# Total Phenolic Contents and Phenolic Acid Constituents in 4 Varieties of Bitter Melons (*Momordica charantia*) and Antioxidant Activities of their Extracts

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**ABSTRACT: Four varieties of bitter melon (*Momordica charantia*), India green (IG), India white (IW), China green (CG), and China white (CW) were analyzed for total phenolics, phenolic acid constituents, and antioxidant activities of their methanolic extracts. Phenolic contents of the oven-dried and freeze-dried tissues ranged from 5.39 to 8.94 and 4.64 to 8.90 mg chlorogenic acid equivalent (CAE)/g on dry weight basis, respectively. Phenolic contents of bitter melon seed, inner tissues, and flesh ranged from 4.67 to 8.02, 4.64 to 8.94, and 5.36 to 8.90 mg CAE/g, respectively. The total phenolic contents of IG, IW, CG, and CW were 4.64 to 6.84, 6.03 to 8.94, 5.39 to 7.81, and 6.07 to 8.90 mg CAE/g, respectively. The main phenolic acids in flesh were gallic acid, gentisic acid, catechin, chlorogenic acid, and epicatechin, which ranged from 8.04 to 39.76, 16.99 to 32.39, 23.06 to 82.45, 4.55 to 15.83, and 16.14 to 44.28 mg/100 g on dry weight basis, respectively, while in inner tissues were gallic acid, gentisic acid, catechin, and epicatechin, which ranged from 2.57 to 18.05, 5.39 to 32.61, 13.54 to 39.74, and 2.96 to 40.91 mg/100 g, respectively. The main phenolic acids contained in seeds were gallic acid, catechin, and epicatechin, which ranged from 4.61 to 18.9, 13.2 to 57.61, and 6.00 to 40.08 mg/100 g, respectively. There was no significant difference in the antioxidant activities of the extracts among varieties ($P = 0.2556$) and between drying methods ($P = 0.1444$). The antioxidant activities of flesh, inner tissue, and seed ranged from 81.7% to 86.5%, 78.8% to 88.4%, and 78.5% to 85.4% inhibition, respectively. Bitter melon is a rich source of phenolic compounds. These natural plant phenolics can be a good source of antioxidants for application in food system.**

**Keywords: bitter melon, phenolics, extract, antioxidant**

## Introduction

Plants contain a large variety of phenolic derivatives including simple phenols, benzoic acid derivatives, flavonoids, phenylpropanoids, tannins, lignans, and lignins. These various substances are essential for the growth and reproduction of plants as well as contribute to plant tissue's defense mechanism against infections or injuries caused by insects and microbial pathogens and environmental stresses such as UV irradiation, fungicide treatment, temperature, or air pollution (Beier and Oertli 1983; Dercks and others 1990; Babic and others 1993; Friedman 1997; Nigg and others 1997).

Many properties of plant products are associated with the presence, type, and content of their phenolic compounds. The beneficial health effects of certain phenolics are significant to producers and consumers of foods. Fruits and vegetables are excellent sources of phenolics. Many studies showed that these phenolic compounds exhibit health-promoting effects such as reducing blood pressure and lowering incidences of cancer and cardiovascular diseases (Huang and Ferraro 1992; Tanaka and others 1993; Balentine and others 1997; Bravo 1998; Surh 1999; Gorinstein and other 2002; Wang and Mazza 2002; Hannum 2004). However, published data on the content of phenolics in fruit and vegetables are still incomplete and restrictive to a few cultivars.

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Many preservatives are added to foods as antioxidant agents. Phenolic compounds are known to possess this property. Antioxidants markedly delay or prevent oxidation of the substrate, when they are present in foods or in the body at low concentrations. Furthermore, beneficial effects of consuming plant foods that have been ascribed to the presence of phenolics are achieved by preventing lipid oxidation, protein cross-linking, and DNA mutation and tissue damage. Antioxidants can be classified as free radical terminators, chelators of metal ions, or oxygen scavengers that react with oxygen in closed system. Phenolic antioxidants are included in the category of free radical terminators. Synthetic food antioxidants currently permitted for use in foods are butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate, dodecyl gallate, and tertiary butylhydroquinone. However, people are concerned to consume foods that contain synthetic compounds as ingredients. Natural antioxidants are primarily plant phenolics and polyphenolic compounds that may occur in all parts of the plant. Exploration of natural phenolic compounds from plants is an alternative to replace synthetic antioxidants to maintain the quality of the foods from oxidative deterioration.

Bitter melon (*Momordica charantia*) or bitter gourd is an important cultivated food crop and widely used as vegetable in Asia. Fruit pulp, seed, and whole plant of bitter gourd have been investigated for their hypoglycemic effects (Ali and others 1993; Srivastava and others 1993; Jayasooriya and others 2000). Bitter melon also has been reported to have other medicinal properties such as anticarcinogenic and hypocholesterolemic (Ganguly and others 2000; Ahmed and others 2001). Like many plants, bitter melon may be a




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source of phenolic compounds. Although the value of bitter melon is realized, information on its phenolic constituents is limited. More detailed investigation on bitter melon phenolics is needed to provide information for their nutraceutical values.

The objectives of this research were to determine the total phenolic contents and phenolic acid constituents of bitter melon tissues from 4 varieties and evaluate the antioxidant activities of their phenolic extracts.

## Materials and Methods

### Materials

Four varieties of bitter melon, India white, India green, China white, and China green, were provided by the Univ. of Arkansas at Pine Bluff (Pine Bluff, Ark., U.S.A.). Fourteen standard phenolic acids were used for phenolic acid constituent determination using HPLC. Eleven phenolic acids including gallic acid, protocatechuic acid, gentistic acid, (+)-catechin, vanillic acid, syringic acid, (–)-epicatchin, *p*-coumaric acid, benzoic acid, sinapinic acid, and o-coumaric acid were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Chlorogenic acid, *t*-cinnamic acid, and *t*-ferulic acid (*trans*-4-hydroxy-3-methoxycinnamic acid) were purchased from Aldrich Chemical Co. (Milwaukee, Wis., U.S.A.). Trifluoroacetic acid, methanol, acetonitrile, and water used for high-performance liquid chromatography (HPLC) analysis were HPLC grade.

### Sample preparation

Flesh, seed coat tissue or inner tissue (IT), and seed (Figure 1) of bitter melons var. India white, India green, China white, and China green were separated soon after the samples were received. The flesh was chopped into approximately 1 to 2 mm thickness using a food processor (Model FP1200, The Black & Decker Corp., Towson, Md., U.S.A.). The separated tissues were then dried in either a freeze-drier (Model 25LE, The Virtis Co., Inc., Gardiner, N.Y., U.S.A.) or an oven (at 60°C for 18 h, about 1 to 1.5 cm thickness of layer on a stainless steel tray), ground using a coffee grinder model KSM 2B (Gillette Canada, Mississauga, Ontario, Canada), and passed through a 60-mesh sieve (W. S. Tyler Inc., Mentor, Ohio, U.S.A.). The fine ground samples were then stored at 4°C for further analysis.

### Total phenolic determination

Total phenolics of the fine ground flesh, inner tissue, and seed of the bitter melons were determined by Folin-Ciocalteau method (Singleton and Rossi 1965). One hundred milligrams of each sample were weighed into a screw-cap test tube and vortexed with 10 mL methanol. The dispersion was heated in a water bath of 65°C for 2 h and allowed to cool at room temperature. To 1 mL of the clear solution in a screw-cap test tube, 1.0 mL of 0.25 N Folin-Ciocalteau's reagent, 1.0 mL of 1 N sodium carbonate, and 7 mL of deionized water were added. The tubes were vortexed and held for 2 h at room temperature. Absorption of the solution at 726 nm was measured using a spectrophotometer (Shimadzu Model UV-1601, Kyoto, Japan). The total phenolic content was expressed as chlorogenic acid equivalents (CAE) in milligram per gram dry material and calculated as follows: total phenolics (mg/g dry weight) = (A × 222.57)/10, where A was absorbance at 726 nm.

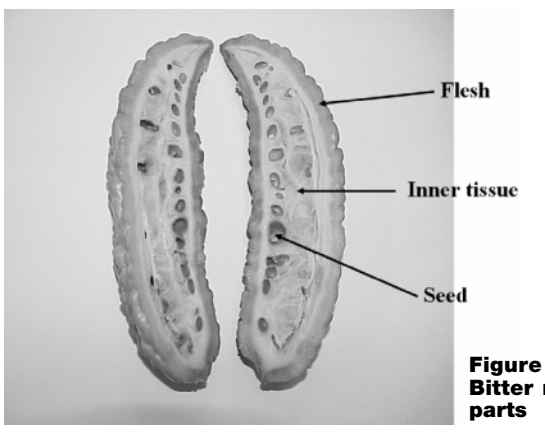


**Figure 1—Bitter melon parts**

### Methanolic extraction of phenolics

Phenolics of the fine ground flesh, inner tissue, and seed of the bitter melon samples were extracted by methanol. Five hundred milligrams of the fine ground sample were weighed into a test tube and 5 mL methanol was added. The tube was then heated for 2 h in a 65°C water bath. During heating, the dispersion was vortexed every 30 min. After extraction, the dispersion was cooled at room temperature and centrifuged (centrifuge model J2-21, Beckman, Fullerton, Calif., U.S.A.) at 3000 × g for 10 min. The residue was rinsed with 1 mL methanol, and the clear solution was combined with the previous supernatant. The combined methanolic extract was then evaporated to dryness under a stream of nitrogen at 60°C. The dried methanolic extract was stored at 4°C for analysis.

### Phenolic acid constituent determination

The phenolic acid constituents of the methanolic extracts were determined using HPLC by the method of Cai and others (2003) with some modification. Twenty milligrams of the extracts were dissolved in 0.2 mL of methanol, and the solution was filtered through a 0.2-μm PVDF Target Syringe Filter (Natl. Scientific, Duluth, Ga., U.S.A.). Phenolics were quantified by a Hewlett-Packard Liquid Chromatograph model 1090 equipped with UV detector (Agilent Technologies, Inc., Palo Alto, Calif., U.S.A.), and absorbance was monitored at 254 nm. A TSK-GEL Super-ODS (Supelco, Bellefonte, Pa., U.S.A.) column was used. The mobile phases consisted of solvent A (0.1% trifluoroacetic acid in acetonitrile), solvent B (0.1% trifluoroacetic acid in HPLC grade water), and solvent C (100% methanol, HPLC grade). Flow rate was set at 1.0 mL/min, and column temperature was maintained at 37°C throughout of the test. The initial solvent composition was 0% solvent A and 100% solvent B. A linear gradient was used to increase solvent A from 0% to 10% within 7 min. This solvent composition was maintained at an isocratic flow for 3 min. The solvent A was then increased from 10% to 40% using a 20-min linear gradient. This composition was maintained for 2 min and returned to the initial composition in 3 min. Solvent C was used for washing the column after each run. A sample size of 6 μL for the intact phenolics was injected for the HPLC analyses. The concentrations of phenolic acids in the sample were calculated from standard curves calibrated using 14 standard phenolics as equal to 20(aA + b)W in mg/100 g sample, where A is peak area, W is weight of extract from 0.5 g dry material, and a and b are the slope and intercept of each calibration phenolic acid standard curve, respectively.

### Antioxidant activity determination

Antioxidant activity was carried out by oxidizing linoleic acid methyl ester (MeLo) in the presence of phenolic extracts as antioxidants as described by Heinonen and others (1998). Two milligrams of the extracts were dissolved in 10 mL methanol. Five hundred microliters of the extract solution were added into 0.2 g MeLo (500 ppm extract in MeLo), and the methanol was evaporated under a stream of nitrogen at ambient temperature. Five hundred microliters of methanol were added into 0.2 g MeLo for blank as a reference. Oxidation of MeLo in the present of extract was carried out in at 40°C for 72 h. Two milligrams of sample aliquots were taken at the starting point (zero

**Table 1—Total phenolic contents of bitter melon tissues from varieties (in mg/g, dried basis)[a]**

| Varieties of bitter melon | Oven-dried (OD) | | | Average from all OD tissues | Freeze-dried (FD) | | | Average from all FD tissues | *P* value |
|---|---|---|---|---|---|---|---|---|---|
| | Flesh | IT[b] | Seed | | Flesh | IT | Seed | | |
| Indian green | 6.52 ± 0.06bcC | 6.84 ± 0.12aB | 6.72 ± 0.08abB | 6.69 | 6.40 ± 0.06cD | 4.64 ± 0.10dD | 4.67 ± 0.02dC | 5.24 | <0.0001 |
| Indian white | 7.06 ± 0.12cB | 8.94 ± 0.36aA | 8.02 ± 0.02bA | 8.01 | 7.21 ± 0.04cC | 7.90 ± 0.09bA | 6.03 ± 0.05dB | 7.05 | <0.0001 |
| China green | 5.39 ± 0.06dD | 7.01 ± 0.07bB | 7.74 ± 0.05aA | 6.71 | 7.81 ± 0.14aB | 5.69 ± 0.25dC | 6.18 ± 0.02cB | 6.56 | <0.0001 |
| China white | 7.75 ± 0.07bA | 6.07 ± 0.16dC | 6.87 ± 0.31cB | 6.90 | 8.90 ± 0.09aA | 6.59 ± 0.23cdB | 6.69 ± 0.19cA | 7.39 | 0.0003 |
| *P* value | <0.0001 | <0.0001 | <0.0001 | | <0.0001 | <0.0001 | <0.0001 | | |

[a]Value are means ± SD of triplicate samples from each of 3-y crops; mean values with different lowercase letters in the same row and different uppercase letters in the same column are significantly different ($P < 0.05$).
[b]IT, inner tissue.

time) and after 72 h of oxidation (at 40°C) and dissolved in 10 mL of 2,2,4-trimethylpentane (isooctane). The conjugated diene absorption of the aliquots was read using a spectrophotometer (Shimadzu Model UV-1601, Kyoto, Japan) at a wavelength of 234 nm. The antioxidant activities were expressed as percentage inhibition of conjugated diene hydroperoxides formation of MeLo after 72 h of oxidation comparing to blank from MeLo as a reference antioxidant as follows: % inhibition = $[(A_{B(72\ h)} - A_{B(0\ h)}) - (A_{E(72\ h)} - A_{E(0\ h)})/(A_{B(72\ h)} - A_{B(0\ h)})] \times 100$, where A is absorbance, E is extract, and B is blank.

### Statistical analysis

All values are reported as means of triplicate samples from each of 3-y crops (2001, 2002, and 2003). Split plot complete randomized design was conducted using JMP 5 software package (SAS 2002) for the total phenolic contents of the bitter melon tissues, and antioxidant activities of methanolic extracts of these drying tissues and Tukey HSD procedure was performed for the significance of differences among varieties, types of tissues, and drying methods at the 5% significance level.

## Results and Discussion

### Total phenolic contents of bitter melon

Total phenolic contents of oven-dried and freeze-dried bitter melon tissues from 4 varieties are given in Table 1. Overall, oven-dried samples were significantly higher than freeze-dried samples ($P < 0.0001$). Phenolic contents of the oven-dried and freeze-dried tissues ranged from 5.39 to 8.94 and 4.64 to 8.90 mg of CAE/g dry material, respectively. Total phenolic acids observed from HPLC analysis (Table 2, 3, and 4) also showed that oven-dried flesh, inner tissue, and seed were higher in the phenolic content than those from freeze-dried samples. When unknown and known phenolic acids were calculated from HPLC analysis (not shown in tables), the total phenolics of oven-dried samples were about two times higher than those of freeze-dried samples (6.74 to 10.32 and 3.29 to 5.94 mg CAE/g dry material, 8.14 to 11.02 and 4.71 to 6.80 mg CAE/g dry material, and 5.52 to 10.05 and 2.03 to 3.62 mg CAE/g dry material in oven-dried and freeze-dried flesh, seed, and inner tissue, respectively). The slightly higher phenolic contents in the oven-dried samples may be caused by the release of bound phenolics. There is a lack of information on the mechanism on the effect of moderate heat in phenolics of plants and vegetables.

Phenolic contents of the bitter melon seed, inner tissue, and flesh ranged from 4.67 to 8.02 mg CAE/g dry material, 4.64 to 8.94 mg CAE/g dry material, and 5.36 to 8.90 mg CAE/g dry material, respectively. Phenolic contents of the flesh were significantly higher than those of the inner tissue and seed ($P < 0.0001$), and phenolic contents of the seed was the lowest among those of all the tissues ($P < 0.0001$).

In many comparative studies, various plant cultivars such as plums, apples, and grapes showed different levels of phenolic content (Javanmardi and others 2003; Kim and others 2003; Lee and others 2003; Pastrana-bonilla and others 2003). This variation, even within the same variety, depends on many factors including environmental factors, maturity, location, and soil condition. The total phenolic contents of these 4 varieties were significantly different ($P < 0.0001$) with the highest being India white followed by China white, China green, and India green. The total phenolic contents of bitter melon from var. India green, India white, China green, and China white were 4.64 to 6.84, 6.03 to 8.94, 5.39 to 7.81, and 6.07 trifluoroacetic acid 8.90 mg CAE/g dry material, respectively.

### Phenolic acid constituents of bitter melon

Phenolic acids constituents of methanolic extracts from bitter melon tissues were quantified by HPLC. The phenolic acid constituents of the sample were identified by their retention times, while quantification was made by comparing the area [mAU*s] of each corresponding peak of the sample and the calibration curves of the standards that were obtained from 3 varying concentrations. The HPLC profiles of 14 standard phenolics are shown in Figure 2a. Figure 2b, 2c, and 2d show the chromatograms of oven-dried and freeze-dried flesh, seed, and inner tissue from 4 varieties of bitter melon. Unknown compounds that had retention times of less than 10 min were present in bitter melon tissues.

Phenolic acid constituents of oven-dried and freeze-dried bitter melon fleshes that were quantified by HPLC using 14 standard phenolic acids are shown in Table 2. The main phenolic acids, which were present in bitter melon flesh, were gallic acid, gentisic acid, catechin, chlorogenic acid, and epicatechin. Gallic acid, gentisic acid, catechin, chlorogenic acid, and epicatechin contents of the bitter melon ranged from 8.04 to 39.76, 16.99 to 32.39, 23.06 to 82.45, 4.55 to 15.83, and 16.14 to 44.28 mg/100 g dry material. Protocatechuic acid, vanillic acid, syringic acid, *p*-coumaric acid, and benzoic acid were present in small amount (less than 10 mg/g dry material) in the flesh of all varieties of the bitter melons. The amounts of these constituents ranged from 2.07 to 8.78, trace to 2.42, 1.77 to 3.67, 1.83 to 8.23, and ND to 5.35 mg/100 g dry material for protocatechuic acid, vanillic acid, syringic acid, and o-coumaric acid, respectively. *trans*-Cinnamic acid was present in trace amount, while sinapic acid and *trans*-ferulic acid were not detected in the flesh from all varieties.

Table 3 shows the phenolic acid constituents of oven-dried and freeze-dried bitter melon seeds from 4 different varieties. Overall, the main phenolic acids in the bitter melon seeds were gallic acid, catechin, and epicatechin. Gallic acid, catechin, and epicatechin contents of bitter melon seeds ranged from 4.61 to 18.9, 13.2 to 57.61, and 6.00 to 40.08 mg/100 g dry material, respectively. Even though it was not detected in oven-dried seed from variety India green, gentisic acid was present in the other oven-dried seeds more than 10 mg/100 g dry material (14.42 to 21.67 mg/100 g dry material). However, gentisic acid was not detected in all freeze-dried seeds. In the bitter melon flesh, there were 2 phenolic acids, sinapic acid and *trans*-ferulic acid, which were not detected in the seed from all varieties. Two phenolic acids, vanillic acid and o-coumaric acid, were found in trace amounts or not detected (ND) in bitter melon

seeds. The amounts of protocatechuic acid, chlorogenic acid, syringic acid, *p*-coumaric acid, benzoic acid, and *trans*-cinnamic acid in bitter melon seeds were 1.02 to 5.75, 1.74 to 5.53, 1.01 to 6.19, 1.44 to 3.55, 1.12 to 4.54, and trace to 4.63 mg/100 g dry material, respectively.

The main phenolic acids in bitter melon inner tissues were gallic acid, gentisic acid, catechin, and epicatechin (Table 4). These phenolic acid contents were 2.57 to 18.05, 6.98 to 32.61, 13.54 to 39.74, and ND to 40.91 mg/100 g dry material as gallic acid, gentisic acid, catechin, and epicatechin, respectively, with the exception of freeze-dried inner tissue of bitter melon var. China white that contained no epicatechin. The minor phenolic acids in the bitter melon inner tissues were protocatechuic acid, vanillic acid, chlorogenic acid, syringic acid, *p*-coumaric acid, benzoic acid, and o-coumaric acid. *trans*-Cinnamic acid was present in trace amount, while no *trans*-ferulic acid and sinapic acid was detected in the inner tissue of all varieties.

The results above indicate that phenolic acid constituents were distributed in various amounts for each phenolic acid among varieties or parts of tissues. The differences among varieties could be due to the variability of each variety against environmental stresses that can induce an increase in phenolic acid constituents as a natural reaction of the plants to survive. Variability of phenolic acid constituents among different tissues could be caused by different biochemical mechanisms in their synthesis during growing period. Some evaluations on the levels of the phenolic components in the various parts of other plants showed differences in the amount of phenolic compounds as well (Pastrana-bonilla and others 2003; Pyo and others 2004; Yilmaz and Toledo 2004).

There is no or undetectable amount of ferulic acid in bitter melon in comparison to cereals such as rice, corn, and wheat, that are rich in ferulic acid (Sosulski and others 1982; Zhou and others 2004). However, bitter melon has higher contents of other phenolic acids such as gallic acid, gentisic acid, catechin, and epicathecin. Berries including grapes are predominant in anthocyanins. These contain a wide variety of phenolic acids and flavonoids including gallic acid, catechin, and epicatechin and some other minor phenolic acids. The various types of phenolics in bitter melon can contribute to antioxidant activity.

## Antioxidant activities of bitter melon extract

Antioxidant activities (in % inhibition) of oven-dried and freeze-dried samples of 4 varieties of bitter melons are shown in Table 5. The level of the extracts used can affect the extent of inhibition of lipid

**Table 2—Phenolic acid constituents of oven-dried and freeze-dried bitter melon fleshes (mg per 100 g of flour, dried basis)[a]**

| | Oven-dried | | | | Freeze-dried | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Phenolic acid | IG | W | CG | CW | IG | IW | CG | CW | *P* value |
| Gallic acid | 10.23 ± 0.21 | 15.85 ± 0.59 | 39.76 ± 0.75 | 20.66 ± 0.29 | 8.04 ± 0.14 | 8.69 ± 0.40 | 13.74 ± 0.23 | 12.42 ± 0.09 | <0.0001 |
| Protocatechuic acid | 3.59 ± 0.17 | 6.14 ± 0.23 | 2.07 ± 0.18 | 5.66 ± 0.09 | 3.85 ± 0.23 | 2.41 ± 0.03 | 3.17 ± 0.05 | 5.51 ± 0.21 | <0.0001 |
| Gentisic acid | 21.43 ± 1.95 | 24.34 ± 1.71 | 24.00 ± 0.64 | 24.65 ± 0.52 | 20.07 ± 0.68 | 16.99 ± 0.81 | 20.34 ± 0.12 | 27.47 ± 1.21 | <0.0001 |
| Catechin | 70.03 ± 3.06 | 82.45 ± 0.66 | 78.67 ± 0.21 | 68.16 ± 2.30 | 23.06 ± 0.47 | 25.26 ± 0.34 | 23.79 ± 0.37 | 34.50 ± 0.78 | <0.0001 |
| Vanillic acid | 2.15 ± 0.12 | 2.42 ± 0.09 | 1.84 ± 0.10 | 2.01 ± 0.00 | 1.25 ± 0.03 | Tr | 1.29 ± 0.03 | 1.35 ± 0.05 | <0.0001 |
| Chlorogenic acid | 10.73 ± 0.68 | 6.42 ± 0.10 | 14.15 ± 0.25 | 8.66 ± 0.07 | 16.37 ± 0.45 | 4.55 ± 0.29 | 15.83 ± 0.19 | 11.64 ± 0.13 | <0.0001 |
| Syringic acid | 2.10 ± 0.35 | 3.15 ± 0.09 | 2.91 ± 0.14 | 2.85 ± 0.06 | 1.77 ± 0.08 | 1.77 ± 0.09 | 2.14 ± 0.15 | 3.67 ± 0.29 | <0.0001 |
| Epicatechin | 21.14 ± 0.74 | 20.98 ± 0.78 | 16.61 ± 0.73 | 24.10 ± 0.56 | 18.90 ± 1.96 | 17.44 ± 0.33 | 32.03 ± 0.96 | 32.38 ± 1.34 | <0.0001 |
| *p*-Coumaric acid | 4.97 ± 0.16 | 3.07 ± 0.10 | 1.83 ± 0.04 | 3.90 ± 0.12 | 8.23 ± 0.17 | 3.85 ± 0.13 | 7.37 ± 0.06 | 8.19 ± 0.33 | <0.0001 |
| *trans*-Ferulic acid | ND | ND | ND | ND | ND | ND | ND | ND | <0.0001 |
| Sinapic acid | ND | ND | ND | ND | ND | ND | ND | ND | <0.0001 |
| Benzoic acid | 3.12 ± 0.18 | 2.99 ± 0.10 | 3.96 ± 0.09 | 3.65 ± 0.05 | Tr | ND | 2.90 ± 0.06 | 3.05 ± 0.11 | <0.0001 |
| o-Coumaric acid | ND | ND | 1.96 ± 0.12 | 2.43 ± 0.02 | 2.18 ± 0.15 | ND | 2.47 ± 0.03 | 2.33 ± 0.11 | <0.0001 |
| *trans*-Cinnamic acid | Tr | Tr | 1.31 ± 0.04 | Tr | Tr | ND | Tr | ND | <0.0001 |
| Total phenolic acids | 149.5 | 167.8 | 189.1 | 166.7 | 103.7 | 81.0 | 125.1 | 142.5 | |
| *P* value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |

[a]Values are means ± SD. of triplicate samples from each of 3-y crops. IG, bitter melon var. India Green; IW, bitter melon var. India White; CG, bitter melon var. China Green; CW, bitter melon var. China white; ND, not-detectable; Tr, trace (less than 1 mg per 100 g dried basis)

**Table 3—Phenolic acid contents of oven-dried and freeze-dried bitter melon seeds (mg per 100 g of flour, dried basis)[a]**

| | Oven-dried | | | | Freeze-dried | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Phenolic acid | IG | IW | CG | CW | IG | IW | CG | CW | *P* value |
| Gallic acid | 8.93 ± 0.25 | 18.90 ± 0.20 | 11.60 ± 0.74 | 6.82 ± 0.51 | 4.61 ± 0.21 | 4.28 ± 0.31 | 5.08 ± 0.22 | 7.14 ± 0.07 | <0.0001 |
| Protocatechuic acid | 4.26 ± 0.02 | 5.75 ± 0.03 | 4.30 ± 0.02 | 4.16 ± 0.01 | 1.02 ± 0.09 | 1.56 ± 0.06 | 2.41 ± 0.06 | 1.89 ± 0.04 | <0.0001 |
| Gentisic acid | ND | 21.67 ± 0.03 | 14.42 ± 0.20 | 16.01 ± 0.29 | ND | ND | ND | ND | <0.0001 |
| Catechin | 49.50 ± 0.53 | 57.61 ± 0.86 | 44.65 ± 0.97 | 55.23 ± 0.92 | 24.85 ± 0.50 | 33.85 ± 0.99 | 36.37 ± 0.56 | 37.00 ± 0.82 | <0.0001 |
| Vanillic acid | 1.05 ± 0.06 | 1.60 ± 0.01 | 1.16 ± 0.03 | 1.27 ± 0.02 | Tr | Tr | Tr | Tr | <0.0001 |
| Chlorogenic acid | 4.08 ± 0.09 | 5.53 ± 0.15 | 3.18 ± 0.22 | 3.03 ± 0.24 | 2.94 ± 0.20 | 3.18 ± 0.15 | 3.56 ± 0.04 | 3.85 ± 0.07 | <0.0001 |
| Syringic acid | 2.31 ± 0.04 | 6.19 ± 0.08 | 3.11 ± 0.01 | 2.88 ± 0.15 | 1.45 ± 0.23 | 3.21 ± 0.02 | 2.29 ± 0.07 | 2.39 ± 0.04 | <0.0001 |
| Epicatechin | 29.89 ± 0.62 | 40.08 ± 1.10 | 31.15 ± 0.49 | 32.50 ± 0.61 | 10.90 ± 0.56 | 17.24 ± 0.16 | 22.75 ± 0.11 | 18.45 ± 0.46 | <0.0001 |
| *p*-Coumaric acid | 3.01 ± 0.27 | 3.55 ± 0.24 | 2.88 ± 0.13 | 2.67 ± 0.12 | 1.47 ± 0.10 | 2.25 ± 0.15 | 2.85 ± 0.24 | 2.72 ± 0.07 | <0.0001 |
| *trans*-Ferulic acid | ND | ND | ND | ND | ND | ND | ND | ND | <0.0001 |
| Sinapic acid | ND | ND | ND | ND | ND | ND | ND | ND | <0.0001 |
| Benzoic acid | 3.74 ± 0.08 | 4.54 ± 0.24 | 3.63 ± 0.10 | 3.92 ± 0.16 | 1.38 ± 0.09 | 2.31 ± 0.01 | 2.70 ± 0.80 | 1.88 ± 0.08 | <0.0001 |
| o-Coumaric acid | 1.82 ± 0.07 | 1.96 ± 0.06 | 1.56 ± 0.02 | 1.44 ± 0.06 | 1.04 ± 0.08 | 1.48 ± 0.03 | 1.41 ± 0.06 | 1.34 ± 0.06 | <0.0001 |
| *trans*-Cinnamic acid | 4.47 ± 0.09 | 4.63 ± 0.02 | 3.95 ± 0.10 | 1.25 ± 0.01 | 1.30 ± 0.09 | 1.23 ± 0.05 | 1.26 ± 0.14 | | <0.0001 |
| Total phenolic acids | 113.1 | 172.0 | 125.6 | 131.2 | 51.0 | 70.6 | 80.7 | 76.7 | |
| *P* value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |

[a]Values are means ± SD of triplicate samples from each of 3-y crops. IG, bitter melon var. India Green; IW, bitter melon var. India White; CG, bitter melon var. China Green; CW, bitter melon var. China white; ND, not-detected; Tr, Trace (less than 1 mg per 100 g dried basis).

**Table 4—Phenolic acid constituents of oven-dried and freeze-dried bitter melon inner tissues (mg per 100 g of flour, dried basis)[a]**

| | Oven-dried | | | | Freeze-dried | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Phenolic acid | IG | IW | CG | CW | IG | IW | CG | CW | *P* value |
| Gallic acid | 8.46 ± 0.80 | 15.59 ± 1.09 | 4.97 ± 0.20 | 10.29 ± 0.16 | 2.57 ± 0.02 | 8.03 ± 7.47 | 3.51 ± 0.10 | 18.05 ± 1.06 | <0.0001 |
| Protocatechuic acid | 5.05 ± 0.48 | 7.82 ± 0.15 | 4.34 ± 0.22 | 4.81 ± 0.08 | 1.49 ± 0.09 | 2.69 ± 0.05 | 2.37 ± 0.09 | 2.59 ± 0.03 | <0.0001 |
| Gentisic acid | 21.78 ± 0.26 | 32.61 ± 2.22 | 18.61 ± 1.51 | 18.19 ± 0.17 | 6.98 ± 0.36 | 13.34 ± 0.69 | 11.81 ± 0.39 | 13.35 ± 0.63 | <0.0001 |
| Catechin | 17.43 ± 1.07 | 25.87 ± 2.09 | 19.49 ± 0.92 | 39.74 ± 1.21 | 13.54 ± 0.05 | 18.87 ± 0.80 | 21.63 ± 0.32 | 20.24 ± 1.92 | <0.0001 |
| Vanillic acid | 1.90 ± 0.12 | 2.19 ± 0.19 | 1.74 ± 0.22 | 1.84 ± 0.03 | 1.38 ± 0.01 | 1.48 ± 0.03 | 1.50 ± 0.03 | 2.15 ± 0.03 | <0.0001 |
| Chlorogenic acid | 6.30 ± 0.31 | 9.91 ± 0.69 | 8.33 ± 0.77 | 3.91 ± 0.06 | 4.11 ± 0.06 | 4.69 ± 0.45 | 7.49 ± 0.12 | 4.17 ± 0.12 | <0.0001 |
| Syringic acid | 1.34 ± 0.18 | 3.66 ± 0.37 | 1.28 ± 0.07 | 1.24 ± 0.03 | Tr | 1.04 ± 0.06 | 1.03 ± 0.05 | 2.08 ± 0.05 | <0.0001 |
| Epicatechin | 19.15 ± 0.39 | 40.91 ± 1.97 | 26.42 ± 0.16 | 8.55 ± 0.23 | 6.51 ± 0.16 | 14.79 ± 0.44 | 15.08 ± 0.29 | ND | <0.0001 |
| *p*-Coumaric acid | 1.88 ± 0.03 | 2.91 ± 0.38 | 1.58 ± 0.05 | 2.53 ± 0.04 | 1.15 ± 0.04 | 1.57 ± 0.02 | 3.99 ± 0.15 | 4.16 ± 0.14 | <0.0001 |
| *trans*-Ferulic acid | ND | ND | ND | ND | ND | ND | ND | ND | <0.0001 |
| Sinapic acid | ND | ND | ND | ND | ND | ND | ND | ND | <0.0001 |
| Benzoic acid | 1.80 ± 0.37 | 2.69 ± 0.18 | 1.90 ± 0.16 | 1.80 ± 0.02 | Tr | ND | ND | ND | < 0.0001 |
| o-Coumaric acid | 1.28 ± 0.05 | 2.08 ± 0.21 | 1.39 ± 0.03 | 1.35 ± 0.01 | 1.02 ± 0.01 | ND | 1.63 ± 0.04 | ND | <0.0001 |
| *trans*-Cinnamic acid | Tr | 1.09 ± 0.03 | Tr | Tr | Tr | Tr | ND | ND | <0.0001 |
| Total phenolic acids | 86.4 | 147.3 | 90.1 | 94.3 | 38.7 | 66.5 | 70.0 | 66.8 | |
| *P* value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |

[a]Values are means ± SD of triplicate samples from each of 3-y crops. IG, bitter melon var. India Green; IW, bitter melon var. India White; CG, bitter melon var. China Green; CW, bitter melon var. China white; ND, not-detected; Tr, Trace (less than 1 mg per 100 g dried basis).

**Table 5—Antioxidant activities (% inhibition) of oven-dried and freeze-dried bitter melon tissues[a]**

| | Oven-dried | | | Freeze-dried | | | |
|---|---|---|---|---|---|---|---|
| Bitter melon | Flesh | IT[b] | Seed | Flesh | IT[b] | Seed | *P* value |
| Indian green | 81.7 ± 3.8bcA | 88.4 ± 0.4aA | 85.1 ± 2.7abA | 83.5 ± 1.6abcA | 86.4 ± 0.8abA | 78.9 ± 1.6cB | 0.024 |
| Indian white | 83.1 ± 2.7abA | 87.2 ± 1.1aAB | 78.5 ± 1.2cB | 82.9 ± 2.0bA | 86.2 ± 0.5abA | 82.5 ± 1.0bcAB | 0.0364 |
| China green | 84.2 ± 2.0abA | 81.2 ± 1.4bcC | 85.4 ± 0.7aA | 83.8 ± 0.8abA | 86.1 ± 1.2aA | 80.0 ± 1.0cAB | <0.0001 |
| China white | 86.5 ± 2.0aA | 84.0 ± 2.6abBC | 79.3 ± 2.6bB | 84.2 ± 0.3abA | 78.8 ± 4.4bB | 83.7 ± 1.9abA | 0.0224 |
| *P* value | 0.2599 | 0.0021 | 0.0044 | 0.6642 | 0.0104 | 0.0131 | |

[a]Value are means ± SD of triplicate samples from each of 3-y crops. Mean values with different lowercase letters in the same row and different uppercase letters in the same column are significantly different ($P < 0.05$)
[b]IT, inner tissue.

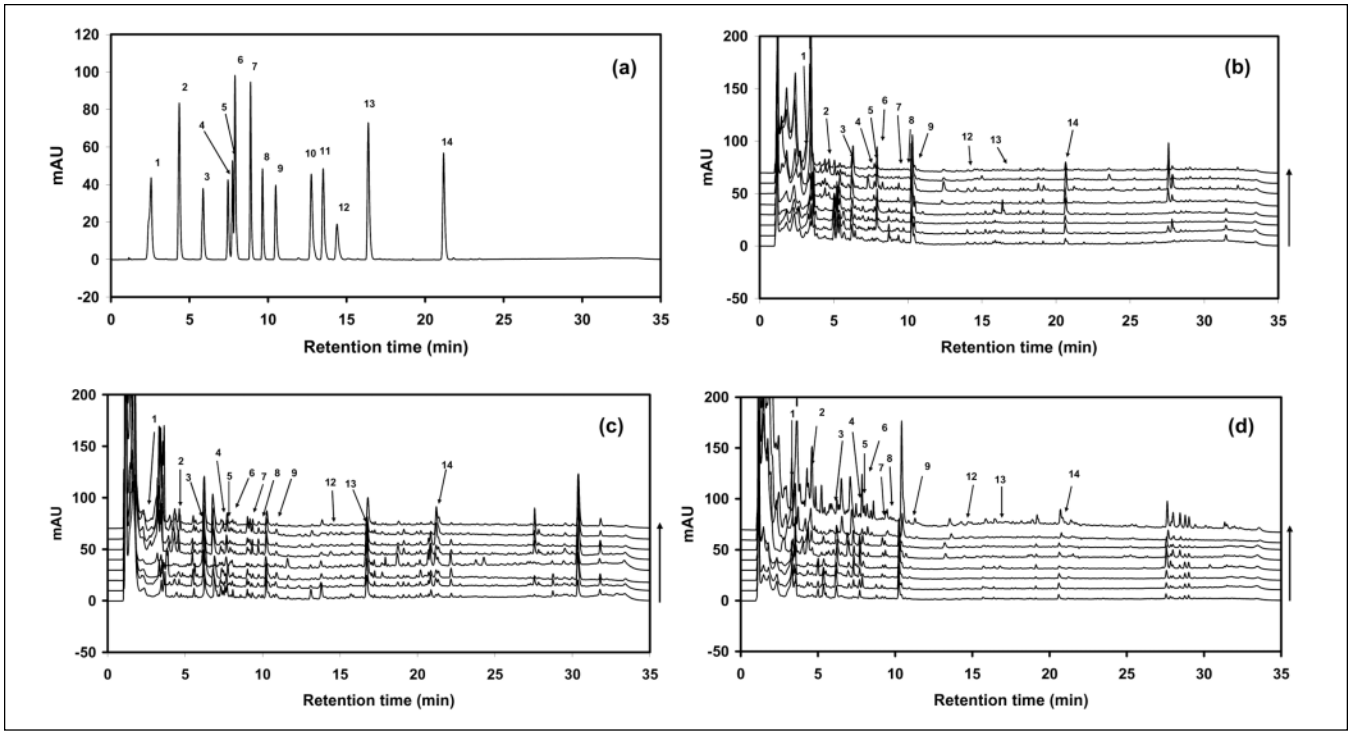


**Figure 2—HPLC profiles of 14 standard phenolic acids (a), 4 varieties of oven and freeze-dried bitter melon flesh (b), seed (c), and inner tissue (d). Peaks: 1, gallic acid; 2, protocatechuic acid; 3, gentisic acid; 4, (±)-catechin; 5, vanillic acid; 6, chlorogenic acid; 7, syringic acid; (–)epicatechin; 9, *p*-coumaric acid; 10 *t*-ferulic acid; 11, sinapic acid; 12, benzoic acid; 13, o-coumaric acid; 14, *t*-cinnamic acid. (b), (c), and (d): from bottom to top: oven-dried India white, India green, China white, and China green, and freeze-dried India white, India green, China white, and China green.**

oxidation. The inhibition values of each extract were observed at a level of 500 ppm extract in methyl linoleate. There was no significant difference in the antioxidant activities of the methanolic extracts from bitter melons among varieties ($P = 0.2556$) and between drying methods ($P = 0.1444$). Even though the total phenolic contents between drying methods or among varieties were significantly different, their similarity in antioxidant activities indicates that antioxidant activity is determined not only by their phenolic contents but also by the types of phenolic acids. Lee and others (2003) evaluated the relative total antioxidant capacity of varying phenolic acids from apple and found the distinction of antioxidant capacity among phenolic acids. The antioxidant activities of methanolic extracts from the bitter melons var. India green, India white, China green, and China white ranged from 78.9% to 88.4%, 78.5% to 87.2%, 80.0% to 86.1%, and 78.8% to 86.5% inhibition, respectively. These extracts had moderate to good inhibition effects on oxidation in comparison to some other plant extracts evaluated by Kähkönen and others (1999). The antioxidant activities of the oven-dried samples and the freeze-dried samples were 78.5% to 88.4% and 78.8% to 86.4% inhibition, respectively. The antioxidant activities of the methanolic extracts from different types of tissues were significantly different ($P < 0.0001$). The antioxidant activities of the methanolic extracts of flesh and inner tissue were not significantly different ($P = 0.0893$), while they were significantly higher than that of seeds ($P < 0.0001$). The antioxidant activities of the methanolic extracts from flesh, inner tissue, and seed ranged from 81.7% to 86.5% inhibition, 78.8% to 88.4% inhibition, and 78.5% to 85.4% inhibition, respectively. Some studies showed a high correlation between antioxidant activity and total phenolics (Reyes and Cisneros-Zevallos 2003; Aljadi and Kamaruddin 2004). This obviously indicates that antioxidant effect of many natural plant extracts is related to their phenolic components. However, some experiments showed that different phenolic compounds demonstrated different antioxidant activity and capacity (Lee and others 2003; Nuutila and others 2003; Gorinstein and others 2004; Yilmaz and Toledo 2004). The different antioxidant activities among types of tissues may be due to not only differences in their phenolic contents but also in their phenolic acid constituents.

## Conclusions

The total phenolic contents of the oven-dried tissues were higher than those of the freeze-dried tissues, which could be due an increase in the amount of simple phenols during oven-drying. Among the tissues, the highest phenolic content was the flesh followed by inner tissue and seed. Overall for oven-dried and freeze-dried among 4 varieties of bitter melon, the highest total phenolic content was India white followed by China white, China green, and India green. The main phenolic acids in the bitter melon flesh and inner tissues were gallic acid, gentisic acid, catechin, and epicatechin, while the main phenolic acids in the bitter melon seeds were gallic acid, catechin, and epicatechin. There was no significant difference on the antioxidant activities of the methanolic extracts from the bitter melons among varieties and between drying methods, but those from different types of tissues were significantly different. The antioxidant activities of the methanolic extracts from flesh and inner tissue were higher than that from seed. Bitter melon is a good source of phenolic compounds. The phenolic extracts showed high inhibition effect to prevent lipid oxidation. Among 4 varieties of bitter melon, India white was the best variety on total phenolic and phenolic acid constituents and would be used for further investigation. These natural plant phenolics in bitter melon have the potential for application in food systems to maintain food quality.

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