Contents of Extractable and Non-extractable Polyphenols in the Leaves of Blueberry

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Abstract

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The contents of extractable polyphenols (EPP), non-extractable polyphenols (NEPP), extractable proanthocyanidins (EPAC), non-extractable proanthocyanidins (NEPAC) and extractable anthocyanidins (EAC) in blueberry leaves were investigated. An experiment was conducted to analyse the effect of solvent types (methanol and ethanol), concentration (50 and 70%) and pH (2 and 6) on the extraction of bioactive compounds. Total extractable polyphenols (TEPP), total extractable proanthocyanidins (TEPAC), and total extractable anthocyanidins (TEAC) were analysed in methanol/ethanol/water extracts, NEPP were determined in acidic hydrolysates and NEPAC were quantified by depolymerisation in HCl/butanol. The results showed that ethanol and methanol did not affect the extraction of TEPP, while methanol was better for TEPAC and TEAC. The contents of TEPP and TEAC in 50% solvent were higher than those in 70% solvent. When solvent pH dropped to 2, the contents of EPP and EPAC were increased, while the EAC content was decreased.

Keywords: leaves of blueberry; extractable polyphenols; non-extractable polyphenols; proanthocyanidins; anthocyanidins

Blueberry is an increasingly important commercial crop in many parts of the world. Many reports have indicated that blueberry fruits have a wide range of beneficial properties to health, including antimicrobial (Singh *et al.* 2010), antiallergenic (Chung & Champagne 2008), antidiabetic (Vuong *et al.* 2009), anticancer (Seeram 2008) and antioxidant ones (Castrejón *et al.* 2008). Blueberries contain many bioactive compounds, like polyphenols, terpenes, dietary fibre. These compounds are also accumulated in large amounts in other plant parts, such as leaves, parks and roots. Many have focused on the blueberry fruit and blueberry products such as wine, juice, and vinegars. However, there is little information on polyphenols (including proanthocyanidins and anthocyanidins) in the blueberry leaves.

The leaves of blueberry have been known to be used in tea drink for diabetics among the alpine peasantry (Allen 1927). More recently, strong oxygen radical absorbance capacity (Ehlenfeldt & Prior 2001), hypolipidaemic effect (Nagao *et al.* 2008) and antileukaemic activity (Skupień *et al.* 2006) of the leaves have been reported. There have been intense researches on the extraction and compounds of polyphenols from blueberry.

Polyphenols are natural and secondary metabolic substances in plants that have strong antioxidant activity. Anthocyanidins are a class of phenolic compounds, which are colour pigments contributing to the antioxidant capacity of blueberry (Lapidot *et al.* 1999). Proanthocyanidins are referred to as condensed tannins, a major group of polyphenols, they are oligomers and polymers

of monomeric flavan-3-ols and flavan-3,4-diols, such as catechin and epicatechin. The proanthocyanidins can be constituted of two types double linkage (A-type and B-type) between the flavan monomeric units, which may be important structural features in the anti-adhesion process (Matsuo *et al.* 2010).

There are also two types of tannins according to their solubility of extraction. Insoluble (non-extractable) polyphenols or proanthocyanidins are the components of cell walls, while soluble (extractable) polyphenols or proanthocyanidins are compartmentalised within the plant cell vacuoles (Beckman 2000). Saura-Calixto *et al*. (2007) pointed out that most data from the literature on "total polyphenol content" refer only to extractable polyphenols (EPP) and ignore non-extractable polyphenols (NEPP). The "total polyphenol content" in foods is actually made up of EPP plus NEPP.

Likewise, the total proanthocyanidins (TPAC) include extractable proanthocyanidins (EPAC) and non-extractable proanthocyanidins (NEPAC). Until now, most reports on phytochemicals of blueberry leaves have mainly focused on the extractable components. In reality, the non-extractable fraction in the food is not negligible and may be released in the human gut once it is released by the action of digestive enzymes (Jenner *et al.* 2005).

The present study thus aimed to evaluate the effect of extraction methods on the contents of extractable and non-extractable polyphenols (proanthocyanidins and anthocyanidins), and to quantify the total extractable polyphenols (TEPP), total extractable proanthocyanidins (TEPAC), and total extractable anthocyanidins (TEAC). The study specially strived to determine the contents of NEPP and NEPAC in the blueberry leaves.

MATERIAL AND METHODS

Material. The fresh leaves of blueberry (*Vaccinium angustifolium* L.) were cultivated and collected from Jinan, China. The leaves were oven-dried at 40°C, pulverised and stored at –20°C until further use.

Reagents. All of chemical reagents (methanol, ethanol, acetone, butanol, sulphuric acid, hydrochloric acid, acetic acid, iron trichloride, potassium chloride, sodium carbonate) were bought from Fisher Scientific Co., Ltd. (Shanghai, China). Vanillin, Folin-Ciocalteu phenol reagent (2 mol/l), and standards of gallic acid, epicatechin, cyanidin-3-glucoside and cyanidin were purchased from Sigma-Aldrich Corp. (Shanghai, China).

Extraction methods. About 500 mg of dried blueberry leaves were mixed with 20 ml of different extracting solvents. The different solvents were as follows: (M1) methanol/water = 70:30 (v/v), pH = 6; (M2) methanol/water = 50:50 (v/v), pH = 6; (M3) methanol/water = 70:30 (v/v), pH = 2; (M4) methanol/water = 50:50 (v/v), pH = 2; (E1) ethanol/water = 70:30 (v/v), pH = 6; (E2) ethanol/water = 50:50 (v/v), pH = 6; (E3) ethanol/water = 70:30 (v/v), pH = 2; (E4) ethanol/water = 50:50 (v/v), pH = 2. pH was adjusted with HCl (2 mol/l).

The solution was sonicated for 1 h and was then centrifuged at 4000 rpm for 5 min to obtain supernatants-1 and pellets-1. The contents of EPP-1, EPAC-1 and EAC-1 from supernatants-1 were determined.

Pellets-1 were resuspended in 20 ml acetone/H_2O solution (70:30, v/v), which was sonicated for 1 h and centrifuged at 4000 rpm for 5 min to obtain supernatants-2 and pellets-2. The contents of EPP-2, EPAC-2 and EAC-2 from supernatants-2 were determined again.

Two hundred mg of dried pellets-2 were treated with 10 ml of butanol/HCl (97.5:2.5, v/v) containing $FeCl_3$ (0.7 g) at 100°C for 1 hour. The mixture was centrifuged at 4000 rpm for 5 min and the supernatants were collected. After the residues were washed twice with butanol (5 ml), supernatants were combined and NEPAC were determined by spectrophotometry at 555 nm. The standard curve was obtained for pure cyanidin (Pérez-Jiménez *et al.* 2009).

One hundred mg of dried pellets-2 were mixed with 10 ml methanol/H_2SO_4 (90:10, v/v), then hydrolysed at 85°C for 20 h, centrifuged at 4000 rpm for 5 min and supernatants-3 were collected (Arranz *et al.* 2009). The contents of NEPP-3 from supernatant-3 were determined by the Folin-Ciocalteu colorimetric method.

Determination of polyphenol content. Polyphenol contents in the extracts were determined by the Folin-Ciocalteu colorimetric method (Slinkard & Singleton 1977). Briefly, the extracts (100 µl) were mixed with 2 ml HPLC grade water, then the Folin-Ciocalteu phenol reagent (200 µl) was added and the solution was mixed. After 3 min, 900 µl of 20% (w/v) sodium carbonate solution were added, and the mixture was incubated for 2 h

in the dark at room temperature. Absorbance of each sample was measured at 765 nm. Polyphenol content was expressed as the percentage of gallic acid in dried leaves.

Determination of proanthocyanidin content. Proanthocyanidin contents in the dried leaves were determined by the vanillin assay in methanol (Broadhurst & Jone 1978). The extracts (1 ml) were mixed with 2.5 ml of H_2SO_4 (20%, v/v), then 2.5 ml of vanillin in methanol (12 g/l) was added and mixed, and the mixture was incubated at 30°C for 15 minutes. Absorbance of each sample was measured at 500 nm and the proanthocyanidin content was expressed as mg epicatechin/g DW (DW = dry weight).

Determination of anthocyanidin content. Anthocyanidin contents were determined by the pH differential method (Chinboga & Francis 1970). The anthocyanidin extracts were diluted 1:6 with 0.4 mol/l KCl-HCl buffer (pH = 1) and 0.4 mol/l sodium acetate buffer (pH = 4.5). The absorbance of both solutions was measured each at 510 nm and 700 nm, respectively. The absorbance difference was calculated as:

$$\Delta A = (A_{510} - A_{700})_{pH = 1} - (A_{510} - A_{700})_{pH = 4.5}$$

The anthocyanidin content was calculated using the molar absorption coefficient (29 600) and molecular weight (449.2) of cyanidin-3-glucoside and expressed as mg cyanidin-3-glucoside/100 g DW.

Statistical analysis. All experiments were carried out in quadruplicate and expressed as mean ± standard error. Data were analysed using analysis of variance (SAS9.0, SAS Inst., Cary, USA) and *t*-test to determine the statistical significance ($P < 0.05$).

RESULTS AND DISCUSSION

Extraction yield of the compounds mainly depends on the solvent and the method of extraction (Goli *et al.* 2004). Some studies have shown that methanol, ethanol, and acetone were commonly chosen solvents for the extraction of polyphenols, proanthocyanidins, and anthocyanidins in blueberries (Arranz *et al.* 2009; Jeong *et al.* 2010). And the ratio of solvents is one of the important factors in the extraction. The pH has been reported to be a critical factor that influences colour degradation in blueberry products (Kalt *et al.* 2000). The experimental approach consisted of two main aspects: (1) determination of extractable compounds using a two-step extraction method. Methanol or ethanol is chosen as the extracting solvent in the first step, and acetone is used as the extracting solvent in the second step. (2) Determination of

Table 1. Contents of total extractable and non-extractable polyphenols and proanthocyanidins in blueberry leaves

Treatment	M1	M2	M3	M4	E1	E2	E3	E4
Solvent	methanol/H_2O				ethanol/H_2O			
Ratio	70/30	50/50	70/30	50/50	70/30	50/50	70/30	50/50
pH	6	6	2	2	6	6	2	2
EPP-1 (%)	$2.26^{B} \pm 0.34$	$2.07^{A} \pm 0.45$	$2.22^{A,B} \pm 0.40$	$2.62^{B,C} \pm 0.56$	$2.27^{A,B} \pm 0.76$	$1.81^{A} \pm 0.24$	$2.24^{A,B} \pm 0.46$	$3.46^{D} \pm 0.37$
EPP-2 (%)	$1.02^{A} \pm 0.25$	$1.34^{A,B} \pm 0.28$	$1.28^{A,B} \pm 0.19$	$1.79^{C} \pm 0.22$	$1.07^{A} \pm 0.17$	$1.81^{C} \pm 0.41$	$1.75^{C} \pm 0.34$	$1.43^{B} \pm 0.30$
TEPP (%)	3.28^{A}	3.41^{A}	$3.50^{A,B}$	4.41^{C}	3.34^{A}	$3.62^{A,B}$	$3.99^{B,C}$	4.89^{D}
NEPP (%)	$3.73^{C} \pm 0.56$	$3.54^{B} \pm 0.38$	$3.37^{B} \pm 0.51$	$3.18^{A,B} \pm 0.26$	$3.72^{C} \pm 0.37$	$3.44^{B} \pm 0.28$	$3.44^{B} \pm 0.35$	$2.81^{A} \pm 0.35$
TPP (%)	$2.81^{A} \pm 0.35$	6.95^{A}	6.87^{A}	$7.59^{A,B}$	7.06^{A}	7.06^{A}	7.43^{A}	7.70^{B}
EPAC-1 (mg/g)	$3.76^{D} \pm 0.33$	$2.51^{C} \pm 0.20$	$4.05^{D,E} \pm 0.41$	$3.78^{D} \pm 0.66$	$2.01^{B} \pm 0.16$	$0.90^{A} \pm 0.38$	$1.95^{B} \pm 0.54$	$4.11^{E} \pm 0.07$
EPAC-2 (mg/g)	$0.92^{A} \pm 0.10$	$1.14^{A} \pm 0.16$	$1.79^{B,C} \pm 0.17$	$2.14^{C} \pm 0.13$	$1.10^{A} \pm 0.17$	$2.11^{C} \pm 0.33$	$1.22^{A} \pm 0.11$	$1.40^{B} \pm 0.21$
TEPAC (mg/g)	4.78^{C}	3.41^{B}	$5.84^{D,E}$	5.92^{E}	3.11^{A}	3.01^{A}	3.17^{A}	5.51^{D}
NEPAC (mg/g)	$10.06^{A} \pm 1.26$	$11.54^{A} \pm 0.38$	$10.07^{A} \pm 1.02$	$10.04^{A} \pm 1.46$	$11.61^{A} \pm 1.89$	$11.56^{A} \pm 1.63$	$11.69^{A} \pm 1.20$	$10.86^{A} \pm 1.83$
TPAC (mg/g)	14.84^{A}	14.95^{A}	15.91^{B}	$15.52^{A,B}$	$15.52^{A,B}$	14.57^{A}	14.81^{A}	16.37^{B}

$^{A–E}$identical letters next to the mean values of enthalpy indicate the absence of significant differences at $P < 0.0$

non-extractable compounds in the residues of the respective extracts.

Polyphenols

As shown in Table 1, methanol/H_2O is used as the extracting solvent, the contents of EPP-1 in blueberry leaves ranged from 2.07 ± 0.45% to 2.62 ± 0.56%. The treatment M4 had the greatest amount of EPP-1, whereas M2 had the lowest. A similar tendency was found in the ethanol treatment. In all the treatments, E4 had the maximum content of EPP-1, and the E2 was the lowest in EPP-1, the difference was almost twofold.

In the second step, we chose acetone as the extracting solvent. The results (Table 1) showed that the EPP-2 content in M1 was the lowest and E2 was the highest. There was no obvious difference among M4, E2 and E3, which have high contents of EPP-2.

The TEPP content was expressed as the EPP-1 plus EPP-2. In the methanol treatment, M4 had the greatest content of TEPP, and M1 had the lowest amount of TEPP. The contents of TEPP in the low pH treatment (pH = 2) were higher than in the high pH treatment (pH = 6) and the contents of TEPP in 70% methanol were lower than in the 50% solution. There was a similar trend in the ethanol treatment.

Most literature data on food polyphenols concern only compounds dissolved in aqueous organic extracts (extractable fractions), but this approach may be limited by the extraction techniques, some polyphenols, especially polyphenols associated with a high molecular weight compounds (non-extractable fractions) may escape the standard extraction methods employed (Saura-Calixto *et al.* 2007). So we chose methanol/H_2SO_4 as the hydrolysing solvent to determine the content of NEPP. Hydrolysable polyphenols are polyesters of a sugar moiety and organic acids. These compounds undergo hydrolytic cleavage to the respective sugar and acid moiety upon treatment with diluted acids. They can be divided into gallotannins and ellagitannins depending on whether the acid component is gallic acid or hexahydroxydiphenic acid (Arranz *et al.* 2009).

As shown in Table 1, the changing tendency of NEPP in the different treatments was opposite to

Table 2. The significant test on the extractable and non-extractable polyphenols and proanthocyanidins

Factors	Solvent (S)	Ratio (R)	pH (P)	S × R	S × P	R × P	S × R × P
EPP-1	ns	ns	*	ns	*	*	*
EPP-2	*	*	*	**	*	*	*
TEPP	ns	*	*	ns	*	*	ns
NEPP	ns	*	*	ns	ns	ns	ns
TPP	ns	ns	ns	ns	ns	ns	ns
EPAC-1	*	*	*	ns	*	*	*
EPAC-2	ns	*	*	*	ns	ns	*
TEPAC	*	ns	*	ns	ns	*	*
NEPAC	ns	ns	ns	ns	ns	ns	ns
TPAC	ns	ns	ns	ns	ns	ns	ns
	Solvent (S)	Ratio (R)	pH (P)	S × R	S × P	R × P	S × R × P
EPP-1	ns	ns	*	ns	*	*	*
EPP-2	*	*	*	*	*	*	*
TEPP	ns	*	*	ns	*	*	ns
NEPP	ns	*	*	ns	ns	ns	ns
TPP	ns	ns	ns	ns	ns	ns	ns
EPAC-1	*	*	*	ns	*	*	*
EPAC-2	ns	*	*	*	ns	ns	*
TEPAC	*	ns	*	ns	ns	*	*
NEPAC	ns	ns	ns	ns	ns	ns	ns
TPAC	ns	ns	ns	ns	ns	ns	ns

*significantly different at $P < 0.05$; ns – not significantly different at $P > 0.05$

TEPP. M1 had the highest amount of NEPP, whereas E4 had the lowest. Saura-Calixto *et al*. (2007) reported that the NEPP content was much higher than the TEPP content in different materials, such as cereals, vegetables, legumes, fruits and nuts. In the present study, the average content of NEPP (3.40%) was a little lower than the average TEPP content (3.81%). The TPP content ranged from 6.87 to 7.70%, and there was no obvious difference in all the treatments. The average content of TPP in blueberry leaves was 7.21%.

The effects of all single or multiple factors on the TPP contents were not significant, while EPP-2 were affected by various factors (Table 2). The type of solvent cannot significantly affect EPP-1, TEPP and NEPP, while there was a significant effect on EPP-2. The ratio of solvents significantly affected EPP-2, TEPP and NEPP except for EPP-1. pH significantly affected polyphenol contents. The interaction of two or three factors was also detected. The contents of TPP and NEPP were not significantly affected by the multiple factors. Solution × ratio (S × R) cannot affect EPP-1, TEPP and NEPP, but it significantly affects EPP-2. Solution × pH (S × P) and ratio × pH (R × P) significantly affected the contents of EPP-1, EPP-2 and TEPP. Solution × ratio × pH (S × R × P) affected only the contents of EPP-1 and EPP-2 (Table 2).

Proanthocyanidins

The vanillin assay in methanol is generally recognised as a useful method for the detection and quantification of proanthocyanidins in plant materials due to its simplicity, sensitivity and specificity (Naczk & Shahidi 2004). The EPAC and NEPAC contents in blueberry leaves are shown in Table 1. In the first stage, the order of EPAC-1 contents in the methanol treatments was M3 > M4 > M1 > M2, whereas the order of EPAC-1 contents in the ethanol-treatments was E4 > E1 > E3 > E2. In all the treatments, the highest content of EPAC-1 was detected in the treatment E4, and the lowest content of EPAC-1 was in the treatment E2.

The extracted EPAC-2 in the second step was notably lower than that in the first step except for the treatment E3. The highest content of EPAC-2 in the second step was detected in the treatment E2. However, in the first step, E2 has the lowest content of EPAC-1. The lowest amount of EPAC-2 was detected in M1. The changed tendency of EPAC-2 was similar to EPP-2.

The TEPAC contents in the different treatments varied from 3.01 to 5.92 mg/g. When methanol was used as the extracting solution, the TEPAC contents in pH = 2 treatments were higher than those in pH = 6 treatments. A similar result was found in the ethanol treatment. In pH = 6 treatments, TEPAC extracted in 70% solution (methanol and ethanol) was higher than that in 50% solution. However, the trend was contrary to pH = 2 treatments. The results showed that pH of the solvent has a higher effect on the EPAC content than the concentration of the solution.

The non-extractable proanthocyanin assay is carried out in a solution of butanol and hydrochloric acid (97.5:2.5, v/v), when in the presence of the acidic solution proanthocyanidins are converted to anthocyanidins. It occurs through autoxidation of carbocations formed by the cleavage of interflacanoid bonds (Naczk & Shahidi 2004). The presence of transition metals enhances the yield of conversion of proanthocyanidins to anthocyanidins. Ferrous and ferric ions were the most effective catalysts in the formation of an-

Table 3. Contents of extractable anthocyanidins in blueberry leaves

Treatment	M1	M2	M3	M4	E1	E2	E3	E4
Solvent	methanol/H_2O				ethanol/H_2O			
Ratio	70/30	50/50	70/30	50/50	70/30	50/50	70/30	50/50
pH	6	6	2	2	6	6	2	2
EAC-1 (mg/100 g)	$36.6^{D} \pm 2.3$	$28.7^{C} \pm 1.7$	$31.8^{C} \pm 1.9$	$27.2^{C} \pm 2.1$	$30.3^{C} \pm 2.5$	$14.2^{A} \pm 1.98$	$19.1^{B} \pm 2.6$	$30.4^{C} \pm 4.1$
EAC-2 (mg/100 g)	$5.6^{A,B} \pm 0.9$	$6.5^{B} \pm 1.0$	$7.0^{B} \pm 0.8$	$8.5^{C} \pm 1.4$	$5.9^{A,B} \pm 0.9$	$16.2^{D} \pm 2.0$	$11.0^{C} \pm 1.9$	$3.7^{A} \pm 0.8$
TEAC (mg/100 g)	42.2^{C}	35.2^{B}	$38.8^{B,C}$	35.7^{B}	36.2^{B}	30.4^{A}	30.1^{A}	34.1^{B}

$^{A–D}$identical letters next to the mean values of enthalpy indicate the absence of significant differences at $P < 0.05$

Table 4. Significance test of extractable anthocyanidins

Factor	Solvent (S)	Ratio (R)	pH (P)	S × R	S × P	R × P	S × R × P
EAC-1	*	*	ns	ns	*	*	*
EAC-2	ns	ns	ns	ns	*	*	*
TEAC	*	*	ns	ns	ns	*	*

*significantly different at $P < 0.05$; ns – is not significantly different at $P > 0.05$

thocyanidins (Porter *et al.* 1986). In the present study, the contents of NEPAC were measured using the butanol/HCl solution containing $FeCl_3$ at the wavelength of 555 nm. As shown in Table 1, the contents of NEPAC in the different treatments ranged from 10.04 ± 1.46 to 11.69 ± 1.20 mg/g, and the change of NEPAC contents was not obvious. The NEPAC contents were twice higher or more than the TEPAC contents. E4 and M4 had the greatest amount of TPAC, and M2 was the lowest in TPAC, which indicated that the extracted TPAC content was significantly affected by pH of the solvent.

As shown in Table 2, the contents of EEPAC and TPAC were not significantly affected by all single or multiple factors. The solvent, ratio, pH, S × P, R × P and S × R × P significantly affected the EPAC-1 contents. EPAC-2 was mainly affected by the factors of ratio, pH, S × R and S × R × P. TEPAC was affected by solvent, pH, R × P and S × R × P.

Anthocyanidins

Quantification of anthocyanidins takes advantage of their characteristic behaviour in acidic media (Naczk & Shahidi 2004). According to Moore *et al.* (1982), the acid may change the native form of anthocyanidins by breaking down their complexes with metals and co-pigments. The AC contents in blueberry leaves are shown in Table 3. In the first stage, the order of the EAC-1 content was M1 > M3 > M2 > M4 in the methanol treatment, whereas the order of the EAC-1 content was E4 > E1 > E3 > E2 in the ethanol treatment. In the methanol treatment, the EAC-1 contents in 70% solution were higher than those in 50% solution. When the solution had the same concentration, the EAC-1 contents in pH = 2 treatment were lower than in pH = 6 treatment. In the ethanol treatments, the EAC-1 contents in 70% solution were much higher than those in 50% solution in pH = 6 treatment. Whereas in pH = 2 treatment, the tendency in the different solutions was opposite to that in pH = 6 treatment. In all the treatments, the highest content was found in the treatment M1, and the lowest was in E2, the difference was about 2.5-fold.

In the second step, E2 has the highest content of EAC-2, and the lowest was found in E4. In all, the EAC-2 contents in the second step were obviously lower than those in the first step except for E2. The tendency of EAC-2 in the second step in the different treatments was contrary to that in the first step.

In the methanol treatment, the TEAC content in M1 was the highest, and in M2 it was the lowest. Whereas in the ethanol treatment, the highest content of TEAC was found in E1 and the lowest was measured in E3. In all the treatments, the highest and the lowest contents of TEAC were detected in M1 and E3, respectively.

We pointed out that proanthocyanidins are converted easily to anthocyanidins in the presence of an acidic solution, such as hydrochloric acid. So it is very difficult to distinguish NEPAC and NEAC, and the content of NEAC was not easily analysed. As a result, we did not determine the content of NEAC, but we determined only the content of NEPAC.

As shown in Table 4, EAC-1 contents were influenced by the factors of solvent, ratio, S × P, R × P, and S × R × P, while pH and S × R cannot obviously affect the EAC-1 contents. EAC-2 was significantly affected by the factors of S × P, R × P and S × R × P. Solvent, ratio, R × P, and S × R × P significantly affected the TEAC content.

CONCLUSIONS

In the present study, different extracting solvents are used to investigate the extractable and non-extractable compounds. The contents of TEPP, TEPAC and TEAC in the blueberry leaves from the different extraction solvents are 3.28–4.89%,

3.01–5.51 and 30.1–42.2 mg/100 g, respectively. The contents of NEPP and NEPAC in the blueberry leaves are 2.81 to 3.73% and 10.0–11.7 mg/g, respectively. The average TPP and TPAC contents are 7.21% and 15.46 mg/g, respectively. The EPP content is approximate to the NEPP content, while the EPAC content is much higher than the NEPAC content. Ethanol and methanol cannot significantly affect the extraction of TEPP, and methanol is better for TEPAC and TEAC. Fifty percent concentration of the solution makes more for TEPP and TEAC than 70% solution, but it does not affect TEPAC. At the low pH of the solvent (pH = 2) the content of TEPP and TEPAC is increased, whereas the TEAC content is decreased.

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