International Food Research Journal 25(5): 1978-1984 (October 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Physical chemical properties and antioxidant capacities of grapefruit juice *(Citrus paradisi)* extracted from two different varieties

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Article history

Received: 5 July 2017
Received in revised form: 12 October 2017
Accepted: 8 November 2017

Keywords

Antioxidant activity
Citrus paradisi
Grapefruit
Flavonoids
Polyphenols

Abstract

This study was undertaken to determine the physical and chemical properties and the antioxidant compounds of grapefruits (Marsh and Star Ruby varieties) grown in South Italy. Flavanones (narirutin, naringin, hesperidin, neohesperidin, and poncirin), flavones (rutin) and aglycones (quercetin, naringenin and hesperetin) have been identified in the grapefruit juices examined. The concentration of total flavonoids ranged between 310 and 390 mg L^{-1} juice. Naringin was found to be the major flavonoid followed by narirutin and poncirin. Their concentrations ranged from 198.10 to 287.97; from 37.07 to 38.87 and from 14.22 to 17.32 mg L^{-1} juice, respectively. Antioxidant activity (DPPH) ranged from 35.25 (Star Ruby) to 46.08% inhibition (Marsh).

Introduction

Citrus fruits are one the world's most important crops, and one of these, grapefruit *(Citrus paradisi),* is interesting for the secondary metabolites that it contains. Secondary metabolites are an important dietary component and are attracting much interest for their physiological effects. Phenols make up one of largest groups of these metabolites.

There are different varieties of grapefruit on the market, including the yellow grapefruit (*Citrus paradisi* Marsh) with its pronounced bitter taste. This variety is most commonly used for the preparation of fresh juice or concentrates. The most common varieties with pale pulp are Duncan, which contains many seed but shows a remarkable resistance to cold winter temperatures, and Marsh, which is a seedless variety.

Over the years, grapefruit growers have tried to improve the flavor by crossing grapefruit with oranges, to produce sweeter and juicier fruits, whose pulp ranges from salmon pink to orange to the dark pulp typical of blood oranges. This hybridization has popularized the grapefruit, as its original acidic and bitter taste was not widely palatable. The cultivars with pigmented pulp, which in most cases are seedless, include the following varieties: Pink Marsh, Star Ruby, Redblush, Flame, Rio Red, Foster and McCarty.

The substantial difference between the yellow and the pink grapefruit, apart from the color of the pulp, is that the pink grapefruit contains a lower content of ascorbic acid, but a higher content of fructose, and thus it is less bitter.

Medical researchers suggest that the metabolites present in grapefruit have an antioxidant effect and reduce atherosclerotic plaque formation (Cerda *et al*., 1994), inhibit breast cancer cell proliferation and mammary cell tumorigenesis (Hertog *et al*., 1993; Hertog *et al*., 1995; So *et al.*, 1996; Guthrie *et al.*, 1998; Nijveldt *et al*., 2001). Grapefruit contains high levels of ascorbic acid and flavonoid antioxidants including naringin and naringenin (Lee, 2000; Peterson *et al*., 2006). Flavonoid properties include anti-inflammatory, antiviral and anticancer activities, an ability to inhibit platelet aggregation, and effects on capillary fragility (Hope *et al*., 1983; Salvayre *et al.,* 1988; Duarte *et al*., 1993; Hanasaki *et al.*, 1994; Cook and Samman, 1996; Sicari *et al*., 2016a; Sicari *et al.*, 2016b).

This paper focuses on the phytochemical properties and antioxidant potentials of two grapefruit varieties Marsh and Star Ruby. The results of this study provide valuable information to growers, distributors and consumers in selecting fresh fruit that are rich in bioactive compounds.

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Materials and Methods

Chemicals

Sigma-Aldrich Chem. Co. (St. Louis, MO, USA) supplied: ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate)], 2,2-diphenyl-1-picryl hydrazyl (DPPH), 6-Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid, 97% (Trolox), Folin Ciocalteu's reagent.

Cyanidin 3-glucoside, narirutin, naringin, hesperidin, neohesperidin, quercetin and rutin were supplied by Extrasynthese (Genay-France). Formic acid, acetonitrile and water all of solvent HPLC grade, were supplied by Carlo Erba Reagents (Milano, Italia). The other reagents were of analytical grade.

Sample analysis

The grapefruits *(Citrus paradisi)*, of two different varieties (Marsh and Star Ruby), were harvested at the end of October 2016 at their full-ripe stage from Reggio Calabria (Italy) and transported to the Food Technology laboratory of the Department of Agriculture, University of Reggio Calabria, Italy, to be analysed. Harvest involved a random sampling from 6 to 8 trees (20-year-old) from each variety. Ten fruits of each variety were washed and then juice was extracted by cutting the fruit in half and careful hand-squeezing in a commercial kitchen juicer. The juice was filtered to remove pulp and seeds. Than it was centrifuged at 3500 rpm for 20 mins, after which the supernatant was passed through a 0.45µm filter (Millipore Corporation, Bedford, USA). The supernatant was kept at -20°C until analysis.

pH, total titratable acidity and total soluble solids

The pH of the juice was determined with an electronic digital pH meter (Crison) and the total titratable acidity (TA) as citric acid percentage of the juice was measured by titration with NaOH (0.1N) to pH 8.1. Soluble solid content (TSS) was measured at 20°C using a digital Atago Model PR 101 α refractometer (Atago Co. Ltd, Milan, Italy), results were reported as Brix degrees (°Brix). All determinations above described were made in triplicate.

Ascorbic acid determination

Analysis was performed in triplicate using a Knauer (Asi Advanced Scientific Instruments, Berlin, Germany) system equipped with two pumps (Smartiline Pump 1000), a Rheodyne injection valve (20 µL), and a photodiode array detector UV/VIS equipped with a semi micro-cell. Data processing was carried out with the support of Clarity Software (Chromatography Station for MS Windows). Ascorbic acid was separated on a Knauer RP C18 column (250 mm × 4.6 mm, 5 µm) in isocratic mode whit a mobile phase of 0.2 M KH_2PO_4.

The flow rate was 0.6 mL/min and the injection volume was 20 µL. The absorbance was monitored at 254 nm. Samples were filtered through a 0.45 µm Millipore filter (GMF Whatman) before injection.

Total phenolic content

The phenolic concentration was determined by the Folin-Ciocalteu colorimetric method (Singleton *et al*., 1999) and results were expressed as mg L^{-1} of gallic acid equivalents (GAE). Briefly, 500 µL of juice were shaken with 1mL of Folin-Ciocalteu's reagent and 10 mL of a 20% in water solution of sodium carbonate solution and made up to 100 mL with ultrapure water. The mixture was shaken and left at room temperature in the dark for 2 hr. After centrifugation, the absorbance was read at 760 nm by using an Agilent 8453 UV/Vis Spectrophotometer G1103A with 89090A Peltier Temperature Control. Samples were analyzed in triplicate.

Total flavonoid content

Flavonoids were extracted with a solution composed by: 5% $NaNO_2$, 0.1 mL of 10% aluminum chloride hexahydrate ($AlCl_3$), and 1M NaOH (Leontowicz *et al*., 2014). The mixture was diluted to 10 mL with water and the absorbance was read at 510 nm. The results were expressed as mg quercetin $\cdot L^{-1}$ juice.

Total anthocyanin content

Total monomeric anthocyanins were determined by a pH differential method (Cheng and Breen, 1991; Nielsen *et al.*, 2003). Briefly, this method is based on the reversible structural change of the anthocyanin chromophore at pH 1.0 (highly colored) and pH 4.5 (colorless). Grapefruit juices were diluted 1:5 with pH 1.0 buffer solution or pH 4.5 buffer solutions. After equilibration at room temperature for 10 minutes, the absorbance was measured at 520 nm (maximum absorbance of Cyd-3-Glu) and 700 nm (for turbidity corrections) on Agilent 8453 UV/Vis Spectrophotometer G1103A with 89090A Peltier Temperature Control (Agilent Technologies, Italy). Measurements were carried out in triplicate. Results were expressed as mg of cyanidin 3-glucoside $\cdot mL^{-1}$ by applying the formula:

$$\text{Cyd-3-Glu equivalents/mL} = A \times MW \times DF/\varepsilon \times l$$

where:

$A = [(A_{520}-A_{700})_{pH\ 1} - (A520-A700)_{pH\ 4.5}]$

MW (Cyd-3-Glu) = 449.2g •mol^{-1}

DF = dilution factor

L = path length of cuvette = 1cm

ε = molar extinction coefficient = 26900L •mol^{-1} •cm^{-1}

Results were expressed as µg of cyanidin 3-glucoside •mL^{-1}.

HPLC analysis of grapefruit juice

Knauer HPLC/DAD system (Asi Advanced Scientific Instruments, Berlin, Germany), equipped with a photodiode array detector UV/VIS and two Smartline pumps, was used to perform HPLC analysis. HPLC-DAD technique is widely used in antioxidant analysis (Giuffrè, 2013; Omoba *et al*., 2015). The mobile phase was a gradient prepared with formic acid in water and formic acid in acetonitrile (La Torre *et al.,* 2006). During analysis, the flow rate was maintained at 1mL min^{-1} and the column temperature was maintained at 30°C. After filtration through a 0.45 µm membrane, a 20 µL aliquot of the sample was injected.

Compounds were separated on a Knauer RP C18 column (250 mm x 4.6 mm, 5 µm). Eluate absorbance was recorded at 280, 254 and 365 nm to monitor peaks. A calibration straight for each standard was obtained. Identification and quantification were carried out based on recorded retention times in comparison with authentic standards. All solutions were filtered through a 0.45µm Millipore filter (GMF Whatman) and injected into the HPLC system. Analyses were performed in triplicate.

Antioxidant capacity

DPPH radical used to determine antioxidant activity

The free radical scavenging capacity against DPPH was determined as suggested by Brand-Williams *et al*. (1995). This method was applied to evaluate the antioxidant activity of the studied sample. In brief, 50 µL of grapefruit juice was added to 2.5 mL of 0.06 mM DPPH methanolic solution. The mixtures was shaken vigorously and left to stand at room temperature in the dark for 20 mins. The decrease in the absorbance from time zero to time 5 mins was determined at 515 nm using an Agilent 8453 UV/Vis Spectrophotometer G1103A (Agilent Technologies). The following equation was used to express results.

$$(\%)\ \text{Inhibition} = (1-A_f/A_0) \times 100$$

where:

A_f = absorbance DPPH +fruit juice at t = 5 mins and

A_0 = absorbance DPPH (control) at t = 0 mins.

Experiments were carried out in triplicate. Juice activity was expressed as % inhibition

ABTS radical used to determine antioxidant activity

The ABTS radical cation decolourisation assay (Re *et al*., 1999) was used to determine the radical scavenging capacity. An ABTS solution was made with 7 mM of ABTS and 140 mM of potassium persulphate, and it was stored at room temperature for 16hr in the dark before use. The ABTS solution was diluted with ethanol (1:80) to an absorbance of 0.70 at λ = 734 nm. After addition of 30 µL of sample or Trolox standard to 2 mL of diluted ABTS solution, absorbance was measured at exactly 6 mins after mixing. The scavenging ability of the sample was calculated according to the following equation:

$$(\%)\ \text{Inhibition} = (1-A_f/A_0) \times 100$$

where:

A_f = absorbance ABTS+ fruit juice at t = 6 mins and

A_0 = absorbance ABTS (control) at t = 0 mins.

Experiments were carried out in triplicate. Juice activity was expressed in % inhibition.

Statistical analysis

Each given value is expressed as the mean of three replicates. Data were subjected to analysis of variance (one way ANOVA) and compared using Tukey's test at $p < 0.05$, by the SPSS software version 17.0 for Windows (SPSS Inc., Chicago, IL).

Results and discussion

The quality parameters, including pH, Total Soluble Solids (TSS), Total Acidity (TA) and TSS/TA ratio of grapefruit juices were shown in Table 1. The pH of the juice samples were between 3.01 and 3.12. Marsh variety had the highest TSS value (13.44%) whereas Star Ruby had value (10.78%). Star Ruby had the highest TA value (1.03%) and Marsh had the lowest value (0.58%). Grapefruit juice is a rich source of vitamin C (ascorbic acid), which is an important antioxidant and the concentration of vitamin C is also a significant indicator of juice quality. Vitamin C plays a key role in the formation of collagen, a primary component of much of the connective tissue in the body. In the present study the focus is on the

Table 1. TA, TSS, TSS/TA ratio and pH of grapefruit juices

Varieties	TSS	TA (g 100 mL^{-1})	pH	TSS/TA
Marsh	13.44 ± 0.20	0.58 ± 0.02	3.01 ± 0.14	23.17±0.21
Star Ruby	10.78 ± 0.16	1.03 ± 0.01	3.12 ± 0.07	10.46±0.83
	**	**	*	**

Data are expressed as mean ± DS. * significance at p<0.05; ** significance at p<0.01.

Table 2. Total flavonoids, total phenolic compounds, total anthocyanins and ascorbic acid contents (mg $^{L-1}$ ± standard deviation) of the grapefruit juices

Varieties	Total flavonoid mg L^{-1}	Total polyphenol mg L^{-1}	Total anthocyanins mg L^{-1}	Ascorbic acid mg L^{-1}
Marsh	390.21±9.32	153.08±2.01	0.42±0.01	680.03±7.03
Star Ruby	310.14±4.12	167.22±0.98	1.87±0.04	455.55±4.02
	**	n.s.	**	**

Data are expressed as mean ± DS. ** significance at p<0.01; n.s. = not significant

antioxidant activity that ascorbic acid exerts on free radicals. Free radical damage has been implicated in the progression of several diverse and important disease states including cancer, cardiovascular disease and cataract formation (García-Closas *et al.*, 2004; Rao *et al*., 2006).

In our study the concentration of ascorbic acid in Marsh and Star Ruby grapefruit were 680.03±7.03 and 455.55±4.02 mg L^{-1} respectively (Table 2), similarly to the ascorbic acid content found in blond orange grown in the same geographic area (Sicari *et al.*, 2017). The total anthocyanic and flavonoid (TFC) content between the two grapefruit varieties were statistically different p<0.01), whereas the polyphenolic content showed difference not significant (Table 2).

Total flavonoids ranged from 390.21±9.32 mg L^{-1} (Marsh) to 310.14±4.12 mg L^{-1} (Star Ruby). Total phenolics in grapefruit juice ranged between 153.08 ±1.82 (Marsh) and 167.22 ±1.64 mg mL^{-1} (Star Ruby). The total anthocyanic content was from 0.42 ±0.05 µg mL^{-1} (Marsh) to 1.87 ±0.03 µg mL^{-1} (Star Ruby). Flavanone are the major flavonoids in grapefruit juice. Five flavanones: narirutin (naringenin 7-β-rutinoside), naringin (naringenin 7-β-neohesperidoside), hesperidin (hesperetin 7-β-rutinoside), neohesperedin (hesperetin 7-β-neohesperidoside) and poncirin (isosakuranetin-7-O-neohesperidoside) were identified in Star Ruby and Marsh grapefruit juices.

Table 3. Flavonoid contents (mg L^{-1}± standard deviation) of the grapefruit juices.

	Marsh	Star Ruby	Sign.
Poncirin	17.32±0.85	14.22±0.54	**
Narirutin	37.07±1.98	38.87±2.08	*
Naringin	287.15±7.54	198.10±5.21	**
Neo-hesperedin	13.48±0.04	12.01±0.14	**
Hesperidin	4.71±0.14	3.77±0.08	**
Naringin/neohesperidin	21.30±0.97	16.49±1.02	**
Rutin	14.71±0.24	12.80±0.85	*
Hesperetin	6.71±1.11	5.63±0.41	*
Naringenin	31.25±3.01	24.32±1.41	**
Quercetin	2.87±0.04	2.10±0.10	*

Data are expressed as mean ± DS. * significance at p<0.05; ** significance at p<0.01

Table 3 shows that, out of the five flavanones, naringin and narirutin were the most abundant in the two varieties, as previously reported (Ortuno *et al.*, 1995; Ross *et al*., 2000; Nogata *et al.*, 2006; Vanamala *et al.*, 2006; Gattuso *et al*., 2007), naringin and narirutin were the most dominant flavanone in grapefruit juices. The concentration of naringin was higher in Marsh juice compared to Star Ruby grapefruit (287.15 ±3.2 and 198.10 ±2.1mg L^{-1} respectively) as reported to Maurer *et al.* (1950) and De Castro *et al*. (2006).

This class of compounds exerted anti-atherosclerotic effects, anti-thrombogenic effects, anti-inflammatory effects, anti-oxidative effects, anti-tumor effects, anti-osteoporotic effects, and anti-viral effects (Hertog *et al.*, 1993; Hertog *et al*., 1995; Nijveldt *et al.*, 2001).

In particular, the activity of naringin has been pharmacologically evaluated in terms of chemoprevention of carcinogenesis, inhibition of human cancer cell proliferation and delay of tumorigenesis (So *et al*., 1996; Tanaka, 1997). The naringin/neohesperidin ratio has been proposed as a quality parameter for a grapefruit juice. The naringin/neohesperidin ratio found for comercial grapefruit juices were 21.30 (Marsh), 16.49 (Star Ruby) (Table 3), and the values were within the range of 14-83, as indicated by Rouseff (1988).

Table 3 shows also that the flavone and aglycone content in all varieties was significantly different ($p < 0.05$). The flavone identified in grapefruit juices was rutin (Quercetin 3-rutinoside), while the aglycones identified were: hesperetin, naringenin (4',5,7-trihydroxyflavanone) and quercetin (3,3',4',5,7-pentaydroxyflavone). Their concentration in the two cultivars was statistically different. The concentrations of flavonoids (flavanones, flavones and aglycones) are in agreement with those reported

Table 4. Antioxidant capacity of grapefruit juices

Varieties	DPPH (% I)	ABTS (% I)
Marsh	46.08±3.21	0.92±0.04
Star Ruby	35.25±2.44	0.61±0.02
	**	**

Data are expressed as mean ± DS. ** significance at p<0.01

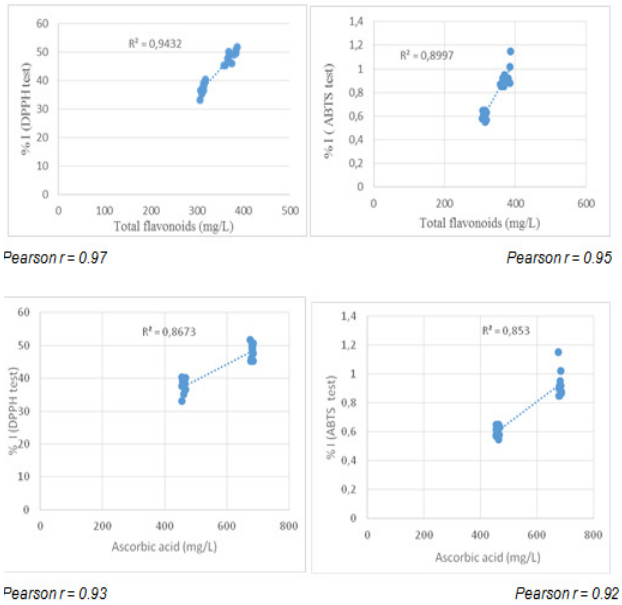

Figure 1. Linear regression plot and Pearson's correlation of DPPH and ABTS values with respect to total flavonoids and ascorbic acid contents of grapefruit juice. All correlations were significant at the 0.05 level.

by other authors (Kawaii *et al.*, 1999; De Castro *et al*., 2006; Wu *et al*., 2007).

DPPH· and ABTS· are stable free radicals, which have been widely accepted as a tool for estimating free radical scavenging activities of antioxidants (Krishnaiah *et al.*, 2011). The samples exhibited a radical scavenging activity against both radicals in a concentration-dependent manner (Table 4).

Phenolic compounds including anthocyanins, flavonoids, and ascorbic acid are known to be responsible for antioxidant activities in fruits, and fruits with higher phenolic contents generally show stronger antioxidant activities.

The varieties influenced the DPPH radical scavenging activity with a range of % inhibition values from 46.08 ±0.1 to 35.25±0.15% (Marsh and Ruby, respectively). Marsh grapefruit was also the most effective in the ABTS test with % inhibition value of 0.92 ±0.08% followed by the Ruby grapefruit sample (% inhibition value of 0.61±0.06%). The ABTS and DPPH assays were used to evaluate the antioxidant capacity of fruits of the two grapefruit varieties, and the obtained values were compared with the amounts of total polyphenol, flavonoid, and anthocyanin determined in each sample. The results obtained between antioxidant capacity assays and antioxidant compounds were compared. As Figure 1 reports a linear regression curves and Pearson correlation coefficients between DPPH•, $ABTS^{\bullet+}$, ascorbic acid and total flavonoids were obtained.

A striking correlation between total flavonoids and DPPH and ABTS test in the grapefruit juices was observed (r = 0.97; r = 0.95). Also, a good correlation was obtained between antioxidant assay values and ascorbic acid content (r = 0.93; r = 0.92) according literature data (Middleton *et al.*, 2000; Rapisarda *et al.,* 2008; Xu *et al*., 2008).

In general, the antioxidant capacity of *Citrus* does not seem to be a property of a single phytochemical compound, but is correlated both to vitamin C and to phenolic constituents.

Conclusion

Grapefruit juice contains several different compounds that can exert beneficial effects on human health. Significant differences regarding the chemical composition, nutritional value, and antioxidant activities among the grapefruit varieties were observed. Grapefruit juice can be considered to be a good dietary source of nutrients and antioxidant compounds, especially flavonoids, anthocyanins and polyphenols. The results revealed a good correlation between total polyphenol, flavonoid, anthocyanin and ascorbic acid contents and the antioxidant capacities, which were statistically significant. Grapefruit juices can be used for their health properties in food products; in fact they can be applied as a source of functional compounds, or as natural preservatives. In conclusion, the grapefruit juice has the potential to be further developed into a nutritionally interesting raw material for food and beverage applications.

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