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Phenolic Compounds and Antioxidant Activity of Extracts from Ultrasonic Treatment of Satsuma Mandarin (*Citrus unshiu* Marc.) Peels

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Ultrasound-assisted extraction (UAE) was used to extract phenolic compounds from Satsuma mandarin (*Citrus unshiu* Marc.) peels (SMP), and maceration extraction (ME) was used as a control. The effects of ultrasonic time (10, 20, 30, 40, 50, and 60 min), temperature (15, 30, and 40 °C), and ultrasonic power (3.2, 8, 30, and 56 W) on phenolic compounds were investigated. High-performance liquid chromatography (HPLC) coupled with a photodiode array (PDA) detector was used for the analysis of phenolic acids after alkaline hydrolysis (bound phenolic acids) and flavanone glycosides. The contents of seven phenolic acids (caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, protocatechuic acid, *p*-hydroxybenzoic acid, and vanillic acid) and two flavanone glycosides (narirutin and hesperidin) in extracts obtained by ultrasonic treatment were significantly higher than in extracts obtained by the maceration method. Moreover, the contents of extracts increased as both treatment time and temperature increased. Ultrasonic power had a positive effect on the contents of extracts. However, the phenolic acids may be degraded by ultrasound at higher temperature for a long time. For example, after ultrasonic treatment at 40 °C for 20 min, the contents of caffeic acid, *p*-coumaric acid, ferulic acid, and *p*-hydroxybenzoic acid decreased by 48.90, 44.20, 48.23, and 35.33%, respectively. The interaction of ultrasonic parameters probably has a complex effect on the extracts. A linear relationship was observed between Trolox equivalent antioxidant capacity (TEAC) values and total phenolic contents (TPC); the correlation coefficient, R^2, is 0.8288 at 15 °C, 0.7706 at 30 °C, and 0.8626 at 40 °C, respectively. The data indicated that SMPs were rich sources of antioxidants. Furthermore, UAE techniques should be carefully used to enhance the yields of phenolic acids from SMPs.

KEYWORDS: Ultrasound-assisted extraction; phenolic acids; antioxidant activity; Satsuma mandarin (*Citrus unshiu* Marc.) peels

INTRODUCTION

Citrus fruits are a significant part of the human diet. Previous investigations have proved that citrus fruits are a good source of phenolics (*1, 2*). Citrus peels, a byproduct during the manufacture of canned citrus fruits, are rich in numerous biologically active compounds such as vitamin C, phenolic acids, and flavonoids (*3, 4*). Phenolic compounds have been found to possess antiallergenic, antiartherogenic, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, cardioprotective, and vasodilatory effects (*5–7*). These compounds have been associated with healthy properties ascribed to their antioxidant activity and free radical scavenging abilities (*8*). The dried citrus peel is taken traditionally as a medicine name *chenpi* in China and Japan.

Phenolic compounds and antioxidant activities in citrus peel have been widely investigated. Bocco et al. (*4*) reported that methanol extracts in citrus peel are rich in flavones and glycosylated flavanones, whereas hydrolyzed extracts contain mainly phenolic acids and flavonols. A comparison between the suppressive activity of fresh and dried peels from Satsuma mandarin (*Citrus unshiu* Marcorv.) showed that methanol extracts in dried peels gave much higher antioxidant activities (*9*). In the papers by Jeong et al. (*10*) and Xu et al. (*11*), heat treatment may contribute to the release of some low molecular weight phenolic compounds and hence increase the antioxidant capacity of extracts in citrus peel. It was observed that the contents of phenolics and antioxidant activities of extracts from citrus peels are dependent on extraction system. As reported by Li et al. (*12, 13*), enzyme-assisted extraction gave higher contents of phenolics and antioxidant activities than solvent extraction. Furthermore, citrus peel condition, extraction tem-

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perature, solvent concentration, enzyme type, enzyme concentration, and species of citrus seem to influence the yields of phenolic compounds and antioxidant activities. However, the enzyme is sensitive to temperature and inactivated easily at the higher temperature, suggesting that application of enzyme-assisted extraction phenolics from citrus peel was limited. Organic extraction such as maceration extraction and Soxhlet extraction have low efficiency in long extraction time and require large volumes of organic solvent, which can result in environmental pollution due to volatilization during concentration steps. Therefore, it is necessary to establish an environmentally friendly method, by which the phenolics could be extracted in a shorter time and in higher efficiency.

Ultrasound-assisted extraction (UAE) is a potential technology due to the cheaper technique and the lower instrumental requirements. The characteristics of UAE are significantly reduced extraction times, decreased extraction temperature, and increased extraction yields. The mechanism for ultrasonic enhancement is mainly attributed to behaviors of the bubbles of cavitation upon the propagation of the acoustic waves (*14*). Collapse of bubbles can produce physical, chemical, and mechanical effects (*15*), which resulted in the disruption of biological cell walls to facilitate the release of extractable compounds and enhance mass transport of solvent from the continuous phase into plant cells (*16, 17*).

Recently, ultrasonic techniques have been widely used in the extraction of various phytochemicals, such as pectin, flavonoids, alkaloids, polysaccharides, oil, and phenols, from different parts of plants and plant seeds (*18–25*). Moreover, application of ultrasound in citrus plants, for example, extraction of volatile compounds from citrus flowers and citrus honey (*26*) and processing of citrus juice (*27*), has shown a positive effect. Our previous research on ultrasound-assisted extraction of hesperidin from penggan peels has also indicated that UAE can improve the yields of hesperidin compared to Soxhlet extraction (*28*). However, to date, there have been few reports on the application of UAE to isolate the phenolic compounds of extracts from Satsuma mandarin peels (SMPs). In the present paper, a comparison between UAE and maceration extraction was performed. Phenolic compounds and antioxidant activities in extracts from SMPs by UAE were investigated.

MATERIALS AND METHODS

Chemicals. Standards of caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, narirutin, and hesperidin were obtained. The chemical structures are shown in **Figure 1**. 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and Folin−Ciocalteu's phenol reagent were purchased from Sigma (St. Louis, MO). Methanol (HPLC grade), glacial acetic acid (HPLC grade), and redistilled water were filtered through a 0.45 μm membrane before use. All other chemical reagents used in experiments were of analytical grade.

Materials. Satsuma mandarin peels were purchased from a citrus-processing plant in Ningbo city, Zhejiang province, China, in October 2006. SMPs were dried in an oven with air circulation at 50 °C, and then they were ground finely in the laboratory with a blade mixer to pass through a 0.45–1 mm screen and stored at −5 °C until use.

Plant Material Treatment. Conventional maceration extraction was performed as a control for comparison with ultrasound-assisted extraction methods. Powders of 5 g of SMP and 100 mL of 80% methanol were put into a 200 mL flask and were treated in a 40 °C water bath without shaking for 1, 5, and 8 h.

The ultrasonic cleaning bath was designed and manufactured by Guangzhou Sonoc Ultrasonic Electronic Equipment Co. Ltd., China, and has a frequency of 20 kHz with a variable-power output, a digital timer, a temperature controller, and a voltage meter. An electric current meter was used for measuring the electrical power consumed. The bottom of the water tank was made in the shape of quadrangular frustum of a pyramid equipped with five sonic generators, one on each side. The schematic diagram of the ultrasonic apparatus is given in a previous paper (*28*). A powder of 2 g of SMP was loaded into a 600 mL flask (8 cm diameter × 14.5 cm height), and 40 mL of 80% methanol was added. UAE was performed at 3.2 W and at 15, 30, and 40 °C for 10, 20, 30, 40, 50, and 60 min. Ultrasonic powers (3.2, 8, 30, and 56 W) were investigated at 15 °C for 20 min.

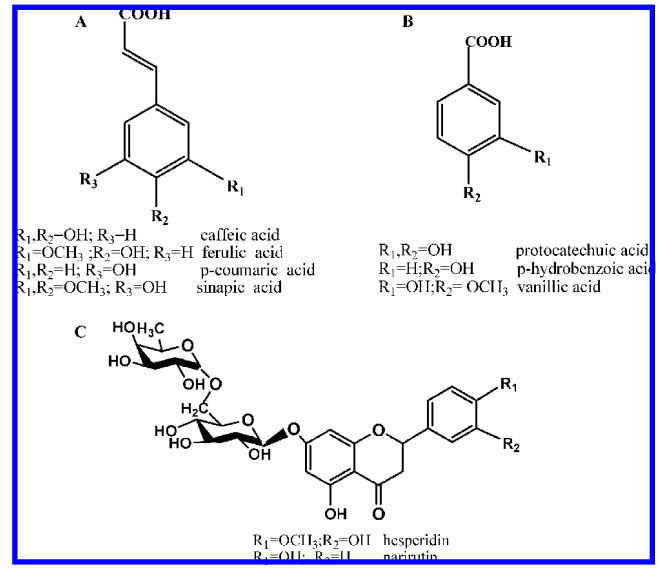

Figure 1. Chemical structures of the phenolic compounds (**A**, cinnamic acids; **B**, benzoic acids) and the flavanone glycosides (**C**).

Extraction of Phenolic Acids and Flavanone Glycosides. Extracts by ultrasonic treatment were filtered off through a 0.45 μm microporous membrane. Then it was taken out for analysis of flavanone glycosides and evaluation of antioxidant capacity.

Phenolic acids were isolated from extracts according to some previously described methods (*29, 30*) with certain modifications. A 10 mL aliquot of the filtrate was evaporated using a rotary vacuum evaporator at 35 °C until approximately 1 mL of filtrate remained. The residue was hydrolyzed with 10 mL of 4 M NaOH for 4 h at room temperature and then acidified to pH 2 with 4 M HCl. The solution acidified was extracted three times with 20 mL of diethyl ether/ethyl acetate (1:1, v/v). The clear organic phase was combined and concentrated to dryness in a rotary vacuum evaporator at 35 °C, and 80% methanol was added to make a final volume of 10 mL. The methanol solution was filtered through a 0.45 μm microporous membrane and used for the identification and quantification of the bound phenolic acids.

High-Performance Liquid Chromatography (HPLC) Analysis. HPLC analysis was performed with a Waters 2695-2996 system (Waters Corp., Milford, MA) equipped with a 515 pump and a photodiode array (PDA) detector. The separation was carried out on a C-18 reversed phase column (250 mm × 4.6 mm, i.d. = 5 μm, Dilma Technologies Co. Ltd.). Column temperature was maintained at 40 °C, and the injection volume for all samples was 10 μL. Elution was performed isocratically with the mobile phase consisting of 4% (v/v) acetic acid in water/100% methanol (80:20, v/v) at a solvent flow rate of 1 mL/min. The column was washed with 100% methanol and equilibrated to initial conditions for 15 min before each injection (*11*). UV–visible spectral measurements were made over the range of 210–400 nm. Chromatograms were recorded at 320 nm for caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid and at 260 nm for protocatechuic acid, *p*-hydroxybenzoic acid, and vanillic acid. Identification of phenolic acids was based on retention times and UV–visible spectra in comparison with standards. Quantification of phenolic acids was achieved by the absorbance recorded in the chromatograms relative to external standards. The concentration of the phenolic acids was calculated from peak area according to calibration curves. The amount

of each phenolic acid was expressed as micrograms per gram of dry weight (μg/g of DW).

The contents of two flavanone glycosides were determined according to a previous method described in ref *11* with some modification. Prepared extract solution was filtered through a Millipore membrane (0.45 μm) before injection. The mobile phase was methanol/4% (v/v) acetic acid in water (37:63, v/v) at a flow rate of 1 mL/min; the column temperature was 40 °C, and the sample volume injected was 10 μL. The optimum detecting wavelength for narirutin and hesperidin was 283 nm. Identification of the flavanone glycosides was performed by comparing the retention times of peaks in extracts to those of flavanone glycoside standards. Flavanone glycoside concentration (expressed as micrograms per gram of DW) was calculated by an external standard method using calibration curves.

Total Phenolic Contents (TPC). The TPC of the SMP samples were measured using a modified colorimetric Folin−Ciocalteu method (*31*). A volume of 0.25 mL of methanol extracts was added to a 25 mL volumetric flask, and additional ddH_2O was added to make a final volume of 10 mL. A reagent blank was prepared using ddH_2O. Folin−Ciocalteu phenol reagent (0.5 mL) was added to the mixture and shaken vigorously. After 5 min, 5 mL of 6% Na_2CO_3 solution was added with mixing. The solution was immediately diluted to volume (25 mL) with ddH_2O and mixed thoroughly and then allowed to stand for 30 min. After that, the absorbance was measured at 760 nm versus the prepared blank. The TPC of sample was expressed as gallic acid equivalent (GAE) milligrams per gram of DW (mg of GAE/g of DW).

Total Antioxidant Activity. The total antioxidant activity of the SMP components was determined using a modified ferric reducing ability assay (FRAP) (*32*). The stock solutions included 200 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM ferric chloride solution. The fresh working solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of ferric chloride solution. Extracts from SMP (100 μL) were allowed to react with 4900 μL of the FRAP solution (working solution) for 30 min in the dark. Then a mixture of readings at the absorption maximum (593 nm) was taken using a Shimadzu UV–visible 2550 spectrophotometer. Trolox solution was used to perform the calibration curves. The result was expressed as Trolox equivalent antioxidant capacity (TEAC) milligrams per gram of DW.

Statistical Analysis. All data were reported as mean ± standard deviation of three replicates. The results were compared by analysis of variance (ANOVA) 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 with the family error rate held at 0.05.

RESULTS AND DISCUSSION

Phenolic Acids and Flavanone Glycosides. Previous investigations showed that the majority of phenolic acids in the citrus fruits are presented in the bound forms (*33, 34*). Therefore, in the present study, the main phenolic acids in SMP were hydrolyzed to analyze their free forms. The phenolic acids including cinnamic acids (caffeic, *p*-coumaric, ferulic, and sinapic acids) and benzoic acids (protocatechuic, *p*-hydroxybenzoic, and vanillic acids), TCE, and two flavanone glycosides such as narirutin and hesperidin from SMP were evaluated. Conventional maceration extraction of phenolic acids from SMP was treated as a control compared to UAE.

Phenolic acids obtained by both ultrasonic treatment and maceration extraction are listed in **Table 1**. With increase of maceration time, the contents of phenolic acids significantly increased. For example, after maceration treatment at 40 °C from 1 to 8 h, the contents of caffeic, ferulic, sinapic, and vanillic acids increased by 154.57, 301.88, 227.45, and 55.95% and TCE increased by 240.67%, respectively. These values were significantly different ($p < 0.05$). However, the values of TCE, after UAE at 30 °C for 10 min, were significantly higher than those

Table 1. Comparison of Flavanone Glycosides, Phenolic Acids of Extracts, and Total Content of Extracts (Micrograms per Gram of Dry Weight) from Satsuma Mandarin Peel with Maceration Extraction a 40 °C for 1, 5, and 8 h and Ultrasound-Assisted Extraction with 8 W at 30 °C for 10 min[a]

extraction method	extraction time	flavanone glycosides		phenolic acids						TCE
		narirutin	hesperidin	caffeic	*p*-coumaric	ferulic	sinapic	protocatechuic	*p*-hydroxybenzoic	vanillic	
ME	1 h	87.80 ± 5.44 dc	323.54 ± 15.76 d	12.46 ± 2.63 c	23.00 ± 1.72 c	189.99 ± 1.37 c	40.40 ± 5.97 c	17.57 ± 0.10 a	14.99 ± 0.22 c	19.23 ± 0.15 c	312.63 ± 12.17 c
	5 h	108.05 ± 15.31 c	493.75 ± 34.38 c	28.28 ± 0.07 b	59.19 ± 1.03 b	735.54 ± 3.55 b	123.15 ± 0.34 b	20.27 ± 0.14 a	22.11 ± 0.19 b	27.36 ± 0.30 b	1015.89 ± 5.63 b
	8 h	152.33 ± 10.45 b	601.22 ± 52.43 b	31.72 ± 1.52 b	63.15 ± 1.91 b	763.54 ± 19.82 b	132.29 ± 0.16 a	20.68 ± 0.93 a	23.50 ± 0.75 b	29.99 ± 0.56 b	1065.03 ± 25.64 b
UAE	10 min	296.73 ± 34.52 a	1077.62 ± 83.95 a	64.28 ± 1.94 a	140.83 ± 23.21 a	1513.21 ± 19.27 a	132.71 ± 3.86 a	15.87 ± 0.64 b	34.11 ± 1.05 a	34.11 ± 0.55 a	1935.12 ± 50.52 a

[a] Mean ± SD of three measurements. Means in each column with different letters are significantly different ($p < 0.05$). ME, maceration extraction; UAE, ultrasound-assisted extraction; TCE, total content of extracts (seven phenolic acids).

by ME at 40 °C for 8 h ($p < 0.05$), indicating that ultrasonic treatments gave higher extraction efficiencies at lower temperature for shorter time when compared to the maceration method.

To improve the efficiency of UAE, establishing appropriate extraction parameters relating to specific vegetal materials is very important (*35*). In our work, extraction time, temperature, and ultrasonic power were chosen as ultrasonic variables.

The effects of UAE at different temperatures from 10 to 60 min on the contents of phenolic acids from SMP are shown in **Table 2**. At relatively lower temperature (15 and 30 °C), the contents of phenolic acids and TCE increased as treatment time increased. For example, after UAE at 30 °C for 20 min, caffeic, *p*-coumaric, ferulic, sinapic, and *p*-hydroxybenzoic acid increased by 50.31, 24.92, 45.12, 32.96, and 29.49% and TCE increased by 41.79%, respectively. Likewise, ultrasonic temperature has a positive effect on the contents of extracts. For example, after UAE from 15 to 40 °C for 10 min, the contents of caffeic, *p*-coumaric, ferulic, sinapic, and *p*-hydroxybenzoic acid increased by 545.47, 357.75, 405.17, 346.27, and 74.80%, respectively. Thus, both treatment time and temperature have significant effects on the contents of extracts, but variations in ultrasonic temperature have always a higher effect on the extraction efficiencies of all detected phenolic compounds than those of ultrasonic time. This is partly because increases of temperature favored extraction by accelerating both the solubility of solute and the diffusion coefficient. However, higher temperature may also induce degradation of some phenolic compounds. Cacace et al. (*36*) showed that an extraction temperature of >50 °C affected the denaturation of membranes and the stability of phenolic compounds. In our investigation, a decrease of the contents of extracts was also found after UAE at relatively higher temperature for extended extraction time. For example, after ultrasonic treatment at 40 °C for 20 min, in the case of the cinnamic acids, the most significant degradation was found to be caffeic acid, which suffered a degradation of 48.90%, and sinapic, *p*-coumaric, and ferulic acids were observed in the range of degradation from 12.89 to 48.23%. In comparison to cinnamic acids, benzoic acids were more stable. For example, the contents of protocatechuic, *p*-hydroxybenzoic, and vanillic acid decreased by 21.16, 35.33, and 2.53%, respectively. Among all of the tested phenolic compounds, the sinapic and vanillic acids have the smallest values of degradation and were found to have methoxylic-type substituents in their aromatic rings. However, despite having one methoxylic-type substituent for ferulic acid, the significant degradation of 48.23% was obtained under the same ultrasonic conditions. As an example, a stability study of 22 phenolic compounds using microwave-assisted extraction showed that considerable degradation of the phenolic compounds was observed as temperature increased. The greater number of hydroxylic-type substituents and the smaller number of methoxylic-type substituents in the aromatic ring of phenolics is easy to degrade (*37*), which is similar to our results, indicating that the degradation of phenolics may depend on the number and type of substituents in their aromatic ring. However, the effects of ultrasound and microwave treatment on the stability of some phenolic compounds were pronouncedly different. In particular, both *p*-coumaric and *p*-hydroxybenzoic acids suffered significant degradation by ultrasound treatment at 40 °C for 20 min, whereas they did not suffer any degradation by microwave at an extremely high temperature of 175 °C, indicating that ultrasound has a greater effect on the stability of phenolic compounds than microwave. In agreement with previous findings, Zhao et al. (*38*) reported that ultrasound and microwave have different effects on the stability of (*all-E*)-astaxanthin. Microwave induced only the conversion of (*all-E*)-astaxanthin to (13*Z*)-astaxanthin, whereas ultrasound probably degraded it to colorless compounds, indicating also that ultrasound led to a stronger effect on the stability of (*all-E*)-astaxanthin than microwave.

Our previous study showed that the contents of phenolic acids in citrus peels decreased after heat treatment at 120 °C for 90 min in comparison with unheated treatment (*11*). In the present work, the decreases of contents of phenolic by ultrasonic treatment were at lower temperature and shorter time in comparison with heat treatment, which probably implies that the physical and chemical effects of UAE on targets were stronger than those of heat treatment. The above results showed that the effects of ultrasonic temperature on the yields and stability of the extracts were more sensitive than ultrasonic time. Moreover, phenolic compounds suffered degradation more easily by ultrasound than by microwave and heat treatment. The mechanism of ultrasonic enhancement was principally ascribed to mechanical and chemical effects of acoustic cavitation collapse, resulting in the disruption of biological cell walls to facilitate the release of content into the system (*39*) and producing local high pressures and high temperatures (*40*), which probably induced the degradation of phenolic compounds. The different effect of ultrasonic treatment on the stability of each phenolic compound is currently under study.

Considering the interactive effect of treatment time, temperature, and power on the phenolic acids, ultrasonic power was performed at lower temperature (15 °C) and appropriate time (20 min) according to the above results obtained. The effect of ultrasonic power on the contents of phenolic acids of extracts from SMP is summarized in **Table 3**. The values obtained showed that ultrasonic power has a positive effect on the contents of phenolic acids. For example, after UAE from 3.2 to 56 W, the content of caffeic, ferulic, sinapic, and vanillic acid increased by 43.65, 49.03, 8.64, and 12.34%, and TCE increased by 41.94%, respectively. All cited phenolic compounds showed stability when ultrasound power from 3.2 to 56 W was employed. In the paper by Sivakumar et al. (*41*), a 3−5-fold improvement of the yields of tannin from myrobalan nuts was obtained with increasing ultrasonic power from 20 to 100 W. Likewise, the weak effect of ultrasonic power on extracting anthraquinones from roots of *Morinda citrifolia* was also reported (*42*). Differences in the type of acoustic field generated by different ultrasonic equipment may be responsible for the different effects of ultrasonic power on the yields of extracts (*43*). Even using the same ultrasonic equipment, the different effect of ultrasonic power on extraction efficiency also was observed, which probably linked the nature of solid (hardness, compactness, and solute distribution) and cavitation behavior in medium (*44*). All of these results suggest that the contents of phenolic compounds are a function of ultrasonic temperature, time, and power.

Effect of ultrasonic treatment on total contents of extracts (TCE), which are the sum of all seven detected phenolic acids in SMP extracts, is shown in **Figure 2**. TCE varied depending on ultrasonic parameters. The extraction yields increased significantly at 15 and 30 °C when the extraction time increased to about 40 and 20 min, respectively ($p < 0.05$), whereas a relatively higher temperature of 40 °C resulted in significant increases of the extracts in a shorter time of 10 min, followed by a vigorous decrease for 20 min ($p < 0.05$).

In fact, the optimum extraction conditions varied between phenolic acids and ultrasonic parameters. The contents of

Table 2. Effect of Ultrasonic Time and Temperature on Flavanone Glycosides, Phenolic Acids of Extracts, and Total Content of Extracts (Micrograms per Gram of Dry Weight) from Satsuma Mandarin Peel with 8 W at 15, 30, and 40 °C from 10 to 60 min[a]

ultrasonic time (min)	*T* (°C)	flavanone glycosides		phenolic acids							TCE
		narirutin	hesperidin	caffeic	*p*-coumaric	ferulic	sinapic	protocatechuic	*p*-hydroxybenzoic	vanillic	
10	15	237.80 ± 15.39 f	821.54 ± 135.01 f	15.24 ± 0.54 f	38.63 ± 2.69 d	448.33 ± 28.88 f	44.48 ± 1.87 d	13.12 ± 0.98 d	27.14 ± 1.69 d	17.12 ± 0.55 d	584.06 ± 37.19 f
	30	296.73 ± 34.52 de	1077.62 ± 83.95 e	64.28 ± 1.94 d	140.83 ± 23.21 b	1513.21 ± 19.27 d	132.71 ± 3.86 c	15.87 ± 0.64 cd	34.11 ± 1.05 c	34.11 ± 0.55 c	1935.12 ± 50.52 d
	40	318.07 ± 8.25 d	1124.97 ± 30.27 e	98.37 ± 7.91 c	176.83 ± 17.36 a	2264.83 ± 193.94 c	198.50 ± 24.07 a	24.34 ± 1.78 a	47.44 ± 2.03 a	43.39 ± 1.56 a	2853.71 ± 248.65 b
20	15	279.72 ± 10.12 ef	864.13 ± 77.43 f	41.12 ± 6.49 f	92.25 ± 5.20 c	1047.99 ± 131.49 f	133.82 ± 20.04 c	16.78 ± 1.79 c	27.05 ± 2.56 d	31.20 ± 4.75 c	1390.21 ± 172.32 f
	30	317.33 ± 18.16 d	1105.34 ± 11.35 e	96.62 ± 0.86 c	175.93 ± 9.12 a	2196.00 ± 85.00 c	176.45 ± 1.60 b	18.27 ± 0.81 b	44.17 ± 2.56 b	36.30 ± 1.36 b	2743.74 ± 101.32 c
	40	328.55 ± 3.39 c	1336.47 ± 17.18 b	50.27 ± 1.92 e	98.67 ± 5.85 c	1172.52 ± 16.12 f	172.92 ± 8.85 b	19.19 ± 0.86 b	30.68 ± 1.12 cd	42.29 ± 1.85 a	1586.55 ± 36.40 f
30	15	289.21 ± 17.24 d	928.88 ± 69.18 e	50.79 ± 3.41 e	95.39 ± 2.55 c	1144.88 ± 34.35 f	140.89 ± 1.61 c	19.87 ± 1.42 b	44.84 ± 2.74 b	45.49 ± 0.41 a	1542.17 ± 46.48 f
	30	324.42 ± 4.07 cd	1197.70 ± 7.72 d	98.22 ± 6.22 c	180.78 ± 12.40 a	2265.75 ± 139.46 c	177.72 ± 0.16 b	18.61 ± 1.03 b	45.84 ± 0.84 ab	39.34 ± 0.72 b	2826.26 ± 160.83 b
	40	334.80 ± 12.86 c	1382.47 ± 130.70 a	55.57 ± 1.40 e	100.02 ± 9.08 c	1308.36 ± 8.26 e	179.45 ± 7.57 a	18.45 ± 0.92 b	30.13 ± 1.32 cd	41.74 ± 2.64 ab	1733.71 ± 31.15 e
40	15	291.32 ± 28.04 d	1201.50 ± 46.03 c	101.82 ± 2.91 ab	175.86 ± 7.18 a	2268.05 ± 100.31 c	183.48 ± 5.69 a	20.23 ± 1.67 ab	27.69 ± 0.58 d	33.28 ± 1.07 c	2810.40 ± 119.41 b
	30	348.25 ± 43.27 bc	1227.82 ± 180.50 c	102.31 ± 4.9 a	186.95 ± 8.52 a	2421.59 ± 121.39 a	184.25 ± 0.82 a	19.25 ± 0.43 b	47.27 ± 1.92 a	41.31 ± 1.06 ab	3002.93 ± 139.01 a
	40	361.61 ± 17.26 b	1368.06 ± 111.37ab	51.38 ± 3.14 e	94.59 ± 1.40 a	1114.59 ± 14.69 f	189.79 ± 21.25 a	18.91 ± 1.57 b	31.81 ± 1.96 c	35.79 ± 1.15 bc	1536.86 ± 45.12 f
50	15	276.29 ± 8.65 f	1140.65 ± 612.99 d	101.90 ± 0.96 ab	181.62 ± 0.38 a	2348.90 ± 7.49 bc	181.25 ± 0.01 a	21.42 ± 0.57 a	28.04 ± 2.21 cd	31.24 ± 0.44 cd	2894.36 ± 12.06 b
	30	368.07 ± 39.08 b	1356.32 ± 2.67 b	104.01 ± 0.83 a	190.37 ± 1.12 a	2448.06 ± 30.46 a	188.04 ± 3.25 a	19.40 ± 0.08 b	48.06 ± 0.42 a	42.09 ± 0.04 a	3040.04 ± 36.20 a
	40	525.97 ± 2.90 a	1421.71 ± 18.25 a	54.65 ± 1.49 e	99.87 ± 6.04 c	1250.90 ± 66.05 e	174.06 ± 4.61 b	19.29 ± 2.11 b	31.32 ± 1.58 c	35.88 ± 0.03 bc	1665.98 ± 81.91 f
60	15	276.38 ± 32.20 f	1180.08 ± 53.91 d	99.03 ± 0.84 c	188.19 ± 2.13 a	2254.85 ± 14.15 c	184.11 ± 4.89 a	20.00 ± 1.16 ab	28.83 ± 0.64 cd	31.10 ± 0.47 c	2806.11 ± 24.28 b
	30	366.60 ± 6.06 b	1391.33 ± 9.18 a	109.06 ± 0.90 a	196.55 ± 2.49 a	2495.94 ± 6.96 a	186.05 ± 1.67 a	19.43 ± 0.52 b	48.19 ± 0.48 a	42.40 ± 0.12 a	3097.60 ± 13.15 a
	40	563.93 ± 3.63 a	1446.05 ± 21.52 a	52.11 ± 7.04 e	102.87 ± 14.49 c	1105.46 ± 76.88 f	177.26 ± 5.14 b	21.60 ± 2.29 a	32.37 ± 3.16 c	34.73 ± 1.26 c	1526.39 ± 110.26 f

[a] Mean ± SD of three measurements. Means in each column with different letters are significantly different ($p < 0.05$). TCE, total content of extracts (seven phenolic acids); *T*, temperature.

Table 3. Effect of Ultrasonic Power on Flavanone Glycosides, Phenolic Acids of Extracts, and Total Content of Extracts (Micrograms per Gram of Dry Weight) from Satsuma Mandarin Peel at 15 °C for 20 min[a]

ultrasonic power (W)	flavanone glycosides		phenolic acids							TCE
	narirutin	hesperidin	caffeic	*p*-coumaric	ferulic	sinapic	protocatechuic	*p*-hydroxybenzoic	vanillic	
3.2	237.80 ± 15.39 b	621.54 ± 135.01 b	41.12 ± 6.49 b	92.25 ± 5.20 c	1047.99 ± 131.49 c	133.82 ± 20.04 c	16.78 ± 1.79 b	27.05 ± 2.56 c	31.20 ± 4.75 a	1390.21 ± 172.32 b
8	358.05 ± 79.39 a	821.74 ± 4.54 a	66.63 ± 1.19 a	130.00 ± 0.32 a	1286.01 ± 33.26 b	171.59 ± 1.46 a	21.85 ± 0.34 a	34.09 ± 1.14 a	33.32 ± 1.60 a	1743.50 ± 39.31 a
30	362.33 ± 13.41 a	831.40 ± 1.92 a	58.71 ± 0.40 a	111.83 ± 0.22 b	1371.36 ± 0.02 b	156.65 ± 1.85 b	20.75 ± 0.26 a	32.40 ± 0.26 a	32.67 ± 0.64 a	1784.4 ± 4.25 a
56	355.33 ± 48.61 a	849.61 ± 65.53 a	59.07 ± 4.53 a	113.99 ± 7.31 b	1565.71 ± 20.53 a	145.33 ± 3.41 bc	20.81 ± 0.27 a	33.29 ± 0.45 a	33.05 ± 0.89 a	1793.25 ± 37.39 a

[a] Mean ± SD of three measurements. Means in each column with different letters are significantly different ($p < 0.05$). TCE, total content of extracts (seven phenolic acids).

Figure 2. Effect of ultrasonic treatment on the total content of extracts (TCE) from Satsuma mandarin peel: (**a**) SMP treated by ultrasound with 8 W at 15, 30, and 40 °C for 10, 20, 30, 40, 50, and 60 min: (**b**) SMP treated by ultrasound with 3.2, 8, 30, and 56 W at 15 °C for 20 min.

phenolic acids detected at 320 nm were the highest at 30 °C for 60 min, whereas phenolic acids detected at 260 nm were the highest at 40 °C for 10 min. Moreover, after UAE at 40 °C from 10 to 60 min, the contents of phenolic acids detected at 320 nm decreased more dramatically than those at 260 nm, again indicating that the latter was more stable than the former. Likewise, the optimum extraction parameters from one phenolic compound to another were different. For example, according to **Table 2**, the most efficient extraction condition for caffeic acid was at 30 °C for 60 min, that for sinapic acid was at 40 °C for 10 min, and that for vanillic acid was at 15 °C for 30 min. Consistent with our results, Cerovic et al. (*45*) reported the optimum extraction contents of hypericin, pseudohypericin, hyperoside, rutin, quercitrin, and hyperforin, obtained from *Hypericum perforatum* L. by ultrasonic treatment, varied from one compound to another.

Narirutin and hesperidin are two major flavanone glycosides in SMP. Ultrasonic treatments can significantly enhance the contents of narirutin and hesperidin compared to maceration extraction (**Table 1**). The values of ultrasonic extraction at 30 °C for 10 min were much higher than those by maceration extraction at 40 °C for 8 h. In addition, the contents of two flavanone glycosides in SMP increased with ultrasonic time, temperature, ultrasonic power (**Tables 2** and **3**). For example, after UAE at 30 °C for 60 min, the contents of narirutin and hesperidin increased by 23.54 and 29.11%; after UAE from 15 to 40 °C for 10 min, the contents of

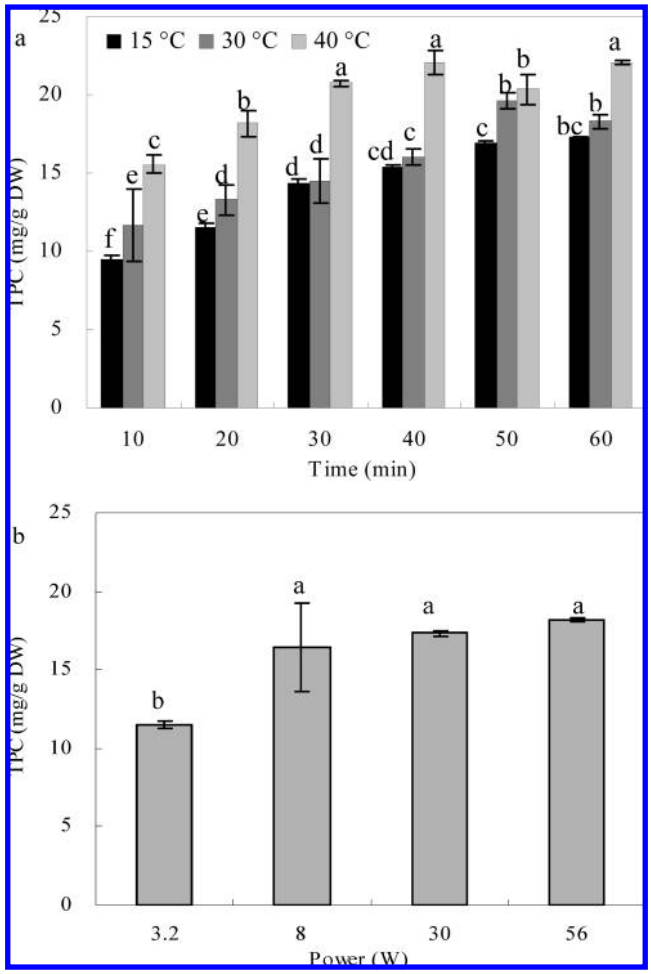

Figure 3. Effect of ultrasonic treatment on total phenolic contents (TPC) of extracts from Satsuma mandarin peel: (**a**) SMP treated by ultrasound with 8 W at 15, 30, and 40 °C for 10, 20, 30, 40, 50, and 60 min; (**b**) SMP treated by ultrasound with 3.2, 8, 30, and 56 W at 15 °C for 20 min.

narirutin and hesperidin increased by 33.76 and 36.93%; and after UAE from 3.2 to 56 W, the contents of narirutin and hesperidin increased by 36.81 and 51.50%, respectively. As compared to the values of phenolic compounds, it can be noted that two flavanone glycosides did not suffer any degradation at the same ultrasonic conditions, and their contents presented a distinctly increased trend. One of the probable reasons for this phenomenon was that phenolic compounds were thermally unstable and suffered pyrolysis due to local high temperature and high pressure produced by cavitation collapse. As has been reported before (*46*), ultrasonic treatment resulted in significant degradation (close to 100%) of phenolics from strawberries, whereas degradation decreased by reducing ultrasound exposure time. On the other hand, the higher values of narirutin and hesperidin could be explained on the basis of their highly thermal stability, at least in the range under study. Previous studies showed that ultrasound did not result in any degradation of hesperidin in citrus peel at 40 °C for 160 min (*28*).

The above results demonstrated that UAE has significant effects on increases of the contents of phenolic acids and flavanone glycosides from SMP compared to the conventional maceration method. However, the higher temperature and longer time resulted in the degradation of some phenolic acids. Therefore, it is important that the application of ultrasound in extraction of unstable bioactive compounds should be carefully

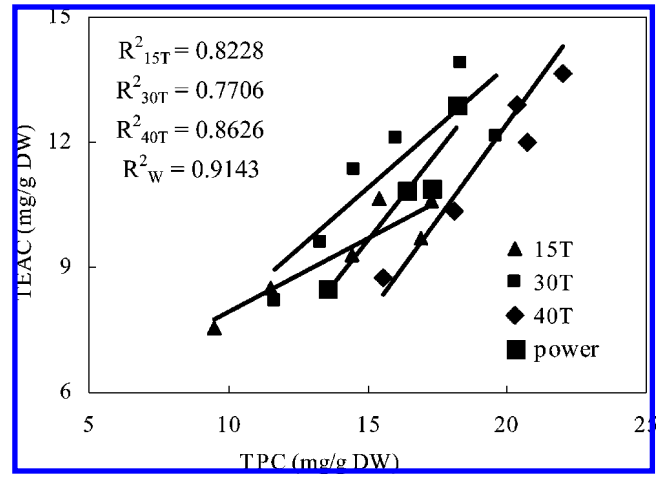

Figure 4. Correlation between TPC and TEAC values.

considered. In this paper, generally, the optimal ultrasonic parameters for phenlic acids from SMP were determined as extraction time of 20 min, temperature of 30 °C, and ultrasonic power of 8 W; and those for flavanone glycosides were extraction time of 60 min, temperature of 40 °C, and ultrasonic power of 8 W.

Total Phenolics and Antioxidant Activity. TPC of extracts was determined by the Folin−Ciocalteu colorimetric method. The effect of ultrasonic treatment on total phenolics is shown in **Figure 3**. Treatment time, temperature, and ultrasonic power all had a significant effect on TPC; for example, after ultrasonic treatment at 15 °C from 10 to 60 min, TPC increased by 81.72%; after ultrasonic treatment at 40 °C from 10 to 60 min, TPC increased by 41.45%. When ultrasonic power was increased from 3.2 to 56 W, TPC increased by 57.90%, which was opposite to the values of phenolic compounds obtained. Reasons for this may be due to TPC evaluating the whole amount of phenolics, that is, all flavonoids and nonflavonoid phenolic compounds in SMP. However, flavanoids showed a higher thermally stability, and their content in SMP is significantly higher than that of phenolic acids. Therefore, the values of TPC by ultrasound have the same increasing trend as those of flavanoids rather than phenolic acids.

The correlation coefficients between TPC and TEAC (FRAP assay), after ultrasonic treatment, are shown in **Figure 4**. The values of TEAC indicated a positive correlation with the values of TPC. A linear relationship in each case was observed between TEAC and TPC. For example, the R^2 correlation coefficient is 0.8288 at 15 °C, 0.7706 at 30 °C, 0.8626 at 40 °C, and 0.9143 at ultrasonic powers of 3.2, 8, 30, and 56 W, respectively, which meant that TPC was the major factor accounting for the antioxidant activity of the SMP extracts.

It has been reported that phenolic compounds and antioxidant activities of extracts from citrus peel significantly increased by heat treatments (*10, 11*). However, the present investigation showed that UAE can significantly enhance the contents of phenolic compounds and antioxidant activities in SMP, implying that the ultrasonic process can be used as a tool for increasing the antioxidant activity of SMP. The stability of phenolics possibly depended on the number and type of substituents in their aromatic ring. Cinnamic acids of extracts from SMP by UAE were susceptible to degradation compared to benzoic acids. The results emphasized the careful application of UAE in the extraction of biologically

active compounds, in particular, some unstable biological phytochemicals.

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