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Antioxidant stability of small fruits in postharvest storage at room and refrigerator temperatures

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ABSTRACT

Strawberries (*Fragaria ananassa*), raspberries (*Rubus idaeus*) and red currants (*Ribes rubrum*), as well as two drupes, cherries (*Prunus avium*), and sour cherries (*Prunus cerasus*), were subjected to two storage temperatures (4 °C and 25 °C) and phytochemicals concentrations (total phenols, flavonoids and anthocyanins) as well as antioxidant capacity (DPPH, ABTS and FRAP assays) were monitored until the fruit visually spoiled. Red currants and strawberries exhibited the highest initial total phenol (TP) contents (322.40 ± 5.56 and 335.47 ± 6.12 mg GAE/100 g FW, respectively) and maintained the highest TP contents throughout storage at both temperatures. Storage of at 25 °C as opposed to 4 °C, facilitated faster spoilage of analyzed fruits. In addition, most fruits stored at 4 °C, exhibited slightly higher antioxidant activity values at the end of storage according to all three antioxidant activity assays as opposed to fruits stored at 25 °C. The dynamic evolution of antioxidant capacity at both temperatures reflected the transient changes in phytochemical composition of small fruits in storage.

1. Introduction

According to numerous published clinical studies a diet rich in fruits and vegetables exerts protective effects against various pathological conditions, including cancer (Wattenberg, 1992), stroke (Joshipura et al., 1999), heart attack (Duthie, Gardner, & Kyle, 2003) and even Alzheimer's disease (Dai, Borenstein, Wu, Jackson, & Larson, 2006). Studies conducted on berry fruits have confirmed that bioactive compounds in berries exhibit a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory, anticancer and cardioprotective (Bomser, Madhavi, Singletary, & Smith, 1996; Heinonen, Meyer, & Frankel, 1998). Protective effects of dark colored berry fruits have been attributed to various classes of phenolic compounds, mostly flavonoids and anthocyanins—concentrated in the skin and responsible for the blue, red and purple colors of berries (Mazza, 2000; Katsube, Iwashita, Tsushida, Yamaki, & Kobori, 2003). And although there seems to be a general agreement about the biologically active compounds present in fruits, there is little information available about postharvest changes taking place in their composition and activity. Storage temperature, in addition to light and oxygen exposure, is one of the key factors influencing stability of phenolic antioxidants in fruits during postharvest storage. Extending the shelf life of berry fruit is often achieved through low-temperature or controlled atmosphere storage, usually in high carbon dioxide atmosphere (Kalt, Forney, Martin, & Prior, 1999).

Several earlier studies focused on the stability of anthocyanins in fruit juices and concentrates at various temperatures pointed to first-order thermal degradation of anthocyanins (Wang & Xu, 2007; Garzón & Wrolstad, 2002), while our recent study of fruit juices in refrigerated storage reported substantial fluctuations in the TP content and antioxidant capacity during 29-day storage (Piljac Zegarac, Valek, Martinez, & Belščak, 2009). However, analyses of antioxidant stability in fruit juices and concentrates cannot be used to draw parallels with fruits in storage, since fruit processing for juice production deactivates enzyme catalyzed processes and postharvest ripening that continue to take place in the fruit even after it has been picked. Is it better to store freshly picked fruit at room temperature or in the refrigerator, if one is after preserving the antioxidant potential of stored fruits? In view of the recent results of other authors, who noted significant fluctuations in antioxidant capacity of fruits and vegetables in storage, and even increases after several days in storage at room temperature (Kevers et al., 2007), it appears that there is no simple answer to that question. The complex reactions taking place within the fruit in the postharvest period, may facilitate formation of compounds with enhanced antioxidant capacity, even at the point when fruit attributes (taste, smell, appearance and texture) have

Abbreviations: ABTS, 2,2′-azinobis (3-ethylbenz-thiazoline-6-sulphonic acid); CE, catechin equivalents; CGE, cyanidin-3-glucoside eqivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing/antioxidant power; GAE, gallic acid equivalents; TA, total anthocyanins; TF, total flavonoids; TP, total phenols; TEAC, trolox equivalent antioxidant capacity.

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already significantly deteriorated. More extensive research in this field is necessary before scientifically founded recommendations about optimal food storage temperatures can be made.

The main objective of this work was to monitor the stability of phenolic compounds (total phenols (TP), total flavonoids (TF) and total anthocyanins (TA), as well as changes in the antioxidant capacity of three berry fruits (strawberries, raspberries and red currants) and two drupes (cherries and sour cherries) during their postharvest storage at room temperature (25 °C) and in the refrigerator (4 °C). Berry fruits and drupes widely available on the market in the summer months and high in anthocyanin content (strawberries (*Fragaria ananassa*), raspberries (*Rubus idaeus*), red currants (*Ribes rubrum*), cherries (*Prunus avium*), and sour cherries (*Prunus cerasus*)), were selected for the study. Since there does not exist a standardized method for antioxidant capacity evaluation, the use of several assays at a time is recommended to obtain a comprehensive view of the antioxidant potency of each biological sample (Prior, Wu, & Schaich, 2005). These methods differ in terms of the assay principles and experimental conditions; consequently, particular antioxidants have varying contributions to the total antioxidant potential in different methods (Cao & Prior, 1998). We employed three methods (Ferric Reducing/Antioxidant Power (FRAP) assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and 2,2′-azinobis (3-ethylbenz-thiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) assays) to determine the antioxidant capacity of fruits in storage and detect both lypophilic and hydrophilic antioxidants present in our samples.

2. Materials and methods

2.1. Chemicals and instruments

Except for $FeSO_4 \times 7H_2O$ (Kamika, Croatia), $NaNO_2$ (Laphoma, Skopje, Macedonia) and Folin–Ciocalteu (Fluka, Madrid, Spain), HPLC-grade chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MQ, USA). Spectrophotometric assays were performed on a double-beam UV–VIS spectrophotometer Bio-Spec-1601 (Shimadzu Corporation, Kyoto, Japan).

2.2. Fruit samples

Fruit trees and shrubs sampled for this experiment were grown in Varaždin county, northern Croatia. The fruits were obtained from the producers at full maturity and the samples were harvested and immediately transported to the laboratory for analyses. Care was taken not to inflict damage to the fruit during harvest, in order to prevent the wounding response and production of phytoalexins. First analyses were performed within 24 h after harvesting and the remaining fruit was separated and subjected to two different storage temperatures, 25 °C (room temperature) and 4 °C (refrigerator). Samples were taken out for analyses of quantification of total phenols, total anthocyanins, total flavonoids as well as antioxidant capacity. Storage was stopped and the experiment ended when fruits presented visual spoilage. Storage duration and analyzed fruit parts are indicated in Table 1.

Table 1
Time in storage before spoilage for small fruits stored in the refrigerator and at room temperature.

Berry	Latin name	Time in storage (days)	
		4 °C	25 °C
Sour cherries	*Prunus cerasus*	30	17
Cherries	*Prunus avium*	17	4
Strawberries	*Fragaria ananassa*	11	4
Red currants	*Ribes rubrum*	30	22
Raspberries	*Rubus idaeus*	9	4

2.3. Extraction

On the day of analysis, extraction was performed by homogenizing three portions of 5 g each of fruit parts on each in a blender (Bosh, Siemens, UFESA) to obtain three biological replicates. The homogenized tissue was mixed with 10 ml of extraction solvent (acetone 70%, water 28%, and acetic acid 2%), shaken for 1 h at 125 rpm on a Cole–Parmer rocking platform (Vernon Hills, IL) at room temperature and then centrifuged at 10,000 *g* for 15 min in Multifuge 3S-R (Kendro, Germany). The supernatant was removed, and the pellet was re-extracted with 10 ml of extraction solvent, shaken for 15 min and centrifuged using the same procedure. The two obtained supernatants from each 5 g portion were pooled, and 70% of the volume was evaporated at 30 °C (Búchi, Switzerland). The volume was then adjusted to 20 ml with water and all analyses were performed the same day.

2.4. Phytochemicals

2.4.1. Total phenol content

The TP content in the extracts was determined spectrophotometrically, using the Folin–Ciocalteu method (Singleton & Rossi, 1965) adapted to small volumes. The added reagent volumes were proportionally reduced so that the final reaction volume amounted to 2 mL and could be prepared in disposable plastic cuvettes. Gallic acid was used as a standard and the results were expressed as milligram of gallic acid equivalents per 100 g of fresh weight (mg GAE/100 g FW).

2.4.2. Total flavonoid content

Total TF content of the plant extracts were determined according to the well-established colorimetric assay (Zhishen, Mengcheng, & Jianming, 1999). Catehin was used as a standard and the results were expressed as miligrams of catechin equivalents per 100 g of fresh weight (FW) (mg CE/100 g FW).

2.4.3. Total anthocyanin content

Anthocyanin quantification was performed by the pH-differential method (Giusti & Wrolstad, 2001). Calculation of the anthocyanins concentration was based on a cyanidin-3-glucoside molar extinction coefficient 26,900 and a molecular mass of 449.2 g/mol. Results were expressed as miligrams (mg) of cyanidin-3-glucoside eqivalents (CGE) per 100 g of fresh weight (FW).

2.5. Antioxidant capacity

2.5.1. Ferric reducing/antioxidant power assay (FRAP assay)

The FRAP assay was used to estimate the reducing capacity of tested extracts, according to the original method of Benzie and Strain (1999). A calibration curve was prepared, using an aqueous solution of ferrous sulphate $FeSO_4 \cdot 7H_2O$ and the results, obtained from three replicate extractions, were expressed as mmol $FeSO_4 \cdot 7H_2O$ per 100 g of fresh weight (mmol Fe^{2+}/100 g FW).

2.5.2. DPPH• radical scavenging assay

Radical scavenging capacity was determined according to the method outlined by Brand-Williams, Cuvelier, and Berset (1995). A calibration curve was prepared, using Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and the results were expressed as mmol Trolox equivalents per 100 g of fresh weight (mmol TEAC/100 g FW).

2.5.3. $ABTS^{\bullet+}$ radical scavenging assay

The Trolox equivalent antioxidant capacity (TEAC) of extracts was also estimated by the $ABTS^{\bullet+}$ radical cation decolorization assay (Re et al., 1999). A calibration curve was prepared, using 6-hydroxy-