



Evaluation of Qualitative Characteristics, Bioactive Compounds and Antioxidant Capacity of 10 Iranian Sour Cherry Genotypes

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ARTICLE INFO

Article history:

Received: 22 February 2015;

Received in revised form:

15 July 2015;

Accepted: 25 July 2015;

Keywords

Prunus cerasus (L.),

Fruit quality properties,

Total antioxidant activity,

Vitamin C, Total phenolics, genotype.

ABSTRACT

The aim of this study was to determine the physicochemical properties in some Iranian genotypes of sour cherry fruits. Knowledge of fruit biochemical properties is very important for perception of product behavior during harvesting, transportation, packaging, storing and regeneration programs. In this study, 10 Iranian sour cherry: SH₁₀₁, SH₁₀₂, SH₁₀₃, ... for SH₁₁₀ were analysed. For evaluation of antioxidant capacity and its relation with total phenolics and flavonoids, total antioxidant capacity {measured with method: ferric-reducing antioxidant potential (FRAP)} and total phenolics and flavonoids in fruits extracts of aforementioned sour cherry genotypes were measured and then, correlation of these parameters were evaluated. The highest °Brix among the studied genotypes corresponded to 'SH₁₀₁' and the highest fruit titratable acidity (TA) correlated to 'SH₁₀₄'. The highest values of vitamin C corresponded to 'SH₁₀₁' and 'SH₁₀₉' had the lowest vitamin C. The highest and lowest values pH corresponded to SH₁₀₆. Results revealed that 'SH₁₀₁' and 'SH₁₁₀' among the studied genotypes had the best antioxidant activity, flavonoid content and fruit quality attributes.

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Introduction

Sour cherry (*Prunus cerasus* L.) is one of the most important stone fruit produced in Asia (Khadivi-khub, 2014). Iran is the fifth major producer of sour cherry with an annual production of 105000 metric tones in 2012 (FAO, 2012). Sour cherry fruit contains organic acids, carbohydrates, phenolic compounds, volatile compounds, pigments, antioxidants amounts of proteins and lipids. However, production and processing units and consumers need to have more information about fruit physicochemical properties. This information is very important for understanding the product behavior during the harvesting, transporting, packaging and storing of fruit crops. The antioxidant compounds and phenolic compounds in fruits and vegetables has been linked with health-promoting benefits, such as antioxidant and anti-cancer activities. There are many scientific papers about their influence on fixation of reactive oxygen, they can prevent the lipoproteins against the oxidation and prevent the outgrowth of cardiovascular diseases. In animal experiments, the consumption of sour cherries could reduce the lipid concentration of the liver (Senem et al., 2013; Ficzek, 2012). It has been hypothesized that this association may be partially attributable to the presence of high content of natural antioxidant compounds in fruit (such as phenolic compounds) which demolish the free radical oxidative agents involved in the cellular damage (Sun et al., 2002). Flavonoids are secondary products of the plant metabolism and play a role in protection against UV radiation, as well as being natural pigments, flavour components and antioxidants. Fruit phenolic compounds also participate in quality parameters such as visual appearance (pigmentation and browning), taste (astringency), and health-promoting properties (free-radical scavengers) of different fruit products (John Shi, 2007; Ficzek, 2012). Vitamin C as an antioxidant has the capacity to scavenge several reactive oxygen species. Other beneficial health effects provided by vitamin C include cardiovascular diseases prevention (Moses et al., 2013).

Nowadays there is an increasing interest in selection of crops with higher antioxidant contents, within blueberries (Prior et al., 1998), strawberries and apples (Scalzoet et al., 2005), plums and peaches (Cevallos-Casals et al., 2006).

The consumption of sour cherry fruit may have potential health benefits due to the range of nutrition component, carotenoids, phenolic compounds and other antioxidant compounds they contain. However, detailed information about the health-promoting components of sour cherry fruit could lead to increase the consumption of this fruit as ingredients in medicine. Because of high consumption and attraction of sour cherry fruit, selection, breeding and realizing of cultivars and genotypes which contain high antioxidant properties is imperative. The aim of this study was to determine the characterize quality, antioxidant capacity, total phenolics and flavonoids the major bioactive compounds in some Iranian genotypes of sour cherry fruits in order to establish a database for utilising these germplasm resources. To our knowledge, this is the one of first study about antioxidant capacity of Iranian sour cherry genotypes.

Materials and methods

Plant materials

Ten sour cherry (*Prunus cerasus* L.) genotypes contained grown in the orchards of Hashtroud region, were harvested at a commercial stage.

Assessment of fruit quality characteristics

Ninety fruits for each genotype were homogenized using household mixer, and the homogenate samples analyzed. That total soluble solids content was measured by using a digital refractometer (Atago Model PR-1, Tokyo). The method for analysis of titratable acidity was based on neutralization of the acids present in the fruit juice with a basic solution (NaOH 0.1N). Values of titratable acidity were expressed as percentage of malic acid on fresh weight basis (AOAC, 1990).

Bioactive Compound Determination

Vitamin C content

Vitamin C content of fruit juice was determined using 2, 6 – Dichloroindophenol titration according to AOAC (1990) method No. 967.21 and results were expressed as mg ascorbic acid 100 g⁻¹ fresh weight (FW).

Total phenolics content

Eight fruits randomly selected from each genotype were crushed and homogenized in a homogenizer. A small fraction of the homogenous mixture was centrifuged at 10 g for 6 min. The supernatant clear juice was analyzed for total phenolics and flavonoids contents and antioxidant activity. The total phenolics content in fruit extracts was determined according to Folin-Ciocalteu's method described by Singleton and Rossi (1965). The extracts were mixed with 0.75 ml of 1:10 diluted Folin-Ciocalteu's reagent and after 5 min at room temperature, 0.75 ml of a sodium bicarbonate solution (60 mg ml⁻¹) was added. This mixture was stored at room temperature for 2 h, and its absorbance was measured at 725 nm. The analysis was performed in triplicate, and phenolics content was expressed as micro molar quercetin equivalents (QE) per 100 µl of extract (µM QE 100 µl⁻¹ extract).

Total flavonoids content

Total flavonoids content of fruit extracts was determined using the colorimetric method reported by Kaijv et al., (2006). The extracts were prepared as mentioned for total phenolics assay and mixed with NaNO₂ (5% w v⁻¹), AlCl₃ (10% w v⁻¹) and NaOH (1 M) and its absorbance was measured at 507 nm, after 5 min. The results were expressed in µM QE 100 µl⁻¹ extract of sample as mean of 4 replicates.

Total Antioxidant Activity Determination

The total antioxidant capacity of fruit extracts was measured by the ferricreducing antioxidant potential (FRAP) method (Benzie and Strain, 1999). FRAP assay measures the ability of the antioxidants contained in a sample to reduce ferric-tripyridyltriazine (Fe³⁺-TPTZ) to a ferrous form (Fe²⁺-TPTZ) which absorbs light at 593 nm. The FRAP solution was prepared freshly by mixing 25 ml of 0.3 M acetate buffer (pH 3.6) plus 2.5 ml of 10 mM TPTZ (2, 4, 6-tripyridyl-Striazine) solution in 40 mM HCl and 2.5 ml of 20 mM ferric chloride (FeCl₃.6H₂O). The sample was incubated in various concentrations (25, 50, 70 and 100 µl ml⁻¹) at 37 °C for 10 min and the absorbance was measured at 593 nm. The antioxidant power was calculated from a standard curve prepared by using different concentrations of FeSO₄ in the range of 100 - 1000 mM. The final FRAP values of samples were the mean of 4 replications. Results were expressed as mmol Fe²⁺ L⁻¹.

Statistical analysis

Statistical analyses were performed using the software SPSS 16. Mean values were compared by Duncan's multiple range tests. Correlation coefficients for polyphenols and antioxidant activity were calculated using the statistical software SPSS 16.

Results

Fruit quality characteristics

The highest total soluble solids (TSS) belonged to 'SH₁₀₁', which had significant difference with other genotypes. The lowest content of total soluble solids was recorded in 'SH₁₀₈' (Table 1). As seen in Table 1, the highest values of titratable acidity belonged to 'SH₁₀₄', and the lowest value of this attribute corresponded to 'SH₁₀₈'. As well as 'SH₁₀₆' contained the highest value of pH and the lowest value of pH corresponded to 'SH₁₀₈'. Regarding vitamin C, significant differences were recorded among the studied genotypes. 'SH₁₀₁' had the highest

content of vitamin C. 'SH₁₀₈' had the next highest vitamin C content among the studied genotypes (Table 1).

Bioactive Compounds and Total

Antioxidant Activity

About the total phenolics and flavonoids contents, significant differences were recorded among studied genotypes (Table 2). Total phenolics contents of the studied genotypes ranged from 2.61 to 4.56 (µM QE 100 µl⁻¹ extract) and flavonoids 2.75 to 3.69 (µM QE 100 µl⁻¹ extract), respectively. 'SH₁₀₈' and 'SH₁₀₄' contained the lowest amount of phenols and flavonoids. The highest phenolics content was found in 'SH₁₁₀', while the highest value of total flavonoids corresponded to 'SH₁₀₈' (Table 2). FRAP values of the studied genotypes ranged from 6.28 to 7.84 mmol Fe²⁺ L⁻¹ (Table 2). The highest of antioxidant activity was recorded to 'SH₁₁₀' while the lowest of antioxidant activity corresponded to 'SH₁₀₈'. The evaluation of the contribution of total phenolics and flavonoids to total antioxidant capacity in fruits was performed by correlation analysis between total antioxidant capacity and phytochemicals.

Discussion

The degree of consumer acceptance was significantly related with total soluble solids, although maximum consumer acceptance was attained at different total soluble solid levels depending on the cultivar (Crisosto and Crisosto, 2005). As mentioned above, the titratable acidity of studied genotypes ranged from 1.87 to 2.38. According reports by Milosevic and Milosevic (2012) the titratable acidity of the high titratable acidity sour cherry fruits ranges above 1.54. Thus all of the studied genotypes in grouping characterized as high titratable acidity sour cherry. The native sour cherry genotypes of the present study showed higher pH and vitamin C content compared to other sour cherry genotypes, which had value of pH ranging from 3.1 to 3.28 and vitamin C content ranging from 10 to 15 mg 100 g⁻¹ FW (Milosevic and Milosevic, 2012; Ebret, 2012; Ferretti et al., 2010). Previous works showed that, the phenolics content of fruit tissues is influenced by numerous pre-harvest parameters, harvesting time, climatic conditions, including genotype, agronomic practices (crop load, culture in greenhouses or fields, biological culture, etc.), processing procedure and degree of ripening (Ficzek, 2012; Valero et al., 2011; Drogoudi et al., 2009; Gonçalves et al., 2004 a, 2004b; Lee and Kader, 2000). Ficzek (2012) showed that a wide diversity of phytochemical levels exist within genera of sour cherry, as the total phenolics concentration expressed as µM QE 100 µl⁻¹ varied from 1.60 to 5.27 for variety sour cherry cultivars. However, the results of the current study showed a significant difference among the studied genotypes on the contents. Sanchez-Moreno et al., (1998) showed the lower values indicate the higher antioxidant activity. 'SH₁₀₄' showed a low value of antioxidant activity. Thus, the mentioned genotype have the highest antioxidant capacity among the studied genotypes. Huang et al., (2008) in the study for characterization for antioxidant capacity reported that genotype plays an important role in determining antioxidant capacity and other phenolics content. Our results are in agreement with reports of other researchers (Popp et al, 2008; ficzek, 2012) who claimed that the antioxidant activity and phenolics content depends on the genotype. According to current study, a linear relationship between total antioxidant capacity and total phenolics content has been reported by Wang and Lin (2000) for blackberry (r=0.961) and raspberry (r= 0.911).

Table 1. Qualitative characteristics of 10 Iranian sour cherry genotypes

Genotypes	TSS(⁰ Brix)	Titrateable acidity (%)	PH	VitaminC (mg 100 g ⁻¹ FW)
SH ₁₀₁	20.13 ^a	1.96 ^g	3.79 ^a	17.19 ^a
SH ₁₀₂	18.03 ^c	2.00 ^f	3.67 ^c	15.21 ^b
SH ₁₀₃	16.40 ^d	2.35 ^{ab}	3.65 ^c	15.28 ^{bc}
SH ₁₀₄	14.20 ^g	2.38 ^a	3.64 ^c	16.13 ^{ab}
SH ₁₀₅	13.61 ^h	2.21 ^c	3.77 ^b	15.20 ^{bc}
SH ₁₀₆	15.60 ^e	2.28 ^{bc}	3.80 ^a	14.83 ^c
SH ₁₀₇	15.70 ^e	2.31 ^b	3.75 ^b	16.30 ^{ab}
SH ₁₀₈	12.41 ⁱ	1.87 ^h	3.38 ^e	16.25 ^{ab}
SH ₁₀₉	14.90 ^f	2.05 ^e	3.79 ^a	14.79 ^c
SH ₁₁₀	18.70 ^b	2.16 ^d	3.41 ^d	15.19 ^{bc}

Each value represents the mean of 4 replicates, Mean followed by the same lower-case letters are not significantly different for $p \leq 1$ by Duncan's multiple range test

Table 2. Total phenolics ($\mu\text{M QE100 } \mu\text{l}^{-1}$ extract), flavonoid contents ($\mu\text{M QE100 } \mu\text{l}^{-1}$ extract) and antioxidant activity [FRAP ($\text{mmol fe}^{2+} \text{ L}^{-1}$)] in 10 Iranian sour cherry genotypes

Genotypes	flavonoid contents ($\mu\text{M QE100 } \mu\text{l}^{-1}$ extract)	Total phenolics ($\mu\text{M QE100 } \mu\text{l}^{-1}$ extract)	antioxidant activity [FRAP ($\text{mmol fe}^{2+} \text{ L}^{-1}$)]
SH ₁₀₁	3.33 ^{bc}	4.31 ^b	7.81 ^a
SH ₁₀₂	3.12 ^d	3.10 ^e	6.76 ^f
SH ₁₀₃	3.21 ^c	3.14 ^d	7.36 ^c
SH ₁₀₄	2.75 ^e	2.69 ^f	6.28 ^g
SH ₁₀₅	3.17 ^d	3.58 ^c	7.06 ^e
SH ₁₀₆	3.11 ^d	3.07 ^e	7.47 ^b
SH ₁₀₇	3.19 ^d	3.30 ^d	7.78 ^a
SH ₁₀₈	3.69 ^e	2.61 ^f	6.29 ^g
SH ₁₀₉	3.50 ^a	3.14 ^e	7.78 ^a
SH ₁₁₀	3.40 ^b	4.56 ^a	7.84 ^a

Each value represents the mean of 4 replicates, Mean followed by the same lower-case letters are not significantly different for $p \leq 1$ by Duncan's multiple range test

The correlation between total phenolics and antioxidant capacity content has also been reported in fruits of cornelian cherry (Hassanpour et al., 2011; Pantelidis et al., 2007), red grape cultivars (Hulya-Orak, 2007), strawberry genotypes (Sara et al., 2008), red-flesh peaches and plums (Cevallos-Casals et al., 2006). Correlation between total flavonoids and total antioxidant capacity was greater than correlation of total phenolics content and antioxidant capacity, which indicates that flavonoids are the most active antioxidant compounds in sour cherry genotypes. Therefore, the phytochemicals responsible for the antioxidant capacity of sour cherry fruit are mainly due to flavonoid compounds and phenolic acids. Consumption of fruits with high phytochemical content and antioxidant activity can prevent chronic degenerative diseases such as atherosclerosis, tumors and cardiovascular diseases. The results of current study suggest that sour cherry fruits represent an important source of antioxidant compounds. Results disclosed that, 'SH₁₀₁' and 'SH₁₁₀' among the studied genotypes had the best antioxidant activity, phenolic content and fruit quality attributes. The mentioned genotypes are 'Red blood flesh' genotypes, and this confirms the results of Vizzotio et al. (2007) who indicated that anthocyanin content, phenolics content and antioxidant activity are higher in red-flesh than in light-colored flesh. The correlation between flesh color and antioxidant power within red fleshed varieties (Szamosi et al., 2007). These authors showed that cultivars with deepest red flesh color at the same time had highest antioxidant activity.

Conclusion

The numerous of studied, showed that human health and nutrition are still one of the most studied and interesting topics. Stone fruits special sour cherry are a significant source of ascorbic acid and phenolic compounds. Natural compounds,

such as flavonoid compounds, phenolic acids, antioxidant and anthocyanin content are nowadays under detailed investigation due to their potentially beneficial effects. In this study, we aimed at characterization of various genotypes of Iranian sour cherry. Antioxidant activity varied greatly among the sour cherry genotypes used in this study and was highly correlated with their contents of phenolic compounds. The present study indicates that the sour cherry grown in the North-West area of Iran is an extremely rich source of phenols, antioxidants and ascorbic acid, demonstrating its potential use as a food additive. It was concluded from the present study that total titratable acidity, pH and total ascorbic acid content of studied genotypes was higher than reported for other sour cherry genotypes in the other country. Therefore, the associations found in this study can give information to consumers in helping to recognize a more nutritional sour cherry. Further studies should be conducted between native genotypes and other genotypes, in order to determine those high in antioxidant properties, which could be used as breeding material. Increasing the phenolic content of Sour cherry by genetic manipulation will increase their antioxidant capacity. However, there is a limit beyond which increased phenolic concentration may cause undesirable levels of astringency in these fruits. Meanwhile, sour cherries should be included in the range of fruits selected by consumers to meet the recommended 30 to 40 servings of fruits per day.

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