1 d at room temperature. All measured parameters including the ratios between cyanidin-3-sophoroside, cyanidin-3-glucoside, cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside were strongly influenced by genotype. Enhanced fruit ripening was reflected by decreased values for titratable acidity, colour parameters and increased concentration of total anthocyanins. After storage, berry colour was darker and fruit considerably lost weight, while total anthocyanins and TEAC were higher in comparison to fresh fruit in both experiments. Soluble solids and total phenols only increased in fruit of different ripening stages of cv. Tulameen but not in the stored ripe fruit of the four cultivars. Titratable acidity, ascorbic acid and the ratio between the individual anthocyanins remained nearly unchanged during the storage period.

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### 1. Introduction

Red raspberry fruit (*Rubus idaeus* L.) are highly appreciated by consumers for their aromatic taste. They provide nutrients and micronutrients essential for health, particular vitamin C and are a significant dietary source of numerous phytochemicals with health benefits, mainly ellagic acid, anthocyanins and phenolics (de Ancos et al., 1999, 2000; Kalt et al., 1999; Wang and Lin, 2000; Mullen et al., 2002a; Beekwilder et al., 2005a). The high concentrations of these components contribute to their high antioxidant activity, which, together with those of blackcurrant, blueberry, blackberry and strawberry, are one of the highest antioxidant activities in fruit (Protagente et al., 2002). Epidemiologically, *in vitro* and *in vivo* studies have given convincing evidence of the beneficial role of a diet rich in fruit and vegetables with high antioxidant activity to maintain human health and to prevent chronic diseases and some forms of cancer. Relevant results are discussed and reviewed in the literature (among others Beattie et al., 2005) and especially for raspberries by McDougall and Stewart (2005), Beekwilder et al. (2005b) and Ross et al. (2007).

Raspberries are often consumed fresh. However, their postharvest life is limited due to their high respiration rate, loss of firmness and freshness, susceptibility to fruit rot and darkening. To overcome these disadvantages, fruit storage at a temperature near 0 °C or with a combination of low temperature and modified atmospheres is common practice. However, postharvest handling of small fruit is often sub-optimal, mainly at the end of the food chain, in distribution trucks and shops (Nunes et al., 2009), and in consumer households where possible poor temperature management affects physical and chemical quality of the fruit.

While the effect of cold storage temperature on nutritional parameters such as anthocyanins, phenolics, ellagic acid, and ascorbic acid, as well as levels of antioxidant activity, of fresh strawberries is well documented (e.g. Kalt et al., 1999; Cordenunsi et al., 2005), only a few studies report on cold storage effects on quality and nutritional characteristics of fresh raspberry fruit under different temperature treatments or storage conditions including controlled atmosphere (Kalt et al., 1999; Haffner et al., 2002; Mullen et al., 2002b).

The interactions of sugar, acids, anthocyanins, total phenolics including ellagitannins, ascorbic acid and the level of antioxidant activity within a species is strongly affected by cultivar but also by fruit maturity at picking (among others Wang and Lin, 2000; Siriwoharn et al., 2004; Beekwilder et al., 2005b; Vicente et al., 2006; Ferreyra et al., 2007). Fully ripe raspberries are more tasteful

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than less ripe fruit (Krüger et al., 2003). However, while the market is mainly focused on fruit firmness, small fruit including raspberries are often picked and sold at an early ripening stage to prolong their marketability. Studies related to changes of phytochemicals with fruit maturity have often been carried out over a wide range of development stages (Beekwilder et al., 2005b; Vicente et al., 2006; Ferreyra et al., 2007). However, the influence of narrow but different maturity stages at harvest on the health promoting quality of raspberries has not yet been thoroughly investigated.

The aim of this study was to evaluate for four cultivars the effects of storage conditions simulating the end of the food chain from harvest to the consumer's table on physical characteristics, sensory parameters (total soluble solids and titratable acidity) and bioactive compounds (anthocyanins, total phenolics, ascorbic acid) as well as antioxidant activity. Additionally, for one cultivar, the interaction of different ripening stages with varying storage conditions were evaluated for these attributes.

## 2. Materials and methods

### 2.1. Fruit material

When picking raspberries, the assessment of ripeness is based on fruit colour. Thus, fruit colour was chosen as the ripening criterion for both experiments.

In Experiment 1, ripe fruit of red raspberry (*R. idaeus*) cv. Resa, Rumiloba, Schönemann and Tulameen were chosen to compare the effects of different storage conditions representing those in which raspberries are often kept from harvest via supermarket to the consumers: (a) freshly picked berries (0 d), (b) berries stored for one day at 20 °C (1 d), and (c) berries stored for three days at 2–4 °C and 85–90% relative humidity followed by a shelf life period of one day at 20 °C (3 d + 1 d). The first three are German cultivars, cv. Tulameen was bred in Canada and is grown now in several countries all over the world. The four cultivars were chosen for their different fruit colours. To avoid fruit-to-fruit variation in ripeness, harvested fruit were classified according to their colour with a portable tristimulus  $L^*a^*b^*$  colorimeter and were considered to be ripe with the following CIE  $L^*a^*b^*$  values: cv. Resa: 30.9/28.1/11.8; cv. Rumiloba: 32.3/31.4/14.6; cv. Schönemann: 26.9/23.0/9.6; cv. Tulameen: 31.5/27.8/12.1. For all cultivars, a deviation of  $\pm 1.5/2.8/1.8$  for  $L^*a^*b^*$  was accepted. Such characterised fruit were picked at two consecutive picking dates, 3–7 d apart during the mid-season harvest period of each cultivar. A sample of 50 berries was used for each harvest date and each combination of cultivar and storage condition. Berries of all treatments were frozen and kept at –20 °C until chemical analyses.

In Experiment 2, fruit of cv. Tulameen were selected for the three ripening stages semi-ripe, ripe and slightly over-ripe, representing fruit which usually can be found in a commercial bin. Therefore, at both harvest dates, they were classified with a portable tristimulus  $L^*a^*b^*$ -colorimeter in the following way: semi-ripe: 36.5/31.9/16.8, deviation  $\pm 2.1/2.9/2.8$ ; ripe 31.5/27.8/12.1, deviation  $\pm 1.5/2.8/1.8$ ; slightly over-ripe 28.6/23.1/8.3 deviation  $\pm 1.3/2.3/1.1$ . Again, fruit samples were picked at two consecutive picking dates 3 d apart during the mid-season harvest period. 50 berries of each harvest date and each ripening stage were subjected to the three storage conditions described above. Berries were frozen at –20 °C, both at the time of harvest and after storage treatments.

### 2.2. Fruit colour

Fruit colour was measured using a portable tristimulus  $L^*a^*b^*$ -colorimeter (Chroma-Meter CR 200, Minolta, Germany), calibrated to a white reflective plate ( $L^* = 97.93$ ,  $a^* = -0.34$ ,  $b^* = 2.27$ , stan-

dard illumination C).  $L^*$  represents colour lightness (0 = black and 100 = white). The  $a^*$  scale indicates in the maximum the red ( $+a^*$ ) and in the minimum the green colour ( $-a^*$ ) while the  $b^*$  axis ranged from yellow ( $+b^*$ ) to blue ( $-b^*$ ). To determine darkening during storage, the colour of each fruit was evaluated just after harvest and again after the different storage periods. Hue (redness) was calculated as  $h = \arctan(b^*/a^*)$  and colour saturation (chroma) as  $C^* = (a^{*2} + b^{*2})^{0.5}$  as recommended by Hung (1990) for colour change measurements in food. A decrease of the hue value indicates a colour change from red to blue. A high chroma value represents a highly saturated and intense colour while a low value stands for dull colours.

### 2.3. Weight loss

Average berry weight of the 0-d stored fruit was measured once. Fruit of the other two storage conditions were weighed before and after storage to determine the weight loss due to respiration and transpiration during storage. Weight loss was expressed as percentage of the original berry weight.

### 2.4. Sample preparation

According to fruit availability, 100–200 g of frozen berries were thawed overnight, ground with a grinder (Braun CombiMax 650), treated with a pectolytic mash enzyme (50  $\mu$ l, Fructozym Color, Erbslöh, Geisenheim) and heated up to 45 °C for 90 min in a water bath. The mash treatment reduces the viscosity and ameliorates the release and the yield of anthocyanins and other polyphenols. The mash was centrifuged at 4.100 g for 10 min. All chemical analyses were done in the supernatant fruit juice.

### 2.5. Chemical analysis

Soluble solid contents were analysed at 20 °C with a digital refractometer (Abbemat, Dr. Kernchen). Titratable acidity was determined by titration of 5 mL extract with 0.3 mol L<sup>-1</sup> NaOH to pH 8.1 and expressed as g L<sup>-1</sup> citric acid. Ascorbic acid was determined reductometrically according to the method of Tanner and Brunner (1987). Raspberry anthocyanins were quantified with HPLC-DAD using a fluorinated RP-18 phase (Fluofix 120 E; 250 – 4.6; 5  $\mu$ m; Dr. Maisch, Ammerbuch, Germany), and gradient elution (eluent A: water 99.5%/phosphoric acid 0.5%; eluent B: acetonitril/water/phosphoric acid 50/49.5/0.5%), according to Rechner et al. (1998). This column allows the simultaneous determination of anthocyanins and of colourless polyphenols, like flavonols, flavanols, and phenol carbonic acids. Total polyphenols were analysed with Folin–Ciocalteu reagent (Singleton and Rossi, 1965), the antioxidant capacity (TEAC) according to the method of Re et al. (1999). Both parameters were not corrected for ascorbic acid.

### 2.6. Statistical analysis

All statistics were performed using SPSS for Windows, version 17.0. Analysis of variance (ANOVA) was done with cultivars and ripening stage as main effects. Picking dates were treated as replicates. Scheffé test was applied to verify statistical differences for the main effects cultivar, storage condition and ripening stage, respectively. Differences at  $p \leq 0.05$  were considered to be significant. To compare the weight loss of the stored fruit with the fresh material, weight loss of the fresh, non-stored fruit was set as zero. Principal component analysis (PCA) was applied to study the relationship between all variables. All data were mean centred and scaled to unit variance prior to PCA.

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