JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Effect of Heat Treatment on the Phenolic Compounds and Antioxidant Capacity of Citrus Peel Extract

GUIHUA XU,†,§ XINGQIAN YE,† JIANCHU CHEN,† AND DONGHONG LIU*,†

Department of Food Science and Nutrition, Zhejiang University, Hangzhou 310029, China, and Department of Food Science, Henan Institute of Science and Technology, Xinxiang 453003, China

This paper reports the effects of heat treatment on huyou (*Citrus paradisi* Changshanhuyou) peel in terms of phenolic compounds and antioxidant capacity. High-performance liquid chromatography (HPLC) coupled with a photodiode array (PDA) detector was used in this study for the analysis of phenolic acids (divided into four fractions: free, ester, glycoside, and ester-bound) and flavanone glycosides (FGs) in huyou peel (HP) before and after heat treatment. The results showed that after heat treatment, the free fraction of phenolic acids increased, whereas ester, glycoside, and ester-bound fractions decreased and the content of total FGs declined ($P < 0.05$). Furthermore, the antioxidant activity of methanol extract of HP increased ($P < 0.05$), which was evaluated by total phenolics contents (TPC) assay, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$) method, and ferric reducing antioxidant power (FRAP) assay. The correlation coefficients among TPC, ABTS, FRAP assay, and total cinnamics and benzoics (TCB) in the free fraction were significantly high ($P < 0.05$), which meant that the increase of total antioxidant capacity (TAC) of HP extract was due at least in part to the increase of TCB in free fraction. In addition, FGs may be destroyed when heated at higher temperature for a long time (for example, 120 °C for 90 min or 150 °C for 30 min). Therefore, it is suggested that a proper and reasonable heat treatment could be used to enhance the antioxidant capacity of citrus peel.

**KEYWORDS: Citrus peel; phenolic acids; flavanone glycosides; HPLC-PDA; total antioxidant capacity**

## INTRODUCTION

As people express more and more concerns about their existence and health in the world, many health-giving foods in nature are attracting increasing attention, among which citrus fruits are one of the hot topics. Citrus fruits have many healthy properties, mainly due to their high content of nutrients, such as vitamin C, carotenoids, and phenolic compounds. These phytochemicals have antioxidant capacity and may protect cells against the oxidative damage caused by free radicals (*1*, *2*).

A number of citrus species have been widely used in traditional Chinese medicine and were officially listed in the Chinese Pharmacopoeia (*3*), which was named *chenpi*. It is well-known that citrus fruits are rich in flavonoids, most of which exist in the form of flavanone glycosides (FGs), namely, narirutin, naringin, hesperidin, and neohesperidin (*4*). Citrus peel is the main waste fraction of citrus fruits, which had been widely studied because they contain numerous biologically active compounds including natural antioxidants such as phenolic acids and flavonoids (*5*, *6*). Phenolic acids have attracted more and more attention for their antioxidant behavior and beneficial health-promoting effects in chronic and degenerative diseases (*7*−*11*).

Phenolic acids account for about one-third of the phenolic compounds in plant foods, and they are presented in free and bound forms. Bound phenolics may be linked to various plant components through ester, ether, or acetal bonds (*12*). It is assumed that many antioxidative phenolic compounds in plants are usually presented as a covalently bound form; therefore, some processing methods were employed to liberate them so as to enhance the antioxidant capacity (*13*, *14*). For instance, it is reported that heat treatment may liberate some low molecular weight phenolic compounds and hence increase the antioxidant capacity of citrus peel (*14*). However, the effect of heat treatment on citrus flavonoids was not involved, and no clear quantity relationships were elucidated, so a further investigation was needed.

Huyou is a hybrid of *Citrus sinensis* (L.) Osbeck and *Citrus grandis* (L.) Osbeck with the commercial name Changshanhuyou tangelo, and it is one of the citrus varieties that is widely planted and consumed in southern China. However, there was little literature about profiles of phenolic compounds and antioxidant capacity of huyou. Therefore, this paper reported on the analysis of huyou peel (HP) in terms of phenolic compounds and antioxidant capacity. Although many papers have already been published in relation to citrus phenolic compounds and their antioxidant activity (*15*−*19*), the change of phenolic acids, FGs, and antioxidant capacity of HP after heat treatment was not reported. High-performance liquid

* Corresponding author (e-mail psu@zju.edu.cn; fax 0086-571-86971165).
† Zhejiang University.
§ Henan Institute of Science and Technology.

10.1021/jf062517l CCC: $37.00 
Published on Web 12/19/2006

**Table 1.** Phenolic Acid Composition and Distribution of Untreated and Heat-Treated Huyou Peel (Micrograms per Gram of Dry Weight)

| | | cinnamics | | | | benzoics | | | |
|---|---|---|---|---|---|---|---|---|---|
| | heat treatment | caffeic | *p*-coumaric | ferulic | sinapic | *p*-hydroxy-benzoic | vanillic | TCB | chlorogenic |
| free | untreated | nd[a] | 5.58 ± 0.18 | 4.56 ± 0.25 | 3.69 ± 0.30 | trace[b] | 54.18 ± 3.95 | 68.64 ± 4.28 f | 126.58 ± 11.67 |
| | heated, 120 °C, 30 min | nd | 21.77 ± 1.53 | 49.40 ± 3.06 | 5.76 ± 0.25 | 4.04 ± 0.71 | 72.54 ± 2.55 | 153.51 ± 2.79 d | 65.96 ± 8.70 |
| | heated, 120 °C, 60 min | nd | 35.30 ± 1.05 | 68.88 ± 2.15 | 6.37 ± 0.92 | 7.76 ± 0.47 | 109.55 ± 2.21 | 227.86 ± 4.78 b | 65.10 ± 2.85 |
| | heated, 120 °C, 90 min | nd | 45.05 ± 3.67 | 69.98 ± 3.86 | 10.66 ± 1.45 | 10.16 ± 0.27 | 160.71 ± 16.8 | 296.56 ± 17.81 a | 35.27 ± 1.38 |
| | heated, 90 °C, 30 min | nd | 6.33 ± 0.35 | 6.85 ± 0.06 | 4.30 ± 0.49 | trace | 63.32 ± 1.54 | 83.71 ± 0.59 e | 104.02 ± 7.21 |
| | heated, 150 °C, 30 min | nd | 54.10 ± 0.35 | 44.88 ± 0.77 | 2.26 ± 0.05 | 14.00 ± 0.52 | 82.00 ± 1.74 | 197.24 ± 3.33 c | 17.47 ± 1.80 |
| ester | untreated | 4.11 ± 0.31 | 24.10 ± 1.98 | 142.68 ± 8.32 | 53.39 ± 3.32 | 10.25 ± 1.20 | 76.05 ± 5.97 | 310.59 ± 12.67 a | nd |
| | heated, 120 °C, 30 min | 4.57 ± 0.74 | 16.77 ± 1.24 | 106.85 ± 4.25 | 44.53 ± 4.02 | 8.77 ± 0.22 | 68.83 ± 2.78 | 250.32 ± 12.61 b | nd |
| | heated, 120 °C, 60 min | 3.77 ± 0.48 | 10.27 ± 0.54 | 66.35 ± 1.67 | 32.01 ± 0.59 | 5.58 ± 0.39 | 47.96 ± 2.67 | 144.99 ± 24.53 c | nd |
| | heated, 120 °C, 90 min | 3.05 ± 0.37 | 7.26 ± 0.60 | 44.99 ± 4.47 | 25.05 ± 2.49 | 4.53 ± 0.83 | 42.80 ± 4.58 | 127.68 ± 13.34 d | nd |
| | heated, 90 °C, 30 min | 5.60 ± 0.77 | 26.86 ± 4.16 | 138.35 ± 11.64 | 48.41 ± 6.05 | 10.19 ± 2.74 | 75.10 ± 3.60 | 304.51 ± 20.24 a | nd |
| | heated, 150 °C, 30 min | 5.20 ± 0.01 | 11.72 ± 0.66 | 57.36 ± 4.72 | 25.61 ± 2.49 | 6.58 ± 0.10 | 58.88 ± 9.06 | 165.35 ± 15.57 c | nd |
| glycoside | untreated | 2.13 ± 0.40 | 52.26 ± 9.16 | 37.35 ± 5.40 | 2.34 ± 0.67 | 6.38 ± 0.83 | 53.70 ± 1.42 | 154.16 ± 8.40 a | nd |
| | heated, 120 °C, 30 min | 2.38 ± 0.16 | 50.55 ± 1.94 | 40.66 ± 0.78 | 2.87 ± 0.48 | 5.97 ± 0.14 | 52.74 ± 3.30 | 155.17 ± 5.96 a | nd |
| | heated, 120 °C, 60 min | 1.94 ± 0.12 | 50.69 ± 1.21 | 30.38 ± 1.22 | 1.69 ± 0.35 | 5.38 ± 0.58 | 44.18 ± 2.93 | 134.27 ± 3.17 b | nd |
| | heated, 120 °C, 90 min | trace | 43.89 ± 6.15 | 16.25 ± 4.52 | trace | 4.60 ± 1.61 | 26.87 ± 10.21 | 91.91 ± 21.45 c | nd |
| | heated, 90 °C, 30 min | 1.58 ± 0.44 | 65.96 ± 6.18 | 34.61 ± 3.26 | 1.80 ± 0.44 | 7.08 ± 0.93 | 44.22 ± 1.07 | 155.25 ± 12.31 a | nd |
| | heated, 150 °C, 30 min | 1.11 ± 0.12 | 56.96 ± 2.13 | 25.90 ± 3.34 | 1.15 ± 0.02 | 6.51 ± 0.05 | 40.51 ± 0.16 | 132.13 ± 1.10 b | nd |
| ester-bound | untreated | trace | 26.04 ± 6.93 | 77.65 ± 13.76 | 5.72 ± 0.63 | 4.19 ± 1.03 | 5.54 ± 1.83 | 119.15 ± 23.98 a | nd |
| | heated, 120 °C, 30 min | trace | 14.84 ± 2.30 | 55.92 ± 9.78 | 3.55 ± 0.61 | 2.56 ± 0.62 | 3.22 ± 0.65 | 80.2 ± 13.90 b | nd |
| | heated, 120 °C, 60 min | trace | 11.98 ± 1.58 | 46.19 ± 5.57 | 2.32 ± 0.22 | 2.03 ± 0.39 | 1.28 ± 0.50 | 66.12 ± 8.28 c | nd |
| | heated, 120 °C, 90 min | trace | 9.92 ± 1.98 | 39.34 ± 4.22 | 1.81 ± 0.49 | 1.94 ± 0.24 | 1.48 ± 0.16 | 54.96 ± 5.20 d | nd |
| | heated, 90 °C, 30 min | trace | 20.65 ± 0.42 | 72.01 ± 0.26 | 7.21 ± 0.52 | 3.50 ± 0.10 | 5.85 ± 0.9 | 110.08 ± 1.15 a | nd |
| | heated, 150 °C, 30 min | trace | 10.31 ± 0.41 | 34.02 ± 0.77 | 4.24 ± 0.15 | 1.65 ± 0.07 | 5.72 ± 0.23 | 57.85 ± 0.88 d | nd |

[a] nd, not detected. [b] trace, content <1 $\mu$g/g of DW.

chromatography (HPLC) coupled with photodiode array detection (PDA) was developed in this study for the separation, characterization, and quantitation of phenolic acids and FGs in HP before and after heat treatment, and the relationship between the antioxidant capacity of HP and their phenolic compounds was also studied.

## MATERIALS AND METHODS

**Chemicals.** Standards of *p*-hydroxybenzoic, vanillic, *p*-coumaric, caffeic, ferulic, sinapic, chlorogenic, narirutin, naringin, hesperidin, neohesperidin, 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS$^{\bullet+}$), and Folin−Ciocalteu phenol reagent were purchased from Sigma. All other chemicals used were of analytical grade.

**Materials.** Huyou fruits in this study were kindly offered by a local farm in Quzhou city, Zhejiang province, southeastern China, and divided into peel and edible parts. HP was dried by hot air at 45 °C for 48 h, and the final water content was below 10%; then they were ground finely and stored at −20 °C for usage.

**Heat Treatment.** Powder of HP (2 g) was placed into a 125 mL flask and heated in an electric oven at 120 °C for 30, 60, and 90 min and at 90 and 150 °C for 30 min. After heating, HP powder was allowed to cool to ambient temperature before extraction.

**Extraction of FGs and Phenolic Acids from HP.** Heated or unheated HP powder (2 g) was extracted three times with 50 mL of 80% methanol and assisted by ultrasonic at room temperature. After centrifugation at 5000 rpm for 10 min, the supernatant was combined, and 80% methanol was added to make a final volume of 150 mL. Then it was taken out for analysis of FGs and evaluation of antioxidant capacity.

Phenolic acids were isolated from the extract according to some previously described methods with certain modifications (*20*−*22*). HP powder (2 g) was extracted three times with 50 mL of 80% methanol and assisted by ultrasonic at room temperature. Then it was centrifuged at 5000 rpm for 10 min. The combined supernatant was evaporated under vacuum at 40 °C to about 20 mL. The aqueous suspension was adjusted to pH 2 (6 M HCl) and centrifuged. The clear supernatant was extracted five times with diethyl ether/ethyl acetate (1:1, v/v) at a solvent to water phase ratio of 1:1 to obtain the free phenolic acid. The ether/ethyl acetate extracts were dehydrated with anhydrous sodium sulfate, filtered, and evaporated to dryness under vacuum at 30 °C. The dry residues were dissolved into 10 mL of methanol. The aqueous phase was first treated by alkaline hydrolysis (4 M NaOH) for 4 h under a nitrogen atmosphere at room temperature. After acidification

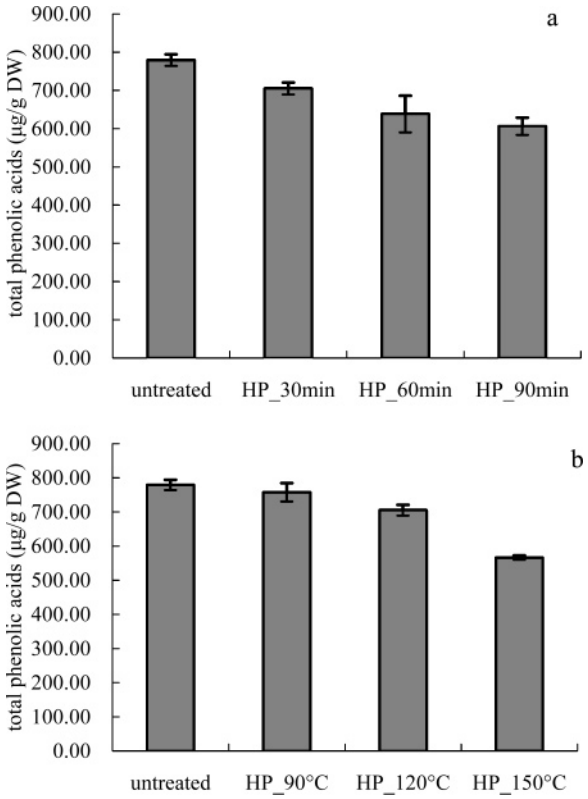


**Figure 1.** Total phenolic acid content of untreated and heat-treated HP (**a**, HP heated at 120 °C for 30, 60, and 90 min; **b**, HP heated at 90, 120, and 150 °C for 30 min).

**Table 2.** Flavanone Glycoside Content of Untreated and Heat-Treated Huyou Peel (Milligrams per Gram of Dry Weight)

| heat treatment | narirutin | naringin | hesperidin | neohesperdin |
|---|---|---|---|---|
| untreated | 2.81 ± 0.50 | 31.57 ± 1.56 | 2.04 ± 0.42 | 24.09 ± 0.86 |
| heated, 120 °C, 30 min | 2.74 ± 0.16 | 31.14 ± 1.39 | 1.83 ± 0.30 | 24.10 ± 0.84 |
| heated, 120 °C, 60 min | 2.31 ± 0.55 | 31.00 ± 1.85 | 1.87 ± 0.24 | 23.58 ± 1.27 |
| heated, 120 °C, 90 min | 2.24 ± 0.23 | 27.82 ± 1.46 | 1.49 ± 0.12 | 21.34 ± 1.12 |
| heated, 90 °C, 30 min | 2.61 ± 0.06 | 30.94 ± 0.01 | 1.83 ± 0.06 | 23.64 ± 0.02 |
| heated, 150 °C, 30 min | 2.40 ± 0.08 | 28.72 ± 0.56 | 1.70 ± 0.06 | 21.33 ± 0.48 |

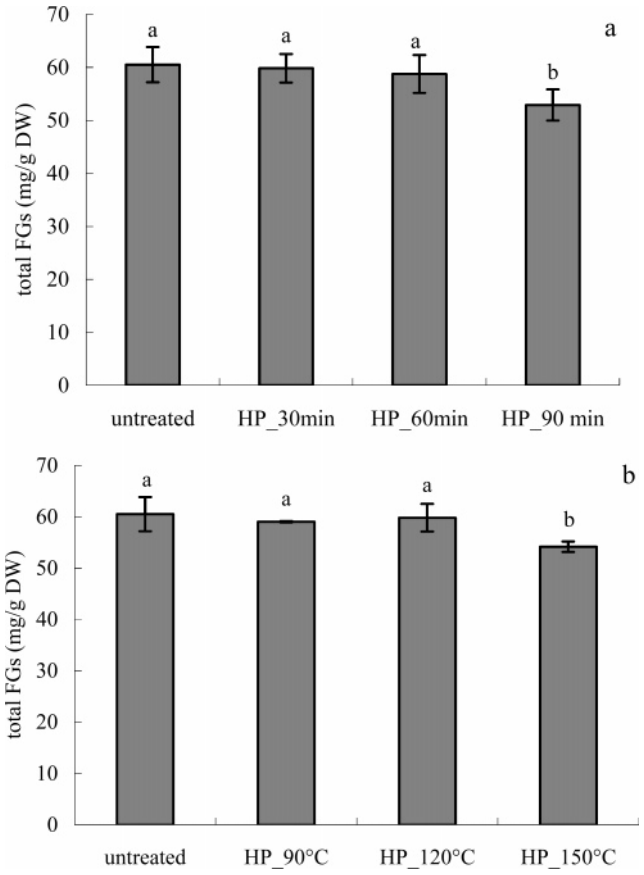


**Figure 2.** Total FG content of untreated and heat-treated HP (**a**, HP heated at 120 °C for 30, 60, and 90 min; **b**, HP heated at 90, 120, and 150 °C for 30 min).

to pH 2 using 6 M HCl, phenolic acids released from the soluble ester were extracted as described above. Afterward, 10 mL of 6 M HCl was added, and the medium was placed under a nitrogen atmosphere and hydrolyzed for 1 h at 85 °C. Then phenolic acids released from soluble glycosides were extracted as described above. The residues from the 80% methanol extractions were hydrolyzed directly with 20 mL of 4 M NaOH and treated as the ester. This fraction was methanol insoluble ester-bound phenolic acids.

**HPLC Analysis.** Phenolic acids of HPLC analyses were carried out on an Alliance 2695 separations module (Waters) linked simultaneously to a PDA 2996 (Waters). Prepared phenolic acid solution was filtered through a Millipore membrane (0.45 $\mu$m) before injection, and 10 $\mu$L was injected on the reversed phase column (250 × 4.6 mm i.d.). The column thermostat was set at 40 °C. Solvent A consisted of 4% acetic acid, and solvent B consisted of methanol (A/B = 20:80) at a flow rate of 1 mL/min, which was in accordance with Subba Rao (*23*) with minor revision. After each run, the column was washed with 100% methanol and equilibrated to initial conditions for 15 min. The PDA detector was set to a scanning range from 210 to 400 nm with a resolution of 1.2 nm. Phenolic acids were identified by the retention time and the UV−vis spectra of standards. Quantification of phenolic acids was carried out by an external standard method using calibration curves, and concentration of phenolic acids was expressed as micrograms per gram of dry weight (DW). Repeatability of the method was evaluated by the intraday data from standards. Three injections were carried out for each sample. The repeatability of intraday analysis ranged from a relative standard deviation (RSD) of 0.57% to a RSD of 2.99% ($n$ = 4). Recovery experiments were performed by adding known amounts of pure standards to samples. Generally the recoveries were good (mean > 80%), and phenolic acids were more stable during alkaline than acid hydrolysis. However, recovery of caffeic acid was quite low (<15%) under base hydrolysis. Coefficients of variation of samples were mostly under 10%.

The contents of narirutin, naringin, hesperidin, and neohesperidin were determined according to a method described elsewhere with some modification (*24*). Ten microliters of extract (appropriately diluted by methanol) was injected into a high-performance liquid chromatography system module 2695 (Waters), and it was filtered through a Millipore membrane (0.45 $\mu$m) before injection. The analysis utilized a Diamonsil C18 column (250 × 4.6 mm i.d.) using methanol/water/acetic acid (37:59:4, v/v/v) as the mobile phase at a flow rate of 1.0 mL/min at a 25 °C oven temperature, and the eluent was monitored at 283 nm for the four FGs. Identification of the FGs was accomplished by comparing the retention times of peaks in fruit samples to those of FG standards. Calculation of FG concentration (expressed as milligrams per gram of DW) was carried out by an external standard method using calibration curves.

**Total Phenolic Contents (TPC).** TPC was determined according to the Folin−Ciocalteu method (*25*). Briefly, 0.2 mL of methanol extract was added to a 25 mL volumetric flask, and additional dd$H_2O$ was added to make a final volume of 10 mL. A reagent blank was prepared using dd$H_2O$. Folin−Ciocalteu phenol reagent (0.5 mL) was added to the mixture and shaken vigorously. After 5 min, 5 mL of 5% $Na_2CO_3$ solution was added with mixing. The solution was immediately diluted to volume (25 mL) with dd$H_2O$ and mixed thoroughly and then allowed to stand for 60 min. After that, the absorbance was measured at 750 nm versus the prepared blank. The TPC of sample was expressed as gallic acid equivalents (GAE) milligrams per gram of DW.

**ABTS Free Radical Scavenging Assay.** The ABTS method is a decolorization assay according to ref *26*. To oxidize the colorless ABTS to the blue-green $ABTS^{\bullet+}$ radical cation, ABTS (7 mM) was mixed with potassium persulfate and kept for 12−16 h at room temperature in the dark. On the day of analysis, the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. After the addition of 0.95 mL of $ABTS^{\bullet+}$ solution to 0.05 mL of extract (diluted 5 times by 80% methanol), the mixture was stirred for 30 s and allowed to stand for 15 min at room temperature, and then the absorbance reading was determined at 734 nm. A calibration curve was made by absorbance reduction and the concentration of Trolox. A control consisted of 0.05 mL of 80% methanol, and 0.95 mL of $ABTS^{\bullet+}$ solution was prepared. The stable ABTS radical scavenging activity of the extracts was expressed as Trolox equivalent antioxidant capacity (TEAC) milligrams per gram of DW. The radical stock solution was prepared fresh daily.

**Ferric Reducing Ability Assay (FRAP).** The ferric reducing ability of each standard solution was measured according to a modified protocol developed in ref *27*. To prepare the FRAP reagent, a mixture of 0.1 M acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM ferric chloride (10:1:1, v/v/v) was made. To 1.9 mL of reagent was added 0.1 mL of extract. Readings at the absorption maximum (593 nm) were taken every 15 s using a Shimadzu UV−visible 2501 spectrophotometer, and the reaction was monitored for up to 10 min. Trolox solution

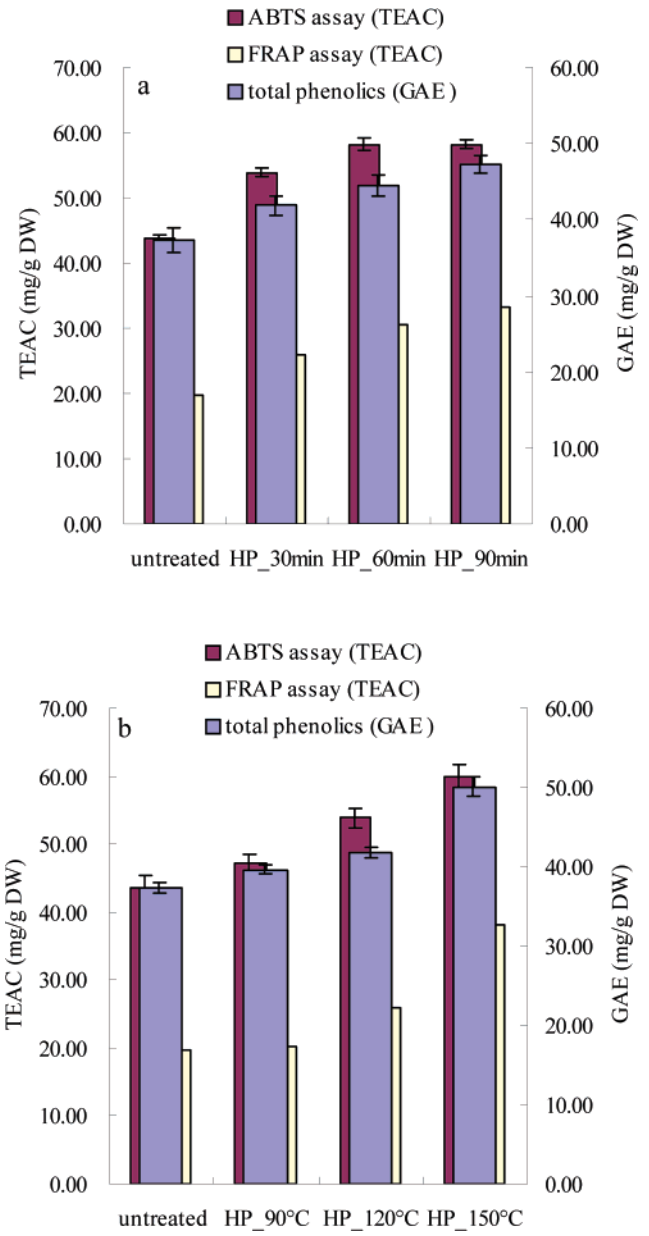


**Figure 3.** TPC, ABTS, and FRAP assays of methanol extract of untreated and heat-treated HP (**a**, HP heated at 120 °C for 30, 60, and 90 min; **b**, HP heated at 90 , 120, and 150 °C for 30 min).

was used to perform the calibration curves. Result was also expressed as TEAC milligrams per gram of DW.

**Statistics.** All samples were prepared and analyzed in triplicate. To verify the statistical significance of all parameters, the values of means ± standard deviation (SD) were calculated. To compare several groups, analysis of variance (ANOVA) was used. The Pearson correlation coefficient ($R$) and $P$ value were used to show correlations and their significance [SPSS for Windows, release 11.5.0 (June 2002, SPSS Inc.)]. A probability value of $P < 0.05$ was adopted as the criterion for significant differences.

## RESULTS AND DISCUSSION

**Phenolic Acid Composition and Distribution of HP.** Phenolic acids in HP were divided into four fractions: free, ester, glycoside, and ester-bound forms. Seven phenolic acids in HP were detected and quantified, namely, benzoic acids (*p*-hydroxybenzoic acid, vanillic acid), cinnamic acids (caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid), and chlorogenic acid (as a representative of phenolic esters). The comparative

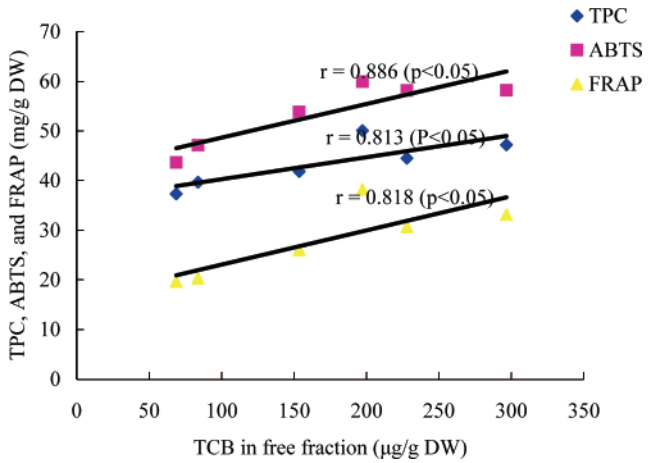


**Figure 4.** Correlations among TCB in free fraction, TPC, ABTS assay, and FRAP assay ($n = 6$).

analyses of phenolic acids in the four fractions indicated that there were rather lower amounts of phenolic acids in the free fraction when compared to the other three parts (**Table 1**). Similarly, it has been reported that the majority of phenolic acids in citrus fruits are presented in bound form (*19*, *28*). Moreover, the ester-bound fraction accounted for a considerable amount of phenolic acids, so in this study we divided phenolic acids into four parts and analyzed them separately.

The content of phenolic acids varied in different fractions, and generally the hydroxycinnamic acid content was in the following order: ferulic acid > sinapic acid > coumaric acid > caffeic acid, which was accordance with the results of refs *5*, *28*, and *29*. Within the four analyzed hydroxycinnamic acids, caffeic acid accounted for the least, and it presented only as ester and glycoside forms. It should be noted that chlorogenic acid, which can be hydrolyzed to caffeic acid under alkaline conditions (*30*), presented largely in the free fraction. Furthermore, the recovery of caffeic acid was very low after alkaline hydrolysis, so the calculated caffeic acid content here was obviously underestimated.

As shown in **Table 1**, cinnamic acids occurred most frequently as esters (except for *p*-coumaric acid), whereas the benzoic acids were presented mainly in the form of esters and glycosides, which supported the report of Herrmann (*31*).

**Effect of Heat Treatment on the Phenolic Acid Distribution of HP.** The effect of heat treatment with different heating times and temperatures on the phenolic acid distribution of HP is demonstrated in **Table 1**. In the free phenolic acid fraction, the content of benzoic acids and cinnamic acids significantly increased after heat treatment ($P < 0.05$). For instance, after heating at 120 °C for 90 min, the content of *p*-coumaric acid increased from 5.58 to 45.05 μg/g of DW, ferulic acid increased from 4.56 to 69.98 μg/g of DW, vanillic acid increased from 54.18 to 160.71 μg/g of DW, and TCB increased from 68.64 to 296.56 μg/g of DW. In addition, chlorogenic acid (an ester of caffeic and quinic acid) was determined in free forms. Nevertheless, its content decreased with heating time and temperature, which indicated that the esterified bond could be cleaved by heat treatment. In ester, glycoside, and ester-bound fractions, the content of phenolic acids also decreased after heat treatment. For example, after heating at 120 °C for 90 min, the content of ferulic acid in the ester fraction decreased from 142.68 to 44.99 μg/g of DW, from 37.35 to 16.25 μg/g of DW in the glycosides fraction, and from 77.65 to 39.34 μg/g of DW in the ester-bound fraction, respectively.

The effect of heat treatment on the content of total phenolic acids, which was the sum of all seven detected phenolic acid in the four fractions, is shown in **Figure 1**. Generally speaking, there was a decline with the heating time and temperature, which indicated that some phenolic acids probably were destroyed by heat treatment, although there was an increase in the free phenolic acid fraction.

The results suggested that after heat treatment, the distribution of phenolic acids was changed due to the cleaving of esterified bond and glycosylated bond, etc. To sum up, the free fraction increased, whereas the ester, glycoside, and ester-bound fractions decreased. Moreover, there was a decrease of total phenolic acid content.

**Effect of Heat Treatment on the FG Composition of HP.** Four major FGs in HP, namely, narirutin, naringin, hesperidin, and neohesperidin, were determined by HPLC, and the effect of heat treatment on the FG composition of HP is shown in **Table 2**. Clearly, after heat treatment, the content of all four FGs declined with heating time and temperature. The effect of heat treatment on the content of total FGs is shown in **Figure 2**. Generally speaking, there was also a decline with the heating time and temperature. For example, total FG content decreased from 60.50 to 52.89 mg/g of DW after heating at 120 °C for 90 min.

It seemed that FGs could be destroyed at higher temperature for a rather long time, and FGs predominated among the citrus flavonoids, so it was not proper to adopt a rather high temperature to enhance the antioxidant of citrus peel, which did not agree the viewpoint of the authors of ref *14*. *Chenpi*, a traditional Chinese medicine, is usually prepared by sun-drying, and its potentially health-promoting effects have been mainly ascribed to citrus flavonoids (*3*); therefore, we suggested that heat treatment at proper and reasonable temperature (for example, 100 °C or so) could be used to prepare *chenpi*.

**Effect of Heat Treatment on the Antioxidant Capacity of HP Extract.** The antioxidant capacity of methanol extract of HP was evaluated according to the ABTS decoloration method and the FRAP assay, and also the TPC of the methanol extract of HP was determined. The results are shown in **Figure 3**. Obviously, the antioxidant capacity of the HP extract increased with heating time and temperature. For example, after being heated at 120 °C for 90 min, the TPC increased from 37.33 to 47.20 GAE mg/g of DW by the Folin−Ciocalteu method, TAC increased from 43.66 to 58.21 TEAC mg/g of DW by the ABTS method, and TAC increased from 19.66 to 33.14 TEAC mg/g of DW by the FRAP assay. According to the results of the ABTS radical scavenging assay, the maximum TAC of HP extract was found after heat treatment of 150 °C for 30 min (59.95 TEAC mg/g of DW), which was in accordance with the result of FRAP (38.2 TEAC mg/g of DW) and TPC (50.07 GAE mg/g of DW). However, the effect of heat treatment at lower temperature (90 °C for 30 min) on TAC was minimal.

The correlation coefficients among TPC, ABTS assay, FRAP assay, and TCB in the free fraction are shown in **Figure 4**. As can be seen, correlation coefficients in each case were significantly high ($P < 0.05$), which meant that the increase of TAC of the HP extract was due at least in part to the increase of TCB in the free fraction. TCB in the free fraction may serve as an indicator of the increase of lower molecular weight phenolic compounds liberated from HP after heat treatment.

There are many plants and herbs that have potential medical uses for mankind, so it is necessary to pay more attention to the methods which can enhance the antioxidant capacity of their extracts. As had been reported before, the antioxidant capacity of citrus peel can be enhanced after heat treatment (*14*). Here we employed HPLC-PDA instead of GC-MS to investigate the change of phenolic acids and FGs after heat treatment to achieve a more precise and comprehensive result. Furthermore, a more complicated extraction method was adopted; as a result, more specific information could be obtained about the effect of heat treatment on the antioxidant capacity of citrus peel. Finally, we suggested that perhaps there were other mechanisms responsible for the enhancement of antioxidant capacity of HP after heat treatment, which were not involved in this research.

## ABBREVIATIONS USED

HP, huyou peel; HPLC-PDA, high-performance liquid chromatography−photodiode array detector; $ABTS^{\bullet+}$, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate); FGs, flavanone glycosides; TPC, phenolics contents; FRAP, ferric reducing antioxidant power; TCB, total cinnamics and benzoics; TAC, total antioxidant capacity.

## LITERATURE CITED

(1) Kris-Etherton, P. M.; Hecker, K. D.; Bonanome, A.; Coval, S. M.; Binkoski, A. E.; Hilpert, K. F.; Griel, A. E.; Etherton, T. D. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.* **2002**, *113* (9) (Suppl. 2), 71−88.

(2) Silalahi, J. Anticancer and health protective properties of citrus fruit components. *Asia Pac. J. Clin. Nutr.* **2002**, *11*, 79−84.

(3) *Pharmacopoeia of People's Republic of China 2000* (in Chinese); Chemical Industry Press: Beijing, China, 2000; pp 108−108.

(4) Rouseff, R. L.; Martin, S. F.; Youtsey, C. O. Quantitative survey of narirutin, naringin, hesperidin, and neohesperidin in citrus. *J. Agric. Food Chem.* **1987**, *35*, 1027−1030.

(5) Bocco, A.; Cuvelier, M. E.; Richard, H.; Berset, C. Antioxidant activity and phenolic composition of citrus peel and seed extracts. *J. Agric. Food Chem.* **1998**, *46*, 2123−2129.

(6) Manthey, J. A.; Grohmann, K. Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. *J. Agric. Food Chem.* **2001**, *49*, 3268−3273.

(7) Lodovici, M.; Guglielmi, F.; Meoni, M.; Dolara, P. Effect of natural phenolic acids on DNA oxidation in vitro. *Food Chem. Toxicol.* **2001**, *39*, 1205−1210.

(8) Olthof, M. R.; Hollman, P. C. H.; Katan, M. B. Chlorogenic acid and caffeic acid are absorbed in humans. *J. Nutr.* **2001**, *131*, 66−71.

(9) Maggi-Capeyron, M. F.; Ceballos, P.; Cristol, J. P.; Delbosc, S.; Le Doucen, C.; Pons, M.; Léger, C. L.; Descomps, B. Wine phenolic antioxidants inhibit AP-1 transcriptional activity. *J. Agric. Food Chem.* **2001**, *49*, 5646−5652.

(10) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure−antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.

(11) Kim, D. O.; Lee, C. Y. Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 253−273.

(12) Robbins, R. J. Phenolic acids in foods: an overview of analytical methodology. *J. Agric. Food Chem.* 2**003**, *51*, 2866−2887.

(13) Seung-Cheol, L.; Seok-Moon, J.; So-Young, K.; Nam, K. C.; Ahn, D. U. Effect of far-infrared irradiation on the antioxidant activity of defatted sesame meal extracts. *J. Agric. Food Chem.* **2005**, *53*, 1495−1498.

(14) Seok-Moon, J.; So-Young, K.; Dong-Ryul, K.; Seong-Chun, J.; Nam, K. C.; Ahn, D. U.; Seung-Cheol, L. Effect of heat treatment on the antioxidant activity of extracts from citrus peels. *J. Agric. Food Chem.* **2004**, *52*, 3389−3393.

(15) Proteggente, A. R.; Saija, A.; De Pasquale, A.; Rice-Evans, C. A. The compositional characterisation and antioxidant activity of fresh juices from sicilian sweet orange (*Citrus sinensis* L. Osbeck) varieties. *Free Radical Res.* **2003**, *37*, 681−687.

(16) Manthey, J. A. Fractionation of orange peel phenols in ultrafiltered molasses and mass balance studies of their antioxidant levels. *J. Agric. Food Chem.* **2004**, *52*, 7586−7592.

(17) Rapisarda, P.; Pannuzzo, P.; Romano, G.; Russo, G. Juice components of a new pigmented citrus hybrid *Citrus sinensis* (L.) Osbeck × *Citrus clementina* Hort. ex Tan. *J. Agric. Food Chem.* **2003**, *51*, 1611−1616.

(18) Yoo, K. M.; Lee, K. W.; Park, J. B.; Lee, H. J.; Hwang, I. K. Variation in major antioxidants and total antioxidant activity of yuzu (*Citrus junos* Sieb ex Tanaka) during maturation and between cultivars. *J. Agric. Food Chem.* **2004**, *52*, 5907−5913.

(19) Gorinstein, S.; Cvikrova, M.; Machacková, I.; Haruenkit, R.; Park, Y.; Jung, S.; Yamamoto, K.; Martinez Ayala, A. L.; Katrich, E.; Trakhtenberg, S. Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits. *Food Chem.* **2004**, *84*, 503−510.

(20) Mattila, P.; Kumpulainen, J. Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. *J. Agric. Food Chem.* **2002**, *50*, 3660−3667.

(21) Krygier, K.; Sosulski, F.; Hogge, L. Free, esterified, and insoluble-bound phenolic acids. I. Extraction and purification procedure. *J. Agric. Food Chem.* **1982**, *30*, 330−334.

(22) Ayaz, F. A.; Hayirlioglu-Ayaz, S.; Gruz, J.; Novak, O.; Strnad, M. Separation, characterization, and quantitation of phenolic acids in a little-known blueberry (*Vaccinium arctostaphylos* L.) fruit by HPLC-MS. *J. Agric. Food Chem.* **2005**, *53*, 8116−8622.

(23) Subba Rao, M. V. S. S. T.; Muralikrishna, G. Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted finger millet (ragi, *Eleusine coracana* Indaf-15). *J. Agric. Food Chem.* **2002**, *50*, 889−892.

(24) El-Shafae, A. M.; El-Domiaty, M. M. Improved LC methods for the determination of diosmin and/or hesperidin in plant extracts and pharmaceutical formulations. *J. Pharm. Biomed. Anal.* **2001**, *26*, 539−545.

(25) Chun, O. K.; Kim, D. O.; Smith, N.; Schroeder, D.; Han, J.; Lee, C. Y. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *J. Sci. Food Agric.* **2005**, *85*, 1715−1724.

(26) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation recolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231−1237.

(27) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.

(28) Peleg, H.; Naim, M.; Rouseff, R. L.; Zehavi, U. Distribution of bound and free phenolic acid in oranges (*Citrus sinensis*) and grapefruits (*Citrus paradisi*). *J. Sci. Food Agric.* **1991**, *57*, 417−426.

(29) Rapisarda, P.; Carollo, G.; Fallico, B.; Tomaselli, F.; Maccarone, E. Hydroxycinnamic acids as markers of Italian blood orange juices. *J. Agric. Food Chem.* **1998**, *46*, 464−470.

(30) Luthria, D. L.; Mukhopadhyay, S. Influence of sample preparation on assay of phenolic acids from eggplant. *J. Agric. Food Chem.* **2006**, *54*, 41−47.

(31) Herrmann, K. Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. *Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 315−347.

**Received for review September 2, 2006. Revised manuscript received November 5, 2006. Accepted November 9, 2006.**

JF062517L