Determination of Flavonoids in Pulp and Peel of Mandarin Fruits

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Summary

The aim of this study was to determine total flavonoids and individually flavanon glycosides as well as antioxidant capacity in pulp and peel of two mandarin groups, namely Satsuma *(Citrus unshiu* Marcovitch) cv. Saigon and Clementine (*Citrus reticulate* var. clementine) cv. Corsica SRA 63. Total flavonoids content was measured using colorimetric method, whereas HPLC-PDA detection was used for the analysis of individual flavanone glycosides (narirutin, naringin and hesperidin). In addition FRAP method was used to determine the antioxidant capacity. The results of colorimetric method showed that there was high concentration of flavonoids in all investigated samples, especially in peels (1156 mg/100 g in Satsuma peel and 804 mg/100 g in Clementine peel). Among the flavonone glycosides, hesperidin, was determined in the highest concentration in both investigated pulps. In peels flavanon glycosides were present in decreasing sequence as follow: narirutin>hesperidin>naringin. According to FRAP method, all samples possess an evident antioxidant capacity especially peels. Correlation between total flavonoids, hesperidin, naringin, narirutin and antioxidant capacity was very high with correlation coefficiants between 0.81 and 0.98.

Key words

flavonoid, flavanon glycosides, mandarin, Clementine, Satsuma, HPLC

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Introduction

Mandarin (*Citrus reticulata* Blanco) is very popular Citrus fruit and its production constantly increases throughout the world. According to FAO data in 2003 year, mandarin was placed on the second place in terms of production amounts after orange (FAO, 2003). However, in Croatia mandarin is produced more than oranges and in 2008 year it is expected to reach 50000 tonnes. *Citrus reticulata* Blanco is the most varied group of Citrus and among them Satsuma (*Citrus unshiu* Markovitch) is one of high importance in Japan, Spain (Rodrigo and Zacarías, 2006) as well as in Croatia. The Clementine mandarin is another group of high importance and recently is becoming one of the most important mandarins in the world (Rodrigo and Zacarías, 2006). Mandarin as other Citrus fruit has nutritional importance due to their particular composition. Among other constituents, flavonoids, especially polymethoxyflavones and flavanones (hesperidin, narirutin and naringin) are identified in Citrus pulp as well as in peel (Mouly et al., 1998; Oufedjikh et al., 1998; Del Caro et al., 2004; Wang et al., 2008). Peterson et al. (2006) gave critical review of flavanons in some citrus fruit from which it is obvious that many scientific papers dealt with flavanon glycosides in orange, but not so many in mandarins. Recently, there are some papers related to flavonoids in mandarins (Abeysinghe et al., 2007; Wang et al., 2008; Xu et al., 2008) but still there is not so many data. Furthermore, there is still lack data about phytochemicals in mandarins grown in Croatia particularly their flavonoids content and antioxidant capacity. The dominant flavanone glycoside in mandarins and sweet oranges (*Citrus sinensis*) is hesperidin, whereas in sour orange (*Citrus aurantium*) three predominant flavanone glycosides are naringin, neohesperidin and neoeriocitrin (Peterson et al., 2006). Naringin is known as bitter compound in Citrus. According to its presence in citrus pulp Citrus fruits could be divided in sweet, without presence, and sour with presence of naringin for instance sweet orange and sour orange (Oufjedikh et al., 1998; Peterson et al., 2006). Flavonoids are known as bioactive compounds with health-related properties (Morton et al., 2000; Garg et al., 2001; Pellegrini et al., 2003). Such components may act as antioxidants (Gorinstein et al., 2004a, b) or as part of other mechanisms contributing to reduce risk of some disease such as cancer or cardiovascular disease (Hiroyuki and Miki, 2001; Manthey et al., 2002). Knekt et al. (2002) reported that intake of orange resulted in reducing incidence of asthma in Finland. The findings of Funaguchi et al. (2007) suggest that narirutin may be an effective new tool in the treatment of bronchial asthma. In general, fruit skin contains a higher concentration of antioxidant substances than the flesh of the fruit (Awad et al., 2001).

The aim of this study was to determine total flavonoids and individual flavanone glycosides as well as antioxidant capacity in pulp and peel of mandarin, specifically of Satsuma *(Citrus unshiu* Marcovitch) cv. Saigon and Clementine (*Citrus reticulata var. clementine*) cv. Corsica SRA 63 grown in Neretva valley, Croatia.

Material and Methods

Materials

Fresh mandarin fruits from Clementine (*Citrus reticulata var. Clementine* cv. Corsica SRA 63) and Satsuma (*Citrus unshiu* cv. Saigon) grown in Neretva Valley, were purchased in local market in Zagreb (Croatia) in January 2007. Mandarins were hand-peeled and edible parts were pulped by blender. Peels and obtained pulps were frozen and kept at -18°C till the analysis.

Methods

Physicochemical parameters, dry matter and pH-value were determined in accordance with standard methods used in literature (Tanner and Brunner, 1979).

Extraction of phenolic compounds was done according to method reported by Abeysinghe et al. (2007). Obtained extracts were used for determination of total flavonoids by using colorimetric method and individual flavanon glycosides by using HPLC with UV/VIS DAD.

Total flavonoids (TF) were determined using a colorimetric method (Abeysinghe et al., 2007) by diethilene glycol. Absorbance at 420 nm was measured using spectrophotometer UV UNICAM HELIOS β. Rutin was used as standard and total flavonoid content was expressed as mg rutin equivalents (RE)/100 g fresh weight (FW), and as mg RE/g dry matter (DM).

Flavanone glycosides. Determination of individual flavanon glycosides was done by HPLC UV/VIS PAD detection according to method described by Abeysinghe et al. (2007). The HPLC analysis was performed by using Varian Prostar system with a ProStar Solvent Delivery Modul 230, injector Rheodyne 7125 and ProStar 330 UV/VIS – Photodiode Array Detector (PDA). Polyphenolics were separated by using Zorbax C18 column, 5 µm (250x4.6 mm I.D.) using two mobile phases consisting of (A) 75 mM citric acid and 25 mM ammonium acetate in water and (B) methanol with ratio of 60:40 (v/v) at a flow rate of 1mL/min. The injection volume was 20 µL and the detection wavelength was set at 282 nm.

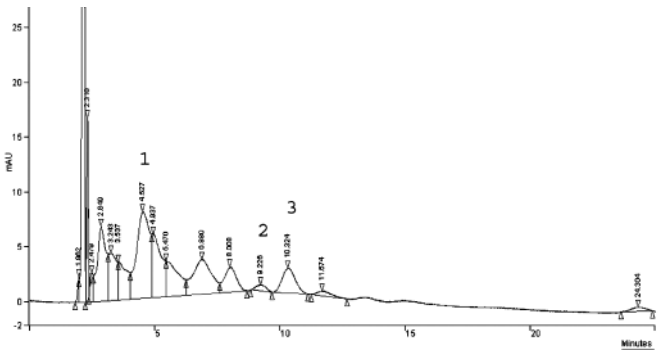

Figure 1. Chromatogram of flavanone glycosides: 1-narirutin, 2-naringin, 3-hesperidin in Satsuma pulp

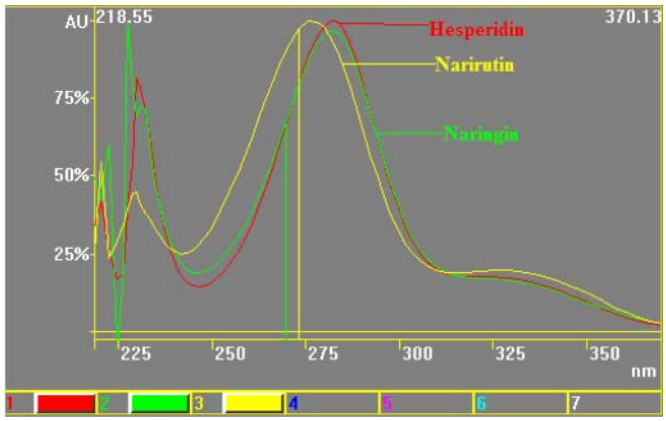

Figure 2. UV-spectra of flavanone glycosides

Among flavonoids, three flavanon gycosides (hesperidin, narirutin and naringin) were identified according to retention time of separated peaks with retention time of available standards (Fig. 1), as well as according to characteristic UV spectra of flavanon glycosides (Fig. 2). Quantification was done according to calibration curve of external standards (naringin and hesperidin). Narirutin was quantified by using calibration curve of hesperidin. The content of individual flavanone glycosides was expressed as mg/kg FW and mg/100 g DM.

Antioxidant capacity of total phenols extracts was determined by ferric ion reducing antioxidant power method (FRAP) (Connor et al., 2002). Results were expressed as mmol Fe^{2+}/kg FM and as mmol Fe^{2+}/100 g DM.

All analysis was done in three parallels and results were expressed as average values ± standard deviations.

Results and discussion

The results of total dry matter, pH value, total and individual flavonoids and antioxidant capacity of mandarin's (Satsuma and Clementine) pulps and peels are shown in Table 1.

Satsuma's and Clementine's pulps had almost the same dry matter while Satsuma peel had a little bit higher dry matter than Clementine's peel. Dry matter of pulps and peels was in accordance with literature data (Senser and Scherz, 1991; USDA, 2005; Levaj et al., 2005) as well as pH value (Del Caro et al., 2004; Levaj et al., 2005).

Total flavonoids content in Satsuma's and Clementine's pulps were almost in the same quantity, approximately 165 mg/100 g FW. The amounts of flavonoids (expressed as mg/g DM) in mandarin's peels were also similar, but obtained values were about 2.5 fold higher than in pulps. The amount of total flavonoids in mandarin peels obtained in presented investigation was in accordance with previously published data (Levaj et al., 2005). At the same time, total flavonoids content of mandarin pulps investigated in this work are approximately three fold higher then it was determined in cv. Kuno (Levaj et al., 2005) and it is higher than values what Abeysinghe et al. (2007) reported (84.5 and 120 mg RE/100 g FW in segments of *Citrus unshiu* and *Citrus reticulata*, respectively).

Three individual flavanon gycosides (hesperidin, narirutin and naringin) were identified in pulp and peel.

In pulp of both investigated mandarins the most abundant was hesperidin what is in accordance with Peterson et al. (2006). In Satsuma's pulp naringin was present in the lowest amount, while in Clementine's pulp the lowest amount of narirutin was determined. Peterson et al. (2006) gave critical review of flavanons in some citrus fruit and according to data in this review hesperidin is the main flavanon glycoside in mandarin, narirutin is present in remarkable lower level, and naringin is not present in mandarin. Del Caro et al. (2004) reported about flavanon glycosides in mandarin, orange and tangelo in decreasing sequence as follow: hesperidin>narirutin>didymin, while naringin was not detected. Further, Abeysinghe et al. (2007) also didn't detect naringin in mandarin while our results on mandarin didn't support such results. In past experience (results is still not published) we also identified naringin in mandarin grown in Neretva Valley although in the low level. Furthermore, content of hesperidin in pulps (in Satsuma 73.33 and in Clementine 51.35 mg/ kg FW) was similar as data from lit-

Table 1. Results of total dry matter, pH value, total flavonoids, individual flavanons (narirutin, naringin and hesperidin) and antioxidant capacity of Satsuma and Clementine

Parameters		Satsuma		Clementine	
		Pulp	Peel	Pulp	Peel
Total dry matter (%)		13.1±0.01 [a]	37.2±0.01	13±0.00	28.64±0.02
pH value		3.62±0.00	-	3.83±0.00	-
Total flavonoids	mg/100 g FW	165.4±2.93	1155.85±9.1	163.83±1.66	804.26±6.5
	mg/g DM	12.63±0.18	31.07±0.44	12.6±0.18	28.1±0.43
Narirutin	mg/kg FW	45±0.63	182.91±2.59	36.76±0.52	167.62±2.37
	mg/100 g DM	34.35±0.49	49.17±0.70	28.27±0.40	58.53±0.83
Naringin	mg/kg FW	18.84±0.27	106.91±1.51	43.25±0.61	135.21±1.91
	mg/100 g DM	14.38±0.20	28.74±0.41	33.27±0.47	47.21±0.67
Hesperidin	mg/kg FW	73.33±1.04	157.48±2.23	51.35±0.73	135.26±1.91
	mg/100 g DM	55.97±0.79	42.33±0.60	39.5±0.56	47.22±0.67
Antioxidant capacity	mmol Fe^{2+}/kg FW	17.61±0.25	87.87±1.24	25.26±0.36	95.09±1.34
	mmol Fe^{2+}/100 g DM	13.44±0.19	23.62±0.33	19.43±0.27	33.2±0.47

[a] – Data presented is in means ± standard deviation (n=3)

Table 2. Correlation coefficient between total flavonoids and individual flavanones and antioxidant capacity

Parameters		Correlation coefficients
Total flavonoids		0.98
Flavanon glycosides	Narirutin	0.87
	Naringin	0.97
	Hesperidin	0.96

erature. Peterson at al. (2006) reported hesperidin presence in range from 4.31 to 47.08 mg/100 g FW while Abeysinghe et al. (2007) determined hesperidin in higher level in *Citrus unshiu* and *Citrus reticulate*, 84.5 and 120 mg/100 g FW, respectively. Del Caro et al. (2004) reported content of hesperidin 0.489 mg/g DM in Palazzelli mandarin. Ortuño et al. (1997) reported 18.7 mg/100 g DM hesperidin in *Citrus reticulata* Blanco. Additionally, Xu et al. (2008) reported much higher level of hesperidin (450.60 mg/L and 337.44 mg/L, respectively) in Wase-Satsuma and Satsuma mandarin juices.

Narirutin was determined in 45.0 and 36.76 mg/kg FW in Satsuma and Clementine what were approximately 1.5 fold lower levels than hesperidin were. In general, such content of narirutin is in the range reported in the literature (Del Caro et al., 2004; Peterson et al., 2004). Del Caro et al. (2004) reported narirutin presence in 0.269 mg/g DM in Palazzelli mandarin, and Peterson et al. (2004) reported in range 0.00-7.70 mg/100 g FW in mandarin while in Wase-Satsuma and Satsuma mandarin juices Xu et al. (2008) also reported much higher level of narirutin (169 and 288 mg/L, respectively).

According to results presented in Table 1 peels contained higher level of investigated flavanones than pulp. The amounts of narirutin in mandarin's peels investigated in this work were the highest (approximately 1.2 folds higher than level of hesperidin) compared to amounts of other flavanon glycosides. These results are not in accordance with previously obtained results for the same cultivars (data are still not published). The reason could be different method of extraction as well as climatic conditions (fruits were harvested in different years), stage of ripeness, time and conditions of storage. (Ortuño et al., 1997; Del Caro et al., 2004).

In the literature there are not many papers dealing with flavonoids in peel but it is known that hesperidin is abundant flavanones in peel (Ma et al., 2008; Wang et al., 2008). Results obtained in this work showed that in peels flavanon glycosides were present in decreasing sequence as follow: narirutin>hesperidin>naringin, additionally in Clementine hesperidin and naringin were determined almost in the same amount.

Generaly, obtained results of Satsuma and Clementine showed that these two mandarins had very similar flavanones content without remarkable differences although Satsuma pulp and peel had higher content of hesperidin and narirutin than Clementine pulp which contained higher level of naringin.

Investigated peels showed much higher antioxidant capacity than pulps and Clementine's peel higher than Satsuma's peel.

According to obtained results it was obvious that there were remarkable high correlation between total flavonoids as well as all individual flavanones and antioxidant capacity. Correlation coeffitions were between 0.87-0.98 in descreasing sequence total flavonoids>naringin> hesperidin and narirutin almost the same (Table 2).

Conclusion

The pulps of both investigated mandarin fruits contained three flavonon glycosides (narirutin, naringin and hesperidin) among which hesperidin was predominant. In mandarin's peels flavanon glycosides were presented in decreasing sequence as follow: narirutin, hesperidin, naringin. According to FRAP method, all samples posses an evident antioxidant capacity especially peels. Correlation between total flavonoids, hesperidin, naringin, narirutin and antioxidant capacity was very high (correlation coefitions varied between 0.81-0.98).

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