Chemical profile and antioxidant capacities of tart cherry products

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ARTICLE INFO

Article history:
Received 3 July 2008
Received in revised form 30 September 2008
Accepted 13 November 2008
Available online xxxx

Keywords:
Prunus cerasus
Anthocyanins
Phenolics
Antioxidant capacities

ABSTRACT

The levels of anthocyanins and other flavonoids, as well as melatonin, in various tart cherry products (frozen and dried cherries, powders from individually quick frozen (IQF) cherry and juice concentrate) from two tart cherry cultivars, Montmorency and ‘Balaton’ were analysed comparatively by HPLC and electrospray mass spectrometry (EMS). Our results show that the major anthocyanin compound in these two tart cherry cultivars is cyanidin 3-glucosylrutinoside, followed by cyanidin 3-rutinoside, cyanidin sophoroside, and peonidin 3-glucoside. Studies on antioxidant activities (total antioxidant status assay) of crude extracts of ten tart cherry products show that these products preserve their antioxidant capacities after processing and storage. We have also compared the antioxidant activities of several single constituents that are present in tart cherry. When TEAC (trollox equivalent antioxidant capacity) values were evaluated conceptually against the cherry phytochemical profile, cyanidin and its derivatives were found to be the significant contributors to the antioxidant systems of tart cherries. It was shown that standard compounds with common aglycon moieties show similar antioxidant activities.

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1. Introduction

Current animal research suggests that tart cherry consumption may confer multiple health benefits (Seymour et al., 2008). However, there is conflicting information about the phytochemistry of anthocyanins and other flavonoids in different cultivars of tart cherry (Prunus cerasus L) and in industrially-processed tart cherry products, such as dry fruits, powders from individually quick frozen (IQF) cherry, frozen cherries, juices, and juice concentrates (Blando, Gerardi, & Nicoletti, 2004; Bonerz, Wurth, Dietrich, & Will, 2007; Chandra, Rana, & Li, 2001; Seeram, Bourquin, & Nair, 2001).

As such, it is unknown if these products have similar phytochemical profiles. This information is critical in order to facilitate and validate further studies on the health effects of consumption of tart cherry products.

Recently, tart cherry has had increasing impact upon the edible fruits market, due to the suspected health benefits associated with regular intake of anthocyanins and related polyphenolics (Beattie, Crozier, & Duthie, 2005; Kim, Heo, Kim, Yang, & Lee, 2005; Piccolcella et al., 2008). The major anthocyanins in tart cherries are derivatives of cyanidin, while in other berries, such as strawberries, pelargonidin glycosides predominate. It is noteworthy, however, that anthocyanins may not be stable during processing or storage. As a result, some anthocyanin derivatives may be rapidly formed which may impact bioavailability and bioactivity.

The finding that tart cherries contain significant levels of anthocyanins may also suggest significant antioxidant activity. One of the best known properties of anthocyanins, in general, is their strong antioxidant activity in metabolic reactions, due to their ability to scavenge oxygen free radicals and other reactive species (ROX). This feature makes anthocyanins a potential tool for use in studies on oxidative stress and its related pathologies. For example, it has been reported in animal studies that tart cherry-enriched diets reduce oxidative stress and inflammation (Seymour et al., 2008). Importantly, these effects were achieved using physiologically relevant amounts of the whole fruit (Seymour et al., 2008).

The antioxidant, melatonin (N-acetyl-5-methoxytryptamine), has been identified in fresh-frozen fruits of ‘Balaton’ and ‘Montmorency’ tart cherries, suggesting that sufficiently high levels of melatonin could alter blood levels of this indole and provide protection against oxidative damage (Burkhardt, Tan, Manchester, Hardeland, & Reiter, 2001).

The biological effectiveness of tart cherries may be due to phytochemical interactions which accomplish complementary effects. Thus, it is not surprising that whole cherry fruit products or mixtures of tart cherry secondary metabolites could be biologically more active than individual components. Such a synergistic effect refers to cases when combinations of bioactive substances exert effects at target sites that are greater than the sum of individual components (Cseke et al., 2006; Lila & Raskin, 2005).

In the present study, we aim to identify and quantify the anthocyanins and other flavonoid phytochemicals, as well as melatonin, in various tart cherry products from two cherry cultivars, namely
‘Montmorency’ and ‘Balaton’. Secondly, we aim to determine their respective antioxidant capacities using the common trolox equivalent antioxidant capacity (TEAC) assay.

2. Materials and methods

2.1. Chemicals

Solvents employed for extraction and HPLC analysis were obtained from Fisher Scientific Co., Pittsburgh, PA. Folin-Ciocalteu phenol reagent, quercetin, kaempferol, melatonin, gallic acid, and propyl gallate were purchased from Sigma Chemical Co., St. Louis, MO. Anthocyanins (cyanidin, cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin 3-glicosylrutinoside, cyanidin 3-sophoroside, peonidin-3-glucoside, peonidin, and pelargonidin) and other flavonoids (isorhamnetin and isorhamnetin-3-rutinoside) were obtained from Extrasynthèse (Genay, France) and Polyphenols Laboratories (Sandnes, Norway).

2.2. Extraction of anthocyanins and other metabolites

Frozen and dried pitted tart cherries, powders from individually quick frozen (IQF) tart cherry and tart cherry juice concentrates were kindly supplied by The Cherry Marketing Institute (CMI) (Lansing, MI). Freeze-dried tart cherry powder (1 g from all products excluding juice concentrate) was extracted with 10 ml methanol/water/acidic acid (85:15:0.5 v/v/v) for anthocyanins and 10 ml methanol/water (80:20 v/v) for other flavonoids and melatonin in 15 ml screw-cap tubes at 4 °C and placed on a gyratory shaker overnight in the dark. The juice concentrate was freeze-dried, after which 1 g dry powder was obtained and extracted as above for the other tart cherry preparations. The samples were then vortexed and sonicated. After filtration through a 0.22 micron filter, the extracts were ready for analysis.

2.2.1. Fractionation of phenolics using C-18 Sep-Pak cartridges

For easier separation of phenolics, a simple fractionation of tart cherry phenolic extracts was performed using preconditioned C-18 Sep-Pak cartridges to separate anthocyanins from non-anthocyanin phenolics, as reported in Kim et al. (2005).

2.3. Total phenolics determination

Total phenolics of freeze-dried tart cherries were measured by spectrophotometric analysis (Kim et al., 2005). Briefly, an aliquot (0.2 ml) of appropriately diluted extract was mixed with 2.6 ml of deionised water. A reagent blank using 2.8 ml of deionised water was also prepared. At zero min, 0.2 ml Folin-Ciocalteu’s phenol reagent was added. After 6 min, 2 ml of 7% Na2CO3 solution was added to the final mixture. The absorbance was measured after 90 min against the prepared blank at 750 nm. Total phenolics in tart cherry were expressed as μg gallic acid equivalents (GAE)/g dry weight biomass.

2.4. Determination of total anthocyanins

The quantification of total anthocyanins in freeze-dried cherry powder was evaluated by the pH differential method (Giusti, Rodriguez-Saona, & Wrolstad, 1999). The extracts of cherries in 0.025 M potassium chloride solution (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) were measured simultaneously at 510 and 700 nm. The content of total anthocyanins was expressed in μg cyanidin 3-glucoside equivalents (CGE) per g of dry weight biomass. A molar absorptivity of 26900 l/mmol cm was used for cyanidin 3-glucoside (molecular weight of 449.2 g/mol).

2.5. HPLC analysis

An aliquot (10 μl) of the extract was analysed by HPLC. The HPLC conditions were as follows: a Phenomenex Luna™ column (Torrance, CA) (5 micron pore size, C-18, 150 mm × 4.60 mm), flow rate of 1 ml per minute, Solvent A = water + 0.1% TFA (trifluoroacetic acid); Solvent B = acetonitrile + 0.1% TFA. HPLC running conditions consisted of a gradient: 0–10 min, 8–18% B in A (linear); 10–18 min, 18–28% B in A (linear); 18–19 min, 28–40% B in A (linear); 19–22 min, 40–60% B in A (linear); 22–23 min, 60–80% B in A (linear); 23–25 min, 8% B in A (isocratic); oven temperature, 40 °C. The quantitative analysis of each compound in the extracts was carried out with a Shimadzu HPLC equipped with a photodiode array detector (SPDM-6A system from Shimadzu, Kyoto, Japan). Each known anthocyanin in the extracts was analysed quantitatively by comparison with the corresponding authentic samples. Detection was set at 520 nm.

HPLC analysis of isorhamnetin, isorhamnetin 3-rutinoside, kaempferol, and quercetin was conducted in the same manner as reported previously (Kirakosyan et al., 2004).

2.6. Liquid chromatography-mass spectrometry (LC–MS) analysis of anthocyanins

An Alliance 2695 HPLC (Waters Corp., Milford, MA) was used to generate a binary gradient with 0.05% TFA in water as the aqueous solvent (A) and 0.05% TFA in acetonitrile as the organic modifier (B). Chromatographic separation of anthocyanins was achieved with a Gemini 5 μm C-18 150 × 2 mm reverse-phase column (Phenomenex) held at 35 °C, using a flow rate of 0.19 ml/min. The column was initially equilibrated to 8% B increased to 18% B over 10 min, 28% B over the next 8 min, 40% B for 1 min, 60% B for 3 min, then returned to initial conditions. Effluent from the HPLC column was directed into the electrospray ionisation probe of a TSQ Quantum Ultra AM triple quadrupole mass spectrometer (ThermoFinnigan, San Jose, CA). Positive ions were generated with a sheath gas and capillary temperature 250 °C. Single reaction monitoring (SRM) was used for mass analysis and quantification. Collision energy was set individually for each compound. Collision gas pressure was held at 1.5 mTorr. Data analysis was performed with Xcalibur quantitation software (version 1.4 SR1, ThermoFinnigan).

2.6.1. Estimation of melatonin by HPLC electrospray mass spectrometry

The powdered cherry samples were extracted in 10 ml of methanol/water (80:20 v/v) at 25 °C for 12 h. After filtration through a 0.22 micron filter, samples were processed through a solid-phase column (C-18 Sep-Pak) equilibrated with 1 ml water and 1 ml 80% methanol + 100 μg/ml propyl gallate. Washing the column was repeated with 1 ml 80% methanol + 100 μg/ml propyl gallate. Samples were subjected to liquid chromatography-electrospray mass spectrometry analysis (LC–EMS) under the following HPLC conditions: column, Gemini 5 μm C-18 150 × 2 mm reverse-phase column (Phenomenex); solvent system, methanol: 0.5% formic acid; flow rate, 0.4 ml/min; column temperature, 20 °C. The chromatographic system consisted of Waters 1525 pumps. The LC eluent was injected into a LCT mass spectrometer (Micromass, Manchester, UK) at a flow rate of 0.4 ml/min. The desolvation temperature was 200 °C; the source temperature was 100 °C. The MS parameters were optimised using melatonin as the reference standard compound at 1 ng/μl. Melatonin: parent ion m/z 234, and product ion m/z 174. The data were collected in centroid mode.
2.7. Antioxidant assays

All standard samples (1 mg) and freeze-dried tart cherry products (1 g) were dissolved in 10 ml methanol/water/acetic acid (85:15:0.5, v/v/v) or methanol/water (80:20, v/v) solvents and filtered through a 0.22 µm filter. Total antioxidant capacity was measured using a modified version of the total antioxidant status assay (Randox, San Francisco, CA) as reported previously (Kirakosyan et al., 2003).

2.8. Statistical analysis of data

Experiments were repeated at least three times, and the data were analysed statistically. All results are given as mean ± standard deviation (SD). Differences between variables were tested for significance by Student’s t-test. A p value of <0.05 was considered to be significant.

3. Results

3.1. Analysis of the levels of total anthocyanins and total phenolics in various tart cherry products

Our results show that all tart cherry products analysed have substantial amounts of total anthocyanins and total phenolics. Generally, tart cherry products contain higher levels of total phenolics than total anthocyanins (Table 1). More total anthocyanins are present in tart cherry products obtained from ‘Balaton’ cultivar than from ‘Montmorency’ cultivar. However, ‘Montmorency’ tart cherry products show higher levels of total phenolics. Our results reveal that frozen tart cherries derived from these two cultivars have the highest levels for both anthocyanins and total phenolics, as compared with other tart cherry products (Table 1). In addition, the products processed with sugar (15% of total fresh weight) show lower concentrations for both anthocyanins and phenolics than products not processed with sugar.

3.2. Tart cherry constituent profile

Using HPLC and electrospray mass spectrometry (EMS) techniques, we performed a comparative analysis of the levels of individual anthocyanins, namely, cyanidin, cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-glucosylrutinoside, cyanidin 3-sophoroside,peonidin 3-glucoside, peonidin, and pelargonidin, as well as other flavonoids, including isorhamnetin, isorhamnetin 3-rutinoside, kaempferol, and quercetin in several tart cherry products. Cyanidins and peonidin 3-glucoside were detected in most samples. Results in Table 2 show that the major anthocyanin compound in ‘Balaton’ and ‘Montmorency’ tart cherries is cyanidin 3-glucosylrutinoside, followed by cyanidin 3-rutinoside and cyanidin sophoroside. These results indicate that total cyanidins in ‘Montmorency’ cherries are about 93% of total anthocyanins present. In ‘Balaton’ cherries, total cyanidins is about 93.5%. In addition, our results show that the other major anthocyanin compound present in fruits of both cultivars is peonidin 3-glucoside.

Tart cherry also produces other kinds of flavonoids that are common in many edible berries. The important colorless polyphenols of tart cherries are neochlorogenic acid, 3-caffeoylquinic acid, and chlorogenic acid (Bonerz et al., 2007). However, they are less bioactive. The flavonols, catechin, epicatechin, procyanidins B1 and B2, are more important bioactive compounds. However, these compounds are non-specific for tart cherries, although some flavonol derivatives have recently been reported in tart cherry (Piccolella et al., 2008). Quercetin 3-glucosylrutinoside, quercetin 3-rutinoside, quercetin 3-glucoside, kaempferol 3-glucoside and isorhamnetin 3-rutinoside may make important contributions to the antioxidant capacity of many edible berries (Bonerz et al., 2007; Li, Liu, Zhang, & Yu, 2008; Piccolella et al., 2008). In our study we mostly paid attention to isorhamnetin, kaempferol and quercetin because of their high antioxidant capacities, as previously reported from other edible berry studies (Li et al., 2008). Interestingly, we did not detect isorhamnetin aglycone in any of the tart cherry products, but isorhamnetin rutinoside seems to be one of the major compounds besides anthocyanins and quercetin in both cultivars (Table 3).

Melatonin (N-acetyl-5-methoxytryptamine) has been reported as one of the important compounds in tart cherry fruits (Burkhardt et al., 2001; Reiter & Tan, 2002). In the current study, melatonin appears to be low in tart cherries, as compared to previously reported results by Burkhard and co-workers (Burkhardt et al., 2001). In addition, ‘Montmorency’ tart cherry contains about four times higher amounts of melatonin, as compared with amounts found in ‘Balaton’ tart cherries (Table 3). Our analyses show that dried tart cherries and tart cherry concentrates do not contain melatonin. Our explanation for this observation is that melatonin is very unstable and may be degraded in these samples after processing and storage.

3.3. Analysis of the antioxidant activities of tart cherry extracts and of individual polyphenol compounds

The anthocyanins and other flavonoids found in tart cherry fruits have been reported to possess various phytotherapeutic activities that are based on their modes of action at different target sites (Kim et al., 2005; Jayaprakasam, Vareed, Olson, & Nair, 2005). Our interest is primarily focused upon their respective antioxidant capacities. Antioxidant activity, as determined by TEAC assay, is presented in Fig. 1. In this study, we measured the antioxidant activities of ten different tart cherry products. The results (Fig. 1) show that crude extracts from frozen cherries have higher

Table 1

<table>
<thead>
<tr>
<th>Cherry products</th>
<th>Total anthocyanins</th>
<th>Total phenolics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montmorency cherry (dried-no sugar added)</td>
<td>173 ± 31</td>
<td>7813 ± 855</td>
</tr>
<tr>
<td>Balaton cherry (dried-no sugar added)</td>
<td>564 ± 65</td>
<td>6343 ± 776</td>
</tr>
<tr>
<td>Montmorency cherry (dried with sugar)</td>
<td>62 ± 5.3</td>
<td>5103 ± 455</td>
</tr>
<tr>
<td>Balaton cherry (dried with sugar)</td>
<td>273 ± 33</td>
<td>3522 ± 512</td>
</tr>
<tr>
<td>Montmorency cherry (frozen)</td>
<td>533 ± 47</td>
<td>12665 ± 1321</td>
</tr>
<tr>
<td>Balaton cherry (frozen)</td>
<td>1741 ± 287</td>
<td>6742 ± 786</td>
</tr>
<tr>
<td>Montmorency cherry concentrate</td>
<td>213 ± 41</td>
<td>4013 ± 578</td>
</tr>
<tr>
<td>Balaton cherry concentrate</td>
<td>722 ± 87</td>
<td>2541 ± 371</td>
</tr>
<tr>
<td>Montmorency cherry (IQF powder)</td>
<td>482 ± 56</td>
<td>10323 ± 1468</td>
</tr>
<tr>
<td>Balaton cherry (IQF powder)</td>
<td>1063 ± 178</td>
<td>772 ± 932</td>
</tr>
</tbody>
</table>

a Total anthocyanins are expressed as µg/g dry weight of cyanidin 3-glucoside equivalents.

b Total phenolics are expressed as µg/g dry weight of gallic acid equivalents.
Table 2

<table>
<thead>
<tr>
<th>Anthocyanin Derivatives</th>
<th>Comparison of Levels of Anthocyanins in Commercial Tart Cherry Products</th>
<th>Values Presented Are Mean ± SD; n = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bal dry no sugar</td>
<td>Bal dry + sugar</td>
</tr>
<tr>
<td>Cyanidin 3-sophoroside</td>
<td>15.7 ± 4.3</td>
<td>46.8 ± 0.8</td>
</tr>
<tr>
<td>Cyanidin 3-glucosylrutinoside</td>
<td>15.5 ± 4.3</td>
<td>10.7 ± 4.3</td>
</tr>
<tr>
<td>Cyanidin 3-glucoside</td>
<td>16.2 ± 4.2</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td>Cyanidin-3-rutinoside</td>
<td>1.6 ± 0.7</td>
<td>nd</td>
</tr>
<tr>
<td>Peonidin-3-glucoside</td>
<td>1.6 ± 0.7</td>
<td>nd</td>
</tr>
<tr>
<td>Pelargonidin</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>nd- not detected.</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

An asterisk indicates a significant difference (p < 0.05).

4. Discussion

In the present study, we compared the antioxidant activities of commercially available tart cherry fruit products as well as the antioxidant activities of individual standard constituents in these products. Some compounds, particularly cyanidin derivatives, kaempferol, quercetin, and melatonin, showed higher antioxidant activities than the other compounds analysed (Fig. 2). Each of these compounds could contribute to the antioxidant effects attributed to tart cherry fruit crude extracts. However, it may be that some minimal level of particular constituents is needed for these molecules to exhibit their antioxidant effects most efficiently.

In a recent report (Bonerz et al., 2007), fruits of five different tart cherry cultivars were processed to make tart cherry juices, in order to analyse their polyphenolics composition and the impact of storage on anthocyanin composition. Anthocyanins were identified as cyanidin derivatives and peonidin 3-rutinoside. These authors claimed that a significant decline in the original anthocyanin concentrations could be observed (70–75%) during 6 months of storage at 20 °C. In addition, these results were in accord with our observation that the antioxidant capacities (TEAC) of tart cherry products were substantial.

In other studies, the anthocyanin profiles found in whole tart cherry fruit extracts were reported to be similar in all tested genotypes (Blando et al., 2004) and that relatively high antioxidant capacity values are obtained for tart cherry fruit extracts. In our study, however, we observed significant differences in anthocyanin and total phenolic levels between the two different genotypes, ‘Montmorency’ and ‘Balaton’.

Piccoloella et al. (2008) have reported on the antioxidant capacity of tart cherry fruit crude extracts, using a specific assay protocol...
Table 3
Comparison of levels of several flavonoids and melatonin in tart cherry products. Data are expressed in μg/g dry weight biomass (melatonin is expressed as ng/g dry weight biomass). Values presented are mean ± SD; n = 3.

<table>
<thead>
<tr>
<th></th>
<th>Isorhamnetin rutinoside</th>
<th>Kaempferol</th>
<th>Quercetin</th>
<th>Melatonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balaton dry no sugar</td>
<td>35.8 ± 6.5*</td>
<td>42.9 ± 6.7*</td>
<td>3.1 ± 0.8*</td>
<td>nd</td>
</tr>
<tr>
<td>Balaton dry with sugar</td>
<td>158.7 ± 44.5*</td>
<td>16.9 ± 4.4</td>
<td>1.9 ± 0.9*</td>
<td>nd</td>
</tr>
<tr>
<td>Montmorency dry no sugar</td>
<td>383.1 ± 62.1</td>
<td>16.9 ± 3.8</td>
<td>7.5 ± 0.9</td>
<td>nd</td>
</tr>
<tr>
<td>Montmorency dry with sugar</td>
<td>203.8 ± 52.1</td>
<td>12.9 ± 4.1</td>
<td>8.8 ± 0.9</td>
<td>nd</td>
</tr>
<tr>
<td>Balaton frozen</td>
<td>250.2 ± 52.3</td>
<td>3.8 ± 0.8*</td>
<td>5.9 ± 0.9</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td>Montmorency frozen</td>
<td>328.9 ± 65.6</td>
<td>13.1 ± 4.4</td>
<td>8.5 ± 0.9</td>
<td>12.3 ± 2*</td>
</tr>
<tr>
<td>Balaton concentrate</td>
<td>163.7 ± 43.2*</td>
<td>5.2 ± 0.9*</td>
<td>2.1 ± 0.7*</td>
<td>nd</td>
</tr>
<tr>
<td>Montmorency concentrate</td>
<td>288.1 ± 52.1</td>
<td>11.9 ± 4.3</td>
<td>6.7 ± 0.9</td>
<td>nd</td>
</tr>
<tr>
<td>Balaton IQF powder</td>
<td>62.9 ± 9.3*</td>
<td>16.8 ± 4.8</td>
<td>56.2 ± 8.2*</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>Montmorency IQF powder</td>
<td>176.6 ± 52.2</td>
<td>85.9 ± 9.9*</td>
<td>292.6 ± 56.5*</td>
<td>7.5 ± 0.9*</td>
</tr>
</tbody>
</table>

An asterisk in the same column indicates a significant difference (p < 0.05).

Fig. 1. Trolox equivalent antioxidant capacity (TEAC) of crude extracts from different tart cherry products. Numbers along the abscissa refer to the following: 1, ‘Montmorency’ dried cherry with sugar; 2, ‘Balaton’ dried cherry with sugar; 3, ‘Montmorency’ dried cherry; 4, ‘Balaton’ dried cherry; 5, ‘Montmorency’ IQF powder; 6, ‘Balaton’ IQF powder; 7, ‘Montmorency’ concentrate; 8, ‘Balaton’ concentrate; 9, ‘Montmorency’ frozen cherry; 10, ‘Balaton’ frozen cherry. Values presented are mean ± SD; n = 3.

Fig. 2. Antioxidant capacity of commercial anthocyanins and other flavonoid standards, as well as melatonin. Numbers along the abscissa refer to the following: 1, cyanidin; 2, cyanidin-3-glucoside; 3, cyanidin-3-rutinoside; 4, cyanidin 3-glucosylrutinoside; 5, cyanidin 3-sophoroside; 6, pelargonidin; 7, peonidin-3-glucoside; 8, peonidin; 9, isorhamnetin rutinoside; 10, isorhamnetin; 11, kaempferol; 12, quercetin; 13, melatonin. Values presented are mean ± SD; n = 3.

Based on the presence of highly reactive radical species (ROX), they show that the most polar extracts in MeOH and EtOAc were able to exercise a massive and dose-dependent increase in antioxidant capacity. In addition, in their study, several new secondary metabolites, namely, epicatechin 3-malate, and epicatechin 3-(1’-methyl) malate, were isolated and characterised. These constituents may play a crucial role in the antioxidant action of tart cherry fruits, especially since (–)-epicatechin shows substantial antioxidant activity in hawthorn leaf crude extracts (Kirakosyan et al., 2003). Based on our results, the antioxidant values of cherry extracts, compared to values for reference standards, suggest that there may be possible synergistic action, in terms of expression of antioxidant activity, between the main polyphenolics present in tart cherry fruits. However, further studies are necessary in order to prove whether or not this is the case.

Acknowledgements
This study was supported by an unrestricted grant provided by The Cherry Marketing Institute (Lansing, MI USA).

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Please cite this article in press as: Kirakosyan, A., et al. Chemical profile and antioxidant capacities of tart cherry products. Food Chemistry (2008), doi:10.1016/j.foodchem.2008.11.042


