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# Phenolics and antioxidant properties of bayberry (*Myrica rubra* Sieb. et Zucc.) pomace

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ABSTRACT

Five cultivars of *Myrica rubra*, Biqi, Wandao, Dongkui, Dingao, and Zaodamei, were collected to analyze the phenolic compounds and evaluate the antioxidant properties of bayberry pomaces. The main anthocyanin was cyanidin-3-*o*-glucoside (3073.3–6219.2 mg/kg dry weight (DW)) and the main flavonol was quercetin-3-*o*-glucoside (296.2–907.9 mg/kg dry weight). Quercetin and myricetin were also found in the bayberry pomaces, and quercetin deoxyhexoside and myricetin deoxyhexoside were tentatively identified. The dominant phenolic acids were gallic acid (102.9–241.7 mg/kg dry weight) and protocatechuic acid (29.5–57.2 mg/kg dry weight). Other phenolic acids such as *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, and ferulic acids were also present in the bayberry pomaces, whereas, chlorogenic acid was only detected in Dongkui (1.58 mg/kg dry weight). The antioxidant activity of Wandao was the strongest of the five cultivars, whereas the activity of Dongkui was the weakest, and a significant positive relationship was observed between antioxidant activity and total phenolic content or total anthocyanins.



## 1. Introduction

Phenolic compounds are secondary metabolites in plants. Over the past 10 years, there has been increasing interest in phenolic compounds and their role in human health and nutrition (Tapiero, Tew, Nguyen, & Mathé, 2002). Some phenolic compounds present in natural products have higher antioxidant activities than those of synthetic antioxidants (Lu & Foo, 2000). These polyphenolic antioxidants can also be used to preserve foods because of their protective effects against microorganisms (Cowan, 1999; Vattem, Lin, & Shetty, 2004).

Residues from the processing of fruits and vegetables, traditionally considered as an environmental problem, are being increasingly recognized as sources for obtaining high-phenolic products. The polyphenolics from waste materials deriving from agro-industrial production may be used as functional food ingredients and as natural antioxidants to replace their synthetic equivalents that have experienced growing rejection.

Pomace is the residue remaining when fruits are processed for juice, wine, or other products. Many studies reported the fruit pomaces contained abundant phenolic compounds (Lu & Foo, 1997; Ruberto et al., 2007), which indicated that these byproducts obtained from the juice and wine industry might be useful raw materials for creating new value-added products. Other studies have been reported that the products contained high phenolics could be recovered from various pomaces, such as grape pomace (Louli, Ragoussis, & Magoulas, 2004) and apple pomace (Schieber et al., 2003).

Bayberry (*Myrica rubra* Sieb. et Zucc.) belongs to the family Myricaceae, which is widespread in tropical, subtropical, and temperate areas of the world. China is the major commercial production area for bayberry (Chen, Xu, & Zhang, 2004; Kuang, Zheng, Li, & Lu, 1979). Bayberries are purple, red, pink, or white in color when ripe, depending on the cultivar. The fruits contain rich nutritional components such as carbohydrates, sugars, organic acids, proteins, minerals, and vitamins, and are popular with local people (Chen et al., 2004; Gong, Wang, Lin, & Liang, 2004; Miao & Wang, 1987). Because they ripen in hot and wet seasons, bayberries can only be kept fresh for 3 days at 20–22 °C and for 9–12 days at 0–2 °C (Xi, Zheng, Qian, & Ying, 1993). To extend their consumption time, the fruits are usually processed into juice or wine, producing about 30–40% (fresh weight) pomace, which is considered to be an unexploited byproduct. Previous studies have shown that bayberry fruits are rich in phenolic compounds and have high antioxidant activity (Bao, Cai, Sun, Wang, & Corke, 2005; Fang, Zhang, & Wang, 2007). However, to the best of our knowledge, there have been no reports of the phenolic compounds and antioxidant activities of bayberry pomaces. This study investigated the phenolic compounds and antioxidant properties of bayberry pomaces made from five cultivars commonly used in local bayberry-juice factories. The relationships between the phenolic compounds and their antioxidant capacities were also assayed.

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## 2. Materials and methods

### 2.1. 1. Materials

Five cultivars of bayberry, Biqi, Wandao, Dongkui, Dingao, and Zaodamei, were harvested at their commercially mature stage in Cixi city, Zhejiang Province, in June, 2006. The samples (~15 kg) were randomly collected from three different orchards, and transported to our laboratory within 5 h at ambient temperature. The mixed sample was separated into pulp and stones, and the pulp was squeezed to juice by a stainless screw squeezer. The fresh pomaces were lyophilized to dryness (LGJ-30, Ningbo Scientific Biotechnology Co., Ltd. China), ground to powder, and stored at –20 °C for analysis. The analyses were carried out in triplicate.

### 2.2. Chemicals

Standards of gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, caffeic acid, ferulic acid, sinapic acid, chlorogenic acid, ellagic acid, quercetin, quercetin-3-*o*-glucoside, myricetin, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), 2,2′-azino-bis-(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), (1,1-diphenyl-2-picrylhydrazyl) (DPPH), 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) and Folin-Ciocalteu phenolic reagent were purchased from Sigma–Aldrich (St. Louis, MO, USA), cyanidin-3-*o*-glucoside was obtained from Extrasynthèse (Genay, France). Acetonitrile, methanol, ethyl acetate, formic acid, ethyl ether, sodium hydroxide, hydrochloric acid and anhydrous sodium sulfate were obtained from Shanghai Chemical Reagent Company, China.

### 2.3. Extraction of phenolic compounds from bayberry pomaces

The lyophilized powder (2 g) was extracted with 40 mL of methanol/water (80:20, v/v) with ultrasonication (40 kHz, 100 W, room temperature) and centrifugation, and the supernatant was collected. The extraction was performed four times, for 20 min each. The combined supernatants were evaporated under reduced pressure at 45 °C, and the residue was redissolved in 100 mL of 80% aqueous methanol (v/v) for analysis.

### 2.4. High-performance liquid chromatography–diode array detector–electrospray-ionization mass spectroscopy analysis of anthocyanins and flavonols

High-performance liquid chromotography–diode array detector–electrospray-ionization mass spectroscopy (HPLC–DAD–ESIMS) analyses were performed on an Agilent 1100 series HPLC system (Agilent Technologies, USA), equipped with an autosampler, binary pump, degasser, and a DAD connected directly to the mass detector (Agilent G2440A SD-Trap-XCT ion trap mass spectrometer) with an ESI source. Bayberry extract (10 μL) was injected into a Diamonsil C-18 column (5 μm, 250 × 4.6 mm i.d., Dikma Technologies, Beijing, China). Solvent A was 0.1% formic acid in water (v/v) and solvent B was 80% acetonitrile in water (v/v). The elution profile consisted of a linear gradient from 0% to 66% B for 30 min, washing (100% methanol) for 5 min, and reequilibration of the column for 10 min with solvent A at a flow rate of 1.0 mL/min. UV–vis absorption spectra were recorded online during the HPLC analysis. Spectral measurements were made over the range 200–600 nm. Flavonols were detected at 360 nm and anthocyanins at 520 nm. The concentrations of anthocyanins and flavonols were expressed as milligram per kilogram of dry weight (DW).

Mass spectra were obtained by electrospray-ionization in positive mode ($ES^+$) for anthocyanins and in negative mode ($ES^-$) for flavonols. The MS parameters were as follows: nebulizer pressure, 50.0 psi ($N_2$); dry gas, $N_2$ (13.0 mL/min); dry gas temperature, 350 °C; spray capillary voltage, 4000 V; skimmer voltage, 40.0 V; ion transfer capillary exit, 100 V; and scan range, $m/z$ 100–1000. Ultrapure He was used as the collision gas. Analyses were carried out using the data-dependent acquirement capabilities of the HPLC/MSD Trap software data system (Agilent).

### 2.5. Analysis of phenolic acids

The phenolic acids were isolated from the extracts using a previously reported method (Mattila, Hellstrom, & Törrönen, 2006; Xu, Ye, Chen, & Liu, 2007), with slight modification. An 80% methanolic pomace extract (50 mL) was evaporated under vacuum at 45 °C to about 15 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 in 5 mL of methanol for the analysis of the free phenolic acids. The aqueous phase was added to 15 mL of 8 M NaOH and hydrolyzed for 4 h under a nitrogen atmosphere at room temperature. After acidification to pH 2 using 6 M HCl, the phenolic acids released from the soluble ester were extracted as described above. The extractable phenolic acids (EPAs) were equal to the sum of the free and ester phenolic acids, and the total phenolic acids were the sum of all EPAs.

The HPLC conditions for the phenolic acid analysis were as follows: The column thermostat was set at 40 °C; solvent A consisted of 4% acetic acid, and solvent B consisted of 80% methanol in water (v/v) at a flow rate of 1 mL/min; the ratio of solvent A to solvent B was 1:4; the injection volume was 10 μL; the DAD was set to a scanning range of 200–400 nm. The phenolic acid contents were calculated with regression equations from standard curves, and are expressed as milligram per kilogram of DW.

### 2.6. Determination of total phenolic content

The total phenolic content (TPC) was determined according to the Folin-Ciocalteu method (Bao et al., 2005). Briefly, 50 μL of extract (80% methanolic pomace extract) was fixed in 5 mL of distilled deionized water. Folin-Ciocalteu reagents (500 μL, 1 M) and $Na_2CO_3$ (500 μL, 20%, w/v) were added, and the mixture was mixed thoroughly and allowed to stand for 60 min at room temperature before the absorbance was measured at 765 nm (Shimadzu UV-2450, Japan). The final results were expressed as gallic acid equivalents (GAE) in milligrams per gram of DW.

### 2.7. Ferric-reducing capacity assay

The extract (5 mL; 80% methanolic pomace extract) was fixed with 25 mL of 80% methanol, and the five-fold diluted samples were used for the analysis of antioxidant activity.

The reducing power of the bayberry pomace extracts was determined as described in a previous report (Benzie & Strain, 1996), with slight modification. To prepare the ferric-reducing antioxidant power (FRAP) reagent, 0.1 M acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM ferric chloride (10:1:1, v/v/v) were combined. The diluted samples (100 μL) were added to 3.9 mL of the prepared FRAP working solution and vortexed. The absorbance was then measured at 593 nm after the mixture had been placed in a warm bath (37 °C) for 10 min. The reducing power of the extracts was expressed as the Trolox equivalent antioxidant capacity (TEAC) in milligrams per gram of DW.

### 2.8. Evaluation of antioxidant activity using the DPPH· method

The scavenging effects of the samples on the DPPH· radical were monitored according to the method of the previously reported (Bao et al., 2005), with slight modification. Briefly, 100 μL of the diluted sample was added to 3.9 mL of DPPH· solution (0.1 mM), vortexed, and then left to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm. A calibration curve was constructed for the decrease in absorbance according to Trolox concentration. The control consisted of 100 μL of 80% methanol and 3.9 mL of DPPH· solution. The stable DPPH· radical-scavenging activity of the extracts was expressed as TEAC in milligrams per gram of DW.

### 2.9. Evaluation of antioxidant activity using the $ABTS^{\cdot+}$ method

The $ABTS^{\cdot+}$ free-radical-scavenging activity of the bayberry pomace extracts was assayed with a method described in detail elsewhere (Bao et al., 2005). The $ABTS^{\cdot+}$ storage solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm on the day of analysis; 50 μL of the diluted samples was added to 50 μL of 80% methanol. The $ABTS^{\cdot+}$ cation solution (3.9 mL) was then added and mixed thoroughly. The reaction mixture was kept at room temperature for 10 min in the dark, and the absorbance was recorded at 734 nm. The results are expressed as the TEAC in milligrams per gram of DW.

### 2.10. Statistical analysis

Values are expressed as means ± standard deviations of triplicate determinations. Total anthocyanins, total flavonols, total phenolic acids, TPC, and total antioxidant activities were analyzed by ANOVA with the LSD test (SPSS for Windows, release 11.5.0).

## 3. Results and discussion

### 3.1. Anthocyanins of bayberry pomaces

Anthocyanins are especially abundant in some berries, including bilberries, black currants, etc (Kähkönen, Hopia, & Heinonen, 2001). The anthocyanins in bilberry were not only abundant but also complex, in that a total of 15 different anthocyanins have been identified (Kähkönen, Heinämäki, Ollilainen, & Heinonen, 2003). However, the anthocyanins were simpler in the bayberry pomaces, because there was only a single peak at 520 nm, as shown in Fig. 1. The same results have been reported for bayberry fruit (Bao et al., 2005). Peak 1 was unambiguously identified as cyanidin-3-*o*-glucoside by comparing its retention time, UV–vis spectroscopic data, and the pseudomolecular ion $[M + H]^+$ with an authentic standard (Table 1).

The result of a quantitative analysis showed that Wandao (6219.2 mg/kg DW) had the highest cyanidin-3-*o*-glucoside content among the five cultivars, followed by Biqi (5489.6 mg/kg DW), Zaodamei (4405.6 mg/kg DW), Dingao (3540.2 mg/kg DW), and Dongkui (3073.3 mg/kg DW) (Table 2). Because only one anthocyanin was detected, the total anthocyanins content was expressed as the cyanidin-3-*o*-gluoside content. Statistical analysis showed that there were significant differences in the cyanidin-3-*o*-glucoside content between the pomaces made from the five cultivars ($p < 0.05$). Anthocyanins are an important antioxidant compound in most berries. Zafra-Stone et al. summarized the functional effects of berry anthocyanins on human health and disease

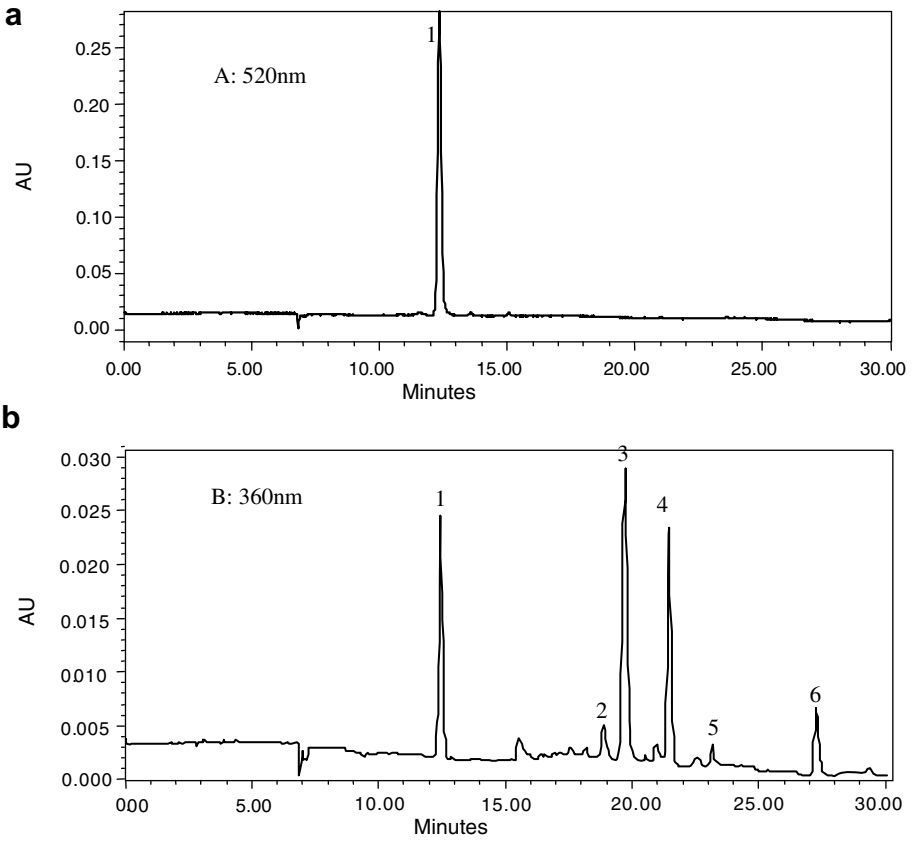


**Fig. 1.** HPLC chromatograph of bayberry pomace (Biqi) detected at 520 nm (A) and 360 nm (B). Peak numbers refer to Table 1.

**Table 1**
Identification of phenolic compounds in bayberry pomace by HPLC–DAD–ESIMS

| Peak No. | Rt | HPLC-DAD (nm) | Molecular weight | HPLC-ESIMS (*m/z*) | Tentative identification | |
|---|---|---|---|---|---|---|
| 1 | 12.228 | 280,520 | 449 | 449,287 | Cyanidin-3-*o*-glucoside [a] | Standard |
| 2 | 18.935 | 263,350 | 464 | 463,317 | Myricetin deoxyhexoside | – |
| 3 | 19.488 | 256,354 | 464 | 463,301 | Quercetin-3-*o*-glucoside | Standard |
| 4 | 21.407 | 257,349 | 448 | 447,301 | Quercetin deoxyhexoside | – |
| 5 | 23.376 | 254,371 | 318 | 317,179 | Myricetin | Standard |
| 6 | 27.193 | 255,369 | 302 | 301,179 | Quercetin | standard |

Rt: Retention time (min).
[a] Cyanidin-3-*o*-glucoside was detected with positive ion mode, others were detected with negative ion model.

**Table 2**
Anthocyanin and flavonols of bayberry pomace (mg/kg DW)

| Cultivars | Anthocyanin | Flavonol | | | | | |
|---|---|---|---|---|---|---|---|
| | Cyanidin-3-*o*-glucoside [a] | Myricetin deoxyhexoside | Quercetin-3-*o*-glucoside | Quercetin deoxyhexoside | Myricetin | Quercetin | Total flavonols [b] |
| Biqi | 5489.6 ± 266.02 u | 53.7 ± 2.62 | 907.9 ± 76.91 | 513.8 ± 11.98 | 31.5 ± 0.43 | 138.3±3.39 | 1647.1 ± 72.33 u |
| Wandao | 6219.2 ± 247.76 v | 67.3 ± 2.80 | 565.1 ± 9.47 | 485.8 ± 18.29 | 33.1 ± 4.44 | 149.6 ± 2.73 | 1260.9 ± 70.46 v |
| Dongkui | 3073.3 ± 70.56 w | 98.6 ± 3.10 | 296.2 ± 7.79 | 139.6 ± 7.77 | 21.7 ± 1.02 | 65.4 ± 3.85 | 616.5 ± 11.42 w |
| Dingao | 3540.2 ± 128.48 x | 78.3 ± 2.93 | 544.5 ± 6.64 | 468.3 ± 11.44 | 50.9 ± 1.18 | 68.9 ± 4.17 | 1215.8 ± 11.07 v |
| Zaodamei | 4405.6 ± 46.60 y | 96.6 ± 1.45 | 613.4 ± 13.92 | 329.6 ± 8.97 | 46.4 ± 2.15 | 98.2 ± 2.12 | 1184.2 ± 26.33 v |

Myricetin deoxyhexoside was quantified as myricetin aglycone. Quercetin deoxyhexoside was quantified as quercetin-3-*o*-glucoside.
[ab] Different letters in the two columns mean significant differences ($p < 0.05$).

prevention (Zafra-Stone et al., 2007). The present result indicates that bayberry pomaces may be good sources of natural anthocyanin pigments, especially the cultivars Biqi and Wandao.

### 3.2. Flavonols of bayberry pomaces

Generally, flavonols exhibit two major absorption peaks in the region 240–400 nm, of which band I (300–380 nm) is considered to be associated with absorption attributable to the B-ring system and band II (240–280 nm) with absorption involving the A-ring benzoyl system. Fig. 1 is the HPLC chromatogram of the Biqi cultivar. Peaks 2–6 in the chromatograms were all within two absorption regions at 240–280 nm and 300–380 nm (Table 1).

Peaks 3, 5, and 6 were identified as quercetin-3-*o*-glucoside, myricetin, and quercetin by comparing their retention times, UV–Vis spectroscopic data, and pseudomolecular ion $[M–H]^-$ with authentic standards (Table 1). The HPLC–MS analysis showed that peak 4 had a pseudomolecular ion $[M - H]^-$ at *m/z* 447 and a fragment ion at *m/z* 301 (Table 1). The latter is a typical mass in the negative mode of the quercetin aglycone, so it was tentatively identified as quercetin deoxyhexoside (447 − 301 = deoxyhexoside, or rhamnoside). Peak 2 presented with a mass typical of myricetin and a fragment ion at *m/z* 317 (Table 1), and was tentatively identified as myricetin deoxyhexoside.

It is very clear that quercetin-3-*o*-glucoside (296.2–907.0 mg/kg DW) is an abundant flavonol in all five cultivars. Quercetin deoxyhexoside (139.6–513.8 mg/kg DW) is another abundant flavonol in bayberry pomaces, and the content of quercetin (65.4–149.6 mg/kg DW) was higher than that of myricetin (21.7–50.9 mg/kg DW). This result shows that the quercetin derivatives are the primary flavonols in the bayberry pomaces. Häkkinen et al. studied the flavonol aglycones of 25 berries from different families, and demonstrated that all edible berries contain quercetin, and that quercetin is the dominant flavonol aglycone in most cultivars (Häkkinen, Kärenlampi, Heinonen, Mykkänen, & Törrönen, 1999).

The total flavonols (616.5–1647.1 mg/kg DW) were lower than the total anthocyanins (3073.3–6219.2 mg/kg DW) in the bayberry pomaces (Table 2). Among the five cultivars, Biqi had the highest, whereas, Dongkui had the lowest total flavonols contents. Statistical analysis showed that the value for the total flavonols in Wandao, Dingao, and Zaodamei did not differ significantly ($p > 0.05$), although they were significantly higher than that of Dongkui, but lower than that of Biqi ($p < 0.05$).

### 3.3. EPAs in bayberry pomaces

Gallic, protocatechuic, *p*-hydroxybenzoic, and vanillic acids belong to the hydroxybenzoic acids, whereas caffeic, *p*-coumaric, ferulic, and sinapic acids belong to the hydroxycinnamic acids (Robbins, 2003). The phenolic acids in the bayberry pomace extracts were divided into two fractions in this assay: free and ester forms. No chlorogenic acid was detected in the ester form because they are hydrolyzed under alkaline conditions. The total EPAs were equal to the sum of the free and ester forms, and the total phenolic acids were the sum of all EPAs. Fang et al. (2007) identified gallic and protocatechuic acids in the bayberry. To date, we have found no published data about other phenolic acids in bayberries.

Berries vary significantly in the content and distribution of their phenolic acids, according to the results of Mattila et al. (2006). Table 3 shows that gallic acid was the dominant EPA in bayberry pomaces (102.9–241.7 mg/kg DW), accounting for 57.1%–74.9% of the total phenolic acids, consistent with the results of Fang et al. (2007). Protocatechuic acid was the second highest EPA (29.5–57.2 mg/kg DW), accounting for 13.2%–27.5% of the total phenolic acids. *p*-Hydroxybenzoic (5.6–10.2 mg/kg DW), vanillic (3.7–7.1 mg/kg DW), caffeic (3.8–4.0 mg/kg DW), *p*-coumaric (6.8–11.6 mg/kg DW), and ferulic acids (4.4–5.8 mg/kg DW) were all detected in the EPAs of the bayberry pomaces, although their contents were relatively low. Similarly, these phenolic acids are widely distributed in other berries (Mattila et al., 2006). Hukkanen et al. found that the chlorogenic acids constitute a significant fraction of the phenolics in some sweet rowanberry cultivars, including Zholtaja and Kubovaja (Hukkanen, Pölönen, Kärenlampi, & Kokko, 2006). In vitro experiments have shown that chlorogenic acid expresses good activity that inhibits damage to the plasmid pUC18 DNA (Shibata, Sakamoto, Oka, & Kono, 1999). However, the content of chlorogenic acids was low in the bayberry pomaces, and they were only detected in Dongkui (1.58 mg/kg DW). Sinapic and

**Table 3**
Phenolic acids of bayberry pomace (mg/kg DW)

| Cultivars | Forms | Gallic | Protocatechuic | *p*-Hydroxy- benzoic | Vanillic | Chlorogenic | Caffeic | *p*-Coumaric | Ferulic | Total phenolic acids [a] |
|---|---|---|---|---|---|---|---|---|---|---|
| Biqi | Free | 91.7 ± 4.86 | 11.8 ± 0.32 | 3.5 ± 0.02 | 0.9 ± 0.01 | ND [a] | 1.8 ± 0.02 | 1.9 ± 0.01 | 1.7 ± 0.02 | _ |
| | Ester | 147.5 ± 7.67 | 30.4 ± 0.63 | 6.7 ± 0.2 | 6.2 ± 0.24 | ND | 1.7 ± 0.06 | 9.8 ± 0.71 | 3.5 ± 0.08 | _ |
| | EPA | 239.1 ± 12.53 | 42.2 ± 0.95 | 10.2 ± 0.18 | 7.1 ± 0.25 | ND | 3.7 ± 0.08 | 11.6 ± 0.70 | 5.2 ± 0.11 | 319.1 ± 12.31u |
| Wandao | Free | 37.3 ± 2.00 | 27.4 ± 0.34 | 3.0 ± 0.07 | 1.8 ± 0.06 | ND | 1.9 ± 0.03 | 2.7 ± 0.11 | 2.0 ± 0.03 | _ |
| | Ester | 81.9 ± 9.06 | 29.8 ± 0.38 | 7.0 ± 1.08 | 2.5 ± 0.04 | ND | 2.1 ± 0.04 | 6.9 ± 0.12 | 2.4 ± 0.05 | _ |
| | EPA | 119.2 ± 7.06 | 57.2 ± 0.72 | 9.9 ± 1.15 | 4.3 ± 0.10 | ND | 3.9 ± 0.72 | 8.6 ± 0.23 | 4.5 ± 0.08 | 207.9 ± 6.75 v |
| Dongkui | Free | 28.1 ± 0.84 | 15.4 ± 0.60 | 2.5 ± 0.12 | 1.7 ± 0.23 | 1.58 ± 0.03 | 1.8 ± 0.06 | 2.3 ± 0.05 | 1.9 ± 0.10 | _ |
| | Ester | 74.8 ± 2.78 | 25.1 ± 1.64 | 3.1 ± 0.08 | 3.0 ± 0.77 | ND | 2.2 ± 0.00 | 7.2 ± 0.21 | 3.3 ± 0.09 | _ |
| | EPA | 102.9 ± 1.95 | 40.5 ± 1.04 | 5.6 ± 0.20 | 4.6 ± 0.54 | 1.58 ± 0.03 | 4.0 ± 0.06 | 9.6 ± 0.26 | 5.8 ± 0.19 | 180.2 ± 0.82 v |
| Dingao | Free | 92.3 ± 4.03 | 12.5 ± 1.23 | 2.7 ± 0.15 | 0.9 ± 0.01 | ND | 1.6 ± 0.00 | 3.8 ± 0.16 | 2.0 ± 0.07 | _ |
| | Ester | 149.3 ± 16.51 | 41.3 ± 4.32 | 6.1 ± 0.62 | 2.8 ± 0.12 | ND | 2.4 ± 0.05 | 3.6 ± 0.14 | 2.4 ± 0.01 | _ |
| | EPA | 241.7 ± 15.54 | 53.8 ± 5.56 | 8.8 ± 0.77 | 3.7 ± 0.13 | ND | 3.9 ± 0.06 | 7.5 ± 0.30 | 4.4 ± 0.08 | 323.7 ± 27.44 u |
| Zaodamei | Free | 42.7 ± 2.89 | 11.7 ± 0.34 | 2.1 ± 0.02 | 2.5 ± 0.13 | ND | 1.4 ± 0.11 | 2.9 ± 0.05 | 2.1 ± 0.07 | _ |
| | Ester | 91.2 ± 6.41 | 17.8 ± 0.08 | 4.2 ± 0.10 | 2.3 ± 0.01 | ND | 1.8 ± 0.01 | 3.9 ± 0.01 | 2.7 ± 0.01 | _ |
| | EPA | 133.9 ± 9.32 | 29.5 ± 0.42 | 6.3 ± 0.12 | 4.8 ± 0.14 | ND | 3.8 ± 0.96 | 6.8 ± 0.05 | 4.8 ± 0.08 | 189.9 ± 8.89 v |

ND: not detected.
[a] Different letters in this column mean significant differences ($p < 0.05$).

**Table 4**
Total phenolic content (TPC) and antioxidant activities of bayberry pomace

| Cultivars | TPC (mg GAE /g DW) | FRAP method (mg TEAC/g DW) | DPPH method (mg TEAC /g DW) | ABTS method (mg TEAC /g DW) |
|---|---|---|---|---|
| Biqi | 45.2 ± 3.22 u | 65.9 ± 1.70 u | 84.3 ± 0.94 uv | 97.1 ± 1.87 uv |
| Wandao | 47.4 ± 1.24 u | 71.4 ± 4.63 u | 91.0 ± 6.63 v | 100.6 ± 2.56 u |
| Dongkui | 27.7 ± 1.37 v | 40.4 ± 2.45 v | 65.7 ± 4.35 w | 78.5 ± 2.17 w |
| Dingao | 31.7 ± 2.11 vw | 49.1 ± 3.67 w | 74.2 ± 2.88 wu | 82.9 ± 3.65 wx |
| Zaodamei | 35.3 ± 2.12 w | 51.2 ± 2.65 w | 82.3 ± 3.16 uv | 89.9 ± 4.31 xv |

Different letters in same column mean significant differences ($p < 0.05$).

ellagic acids were not detected in the bayberry pomaces in this assay.

The total phenolic acids (180.2–323.7 mg/kg DW) were much lower than the anthocyanins and total flavonols in all five cultivars. Statistical analysis showed that there was no significant difference between Wandao, Dongkui, and Zaodamei ($p > 0.05$).

### 3.4. TPC of bayberry pomaces

Kähkönen et al. studied the TPC and antioxidant activities of 92 plant materials, and their results showed remarkably high TPC (GAE > 40 mg/g DW) and antioxidant activities in berry fruits, such as aronia and crowberry (Kähkönen et al., 1999). Similarly, the bayberry fruit has high TPC (GAE = 3.6–4.5 mg/g FW; Fang et al., 2007). Table 4 shows that the bayberry pomaces also had high TPC. Wandao had the highest TPC (47.4 mg/g DW) among the five cultivars. The other cultivars, in descending order by TPC, were Biqi (45.2 mg/g DW), Zaodamei (35.3 mg/g DW), Dingao (31.7 mg/g DW), and Dongkui (27.7 mg/g DW). However, the potential interference caused by other substances, such as reducing sugars, was not taken into account in this assay. Compared with other fruit byproducts, the TPC of bayberry pomaces was higher than that of apple pomace (10.16 mg/g DW; Sudha, Baskaran, & Leelavathi, 2006), and the TPC of Wandao and Biqi was similar to that of freeze–dried red grape pomace (43 mg/g DW; Larrauri, Rupérez, & Saura-Calixto, 1997). Louli et al. (2004) suggested that a high-phenolic product could be obtained from grape pomace. The high TPC of the bayberry pomaces implies that they might be good bio-resources for value-added products. Statistical analysis showed that the TPCs of Wandao and Biqi were significantly higher ($p < 0.05$) than those of Dongkui, Dingao, and Zaodamei (Table 4), which indicates that the choice of cultivar is an important factor in bayberry pomace reutilization.

### 3.5. Antioxidant activities of bayberry pomaces

The antioxidant effects of phenolics are strongly dependent on the choice of the raw material, because the antioxidant activity differs between different phenolic constituents (Rice-Evans, Miller, & Paganga, 1996). With various radical-scavenging assays, different berry cultivars have been reported to have good antioxidant activities, although the results had a little differed slightly (Kähkönen et al., 2001; Zheng & Wang, 2003).

The bayberry pomaces had good reducing power and free-radical-scavenging capacities (Table 4). Among the five cultivars, Wandao had the highest reducing power (71.4 mg TEAC/g DW), and scavenging $DPPH^{\cdot}$ (91.0 mg TEAC/g DW), and $ABTS^{\cdot+}$ (100.6 mg TEAC/g DW) capacities, whereas, Dongkui had the lowest reducing power (40.4 mg TEAC/g DW), and scavenging $DPPH^{\cdot}$ (65.7 mg TEAC/g DW) and $ABTS^{\cdot+}$ (78.5 mg TEAC/g DW) capacities. The other three cultivars with reducing power and free-radical-scavenging capacities were Biqi, Zaodamei, and Dingao, in descending order. Statistical analysis showed that there were no significant differences between Biqi and Wandao, or Zaodamei and Dingao in reducing power ($p > 0.05$). The results of the statistical analysis of these free-radical-scavenging capacities were similar: there were no significant differences between Biqi and Wandao, Dongkui

**Table 5**
Correlation coefficient among TPC, total anthocyanins, total flavonols, total phenolic acids and total antioxidant activities

| | FRAP method | DPPH method | ABTS method |
|---|---|---|---|
| Total phenolic | 0.9359 | 0.8802 | 0.9320 |
| Total anthocyanins | 0.9013 | 0.7906 | 0.9125 |
| Total flavonols | 0.5431 | 0.4722 | 0.5212 |
| Total phenolic acids | 0.0936 | 0.0755 | 0.0726 |

and Dingao, or Dingao and Zaodamei in scavenging $DPPH^{\cdot}$ or $ABTS^{\cdot+}$. This study investigated these cultivars only with reducing-power and radical-scavenging tests in vitro. It must be emphasized that the evaluation of in vitro antioxidants in a simple test model does not necessarily indicate their activity in the environment in vivo.

### 3.6. Correlation of TPC, anthocyanins, total flavonols, and total phenolic acids with antioxidant activities

Correlation coefficients for TPC, anthocyanins, total flavonols, and total phenolic acids with the FRAP assay, DPPH assay, and ABTS assay are shown in Table 5. These results show that TPC and anthocyanins correlate highly with antioxidant activities ($R^2 > 0.7906$). The flavonols might have also contributed to the pomace antioxidant activities, but the correlation coefficients were relatively low ($R^2 \sim 0.5$). However, the correlation coefficients of the total phenolic acids to the antioxidant capacities were less than 0.1, which indicates that these kinds of phenolics have little effect on the antioxidant capacities of the pomaces.

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