Identification of phenolic compositions and the antioxidant capacity of mandarin juices and wines

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Abstract This research was undertaken to determine the phenolic composition and antioxidant capacity of juices and wines obtained from Robinson, Fremont and Satsuma mandarins. High-performance liquid chromatography coupled with diode-array detection was used for identifying and quantifying phenolic compounds. The total amount of phenolic compounds ranged from 36.6 to 132.6 mg/L for the mandarin juice, and from 14.1 to 54.5 mg/L for the wines. In the juices and wines, the major hydroxybenzoic acid was vanillic acid; the major hydroxycinnamic acid was ferulic acid; and the major flavanone was hesperidin. The antioxidant activity was measured using the DPPH and ABTS radical scavenging assays, and the antioxidant capacity of mandarin juices was found to be higher than that of wines. Results of this study indicated that these mandarin wines had a composition similar to other beverages, thus demonstrating that these fruits have the potential to be used to produce fermented beverages.

Keywords Antioxidant activity · Fruit wine · HPLC · Mandarin juice · Phenolic compounds

Introduction

In recent years, because of an increased interest in human health, nutrition and disease prevention, consumers have increased their demand for functional foods including fruits and their products such as wine. It has been well documented that a higher intake of fruits and vegetables is related with a lower incident of many degenerative diseases, such as cardiovascular disease and cancer (Peterson et al. 2006; Xu et al. 2008; Hayat et al. 2010; Oboh and Ademosun 2011; Loganayaki et al. 2011; Annegowda et al. 2011). Among various natural healthy fruits, citrus fruits prevail abundantly around the world, and mandarins are among the most popular citrus fruits. Mandarins are a good source of organic acid and phenolic compounds. Their nature and concentration largely affect taste characteristics and organoleptic quality (Peterson et al. 2006; Gattuso et al. 2007).

Phenolic compounds constitute a large group of secondary plant products with an aromatic ring bearing one or more hydroxyl substituents. Phenolic acids are one major class of phenolic compounds found widely in citrus fruits. The four most common hydroxycinnamic acids are *p*-coumaric, caffeic, ferulic, and sinapic acids, while the corresponding hydroxybenzoic acids are *p*-hydroxybenzoic, protocatechuic and vanillic acids. Another peculiar characteristic of citrus juice is the high concentration of flavanones. Flavanones in citrus fruits occur mainly under their glycosides form. Narirutin and hesperidin are known as the main flavanones in mandarin juices (Gorinstein et al. 2004; Gattuso et al. 2007; Sanchez-Moreno et al. 1998).

Wine is defined as an alcoholic beverage, which is produced by the fermentation of fresh grapes or must. Grapes and apples are the crops most widely grown for the

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production of juices for winemaking. There are many studies in the literature that demonstrate the feasibility of using different fruits to produce alcoholic beverages (Duarte et al. 2009; Kelebek et al. 2009; Dias et al. 2003, 2007; Reddy and Reddy 2005; Selli et al. 2004; Soufleros et al. 2001).

Mandarins are the second most popular citrus fruit grown in Turkey after oranges with an annual production of 846390 tonnes in 2009 (FAO 2011). Most Turkish mandarin production is conducted in the Mediterranean region (76%), in which Adana and Hatay are important producing provinces. In the Mediterranean region of Turkey, the most commonly grown mandarin cultivars are Satsuma, Fremont, Robinson and Nova (Demirköser et al. 2009). Although they are important cultivars, phenolic compositions and antioxidant activity of Robinson, Fremont and Satsuma juices and wines have not been investigated before. Therefore, this research was undertaken to determine the phenolic composition and antioxidant capacity of the juices and wines obtained from the cvs. Robinson, Fremont and Satsuma.

Material and methods

Chemicals

Milli-Q water (Millipore, Bedford, MA) was used throughout the study. HPLC-grade acetonitrile and formic acid (Merck, Darmstadt, Germany) were used after filtration through a 0.45- μ m pore size membrane filter. 2,2'-azino-bis-(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulfate, 2,2-diphenyl-1-picryl hydrazyl (DPPH[•]) was obtained from Sigma-Aldrich (St. Louis, MO) and ascorbic acid was purchased from Merck (Darmstadt, Germany). Phenolic acids (protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, chlorogenic, *p*-coumaric, ferulic, and sinapic acids) and flavanones (narirutin, hesperidin and didymin) were purchased from Sigma-Aldrich (Steinheim, Germany).

Mandarins

The mandarins, Satsuma (*Citrus unshiu* Marcov.), Robinson (*Citrus reticulata* Blanco x (*Citrus paradisi* Macf. x *C. reticulata*)) and Fremont (*Citrus reticulata*), were harvested at optimum maturity (using soluble solids concentration/titratable acidity ratio) from the Cukurova University experimental orchard. The mandarins were then transported to the biotechnology laboratory of the Department of Food Engineering, University of Cukurova, Adana, Turkey. Harvest involved a random sampling from 7 to 9 trees. Studies were carried out with 150 kg of mandarins from each variety.

Wine making

Mandarin wines were produced as described previously (Selli et al. 2002, 2004). The mandarin juices were extracted by cutting the fruit in half and carefully hand-squeezing in a kitchen juicer. The juice was then passed through a strainer to remove pulp and seeds, and then 50 mg/l sulphur dioxide (SO₂) was added. The mandarin juices were then transferred into stainless steel tanks (50 L) for fermentation using spontaneous yeasts. During the alcoholic fermentation, 185 g/l sugar was also added in order to obtain a higher ethanol level. Fermentation was followed by drop in sugar content of juices (Fig. 1). After fermentation, the wine was racked with an addition of 50 mg/l SO₂. Finally, wines were bottled into 750 ml bottles and stored at 15 °C until analysis.

Standard chemical analysis

The total titratable acidity (TA) was assessed by titration with sodium hydroxide (0.1 N) and expressed as citric acid %. The pH value was measured using a digital pH meter (WTW Inolab pH-L1, Germany). Total soluble solids (TSS) were measured as °Brix using a refractometer (Carl Zeiss, Jena, Germany) (AOAC 1990). The ascorbic acid content was determined by diluting known volume of juice with 3% metaphosphoric acid as buffer and titrating it against 2,6-dichlorophenol indophenol dye solution until the stable faint pink color was obtained (AOAC 1990; Rapisarda and Intellisano 1996). Total phenolics were determined colorimetrically using Folin-Ciocalteu reagent as described by Velioglu et al. (1998). Density, sugar, extract, and free and bound SO₂ were measured by the method of AOAC (AOAC 1990).

Liquid chromatographic analysis of phenolic compounds

Samples were filtered through a 0.45- μ m pore size membrane filter before injection. An Agilent 1100 HPLC system

Fig. 1 The course of alcoholic fermentation ($n=3$)

(Agilent Technologies, Palo Alto, CA-USA) operated by Windows NT based ChemStation software was used. The HPLC equipment was used with a diode array detector (DAD). The system consisted of a binary pump, degasser and auto sampler. The column used was a Beckman Ultra-sphere ODS (Roissy CDG, France): 4.6×250 mm, 5 µm equipped with a precolumn 4.6×10 mm (same granulometry). The mobile phase consisted of two solvents: Solvent A, water/formic acid (95:5; v/v) and Solvent B, acetonitrile/solvent A (60:40; v/v). Phenolic compounds were eluted under the following conditions: 1 mL min⁻¹ flow rate with the temperature set at 25 °C; isocratic conditions from 0 to 10 min with 0% Solvent B; gradient conditions from 0% to 5% Solvent B in 30 min; from 5% to 15% Solvent B in 18 min; from 15% to 25% Solvent B in 14 min; from 25% to 50% Solvent B in 31 min; and from 50% to 100% Solvent B in 3 min. The elution was followed by washing and reconditioning the column. The ultra-violet-visible spectra (scanning from 200 nm to 600 nm) were recorded for all peaks. Triplicate analyses were performed for each sample. The identification of phenolic compounds were obtained by using authentic standards and by comparing the retention times and ultra-violet-visible spectra with those found in the literature (Merken and Beecher 2000; Gattuso et al. 2007; Kelebek et al. 2008). Quantification was performed by external calibration with standards. A calibration curve was prepared using standards to determine the relationship between the peak area and concentration. Concentration of these compounds was expressed as mg per liter of juices and wines.

Antioxidant activity

DPPH assay The DPPH assay was performed according to the method developed by Brand-Williams et al. (1995) as slightly modified by Kim et al. (2002). A solution of 1 mM DPPH in 80% (v/v) methanol was stirred for 40 min. Absorbance of the solution was adjusted to 0.650 at 517 nm using 80% (v/v) methanol. Then, 50 µl of the standard or sample were mixed with 2.95 ml of DPPH solution and incubated for 30 min in the dark covered with aluminum foil. The decrease of absorbance was monitored at 517 nm at 30 min by a UV-Visible spectrophotometer (Shimadzu UV-1201, Kyoto-Japan). A control consisted of either 50 µl of acidified distilled deionized water in 2.95 mL of DPPH solution for vitamin C standard or 50 µl of 50% (v/v) methanol in 2.95 ml of DPPH solution for samples. The DPPH scavenging activities of samples were expressed as mg vitamin C equivalent (VCE)/100 ml. All tests were performed in triplicate. The mean and standard deviation ($n=3$) were calculated.

ABTS assay Antioxidant activity was also measured using an improved ABTS method as described by Re et al. (1999)

and Pellegrini et al. (2003). The ABTS radical cation solution was prepared through the reaction of 7 mM ABTS and 2.45 mM potassium persulphate, after incubation at 23 °C in the dark for 12–16 h. The ABTS solution was then diluted with 80% ethanol to obtain an absorbance of 0.700 ± 0.005 at 734 nm. ABTS solution (3.9 mL; absorbance of 0.700 ± 0.005) was added to 0.1 ml of the test sample and mixed vigorously. The reaction mixture was allowed to stand at 23 °C for 10 min and the absorbance at 734 nm was immediately recorded. A standard curve was obtained by using vitamin C standard solution at various concentrations (ranging from 0 to 50 µM) in 80% ethanol. The absorbance of the reaction samples was compared to that of the vitamin C standard and the results were expressed in terms of vitamin C equivalents (VCE).

Statistical analysis

The results of the three replicates were compared using the analysis of variance (ANOVA) and Pearson correlation coefficients using SPSS for Windows [version 15.0.0 (6 Sep 2006, SPSS Inc.)]. Duncan's multiple-range tests were used to compare the significant differences of the mean values at $p\leq0.05$.

Results and discussion

Chemical composition of the mandarin juices and wines

The chemical composition of the mandarin juices and wines are given in Table 1. The total acidity was 1.04, 0.89 and 1.02% in Satsuma, Robinson and Fremont, respectively. The total acidity in juices of different mandarin varieties ranged from 0.94 to 1.87% (Xu et al. 2008). The TSS/TA ratio was also an important parameter, associated with the quality characteristics of the citrus fruits. Robinson had the highest TSS/TA ratio (14.5), and Satsuma had the lowest ratio (11.9). The ratios obtained for the studied samples were in good agreement with data previously reported by Xu et al. (2008) and Yu et al. (2007).

Citrus juices, especially mandarin juice, are a rich source of ascorbic acid, which is an important antioxidant and also a significant indicator of mandarin juice quality (Selli et al. 2004). Concentrations of ascorbic acid in cvs. Satsuma, Robinson and Fremont were found to be 372.6, 496.2 and 398.8 mg/L, respectively (Table 1). The ascorbic acid content of the wines was lower than the juices. This decrease in the ascorbic acid levels of the mandarin wines can be explained by the oxidation of these parameters (Pareek et al. 2011). Oxidation of ascorbic acid may lead to the formation of dehydro ascorbic acid, which cannot be detected by the method used (Puttongsiri and Haruenkit 2010). Nagy and Smoot (1977) reported concentrations of total acidity

Table 1 General composition of mandarin juices and wines

Analysis	Satsuma	Robinson	Fremont
Juice composition			
Density (20 °C/20 °C)	1.052±0.01 ^a	1.055±0.02 ^a	1.057±0.01 ^a
Total acidity (TA,%)	1.04±0.02 ^a	0.89±0.01 ^b	1.02±0.03 ^a
pH	3.5±0.01 ^a	3.6±0.01 ^a	3.4±0.0 ^a
Total soluble solids (TSS)	12.4±0.00 ^b	13.0±0.01 ^a	12.8±0.0 ^{ab}
TSS/TA ratio	11.9±0.01 ^c	14.5±0.01 ^a	12.5±0.0 ^b
Ascorbic acid (mg/L)	372.6±10.1 ^c	496.2±12.1 ^a	398.8±11.2 ^b
Total phenolics (GAE, mg/L)	750.3±3.36 ^a	426.1±4.74 ^c	486.3±7.71 ^b
Extract (g/L)	111.9±1.18 ^b	129.3±2.04 ^a	126.2±1.2 ^a
Wine composition			
Density (20 °C/20 °C)	1.008±0.01 ^a	1.015±0.01 ^a	1.021±0.02 ^a
Ethanol (v/v%)	12.7±0.04 ^a	12.6±0.02 ^a	12.6±0.01 ^a
Total acidity(%)	0.89±0.03 ^a	0.76±0.02 ^b	0.85±0.03 ^a
pH	3.4±0.01 ^a	3.6±0.01 ^a	3.5±0.01 ^a
Volatile acidity ^x (g/L)	0.23±0.06 ^a	0.20±0.12 ^b	0.18±0.08 ^c
Total sugar (g/L)	72.2±0.25 ^a	71.6±1.2 ^a	71.3±0.03 ^a
Ascorbic acid (mg/L)	203.2±2.5 ^b	225.6±2.7 ^a	202.6±1.5 ^b
Total phenolics (GAE, mg/L)	421.3±0.07 ^a	117.4±2.19 ^c	237.5±3.82 ^b
Extract (g/L)	78.9±2.1 ^a	78.6±2.2 ^a	77.9±1.2 ^a
Free SO ₂ (mg/L)	15.2±0.59 ^b	16.5±1.2 ^a	15.7±0.48 ^{ab}
Bound SO ₂ (mg/L)	88.7±1.4 ^a	89.2±2.5 ^a	90.3±1.6 ^a

TSS Total soluble solids; TA Total acidity. Values represent means of triplicate ($n=3$). ^xas acetic acid

^{a,b,c} Different superscripts in the same row indicate statistical differences at the 0.05 ($p<0.05$)

(%) from 0.77 to 1.11, and total soluble solids from 10.2 to 12.6 in Valencia and Hamlin orange juices. The total acidity of the mandarin wine was higher than that of normal wine. Sugar was added partly to balance the taste sensation of high acidity in mandarin wine. The general composition of the wine was in accordance with previous studies carried out on mandarin wine (Selli et al. 2004).

The concentration of total polyphenols determined by Folin–Ciocalteu method ranged from 426.1 to 750.3 mg of gallic acid equivalent/L for the mandarin juices, and from 117.4 to 421.3 mg of gallic acid equivalent/L for the wines (Table 1). The total content of phenolics in several juices obtained from different *Citrus* varieties ranged from 801 (Mandarin) to 1551 (Pummelo) mg of gallic acid equivalents per gram (Xu et al. 2008). The concentration of total polyphenols determined by Folin–Ciocalteu method was higher than the concentration obtained by HPLC method. This can be explained by the lack of selectivity of Folin–Ciocalteu reagent, which reacts not only with phenols but also with other reducing compounds such as carotenoids, amino acids, sugars and vitamin C (Vinson et al. 2001).

Phenolic compositions of the mandarin juices and wines

A total of 11 phenolic compounds were identified and quantified in mandarin juices and wines, including hydroxybenzoic acids (3), hydroxycinnamic acids (5) and flavanones

(3) (Fig. 2). The total amount of phenolic compounds ranged from 36.6 to 132.6 mg/L for the mandarin juices, and from 14.1 to 54.5 mg/L for the wines (Table 2). As can be seen in Table 2, the total phenolic content of the juices was about two-fold higher than that of the wines. These changes were possibly due to the transformation of phenolic compounds into condensed forms that possessed slightly different chemical properties.

Hydroxybenzoic acids Three different hydroxybenzoic acids (protocatechuic, *p*-hydroxybenzoic and vanillic acid) were detected and quantified in the juices and wines (Table 2). Satsuma juice presented the highest total hydroxybenzoic acid content (2.9 mg/L). Vanillic acid accounted for the largest proportion of the total hydroxybenzoic acid contents. The highest level of vanillic acid was detected in Satsuma juice (1.3 mg/L), followed by Fremont juice (0.86 mg/L) and lastly Robinson juice (0.74 mg/L). In a previous study, the vanillic acid content varied with the variety of citrus fruit: Satsuma (Mandarin) juice had the highest (3.4 mg/L), while Miyou (Pummelo) juice had the lowest (0.6 mg/L) contents (Xu et al. 2008). *p*-Hydroxybenzoic acid was the second most abundant hydroxybenzoic acid and its concentration ranged from 0.55 to 0.92 mg/L for the juices and from 0.18 to 0.35 mg/L for the wines. The hydroxybenzoic acid contents in the mandarin juices were in agreement with the previously reported data from *Citrus* juices (i.e. 0.69–3.65 g/L for vanillic

Fig. 2 HPLC-DAD chromatogram at 280 and 320 nm of the Satsuma mandarin juice. (1: protocatechuic acid; 2: *p*-hydroxybenzoic acid; 3: vanillic acid; 4: caffeic acid; 5: chlorogenic acid; 6: *p*-coumaric acid; 7: ferulic acid; 8: sinapic acid; 9: narirutin; 10: hesperidin; 11: didymin)

acid, 0.74–1.77 g/L for *p*-hydroxybenzoic acid and 0.55–0.86 g/L for protocatechuic acid) (Xu et al. 2008).

Hydroxycinnamic acids The five hydroxycinnamic acids identified in the analysis were caffeic, chlorogenic, *p*-coumaric, ferulic and sinapic acid. The total amount of phenolic compounds ranged from 9.2 to 36.5 mg/L for the

mandarin juice, and from 3.4 to 14.6 mg/L for the wines. Ferulic acid was the most dominant hydroxycinnamic acid in the mandarin juices (5.4–20.8 mg/L) and the wines (2.1–8.1 mg/L), as it accounted for the largest proportion of the total hydroxycinnamic acid contents (Table 2). Sinapic acid was the second most abundant hydroxycinnamic acid, followed by chlorogenic, caffeic, and *p*-coumaric acids in the juices and

Table 2 Phenolic compositions (mg/L $\pm$ standard deviation) of the mandarin juices and wines

	Juices			Wines		
	SJ	RJ	FJ	SW	RW	FW
Hydroxybenzoic acids						
Protocatechuic acid	0.66 $\pm$ 0.01 ^a	0.35 $\pm$ 0.01 ^c	0.44 $\pm$ 0.01 ^b	0.26 $\pm$ 0.01 ^a	0.14 $\pm$ 0.01 ^b	0.17 $\pm$ 0.02 ^b
<i>p</i> -Hydroxybenzoic acid	0.92 $\pm$ 0.02 ^a	0.55 $\pm$ 0.03 ^c	0.68 $\pm$ 0.03 ^b	0.35 $\pm$ 0.01 ^a	0.20 $\pm$ 0.01 ^b	0.18 $\pm$ 0.01 ^b
Vanillic acid	1.3 $\pm$ 0.02 ^a	0.74 $\pm$ 0.02 ^c	0.86 $\pm$ 0.01 ^b	0.56 $\pm$ 0.01 ^a	0.29 $\pm$ 0.02 ^c	0.37 $\pm$ 0.01 ^b
Total	2.9 $\pm$ 0.0 ^a	1.6 $\pm$ 0.06 ^c	2.0 $\pm$ 0.01 ^b	1.2 $\pm$ 0.01 ^a	0.63 $\pm$ 0.02 ^c	0.72 $\pm$ 0.01 ^b
Hydroxycinnamic acids						
Caffeic acid	2.7 $\pm$ 0.03 ^a	0.71 $\pm$ 0.01 ^c	1.6 $\pm$ 0.04 ^b	1.1 $\pm$ 0.05 ^a	0.22 $\pm$ 0.00 ^c	0.71 $\pm$ 0.00 ^b
Chlorogenic acid	5.0 $\pm$ 0.66 ^a	0.88 $\pm$ 0.03 ^c	2.4 $\pm$ 0.04 ^b	2.1 $\pm$ 0.21 ^a	0.34 $\pm$ 0.02 ^c	1.0 $\pm$ 0.01 ^b
<i>p</i> -Coumaric acid	2.3 $\pm$ 0.37 ^a	0.17 $\pm$ 0.04 ^b	0.37 $\pm$ 0.04 ^b	0.90 $\pm$ 0.05 ^a	0.07 $\pm$ 0.01 ^b	0.16 $\pm$ 0.02 ^b
Ferulic acid	20.8 $\pm$ 5.55 ^a	5.4 $\pm$ 0.16 ^b	6.6 $\pm$ 0.25 ^b	8.1 $\pm$ 1.16 ^a	2.1 $\pm$ 0.14 ^b	2.9 $\pm$ 0.18 ^b
Sinapic acid	5.7 $\pm$ 0.21 ^a	2.0 $\pm$ 0.03 ^c	2.8 $\pm$ 0.03 ^b	2.4 $\pm$ 0.01 ^a	0.66 $\pm$ 0.02 ^c	1.2 $\pm$ 0.04 ^b
Total	36.5 $\pm$ 6.7 ^a	9.2 $\pm$ 0.05 ^b	13.8 $\pm$ 0.24 ^b	14.6 $\pm$ 1.3 ^a	3.4 $\pm$ 0.10 ^c	6.0 $\pm$ 0.25 ^b
Flavanones						
Narirutin	18.3 $\pm$ 0.51 ^a	6.6 $\pm$ 0.14 ^b	17.9 $\pm$ 0.13 ^a	7.3 $\pm$ 0.06 ^b	2.6 $\pm$ 0.15 ^c	7.9 $\pm$ 0.13 ^a
Hesperidin	74.1 $\pm$ 0.96 ^a	18.7 $\pm$ 0.44 ^c	40.5 $\pm$ 0.04 ^b	31.1 $\pm$ 0.64 ^a	7.3 $\pm$ 0.44 ^c	17.7 $\pm$ 0.45 ^b
Didymin	0.75 $\pm$ 0.01 ^a	0.50 $\pm$ 0.03 ^b	0.44 $\pm$ 0.02 ^c	0.31 $\pm$ 0.01 ^a	0.21 $\pm$ 0.02 ^b	0.19 $\pm$ 0.01 ^b
Total	93.2 $\pm$ 0.46 ^a	25.8 $\pm$ 0.60 ^c	58.9 $\pm$ 0.07 ^b	38.7 $\pm$ 0.60 ^a	10.1 $\pm$ 0.60 ^c	25.8 $\pm$ 0.59 ^b

SJ Satsuma juice; RJ Robinson juice; FJ Fremont juice; SW Satsuma wine; RW Robinson wine; FW Fremont wine. Values represent means of triplicate ($n=3$). Each group (juices/wines) is statistically compared within themselves

^{a,b,c} Different superscripts in the same row indicate statistical differences at the 0.05 ($p<0.05$)

the wines. The juices contained chlorogenic, caffeic and *p*-coumaric acids in quantities of up to 5.0, 2.7 and 2.3 mg/L, respectively (Table 2). Meanwhile, the mandarin wines contained about half the quantities of these acids, with amounts of up to 2.1, 1.1 and 0.90 mg/L, respectively. The levels of hydroxycinnamic acid content in the mandarin juices were in agreement with previously reported data from mandarin juices (i.e. 2.7–6.6 g/L for caffeic acid, 1.3–7.2 g/L for *p*-coumaric acid, 16.5–45.9 g/L for ferulic acid and 2.8–6.1 g/L for sinapic acid) (Xu et al. 2008). Rapisarda et al. (1998) found that ferulic acid (3.77 mg/100 ml) was the main phenolic acid in Liucheng (Valencia late) juices. Caffeic acid (0.21 mg/

100 ml), sinapic acid (0.90 mg/100 ml), and *p*-coumaric acid (0.80 mg/100 ml) were also quantified.

Flavanone Flavanone is the major flavonoid in mandarin juices and wines. Three flavanones, narirutin, hesperidin and didymin, were identified in the mandarin juices and wines. Table 2 shows that, of the three flavanones, hesperidin was the most abundant in the juices and wines. The levels of hesperidin reported here for the mandarin juices (18.7 to 74.1 mg/L) are in good agreement with the levels previously reported by Gattuso et al. (2007), 8–458 mg/L, while lower than that found by Xu et al. (2008). Narirutin was the second most

Table 3 Antioxidant activities of the mandarin juices and wines

	Juices			Wines		
	SJ	RJ	FJ	SW	RW	FW
DPPH	48.8 $\pm$ 0.91 ^a	33.9 $\pm$ 0.20 ^c	41.5 $\pm$ 0.83 ^b	26.7 $\pm$ 0.40 ^a	15.9 $\pm$ 0.34 ^c	20.8 $\pm$ 0.83 ^b
ABTS	59.3 $\pm$ 0.23 ^a	44.6 $\pm$ 0.84 ^c	52.6 $\pm$ 0.81 ^b	35.6 $\pm$ 0.57 ^a	21.3 $\pm$ 0.18 ^c	27.9 $\pm$ 0.76 ^b

SJ Satsuma juice; RJ Robinson juice; FJ Fremont juice; SW Satsuma wine; RW Robinson wine; FW Fremont wine. Values represent means of triplicate ($n=3$). Each group (juices/wines) is statistically compared within themselves

^{a,b,c} Different superscripts in the same row indicate statistical differences at the 0.05 ($p<0.05$)

Table 4 Spearman's rank order coefficients for the correlation between antioxidant capacities measured by DPPH and ABTS assays and phenolic contents of the mandarin juices and wines

	DPPH	ABTS
DPPH	1	0.972
ABTS	0.972	1
Hydroxybenzoic acids		
Protocatechuic acid	0.965	0.993
<p>-Hydroxybenzoic acid</p>	0.923	0.923
Vanillic acid	0.972	0.986
Hydroxycinnamic acids		
Caffeic acid	0.909	0.874
Chlorogenic acid	0.818	0.804
<p>-Coumaric acid</p>	0.762	0.748
Ferulic acid	0.797	0.825
Sinapic acid	0.923	0.923
Flavanones		
Narirutin	0.748	0.755
Hesperidin	0.923	0.937
Didymin	0.874	0.895

Correlation is significant at $p < 0.05$ level

abundant flavanone and its concentration ranged from 6.6 to 18.3 mg/L for the mandarin juices, and from 2.6 to 7.9 mg/L for the wines. Narirutin levels in mandarin juices have been reported at 1–90 (Gattuso et al. 2007) and 24–288 mg/L in different varieties (Xu et al. 2008). Didymin was the least abundant flavanone in each of the samples, ranging from 0.19 g/L in the Fremont wine to 0.75 g/L in the Satsuma juice. Gattuso et al. (2007) reported that levels of didymin content ranged from 0.5 to 31.0 g/L in different mandarin varieties.

Antioxidant activity of mandarin juices and wines

Antioxidant capacity was measured by two methods namely, ABTS and DPPH assays. Table 3 presents the results of the antioxidant activities obtained by the mandarin juices and wines. As can be seen, the antioxidant capacity of phenolic compounds were better reflected by ABTS assay than DPPH assay and ABTS assay produced higher values than DPPH assays. These data suggest that ABTS assay may be more useful than DPPH assay for detecting the antioxidant capacity of phenolic compounds in fruits.

DPPH assay DPPH scavenging method have been used to evaluate the antioxidant activity of compounds due to the simple, rapid, sensitive, and reproducible procedure. Table 3 presents the results of the antioxidant capacity. The antioxidant activity ranged from 33.9 to 48.8 mg VCE/100 ml for the mandarin juices, and from 15.9 to 26.7 mg VCE/100 ml

for the wines. As shown in Table 3, Satsuma showed higher antioxidant activity compared to Robinson and Fremont. Our results for juices were comparable to those observed for citrus juices by other authors (Lim et al. 2007; Floegel et al. 2011). Antioxidant activity of the juices was about two-fold higher than that of the wines. The decrease in the antioxidant activity of the mandarin wines was due possibly to the transformation and oxidation of phenolic compounds and ascorbic acids.

ABTS assay ABTS is frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods. The antioxidant activity ranged from 44.6 to 59.3 mg VCE/100 ml for the mandarin juices, and from 21.3 to 35.6 mg VCE/100 ml for the wines (Table 3). The highest values were measured in Satsuma and the lowest in Robinson juices and wines. The antioxidant capacity in several juices obtained from different fruit varieties ranged from 22.8 (Apple juice) to 120.7 (Grape juice) mg VCE/100 ml (Floegel et al. 2011).

The total phenolic contents and antioxidant capacities as measured by DPPH and ABTS assays were compared (Table 4). Our results show strong correlations both between antioxidative capacity and total phenolic contents. Correlations between the ABTS and DPPH assays were also evaluated (Table 4). We found significant correlations ($p < 0.05$) between ABTS and DPPH ($r = 0.972$). Floegel et al. (2011) reported that antioxidant capacity by ABTS assay was strongly positively correlated to that by DPPH assay ($r = 0.949$, $p < 0.001$). Compared with antioxidant capacity by DPPH assay, antioxidant capacity measured by ABTS assay showed a stronger correlation with ORAC (for ABTS: $r = 0.593$ and for DPPH: $r = 0.539$, $p < 0.001$, respectively) (Floegel et al. 2011). In another study, Dudonné et al. (2009) reported a strong positive correlation between ABTS and DPPH assays with a Pearson correlation coefficient ($r = 0.906$).

Conclusion

In this study, phenolic contents and antioxidant activity of the Satsuma, Robinson and Fremont mandarin juices and wines have been examined. The results indicated that the total antioxidant activity and phenolic contents of mandarin juices were higher than mandarin wines. Our results show strong correlations between antioxidative capacity and phenolic content (ranging from 0.748 to 0.993, $p < 0.05$). The results of this study indicated that these mandarin wines had a composition similar to other beverages demonstrating that these fruits have the potential to be used to produce fermented beverages. Furthermore, use of mandarins in the production of fruit wines is a viable alternative that allows for the use of harvest-surplus fruits, resulting in the introduction of new products into the market.

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