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Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of four species

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Abstract

Different edible tissues of citrus fruit, namely juice sacs (JS), segment membrane (SM), and segment (Seg), of four species, were examined for contents of bioactive compounds and total antioxidant capacities (TAC) by ferric reducing antioxidant power (FRAP) assay. Two flavanones (naringin and hesperidin) were identified by HPLC; hesperidin accounted for 18.5–38.5% of the total phenolics in the species *Citrus unshiu*, *Citrus reticulata*, and *Citrus sinensis*, while naringin was only found in *Citrus changshanensis* and it accounted for 53.7% of the total phenolics in SM of this species. In SM of all selected species, the contents of phenolic compounds and TAC were significantly higher than those in JS and Seg. Highest total phenolics, total flavonoids, naringin, and TAC were found in SM of *C. changshanensis,* while the highest carotenoid content was found in JS of *C. reticulata*. The contribution of vitamin C to TAC ranged from 26.9% to 45.9% in JS and Seg of all selected species. In SM, however, a high contribution from hesperidin was observed in *C. unshiu* (54.0%), *C. sinensis* (46.7%) and *C. reticulata* (30.0%). The results indicated that SM of citrus fruit were high in contents of bioactive compounds and TAC; it is thus recommended to consume citrus fruit with all edible tissues rather than juice or JS alone.

Keywords: Antioxidant capacity; Bioactive compounds; Citrus; Edible tissues

1. Introduction

Nutritional studies are now concentrating on examining food for their protective and health-promoting potential. Epidemiological studies have reported that there is a significant positive association between consumption of fruit and vegetables and reduced risk of chronic diseases, such as cancer, cardiovascular disease, diabetes and Alzheimer's disease (Liu, 2003; Temple, 2000; Willett, 2002). Phytochemicals, such as alkaloids, phenolics, carotenoids, and various nitrogenous compounds, in fruit and vegetables, are reported to account for various bioactivities, e.g. antioxidant, antiproliferation, anti-fungal, antibacterial and antiviral activities (Dillard & German, 2000). Fruit are especially rich in natural antioxidants and these compounds can reduce oxidative damage in the human body, which would otherwise increase the risk of chronic diseases (Liu, 2003).

Citrus (*Citrus L.*) is one of the most important world fruit crops and is consumed mostly as fresh produce or juice because of its nutritional value and special flavour. Consumption of citrus fruit or juice is found to be inversely associated with several diseases (Joshipura et al., 2001). Knekt et al. (2002) reported that intake of orange resulted in reducing incidence of asthma in Finland. Citrus fruit extracts are also found to have activities, such as anti-inflammatory, anti-tumor, anti-fungal and blood clot inhibition activities (Middleton & Kandaswami, 1994; Olson, 1988; Yehoshua, Rodov, Fang, & Kim, 1995). The health benefits of citrus fruit have mainly been attributed to the presence of bioactive compounds, such as phenolics (e.g.

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flavanone glycosides, hydroxycinnamic acids) (Rossa, Ziskac, Ke Zhaod, & ElSohlya, 2000), vitamin C (Knekt et al., 2004), and carotenoids (Craig, 1997). So far, studies on bioactive compounds and TAC of citrus have mainly focussed on comparison of fruit peel, whole segment (Gorinstein et al., 2004a; Yoo, Lee, Park, Lee, & Hwang, 2004) and juice (Gardner, White, McPhail, & Duthie, 2000; Gorinstein et al., 2004b). There have been no reports comparing the nutritional and health-promoting values of different edible tissues of citrus fruit. The objective of this study was to determine the contents of bioactive compounds and the antioxidant capacity in different edible tissues, namely juice sacs (JS), segment membrane (SM), and segment (Seg), of fruit of four citrus species.

2. Materials and methods

2.1. Fruit and sample preparation

Fruit of four citrus species were obtained from Quzhou Citrus Institute in Zhejiang, China, in 2005. They can be categorized into three types: mandarin type (*Citrus unshiu* and *Citrus reticulata*), orange type (*Citrus sinensis*) and hybrid type (*Citrus changshanensis*).

Different edible tissues of citrus fruit (JS, SM and Seg), of each species were manually separated. They were frozen in liquid nitrogen and then stored at −20 °C prior to analysis. A completely randomized design with three replicates was used in the experiment.

2.2. Chemicals and reagents

Ferric chloride ($FeCl_3 \cdot 6H_2O$), 2,4,6-tripyridyl-2-triazine (TPTZ), chlorogenic acid, rutin and Folin-Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox), were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Naringin and hesperidin were purchased from the National Institute for the Control of Pharmaceutical and Biological Products in China. All other reagents used were of analytical grade.

2.3. Extraction

The phenolic compounds were extracted according to the method reported by Gorinstein et al. (2004b) with slight modifications. The frozen fruit was ground into fine powder in a mortar with liquid nitrogen and 0.5 g of ground sample was accurately weighed in a screw-capped tube. The phytochemicals were extracted with 5 ml of 1.2 M HCl in 80% methanol/water and vortexed for 1 min. The samples were then heated at 90 °C for 3 h, with vortexing every 30 min. After the samples reached room temperature, they were diluted to 10 ml with methanol and centrifuged at 10,000*g* for 5 min to remove the solid fraction. The supernatant was used for determination of total phenolics, total flavonoids, and two flavanones (naringin and hesperidin).

2.4. Determination of naringin and hesperidin contents

The naringin and hesperidin contents of fruit extracts were determined according to the method of Kanaze, Gabrieli, Kokkalou, Georgarakis, and Niopas (2003) with modifications by using reversed-phase HPLC coupled to a 166 UV detector (Beckman, USA). The chromatographic separation was performed on an ODS C18 column (4.6 × 150 mm) (Beckman USA). The mobile phase consisted of 75 mM citric acid and 25 mM ammonium acetate in double-distilled water (A) and methanol (B) with a ratio of 60:40 (v/v) at a flow rate of 1 ml/min. The injection volume was 20 μl and the detection wavelength was set at 282 nm. The retention times and spectra were compared to those of authentic standards. The contents of naringin and hesperidin were expressed in mg/100 g FW.

2.5. Determination of total phenolic content

Total phenolic contents of the fruit extracts were measured using a modified colorimetric Folin-Ciocalteu method (Cai, Luo, Sun, & Corke, 2004). Four millilitres of distilled water and 0.5 ml of properly diluted fruit extract were placed in a test tube. Folin-Ciocalteu reagent (0.5 ml) was added to the solution and allowed to react for 3 min. The reaction was neutralized with 1 ml of saturated sodium carbonate. Absorbance at 760 nm was read after 2 h, using a spectrophotometer (Beckman Coulter DU-800, USA). Chlorogenic acid was used as standard and data were expressed as mg chlorogenic acid equivalents (CAE)/100 g FW.

2.6. Determination of total flavonoid content

The total flavonoid content of the samples was measured using a modified colorimetric method (Yuan, Liu, & Hu, 1996). Properly diluted fruit extract (0.5 ml) was added to a test tube containing 3.5 ml of absolute ethanol. After addition of 4 ml of 90% diethylene glycol and thorough mixing, the reaction was initiated by adding 0.1 ml of 4 M sodium hydroxide. Absorbance at 420 nm was measured after 10 min of incubation at 40 °C using a spectrophotometer (Beckman Coulter DU-800, USA). Rutin was used as the standard and total flavonoid content was expressed as mg rutin equivalents (RE)/100 g FW.

2.7. Determination of vitamin C content

Vitamin C was extracted and determined according to a procedure reported by Gardner et al. (2000) with modifications. Vitamin C in citrus fruit tissues was extracted from 1 g of ground samples, using 10 ml of 1% (w/v) oxalic acid. After sonication (10 min) and centrifugation (10,000*g*, 4 °C, 10 min), the supernatant was injected into a reversed phase HPLC system (Beckman, USA) with UV detection. The conditions of the system were: ODS C18 column (4.6 × 250 mm) (Beckman USA); flow rate of 1.0 ml/min;

injection volume of 20 μl; detector wavelength set at 251 nm. Ammonium acetate buffer (0.02 M, pH 5.4) with 1 mM octylamine (dissolved in methanol) was used as mobile phase. The vitamin C content was expressed as mg /100 g FW.

2.8. Determination of carotenoid content

Total carotenoids were extracted and determined, using the method adopted by Fraser, Pinto, Holloway, and Bramley (2000) with modifications. Four millilitres of chloroform were added to a test tube containing 1 g of ground fruit tissue and 2 ml of methanol. Then, 2 ml of 10% sodium chloride were added and vortexed. The mixture was centrifuged at 8000*g* for 15 min at 4 °C and the coloured chloroform layer was collected in a new tube. The chloroform extraction was repeated three times until the chloroform layer became colourless. The chloroform layers were pooled and evaporated to dryness in a rotary evaporator. The residue was resuspended in 2 ml of 6% methanolic KOH solution (w/v) and samples were saponified for 30 min at 60 °C. The samples were cooled in an ice bath and 4 ml of chloroform were added to each tube. The suspensions were washed thrice with 4 ml of water by vigorous vortexing, followed by centrifugation at 8000*g* for 15 min at 4 °C. Then chloroform layer was carefully separated and absorbance at 450 nm was measured using a spectrophotometer (Beckman Coulter DU-800, USA). The carotenoid content was expressed as mg β-carotene equivalents (BCE)/100 g FW.

2.9. Quantification of TAC

TAC was determined using the FRAP assay (Benzie & Strain, 1996; Connor, Luby, Hancock, Berkheimer, & Hanson, 2002) with modifications. The FRAP reagent was freshly prepared by mixing 25 ml of 300 mM sodium acetate buffer (pH 3.6), 2.5 ml of 10 mM TPTZ solution, and 2.5 ml of 20 mM ferric chloride solution. The absorbance at 593 nm was measured 24 h after the mixing of 100 μl of fruit extract (or antioxidant standard) with 900 μl of FRAP reagent, using a spectrophotometer (Beckman Coulter DU-800, USA). The TAC was expressed as μmol trolox equivalents (TE)/g FW.

2.10. Statistical analysis

Statistical comparisons of the mean values were performed by analysis of variance (ANOVA), followed by Duncan's multiple-range test ($P < 0.05$), using SAS 8.3 software (SAS Ins. Inc., Cary, USA).

3. Results and discussion

3.1. Identification and quantification

Phenolics are secondary metabolic products, which mainly include flavonoids (namely flavanones, flavonols, flavones, isoflavonoids, flavanols, anthocyanidins), phenolic acids, stilbenes, coumarins and tannins (Liu, 2004). In addition to vitamin C and carotenoids, a variety of phenolic compounds, namely flavanone glycosides, hydroxy-cinnamic acids, are present in citrus fruit as bioactive compounds (Caro, Piga, Vacca, & Agabbio, 2004; Gorinstein et al., 2004a). Naringin and hesperidin, so-called citrus flavonoids, are two major flavanone glycosides present in citrus fruits (Caro et al., 2004; Kanaze et al., 2003; Ortuno et al., 1997). HPLC, using either normal or reversed-phase columns, has been reported to identify and quantify flavonoids in citrus (Bronner & Beecher, 1995; Kanaze et al., 2003; Kanaze, Kokkalou, Georgarakis, & Niopas, 2004). In this study, the methanolic extracts of different edible tissues of four citrus species were analyzed by a modified reversed-phase HPLC system with a better resolution and low detection limits of 0.01 μg/ml and 0.03 μg/ml for naringin and hesperidin, respectively. The chemical structures and HPLC profiles of naringin and hesperidin are shown in Fig. 1.

There was significant variation in the contents of naringin and hesperidin in different edible tissues of all selected citrus species (Table 1). Naringin was detected only in *C. changshanensis* and its contents in JS, Seg and SM were 19.3, 75.9 and 492 mg/100 g FW, respectively. The naringin content in SM accounted for 53.7% of total phenolics in *C. changshanensis*, indicating that naringin is a major phenolic compound present in SM of *C. changshanensis*. Naringin could not be detected in the other three species, which was consistent with the results of mandarin and orange type of citrus fruit reported by Ortuno et al. (1997) and Caro et al. (2004).

Hesperidin was found in the edible tissues of all selected citrus species except in JS and Seg of *C. changshanensis* (Table 1). The contents of hesperidin were also significantly higher in SM than in JS and Seg of all the four species, with the highest hesperidin content found in SM of *C. unshiu* (293 mg/100 g FW). As far as the JS and Seg are concerned, *C. sinensis* had the highest hesperidin content, with 63.0 and 105 mg/100 g FW, respectively. In SM, a comparatively low content of hesperidin was detected in *C. changshanensis* (38.0 mg/100 g FW), when compared to either the hesperidin content of different species or the naringin content of the same species (Table 1). Hesperidin accounted for 18.5–38.5% of the total phenolics in the species *C. unshiu*, *C. reticulata*, and *C. sinensis*, indicating that it is a main component of phenolics present in these three citrus species.

Previous studies have shown the presence of higher contents of phenolic compounds in fruit peel than segment of citrus (Gorinstein et al., 2001). So far, there are no studies on comparison of contents of phenolic compounds in different edible tissues of citrus. Significant differences in the contents of total phenolics and total flavonoids among different edible tissues of selected citrus species were observed in present study (Table 2). Of all selected species, the contents of total phenolics and total flavonoids were signifi-

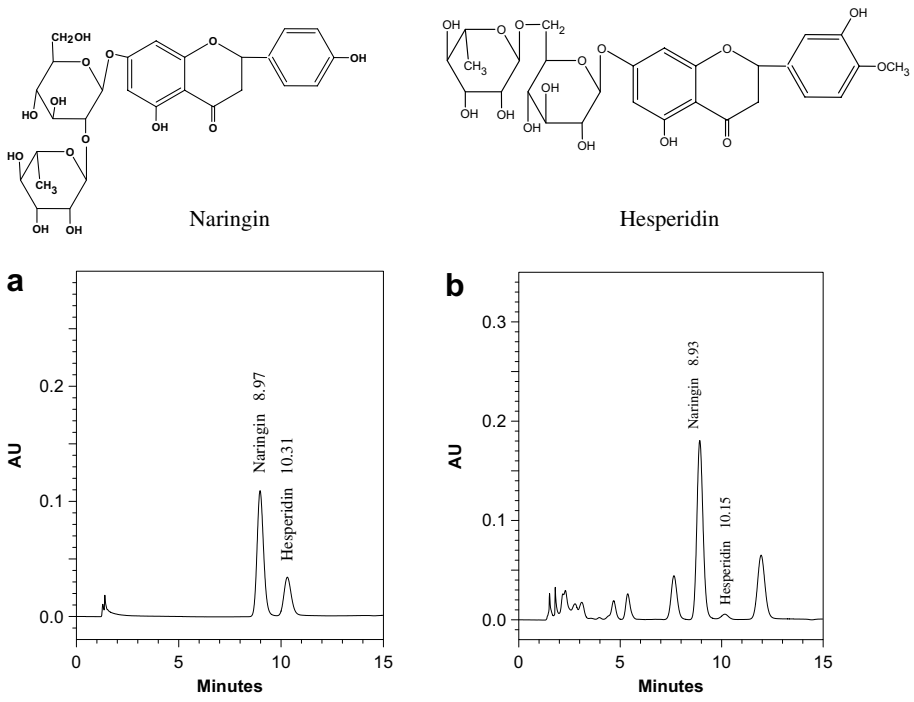

Fig. 1. HPLC profiles of naringin and hesperidin. (a) Standards; (b) Samples isolated from SM of *C. changshanensis* fruit.

Table 1
Contents of naringin and hesperidin in different edible tissues of citrus fruit of four species

Citrus species	Edible tissues	Flavanone content[1]	
		Naringin	Hesperidin
C. unshiu	JS	nd	40.0 g
	SM	nd	293 a
	Seg	nd	74.3 e
C. reticulata	JS	nd	49.3 fg
	SM	nd	161 c
	Seg	nd	58.9 f
C. sinensis	JS	nd	63.0 ef
	SM	nd	246 b
	Seg	nd	105 d
C. changshanensis	JS	19.3 c	nd
	SM	492 a	38.0 g
	Seg	75.9 b	nd

Means with different letters within the same column represent significant differences at $P < 0.05$.
[1] mg/100 g FW.

cantly higher in SM than in JS and Seg, with the highest contents of total phenolics and total flavonoids found in SM of *C. changshanensis* (916 mg CAE/100g FW and 713 mg RE/100 g FW, respectively). These results provide important information for a comprehensive utilization of citrus SM in citrus juice manufacturing.

Significant differences in the contents of carotenoids and vitamin C were also observed in selected citrus species and among different edible tissues (Table 2). Carotenoid content was significantly higher in mandarin type species, such as *C. unshiu* and *C. reticulata*, than that in the orange type, *C. sinensis* and hybrid type, *C. changshanensis*. Fanciullino et al. (2006) also recorded that mandarin type citrus had higher contents of carotenoids than orange or pomelo types of citrus. In addition, JS and Seg of all selected citrus species had significantly higher carotenoid contents than had SM. The vitamin C content of JS and Seg of all selected species ranged from 25.4 to 45.3 mg/100 g FW.

3.2. Total antioxidant capacity

The TAC varied significantly among different edible tissues of four citrus species (Fig. 2). TAC in SM was significantly higher than in JS and Seg for all selected citrus species, with the highest TAC found in the SM of *C. changshanensis* (14.7 μmol TE/g FW). TAC showed significant correlations with the contents of total phenolics ($r = 0.89$, $P < 0.001$) and total flavonoids ($r = 0.82$, $P < 0.001$). The highest TAC in SM could be attributed to the high total phenolic and total flavonoid contents in SM. As far as the JS and Seg are concerned, *C. changshanensis* and *C. sinensis* showed the highest TAC values, respectively. Such results indicated the effects of both different edible tissues and different species on the antioxidant capacity of citrus fruit.

3.3. Contributions of naringin, hesperidin and vitamin C to TAC

The contributions of naringin, hesperidin and vitamin C to the TAC in different edible tissues of selected citrus species were analyzed (Fig. 3). Data were calculated from the percentage of TAC contributed by each bioactive

Table 2
Contents of total phenolics, total flavonoids, carotenoids and vitamin C in different edible tissues of citrus fruit of four species

Citrus species	Edible tissues	Total phenolics[1]	Total flavonoids[2]	Carotenoids[3]	Vitamin C[4]
C. unshiu	JS	184 i	47.0 g	1.02 c	25.4 f
	SM	779 c	332 b	0.34 f	32.6 e
	Seg	230 h	84.5 e	0.74 d	26.1 f
C. reticulata	JS	251 g	64.8 f	1.36 a	45.3 a
	SM	801 b	278 c	0.62 e	14.2 h
	Seg	318 e	120 d	1.21 b	38.9 c
C. sinensis	JS	217 h	78.0 ef	0.30 f	32.9 e
	SM	752 d	274 c	0.16 h	35.2 d
	Seg	273 f	121 d	0.25 g	35.2 d
C. changshanensis	JS	210 h	66.6 ef	0.35 f	41.6 b
	SM	916 a	713 a	0.14 h	15.7 g
	Seg	280 f	136 d	0.30 f	32.7 e

Means with different letters within the same column represent significant differences at $P < 0.05$.
[1] mg CAE/100 gFW.
[2] mg RE/100 gFW.
[3] mg BCE/100 gFW.
[4] mg/100 gFW.

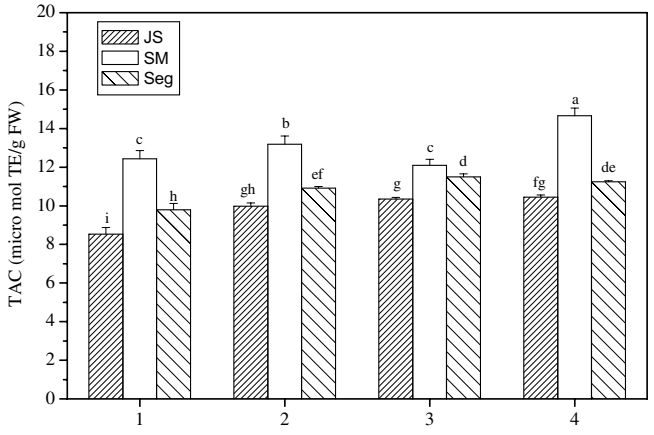

Fig. 2. TAC of different edible tissues of citrus fruit of four species. Means with the same letter represent non-significant differences ($P < 0.05$). (1) *C. unshiu*; (2) *C. reticulata*; (3) *C. sinesis*; (4) *C .changshanensis*.

compound present in different tissues of citrus species, where the total antioxidant capacity of each fruit sample was assigned as 100%. Based on the FRAP assay in the present study, the antioxidant capacity caused by the vitamin C standard was higher than those caused by naringin and hesperidin, which may be due to the variation in their chemical structures (Burda & Oleszek, 2001). Several studies have shown that vitamin C is not the predominant antioxidant in fruits, such as apple (0.40–0.80%), grape (0.35%), pear (0.67%), and pineapple (0.80–5.20%) (Gardner et al., 2000; Sun, Chu, Wu, & Liu, 2002). In our study, vitamin C was found as a main contributor of TAC in JS and Seg of all selected citrus species since its contribution ranged from 26.9% to 45.9% (Fig. 3). In reported citrus research, it was found that orange and grapefruit contributed 8.16% and 8.57%, respectively (Sun et al., 2002). However, Gardner et al. (2000) reported vitamin C as a major antioxidant in citrus juice and found that it contributed 65–100% to the TAC. Also, Yoo et al. (2004) recorded a 90.4% contribution of vitamin C in citrus fruit. This indicates a wide variation in vitamin C contribution in different fruit species and even different cultivars within citrus species.

The percentage contribution of hesperidin to the TAC ranged from 5.9% to 54.0% for all tissues that contained it. The contribution of hesperidin to TAC was higher than that of vitamin C in SM of all selected citrus species except *C. changshanensis*. In addition, a very low

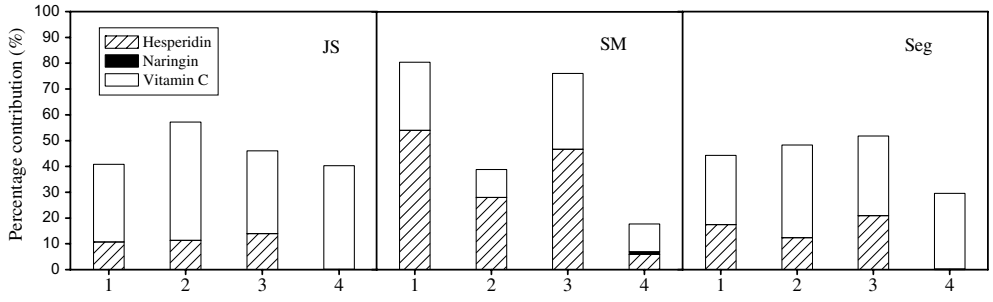

Fig. 3. Percentage contributions of hesperidin, naringin and vitamin C to the TAC in different edible tissues of citrus fruit of four species. (1) *C. unshiu*; (2) *C. reticulata*; (3) *C. sinesis*; (4) *C .changshanensis*.

percentage contribution by naringin to TAC was found in JS (0.05%), Seg (0.20%) and SM (0.97%) of *C. changshanensis* species. The antioxidant capacity caused by the naringin standard was even much lower than that caused by the hesperidin standard, which may be due to the differences in both the number and position of hydroxyl groups present in their chemical structures (Burda & Oleszek, 2001). As β-carotene was found to be un-reactive with FRAP (Gardner et al., 2000), the contribution of the low content of carotenoids detected to the total antioxidant capacity of citrus was not analyzed in the present study.

In this study, the total contribution of naringin, hesperidin and vitamin C to the TAC ranged from 17.7% to 80.5% in different fruit tissues of four species, indicating that TAC may be contributed by other bioactive compounds as well.

4. Conclusions

Our study, on four citrus species, of bioactive compounds and antioxidant capacity, showed effects of different edible tissues and species on the content of dietary phytochemicals and health-promotion value of selected citrus fruit. SM contained significantly higher amounts of total phenolics, total flavonoids, two flavanones (naringin and hesperidin), and higher antioxidant activity than did Seg and JS of four citrus species. While vitamin C contributed significantly to the TAC in JS and Seg of all citrus species, hesperidin is the major contributor in SM of *C. unshiu*, *C. reticulata* and *C. sinensis* species. Our results indicate that SM of citrus fruit is higher in contents of bioactive compounds and TAC. It is thus recommended to consume citrus fruit with all edible tissues rather than juice or JS alone.

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