Food Chemistry 112 (2009) 676–684

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The influence of light and maturity on fruit quality and flavonoid content of red raspberries

Shiow Y. Wang [a,*], Chi-Tsun Chen [b], Chien Y. Wang [b]

[a] Genetic Improvement of Fruit and Vegetable Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705-2350, United States

[b] Produce Quality and Safety Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705-2350, United States

ARTICLE INFO

Article history:
Received 13 March 2008
Received in revised form 3 June 2008
Accepted 16 June 2008

Keywords:
Antioxidant activity
Anthocyanins
Total phenolics
Flavonoids
Sugars
Organic acids
Rubus idaeus subsp.

ABSTRACT

The effects of various fruit maturities and different light intensities on berry fruit quality, antioxidant capacity and phytonutrient levels in red raspberries (*Rubus ideaus* L.) were determined. At harvest, immature berries contained significantly lower levels of sugars and acids than ripe berries. When berries were harvested at 5% or 20% maturity, they never developed the levels of soluble solids content (SSC) and titratable acid (TA) values observed in ripe berries at harvest. However, fruit harvested at 50% or more advanced maturity had the capacity of attaining comparable levels of SSC, TA and sugars as those harvested at 100% maturity. When 5% and 20% berries were stored under light, higher level of SSC and lower levels of TA values were observed than those kept in the dark. However, light condition showed little effect in 50% and 80% maturity fruit after 4 days at 24/16 °C (day/night). Ripe raspberries (100%) had stronger antioxidant activities and higher total anthocyanin content when compared with the pink stage (50% maturity). Fruit harvested at greener stages (5% and 20%) also consistently showed higher antioxidant activities and total phenolics than those harvested at 50%. Cyanindin-based anthocyanins increased during postharvest period. On the other hand, other polyphenols such as ellagic acid, quercetin 3-glucoside, quercetin derivative, and kaempferol 3-glucuronide were initially present at high levels but decreased drastically during storage. Red raspberries harvested at different developmental stages continued their development during storage even under the dark conditions. The antioxidant activity of red raspberries was directly related to the total amount of phenolics and flavonoids. Results of this study indicate that red raspberries harvested at 50% or more advanced maturity could develop comparable quality and antioxidant levels as those harvested at full maturity.

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1. Introduction

Berry fruits are extremely perishable and have a short market life. Several studies have shown that strawberry fruits harvested slightly under-ripe are firmer, have less decay, a longer shelf-life and would ship better than fully ripe strawberries (Mitchell, Maxie, & Greathead, 1964; Pritts, Bartsch, Worden, & Jorgensen, 1976). It has been reported that strawberries harvested at early stages of colour development (white stage), can become red during storage similar to commercially ripe fruit (Kalt, Prange, & Lidster, 1993; Miszczak, Forney, & Prange, 1995; Sacks & Shaw, 1993; Spayd & Morris, 1981; Woodward, 1972). Although highbush blueberries harvested at white and pink stages of development never attained a pigment level as high as that of the 100% blue or ripe fruit at harvest, anthocyanins continued to form during storage (Kalt et al., 2003). This indicates that strawberries and highbush blueberries harvested at certain stages of maturity can synthesize pigment during storage under favourable conditions that are temperature dependent (Austin, Shutak, & Christopher, 1960; Kalt et al., 2003) and can occur in darkness, but light can slightly increase the rate of formation (Austin et al., 1960).

Anthocyanins are the major contributors to the red colour pigment in berry fruits and are also used by consumers to judge the quality of a fruit. The synthesis of anthocyanins depends on many ecological and physiological factors, but also on berry species and cultivars. Light has been shown to be the most important environmental factor influencing anthocyanin biosynthesis in plants (Grisebach, 1982). Red pigmentation of berry fruits also can be improved after harvesting using artificial light illumination (Austin et al., 1960).

Raspberries (*Rubus ideaus* L.) are a member of the Rosaceae family, grown as a perennial crop. Raspberries are a compound fruit made up of many drupelets and a hollow center where the fruit detaches from the receptacle. For fresh market, red raspberries are best harvested when bright-red and can be stored at 0 °C only for

* Corresponding author. Tel.: +1 301 504 5776; fax: +1 301 504 5062.
E-mail address: shiow.wang@ars.usda.gov (S.Y. Wang).

0308-8146/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2008.06.032

a few days. Raspberries are soft, juicy with a distinct aroma and are a good source of natural antioxidants. In addition to vitamins and minerals, raspberries are also rich in anthocyanin, phenolic acids, and other flavonoids (Heinonen, Meyer, & Frankel, 1998; Wang, Cao, & Prior, 1996; Wang & Lin, 2000). A previous study showed that raspberries exhibit high oxygen radical absorbance capacity (ORAC) against peroxyl radicals (ROO**.**), superoxide radicals (O_2^{-}), hydrogen peroxide (H_2O_2), hydroxyl radicals (**.**OH), and singlet oxygen ($^1O_2$) (Wang & Jiao, 2000; Wang & Lin, 2000). Anthocyanin content in raspberries increased as berries matured, and total phenolic content decreased from the green to the pink stage followed by a significant increase in total phenolics from the pink stage to the ripe stage (Wang & Lin, 2000). However, the postharvest quality and antioxidant capacity of raspberry fruit harvested at various maturity stages and stored under different light intensities have not been described.

The objective of this study was to determine how fruit maturity (5%, 20%, 50%, 80%, and 100% maturity level) and different light intensities (56 ± 0.5 (H), 31 ± 0.2 (L) μmol m^{-2} s^{-1} and dark (D)) influenced fruit quality and the content of total phenolics, anthocyanins and flavonoids as well as antioxidant capacity in red raspberries. The study was undertaken in an attempt to find suitable harvest maturity and storage conditions for red raspberries so that the fruit would be firmer and could be shipped better than the fully ripe berries but still attain similar quality after harvest.

2. Materials and methods

2.1. Chemicals

2, 2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA Inc, (Richmond VA). Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 2, 2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH) and disodium fluorescein were obtained from Aldrich Chemical Co. (Milwaukee, WI). Acetonitrile, methanol, acetone and water were of HPLC grade and were purchased from Baxter (Muskegon, MI). All anthocyanins and aglycons were obtained from Indofine Chemical Co. Inc., (Somerville, NJ). Other authentic standards were obtained from Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Newark, NJ).

2.2. Plant materials and treatments

Red raspberries cultivar Caroline (*Rubus idaeus* subsp.) used in this study were grown at a farm near Beltsville, Maryland, USA, and were hand–harvested at five different stages based on their surface red colour at harvest: (1) 0–5% red, (2) 20% red, (3) 50% red, (4) 80% red, (5) 100% red. Berries were sorted to eliminate damaged, shriveled and decayed samples. Selected berries were randomized and used for the experiments. Fruit that was 100% red was evaluated only at the time of harvest. The other 4 maturities were evaluated at harvest, and again after 1, 2, 3, and 4 days of storage under 3 different light intensities. Thirty-five fruits from each were placed in 1 L polystyrene trays. Trays containing berries were stored in a growth chamber (model 23 L; Conviron, Winnipeg, Manitoba, Canada) maintained at 24 °C during the day (0700–1900) and 16 °C at night (1900–0700), with relative humidity at 75% and under three different light intensities. Light was supplied by fluorescent lamps for 12 h day^{-1} (0700–1900). Light intensity was monitored using a solar monitor (LI-1776; LI-COR, Lincoln, Neb., USA). Four trays (each contained 35 fruit) from one of four maturity stages (80%, 50%, 20% and 5%) were exposed to photosynthetically active radiation (PAR) level of 56 ± 0.5 μmol m^{-2} s^{-1} (H), another 4 containers of berries as described above were exposed to PAR level of 31 ± 0.2 μmol m^{-2} s^{-1} (L), and another four containers were kept in the dark (D). Berries were sampled after 0, 1, 2, 3, and 4 days of storage for chemical analysis.

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

The SSC of the fruit was determined on a digital refractometer Palette 100 PR-100 (ATAGO-Spectrum Technologies, Plainfield, IL) standardized with distilled water. Titratable acid (TA) was determined by diluting each 5 mL aliquot of raspberry juice to 100 mL with distilled water, then titrating to pH 8.2 using 0.1 N NaOH. Acidity was expressed as mg citric acid/100 mL juice.

2.4. Analysis of sugars and organic acids

Three 5 g sub-samples of red raspberries were extracted twice with 15 mL of imidazole buffer (20 mM, pH 7.0) using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). The extraction, purification, and derivatization procedures for nonstructural sugars and organic acids have been described previously (Wang, Ji, & Faust, 1987). A Hewlett–Packard 5880 gas chromatograph equipped with a flame ionization detector and a fused silica capillary column (dimethylsilicone fluid, 12.5 m × 0.2 mm) was used for separation of sugars and organic acids. Sugars and organic acids were quantified by comparing peak area with those of standards.

2.5. Total anthocyanin and total phenolic content

Three 5 g sub-samples of fresh berries were extracted with 25 mL 80% acetone containing 0.2% formic acid. Total anthocyanin content in fruit extracts was determined using the pH differential method (Cheng & Breen, 1991). Absorbance was measured using a Shimadzu Spectrophotometer (Shimadzu UV-160, Kyoto, Japan) at 510 nm, and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510}-A_{700})_{\text{pH 1.0}}-(A_{510}-A_{700})_{\text{pH 4.5}}]$ with a molar extinction coefficient of cyanidin-3-glucoside (29,600) Results were expressed as milligrams of cyanidin-3-glucoside equivalent per 100 g of fresh weight (mg/100 g fw). Total soluble phenolics in the berry fruit extracts were determined with Folin–Ciocalteu reagent by the method of Slinkard & Singleton, 1972. Results were calculated using regression equation of gallic acid (20–200 μM) and expressed as mg gallic acid equivalents (GAE) per 100 g fresh weight (mg GAE/100 g fw).

2.6. Oxygen radical absorbance capacity (ORAC) assay

Three 5 g sub-samples of fresh berries were extracted with 25 mL 80% acetone containing 0.2% formic acid. The ORAC assay was carried out according to Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002) using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system. A FL800 microplate fluorescence reader (Bio–Tek Instruments, Inc., Winooski, VT) was used with fluorescence filters for an excitation wavelength of 485 ± 20 nm and an emission wavelength of 530 ± 25 nm. The plate reader was controlled by software KC4 3.0 (revision 29) (Bio–Tek Instruments, Inc., Winooski, VT). Sample dilution was accomplished by a Precision 2000 automatic pipetting system managed by precision power software (version 1.0) (Bio–Tek Instruments, Inc., Winooski, VT). The ORAC values were determined by calculating the net area under the curve (AUC) of the standards and samples (Huang et al., 2002). The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final ORAC values were calculated using the regression equation between Trolox concentration (6.25–50 μM) and the net AUC and were expressed as micromole Trolox equivalents per gram of fresh weight (Huang et al., 2002).

2.7. 2, 2-Di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) assay

To determine the antioxidant activity of different extracts, 2, 2-di (4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH) radicals were used and the method described by Cheng, Moore, and Yu (2006) was followed with some modifications. A high-throughput instrument platform consisting of a robotic eight-channel liquid handling system and a microplate with a FL800 microplate UV-visible spectrometer reader (Bio–Tek Instruments, Inc., Winooski, VT) was utilized for this assay. The automated sample preparation was performed using a Precision 2000 instrument with an automatic pipetting system managed by precision power software (version 1.0) (Bio–Tek Instruments, Inc.). The plate reader was controlled by software KC4 3.0 (revision 29). Five grams of fruit sample were extracted with 25 mL of 50% acetone, and 50 μL of this extract were diluted with 150 μL of 50% acetone. Then 40 μL of this diluted extract were used for assay. An aliquot (160 μL) of the DPPH solution (3.3 mg/50 mL 100% ethanol) was added to each well. The mixtures were shaken gently and allowed to stand for 40 min in the dark. The decrease in absorbance was measured at 515 nm against a blank (50% acetone) without extract using a FL800 microplate UV-visible spectrometer reader (Bio–Tek Instruments, Inc., Winooski, VT). The final DPPH values were calculated using the regression equation for the standard gallic acid at different concentrations (6.25–50 μM) and were expressed as micromole gallic acid equivalents per gram of fresh weight (μmol GAE/g fw).

2.8. HPLC analysis of red raspberry flavonoids

High performance liquid chromatography (HPLC) was used to separate and determine individual anthocyanins and phenolic compounds in raspberry fruit tissue. Three 5 g sub-samples of fresh berries were extracted twice with 20 mL of 80% acetone containing 0.2% formic acid using a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY) for 1 min. Extracts (40 mL) were combined and concentrated to 1 mL using a Buchler Evapomix (Fort Lee, NJ) in a water bath at 35 °C. The concentrated sample was dissolved in 10 mL of acidified water (3% formic acid) and then passed through a C_{18} Sep-Pak cartridge (Waters Corp., Milford, MA), which was previously activated with methanol followed by water and then 3% aqueous formic acid. Anthocyanins and other phenolics were adsorbed onto the column while sugars, acids, and other water–soluble compounds were eluted. The anthocyanins and other phenolics were then recovered with 2 mL of acidified methanol containing 3% formic acid. The methanol extract was passed through a 0.45 μm membrane filter (Millipore, MSI, Westboro, MA) and 20 μL of it were analyzed by HPLC. The samples were analyzed using a waters (Waters Corp., Milford, MA) HPLC system equipped with two pumps (600 E system Controller) coupled with a photodiode array detector (Waters 990 Series). Samples were injected at ambient temperature (20 °C) onto a reverse phase NOVA–PAK C_{18} column (150 × 3.9 mm, particle size 4 μm) with a guard column (NOVA–PAK C_{18}, 20 × 3.9 mm, particle size 4 μm) (Sentry guard holder universal) (Waters Corp., Milford, MA). The mobile phase was acidified water containing 2.5% formic acid (A), and acetonitrile (B). The flow rate was 1 mL/min, with a gradient profile consisting of A with the following proportions (v/v) of B: 0 min, 3%, 1–10 min, 3–6% B; 10–15 min, 6% B; 15–35 min, 6–18% B; 35–40 min, 18–20% B; 40–45 min, 20–100% B; 45–50 min, 100% B. The phenolic compounds in fruit extracts were identified by their UV spectra, recorded with a diode-array-detector and by chromatographic comparison with authentic markers. Individual flavonol and anthocyanins were quantified by comparison with external standards of ellagic acid, quercetin, kaempferol and cyanidin 3-glucoside. Scanning between 250 and 550 nm was performed and data were collected using the Waters 990 3-D chromatography data system.

2.9. Statistical analysis

Data were subjected to analysis of variance using NCSS (NCSS 2007, Kaysville, UT) ORAC and DPPH values, total phenolics, and total anthocyanins were evaluated by the Tukey–Kramer Multiple-Comparison test used in NCSS. Differences at $P \leqslant 0.05$ were considered significant. Correlation coefficients were calculated using the software, Microsoft Excel (Microsoft, 2003, Roselle, IL), and are reported as R^2 values.

3. Results and discussion

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

Flavour is derived from the interactive taste and aroma of many chemical constituents. SSC and TA contribute to fruit flavour. High sugars and high acids are required for good berry flavour (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). Red raspberries SSC and TA values varied among different maturity levels at harvest. The SSC were in the range of 7.5–10.9%, and the TA was 1.39–1.77% in berries from 5 to 100% maturity. At harvest, the 100% mature fruit had the greatest SSC, but lower TA values (Fig. 1). The effects of different maturities (80%, 50%, 20%, and 5%) and 4 days at 24/16 °C (day/night) under different light intensity storage conditions (56 ± 0.5 (H), 31 ± 0.2 (L) μmol m^{-2} s^{-1} and dark (D)) on SSC and TA values are presented in Fig. 1. Berries harvested at 5% and 20% maturity never developed the levels of SSC and TA value observed in red berries at harvest. However, after 4 days at 24/16 °C (day/night), SSC of 50% and 80% mature berries increased to a level comparable or above that of 100% mature fruit at harvest regardless of the light intensity exposure (Fig. 1). Similarly, TA of 50 and 80% mature berries decreased to a level comparable to that of 100% mature fruit at harvest. Higher levels of SSC and lower levels of TA values were observed when 5% and 20% berries were stored under the light conditions compared to the dark condition. Light conditions showed no effect on SSC and TA levels on 50% and 80% mature fruit after 4 days at 24/16 °C (day/night) (Fig. 1). Kalt et al. (1993) also showed that light had no effect on SSC content on red ripe harvested strawberries stored at 5–30 °C, but higher levels of SSC were observed when white berries were stored in the light. SSC and TA content in the fruit were negatively correlated ($R^2 = 0.8146$). The increases in SSC are probably not due to the conversion of starch to soluble sugars since red raspberries, similar to strawberry fruit, accumulate very little starch during development (Souleyre et al., 2004). Instead, the increases of SSC during storage in berries in all maturities might be explained by solubilization of cell wall polyuronides and hemicelluloses (Huber, 1984; Nogata, Ohta, & Voragen, 1993).

3.2. Sugars and organic acids

Fructose, glucose, and sucrose were found to be the three major sugars in raspberries, their concentration varied based on the degree of ripeness or condition of postharvest storage (Fig. 2). In general, fruits contained lower sucrose than fructose and glucose. The low sucrose content in the fruit is probably due to enzymatic hydrolysis after translocation from the leaves. Since fructose is characteristically sweeter than glucose or sucrose, its concentration is a desirable organoleptic trait (Doty, 1976; Pangborn, 1953; Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1970), and the majority of consumers prefer sweeter fruit. Fructose and glucose

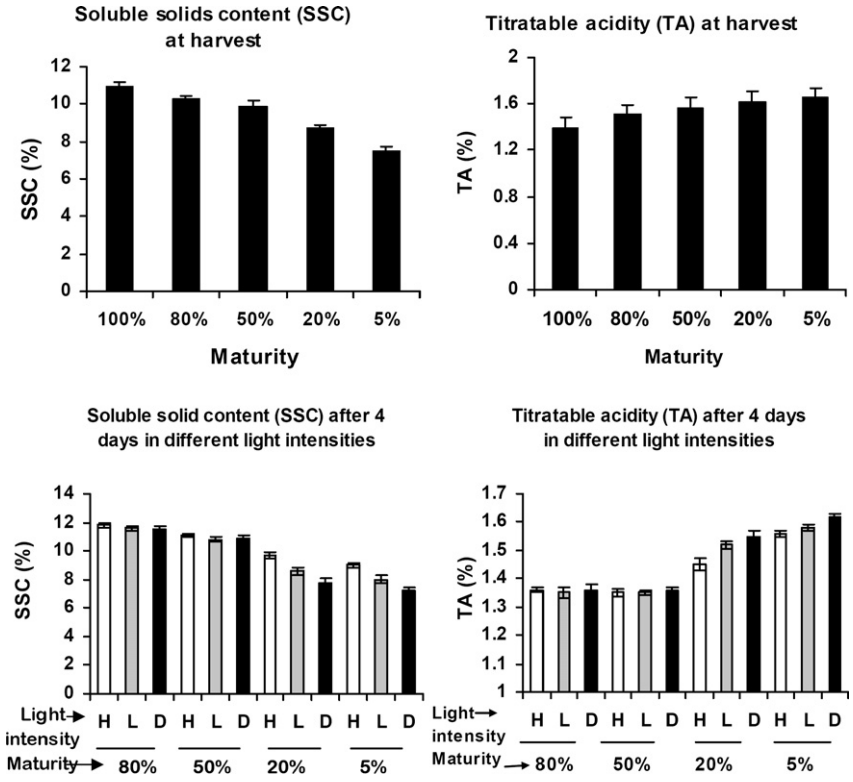

Fig. 1. Soluble solid content (SSC), and titratable acidity (TA) values in red raspberries harvested at different maturities (100%, 80%, 50%, 20%, and 5%) and after 4 days at 24/16 °C (day/night) under different light intensities (photosynthetically active radiation (PAR) level of 56 ± 0.5 μmol m^{-2} s^{-1} (H); 31 ± 0.2 μmol m^{-2} s^{-1} (L), or in dark (D)). Data were expressed as mean ± SD, $n = 3$.

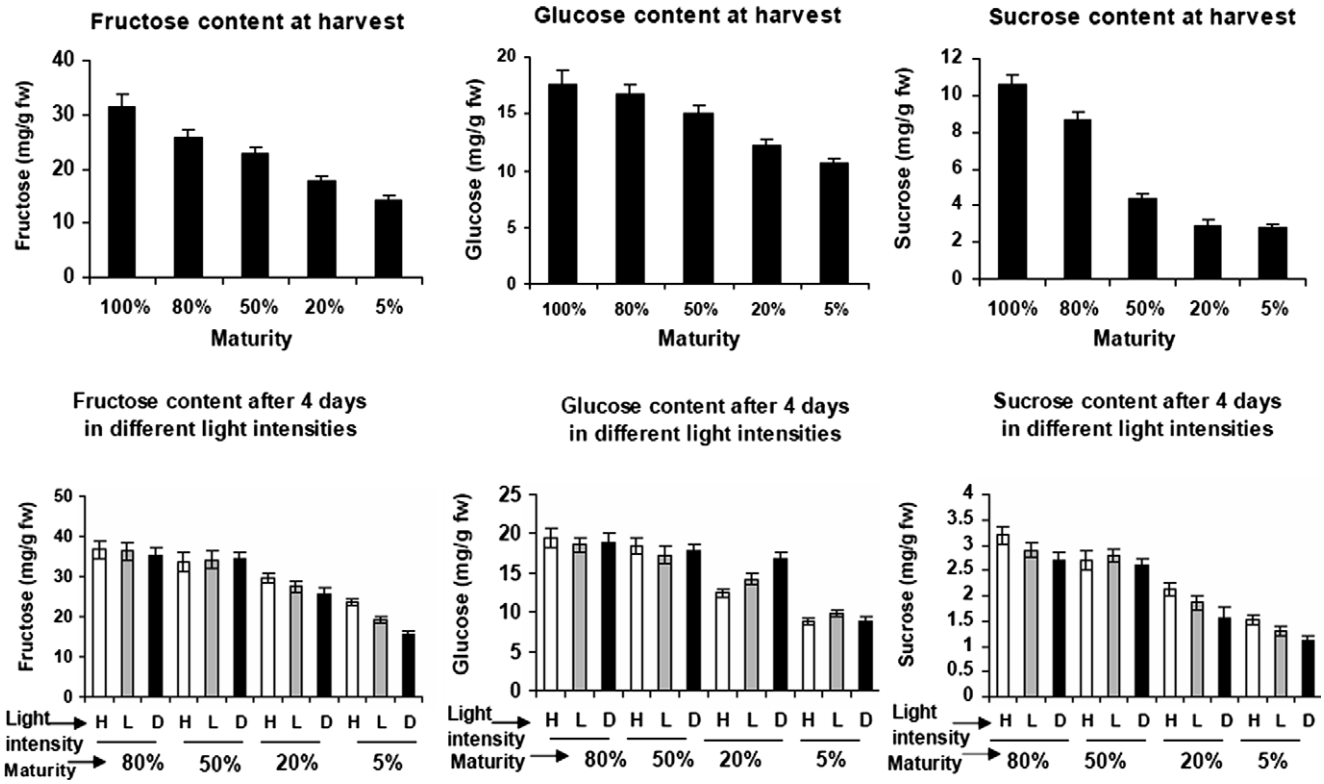

Fig. 2. Fructose, glucose and sucrose content in red raspberries harvested at different maturities and after 4 days at 24/16 °C (day/night) under different light intensities (photosynthetically active radiation (PAR) level of 56 ± 0.5 μmol m^{-2} s^{-1} (H); 31 ± 0.2 μmol m^{-2} s^{-1} (L), or in dark (D)). Data was expressed as mean ± SD, $n = 3$.

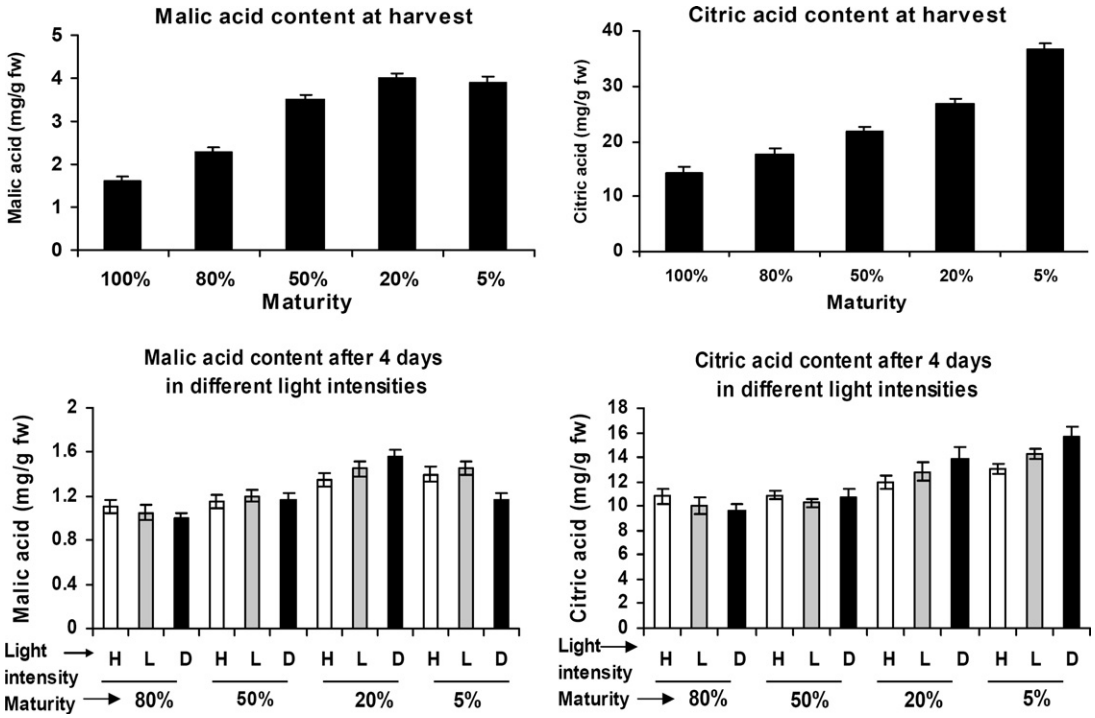

Fig. 3. Organic acid, malic acid and citric acid in red raspberries harvested at different maturities and after 4 days at 24/16 °C (day/night) under different light intensities (photosynthetically active radiation (PAR) level of 56 ± 0.5 μmol m^{-2} s^{-1} (H); 31 ± 0.2 μmol m^{-2} s^{-1} (L), or in dark (D)). Data were expressed as mean ± SD, $n = 3$.

were positively correlated with SSC content in the fruit. The R^2 between fructose and SSC was 0.8108, and between glucose and SSC was 0.9236. Similar to changes in SSC (Fig. 1), both fructose and glucose in 50% and 80% maturity fruit after 4 days at 24/16 °C (day/night) in all light intensity conditions increased to levels comparable or higher than that of 100% maturity fruit at harvest (Fig. 2). Sugar content in 5% and 20% maturity fruit also increased after 4 days at 24/16 °C (day/night), but the levels did not reach that of fully mature fruit at harvest.

Organic acids are minor components of red raspberries, but they contribute important attributes to flavour that, in combination with sugar, have an impact on sensory quality of red raspberries. Citric acid was the major organic acid found in red raspberries (Fig. 3). Malic acid was also detected but at a lesser amount. Both citric and malic acids were present at high levels when the fruit were at the immature stages (5% and 20%). The acid levels decreased with increasing maturity (Fig. 3). High concentrations of organic acids and a low pH in most fruits are critical for fruit

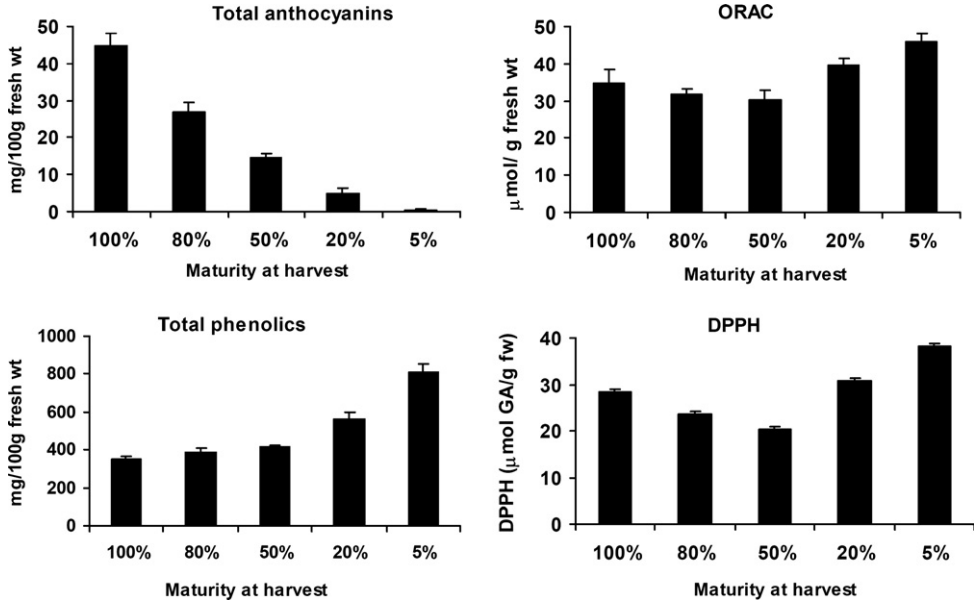

Fig. 4. Total anthocyanins, total phenolics and antioxidant activity (oxygen radical absorbance capacity (ORAC) and 2, 2-di (4-*tert*-octylphenyl) -1-picrylhydrazyl (DPPH)) in red raspberries harvested at different maturities. Data were expressed as mean ± SD, $n = 3$.

preservation (Viljakainen, Visti, & Laakso, 2002). Organic acids also help to stabilize ascorbic acid and anthocyanins.

3.3. Total phenolics, total anthocyanins, and antioxidant capacity against peroxyl radicals (ORAC) and 2, 2-di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) radicals

Antioxidant capacity and polyphenolic content varied considerably among different stages of maturity. In red raspberry fruit, anthocyanin content steadily increased with fruit maturity, but the total phenolic content showed a decrease from the 5% green stage to the 100% ripe stage (Fig. 4). Fruit harvested at their greener stages (5% and 20%) consistently yielded higher antioxidant activities and total phenolics than those harvested during the 50–80% mature stages (Fig. 4). This may be due to an abundant of procyanidin content in the green fruit. From previous research, we also found that blackberries, black raspberries and strawberries had the highest ORAC values and total phenolic content during the green stages (Wang & Lin, 2000). Fruit harvested at pink stage (50% maturity) had the lowest ORAC and DPPH values. Following the pink stage, many phytonutrients are synthesized in parallel with the overall development and maturation of the fruit. The fully mature red raspberries (100% maturity) had stronger antioxidant activities compared to 50% mature fruit which was demonstrated by higher ORAC and DPPH values and total anthocyanin content. There was a positive correlation between ORAC value or DPPH with the total phenolic content at harvest for 5%, 20%, 50%, 80%, and 100% maturity with R^2 = 0.8241 and 0.8209, respectively. No significant correlation existed between the ORAC values or DPPH with the anthocyanin content. However, the DPPH-radical scavenging

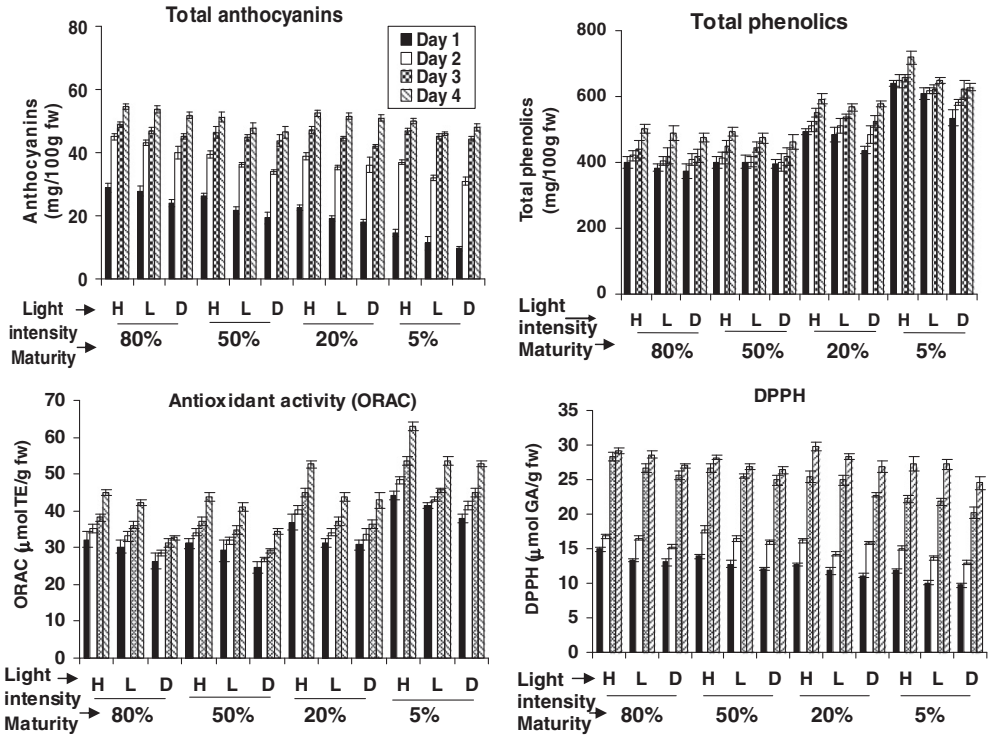

Fig. 5. Total anthocyanins, total phenolics, and antioxidant activity (ORAC and DPPH) in red raspberries harvested at different maturities and after 4 days at 24/16 °C (day/night) under different light intensities (photosynthetically active radiation (PAR) level of 56 ± 0.5 μmol m^{-2} s^{-1} (H); 31 ± 0.2 μmol m^{-2} s^{-1} (L), or in dark (D)). Data were expressed as mean ± SD, n = 3.

Table 1
Effect of different maturities on ellagic acid, quercetin 3-glucuronide, quercetin derivative, kaempferol 3-glucuronide, cyanidin 3-sophoroside, cyanidin 3-glucoside, cyanidin 3-glucosylrutinoside, and cyanidin 3-rutinoside content in red raspberries (cv. Caroline) at harvested[a]

Maturity (%)	Ellagic acid[b]	Quercetin 3-glucuronide[c]	Quercetin derivative[c]	Kaempferol 3-glucuronide[c]	Cyanidin 3-sophoroside[d]	Cyanidin 3-lucoside[d]	Cyanidin 3-glucosylrutinoside[d]	Cyanidin 3-rutinoside[d]
100	45.3 ± 3.2	25.3 ± 1.8	4.3 ± 0.3	5.6 ± 1.0	58.7 ± 8.1	382.4 ± 23.0	326.2 ± 19.2	82.5 ± 6.2
80	53.6 ± 4.3	30.4 ± 2.5	4.7 ± 0.5	5.8 ± 0.5	52.1 ± 7.1	213.7 ± 23.9	172.6 ± 11.1	77.4 ± 5.8
50	64.5 ± 5.4	35.6 ± 2.0	5.5 ± .1.3	6.5 ± 2.2	49.5 ± 5.8	107.5 ± 10.5	58.2 ± 7.0	62.5 ± 4.3
20	82.8 ± 11.2	64.9 ± 3.2	19.8 ± 1.2	11.7 ± 2.3	24.2 ± 6.2	17.2 ± 8.1	18.4 ± 6.7	27.6 ± 3.1
5	295.3 ± 17.2	138.6 ± 16.1	63.5 ± 3.6	71.6 ± 8.5	2.4 ± 1.3	3.2 ± 1.5	1.2 ± 0.6	0.8 ± 0.3
Significance[e] Maturity	*	*	*	*	*	*	*	*

[a] Data expressed as mean ± SD, n = 3.
[b] Data expressed as micrograms of ellagic acid equivalents per gram of fresh weight.
[c] Data expressed as micrograms of quercetin 3-glucoside equivalents per gram of fresh weight.
[d] Data expressed as micrograms of cyanidin 3-glucoside equivalents per gram of fresh weight.
[e] *, Significant at $p < 0.05$.

activity was correlated with ORAC values with a R^2 of 0.8601. This indicates that antioxidant capacity of red raspberries could be measured either by the ORAC or DPPH-radical scavenging assay. The advantage of the ORAC assay over other methods has been reviewed by Cao and Prior (1999).

Pre- and postharvest environmental conditions, such as temperature and light, influence the anthocyanin and total phenolic concentration of fruits (Hall & Stark, 1972). Total anthocyanin contents increased significantly with storage (Fig. 5). Increases in anthocyanin content during storage have also been reported for strawberries (Kalt et al., 1993), lowbush blueberries (Kalt & McDonald, 1996), rabbiteye blueberries (Basiouny & Chen, 1988) and raspberries (Mazza & Miniati, 1993). All fruit harvested at different maturities, after storage at 24/16 °C for 4 days, turned red including those harvested at 5% maturity. The differences in red colour and total anthocyanins among different maturities were not appreciable after 4 days at 24/16 °C (Fig. 5). High light intensity enhanced red colour development, especially for the immature fruit (5% and 20%). This indicated that red raspberries harvested before full maturity could synthesize pigment during storage under favourable conditions. Austin et al. (1960) found a small effect of light on the rate of postharvest colour development in Sparkle strawberries at 29 °C while the effect was negligible at 13 °C. Kalt et al. (1993) observed that light increased the surface colour rating of white Blomidon strawberries; however, the harvested white and pink stages of highbush blueberries never attained a pigment level as high as that of the 100% blue or ripe fruit at harvest (Kalt et al., 2003). Total phenolic content also increased in fruit of all maturities during storage. Light intensity did not affect the changes of total phenolics in 50% and 80% maturity fruit, but for immature berries (5% and 20% maturities), fruit exposed to higher light intensities had higher total phenolics than those exposed to lower light intensities, especially during the first 2 days of storage (Fig. 5). During storage, decreases in titratable acidity and organic acids may provide carbon skeletons for the synthesis of phenolics, including both anthocyanin and non-anthocyanin phenolics (Mazza & Miniati, 1993). The synthesis of both anthocyanins and non-anthocyanins may have contributed to the increase in antioxidant activity in red raspberries after storage (Fig. 5). A positive relationship existed between antioxidant activities and phenolic content in raspberries.

3.4. HPLC analysis of red raspberries flavonoids

Phenolics in red raspberry extracts were presented in Table 1. Cyanidin 3-glucoside, cyanidin 3-glucosylrutinoside, and cyanidin 3-rutinoside were the predominant anthocyanins in ripe red raspberries, whereas ellagic acid and quercetin 3-glucuronide were the most abundant in green raspberries (Table 1). Anthocyanins such as cyanidin 3-sophoroside, cyanidin 3-glucoside, cyanidin 3-glucosylrutinoside, and cyanidin 3-rutinoside increased during fruit ripening (Table 2). Other polyphenols such as ellagic acid, quercetin 3-glucuronide, quercetin derivative, and kaempferol 3-glucuronide decreased significantly during fruit ripening (Tables 1 and 2). The amount of anthocyanins produced during storage was dependent upon the maturity of berries at harvest and 50–80% maturity red raspberry fruit accumulated anthocyanins during storage to levels higher than those of ripe fruit (100% maturity) at harvest (Figs. 4 and 5). However, phenolic content decreased while cyanidin-base anthocyanins increased in fruit harvested at 5% or 20% maturity. Light intensity had a positive effect on flavonoid content in the fruit. Increase flavonoid content occurred in darkness, but light slightly increased their content (Table 2).

Anthocyanins are the largest group of flavonoids which provide beneficial effects to human health. Anthocyanidins are strong

Table 2
Effect of different light intensities on ellagic acid, quercetin 3-glucuronide, quercetin derivative, kaempferol 3-glucuronide, cyanidin 3-sophoroside, cyanidin 3-glucoside, cyanidin 3-glucosylrutinoside, and cyanidin 3-rutinoside content in red raspberries (cv. Caroline) harvested at various maturities and stored at 24/16 °C (day/night) for 4 days[a]

Maturity (%)	Light intensity	Ellagic acid [b]	Quercetin 3-glucuronide[c]	Quercetin derivative[c]	Kaempferol 3- glucuronide[c]	Cyanidin 3-sophoroside[d]	Cyanidin 3-glucoside[d]	Cyanidin 3-glucosylrutinoside[d]	Cyanidin 3-rutinoside[d]
80	High	53.7 ± 3.2[a]	28.5 ± 0.8	8.8 ± 0.3	9.6 ± 1.0	85.8 ± 8.1	478.4 ± 9.8	386.9 ± 9.2	102.2 ± 9.7
80	Low	48.6 ± 3.3	32.3 ± 1.5	5.6 ± 0.5	8.5 ± 0.5	76.1 ± 7.1	466.5 ± 3.9	371.3 ± 11.1	97.4 ± 10.5
80	Dark	50.1 ± 2.5	24.5 ± 1.0	5.4 ± .0.3	5.4 ± 2.2	71.7 ± 5.8	442.7 ± 9.6	375.3 ± 9.8	86.9 ± 5.3
50	High	55.1 ± 2.0	29.8 ± 1.1	5.1 ± 0.2	6.7 ± 1.3	70.1 ± 7.2	415.2 ± 8.6	381.8 ± 8.5	98.1 ± 3.1
50	Low	47.3 ± 1.8	25.5 ± 6.1	4.5 ± 0.6	5.6 ± 0.1	61.4 ± 6.3	397.6 ± 6.5	359.6 ± 6.8	89.6 ± 5.3
50	Dark	51.6 ± 5.6	23.9 ± 0.4	5.6 ± 0.7	7.5 ± 0.3	54.6 ± 8.5	386.2 ± 7.9	356.9 ± 9.4	89.6 ± 2.2
20	High	78.8 ± 2.0	24.6 ± 5.5	5.8 ± 0.8	6.9 ± 0.3	84.9 ± 9.9	413.2 ± 2.3	397.3 ± 9.2	94.5 ± 4.0
20	Low	73.6 ± 1.0	28.4 ± 0.5	5.1 ± 0.1	6.1 ± 0.1	85.5 ± 5.9	397.6 ± 8.6	391.2 ± 6.3	96.2 ± 0.7
20	Dark	79.3 ± 2.3	23.7 ± 2.2	4.1 ± 0.5	6.0 ± 0.3	80.6 ± 9.7	415.4 ± 9.5	380.2 ± 12.2	81.8 ± 3.1
5	High	86.2 ± 2.2	37.6 ± 1.2	6.0 ± 0.3	10.3 ± 0.2	68.8 ± 4.4	423.5 ± 8.3	364.5 ± 9.5	82.9 ± 2.4
5	Low	74.9 ± 0.6	36.8 ± 4.9	6.6 ± 1.5	8.6 ± 0.8	65.0 ± 2.1	398.7 ± 9.7	357.9 ± 15.4	82.7 ± 1.2
5	Dark	73.1 ± 2.5	33.9 ± 1.1	6.8 ± 0.9	9.2 ± 0.7	72.7 ± 1.9	406.3 ± 9.8	338.9 ± 11.4	86.9 ± 3.8
Significance[e]									
Maturity (M)	*	*	ns	ns	ns	*	*	*	*
Light intensity (L)	*	ns	ns	ns	ns	*	*	*	*
M × L	*	*	ns	ns	ns	*	*	*	*

[a] Data expressed as mean ± SD, $n = 3$.
[b] Data expressed as micrograms of ellagic acid equivalents per gram of fresh weight.
[c] Data expressed as micrograms of quercetin 3-glucoside equivalents per gram of fresh weight.
[d] Data expressed as micrograms of cyanidin 3-glucoside equivalents per gram of fresh weight.
[e] *, ns, Significant or non-significant at $p < 0.05$.

antioxidants with free radical scavenging properties attributed to the phenolic hydroxyl groups attached to ring structures (Rice-Evans & Miller, 1996; Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995; Wang, Cao, & Prior, 1997). The hydroxyl radical scavenging activities of flavonoids increase with the number of hydroxyl groups substituted on the B-ring, especially at C-3′ (Rajalakshmi & Narasimhan 1996; Ratty & Das, 1988). Flavonoids have been shown to protect against free-radical damage and low-density lipoprotein oxidation, platelet aggregation, and endothelium-dependent vasodilatation of arteries (Cao, Sofic, & Prior, 1997; Heinonen et al., 1998). Epidemiologic studies have shown a correlation between an increased consumption of antioxidants and a reduced risk of cardiovascular disease and certain types of cancer (Cao et al., 1997; Heinonen et al., 1998; Rice-Evans & Miller, 1996).

Ellagic acid, quercetin 3-glucoside, quercetin derivative and kaempferol 3-glucuronide were also found in raspberry (Tables 1 and 2). Ellagic acid is a naturally occurring phenolic constituent of many plant species (Daniel et al., 1989) and has shown significant inhibition of colon, esophageal, liver, lung, tongue, and skin cancers in rats and mice by *in vitro* and *in vivo* antimutagenic and anticarcinogenic activity against chemical-induced cancers (Okuda, Yoshida, & Hatano, 1989). Kaempferol and quercetin are potent quenchers of ROO·, $O_2^{\cdot-}$ and $^1O_2$ (Larson, 1988). Quercetin and other polyphenols have been shown to play a protective role in carcinogenesis by reducing the bioavailability of carcinogens (Starvic, Matula, Klassen, Downie, & Wood, 1992). Quercetin had a higher antioxidant capacity compared to kaempferol against peroxyl radicals (Ratty & Das, 1988). The antioxidant capacities measured by the ORAC assay for quercetin and kaempferol were 3.29 and 2.67 μM Trolox equivalents, respectively, (Cao et al., 1997). Quercetin has also been shown to inhibit the proliferation of azoxymethanol-induced colonic epithelial tumor cells in mice (Deschner, Ruperto, Wong, & Newark, 1991). We found that cyanidin-3-rutinoside from raspberries induced apoptosis in HL-60 cells in a dose- and time-dependent manner (Feng et al., 2007). Paradoxically, this compound induced accumulation of peroxides which is involved in the induction of apoptosis in HL-60 cells. In addition, cyanidin-3-rutinoside treatment resulted in reactive oxygen species (ROS)-dependent activation of p38, MAPK and JNK, which contributed to cell death by activating the mitochondria pathway. However, cyanidin-3-rutinoside treatment did not increase ROS accumulation and had no cytotoxic effects in normal human peripheral blood mononuclear cells (Feng et al., 2007). These results indicate that anthocyanins, such as cyanidin-3-rutinoside, could have a selective toxicity toward tumor cells, which correlates with their ability to promote oxidative stress in these cells.

Raspberries are rich sources of the antioxidant compounds and these phytochemicals are capable of inhibiting several important stages in colon carcinogenesis *in vitro* (Coates et al., 2007; Juranic et al., 2005). Feeding Fisher 344 rats with freeze–dried black raspberry, blackberry or strawberry, inhibited the number of esophageal tumors in *N*-nitrosomethylbenzylamine (NMBA)-treated animal by 40–60% compared to NMBA control. This inhibition correlated with reductions in the formation of the NMBA-induced O^6-methylguanine adducts in esophageal DNA and reduced DNA damage. Black raspberries and strawberries were also found to inhibit NMBA-induced esophageal tumorigenesis by 30–40% when administered in the diet after treatment of the animals (male F344 rat) with NMBA, indicating that these berries inhibit tumor promotion and progression events as well as tumor initiation (Carlton et al., 2001; Kresty et al., 2001; Reinemann, Aziz, Nines, Gordon, & Stoner, 2004). Raspberries were also found to prevent the development of colon cancer by up to 60% and of adenocarcinomas up to 80% in rodent colon after treatment of F-344 rats with azoxymethane (Harris et al., 2001).

4. Conclusions

The concentration of phenolic compounds within the berries is important for their beneficial effects and quality. Red raspberries contained several flavonoids with potent antioxidant properties. The amount of phenolic compounds significantly changed during the maturation process. Cyanidin-based anthocyanins increased during fruit ripening, but other polyphenols such as ellagic acid, quercetin 3-glucoside, quercetin derivative, and kaempferol 3-glucuronide significantly decreased during fruit ripening. Red raspberries harvested at different developmental stages continued their development during storage and light slightly increased speed and degree of colouration at 20 °C in red raspberries. Thus, the content of individual health-promoting compounds in raspberry could vary significantly according to their developmental stage and can also improve colour development after fruit harvest and during storage. Red raspberries represent a diverse source of potentially healthy antioxidants and thus can provide a useful component in our daily diet.

Taken together, red raspberries harvested at 50% or higher maturity have the capability of attaining comparable levels of SSC, sugars, anthocyanins, phenolics, and antioxidant activities during storage as those harvested at full maturity. Thus, raspberry fruit could be harvested at a stage as early as 50% maturity when the fruits are firmer and less susceptible to mechanical injury during harvesting and transportation, and could still develop into a quality parallel to that of fully mature fruit.

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