Phenolic Acids of Borage (*Borago officinalis* L.) and Evening Primrose (*Oenothera biennis* L.)

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ABSTRACT: The composition of phenolic acids, both free and liberated from esters and glycosides, was determined in evening primrose and borage seeds by GC and MS. The free phenolic acid fraction was predominant in these seeds. Protocatechuic acid was the principal phenolic acid of the free and esterified phenolic acids in evening primrose seeds. Ferulic acid represented a high proportion of the free phenolic acids, but hydroxycaffeic acid was the major constituent of phenolic acids liberated from esters of borage seeds.

Paper no. J10057 in *JAOCS 79*, 335–338 (April 2002).

KEY WORDS: Borage, evening primrose, phenolic acids, polyphenols.

There is a growing interest in substituting synthetic food antioxidants such as BHA, BHT, propyl gallate (PG), and TBHQ with natural alternatives. Fruits, vegetables, leaves, seeds, roots, and barks have been targeted as potential sources of natural antioxidants (1,2).

The seeds of borage (*Borago officinalis* L.*)* and evening primrose *(Oenothera biennis* L.) plants are good sources of the EFA γ-linolenic acid (18:3n-6). Evening primrose seeds contain from 10 to 17% oil, and γ-linolenic acid may constitute up to 10% of the FA (3). On the other hand, the content of oil in borage seeds, containing up to 25% γ-linolenic acid, ranges from 17 to 25% (4). Although this FA is quite prone to oxidation, the oil in these seeds resists oxidation. This suggests that these oleaginous seeds must contain some potent antioxidants (5). Lu and Foo (6) and Shahidi *et al.* (7) demonstrated that the antioxidative activity in evening primrose may be due to the presence of phenolic compounds. Balasinska and Troszynska (8) reported that phenolics extracted from evening primrose seeds may protect lipids from oxidation caused by free radicals. Therefore, the meals after extraction of oils may be potential sources of natural food antioxidants.

The available information on phenolic compounds of evening primrose and borage seeds is diverse and fragmentary. Lu and Foo (6) reported the presence of a procyanidin gallate oligomer in evening primrose seeds. Later, Shahidi *et al.* (7) demonstrated that evening primrose seeds contain catechins as well as dimers and trimers of proanthocyanidins. Subsequently, Balasinska and Troszynska (8) reported the presence of a phenolic acid in an acetone/water extract of evening primrose seeds. However, the authors did not attempt to identify the phenolic acid. Recently, Wettasinghe *et al.* (9) identified rosmarinic, syringic, and sinapic acids in ethanolic extracts of borage meals.

In this study we shall report the composition of free, soluble ester and soluble glycoside phenolic acids present in defatted borage and primrose seeds.

MATERIALS AND METHODS

Materials. Seeds of borage (*B. officinalis* L.) and evening primrose (*O. biennis* L.), obtained from the Institute of Horticulture of the University of Warmia and Mazury (Olsztyn, Poland) were ground, extracted with hexane for 12 h using a Soxhlet apparatus, and then dried at room temperature.

Preparation of crude phenolic extract. Soluble phenolics were extracted six times from defatted ground seed into aqueous 80% (vol/vol) methanol (at a ratio of 1:1, wt/vol) at room temperature for 1 h using an orbital shaker at 250 rpm. The mixture was centrifuged at 1750 × *g* for 10 min, and the supernatants were collected, combined, evaporated to near dryness under vacuum at ≤40°C, and lyophilized.

Fractionation of phenolic acids. Phenolic acids present in the crude extract were fractionated into free and bound forms according to the procedure described by Kozłowska *et al.* (10) and Zadernowski (11). A 0.5-g sample of dried crude phenolic extract was suspended in 50 mL of triply distilled water, acidified to pH = 2 using 6 N HCl, and extracted five times with diethyl ether (1:1, vol/vol) at room temperature. The ether extracts of phenolic acids (referred to as free phenolic acids) were combined and evaporated to dryness under vacuum at ≤40°C. The water phase was adjusted to pH = 7 with 2 M NaOH and then evaporated to near dryness under vacuum at ≤40°C. The residue was treated with 20 mL 4 N NaOH under nitrogen for 4 h at room temperature. The reaction mixture was then acidified with 6 N HCl to pH = 2 and extracted with diethyl ether as described above. The ether extracts of phenolic acids are referred to as phenolic acids liberated from ester bonds. Following this, the water phase was

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again adjusted to pH = 7 with 2 M NaOH and then evaporated to near dryness under vacuum at ≤40°C. The residue was heated with 50 mL of 2 M HCl for 30 min at 95°C, cooled to room temperature, and extracted with diethyl ether as described above. These ether extracts of phenolic acids are referred to as phenolic acids liberated from glycosidic bonds.

Purification of phenolic acid fractions. Each of the residues of phenolic acid fractions, obtained as described above, was dissolved in 50 mL of 5% $NaHCO_3$ (pH = 8) and extracted five times with diethyl ether to remove residual fatty material. The water phase was then acidified with 6 N HCl to pH = 2 and extracted with diethyl ether as described above. The dry residues of phenolic acids were dissolved in 5 mL of 80% (vol/vol) methanol.

Formation of trimethylsilyl derivatives. To a 0.5-mL methanolic solution of purified phenolic acids in a reaction vial was added 20–50 μL of *N*,*O*-bis(trimethylsilyl)acetamide (Sigma) the volume depending on the phenolic acid concentrations. The vial was then tightly closed and left at room temperature for 24 h.

GC–MS identification of phenolic acids. The trimethylsilyl derivatives of phenolic acids were identified using GC–MS methodology as described by Zadernowski (11), Horman and Viani (12), Tian and White (13), and Xing and White (14). GC–MS analysis was carried out on a Hewlett-Packard 5890 Series II gas chromatograph interfaced with a Hewlett-Packard 5970 mass-selective detector (Kennett Square, PA). Separations were performed using a 30 m × 0.25 mm i.d. SPB-1 fused-silica capillary column coated with a 0.25-μm film of poly(dimethylsiloxane) as the stationary phase (Supelco Inc., Bellefonte, PA). Helium was used as the carrier gas at an average flow rate of 28 cm^3 per min. The injector and the transfer line temperatures were kept at 240°C. The oven temperature program used was 120–260°C at a rate of 20°C per min. Initial and final temperatures were held for 2 and 10 min, respectively. The injections were carried out in a split mode with a split ratio of 20:1. The mass spectrophotometer was operated with an ionization voltage of 235 eV and electron multiplier voltage of 1700 V and was scanned from 50 to 500 *m/z* at 0.8 s per scan. The volume of injected samples ranged from 1 to 2 μL, depending on the sample.

Quantitation of phenolic acids. The phenolic acids were quantified as described by Zadernowski (11) using a Hewlett-Packard 5890 Series II gas chromatograph equipped with an FID. Separations of trimethylsilyl derivatives of phenolic acids were performed as described in the previous paragraph. *n*-Tetracosane was used as an internal standard. The contents of the phenolic acids are expressed as mg/kg defatted seed on a dry weight basis.

Chemical analysis. The total content of phenolics in crude extracts was estimated by the Folin–Ciocalteau assay (15) and were expressed in mg (+)-catechin equivalents per gram of extract.

Data treatment. The results presented in the tables are mean values of duplicate experiments (with at least three replicates per experiment). No statistically significant difference (*t*-test; $P > 0.05$) was found among experiments for each treatment.

RESULTS AND DISCUSSION

In this study, we selected an 80% (vol/vol) methanol/water solvent system for the extraction of phenolics from evening primrose and borage meals. This solvent system is commonly used for the extraction of phenolic acids and their derivatives from plant materials (15,16). Crude phenolic extracts constituted 22 and 16% of dry matter of evening primrose and borage meals, respectively. The total content of phenolics in the extracts, expressed as (+)-catechin equivalents, amounted to 200 and 160 mg of phenolics per g of evening primrose and borage extracts, respectively. The total phenolic contents were similar to those reported by Balasinska and Troszynska (8) for an aqueous extract from evening primrose.

The total content of phenolic acids in borage and evening primrose meals (Tables 1,2) was similar to those found in

TABLE 1
Phenolic Acids in Evening Primrose Seeds (mg per kg defatted seeds)

	Liberated from			
Acid	Free	Esters	Glycosides	Total
p-Hydroxyphenyl acetic	—[a]	1.03 ± 0.18	0.26 ± 0.05	1.29 ± 0.19
p-Hydroxybenzoic	4.12 ± 0.25	0.38 ± 0.07	0.29 ± 0.10	4.79 ± 0.26
2-Hydroxy-4-methoxybenzoic	6.52 ± 0.30	—	0.83 ± 0.28	7.35 ± 0.41
Caffeic	6.48 ± 0.29	0.80 ± 0.14	0.23 ± 0.05	7.51 ± 0.33
Hydroxycaffeic	—	0.77 ± 0.18	—	0.77 ± 0.18
m-Coumaric	4.90 ± 0.45	0.83 ± 0.21	—	5.73 ± 0.50
p-Coumaric	1.32 ± 0.10	1.96 ± 0.23	0.06 ± 0.06	3.34 ± 0.25
Ferulic	4.08 ± 0.30	0.72 ± 0.09	0.22 ± 0.06	5.02 ± 0.32
Gallic	1.87 ± 0.22	7.03 ± 0.82	5.91 ± 1.56	14.81 ± 1.78
Protocatechuic	50.28 ± 0.77	10.96 ± 0.34	2.16 ± 2.42	63.40 ± 2.56
Vanillic	5.22 ± 0.28	0.06 ± 0.02	0.83 ± 0.28	7.35 ± 0.41
Veratric	—	0.41 ± 0.03	0.47 ± 0.15	0.88 ± 0.15
Homoveratric	—	0.43 ± 0.06	—	0.43 ± 0.06
Salicylic	1.15 ± 0.04	1.40 ± 0.18	—	2.55 ± 0.18
Total	85.94 ± 1.12	26.78 ± 1.01	11.26 ± 2.92	123.98 ± 3.29

[a]—, not detected.

TABLE 2
Phenolic Acids in Borage Seeds (mg per kg defatted seeds)

Acid	Liberated from			
	Free	Esters	Glycosides	Total
p-Hydroxybenzoic	13.49 ± 2.47	0.69 ± 0.09	0.29 ± 0.11	14.47 ± 2.47
2-Hydroxy-4-methoxybenzoic	6.67 ± 1.18	2.25 ± 0.41	—[a]	8.92 ± 1.25
Caffeic	—	1.52 ± 0.21	0.23 ± 0.06	1.75 ± 0.22
Hydroxycaffeic	—	65.43 ± 3.95	—	65.43 ± 3.95
Cinnamic	1.79 ± 0.39	0.64 ± 0.10	0.02 ± 0.01	2.45 ± 0.40
o-Coumaric	—	0.11 ± 0.03	0.43 ± 0.11	0.54 ± 0.11
p-Coumaric	1.52 ± 0.26	—	0.06 ± 0.02	1.58 ± 0.26
Ferulic	50.18 ± 1.44	0.40 ± 0.21	0.22 ± 0.06	50.80 ± 1.45
Gallic	3.65 ± 0.38	0.59 ± 0.12	0.91 ± 0.41	5.15 ± 0.57
p-Hydroxyphenyl lactic	17.80 ± 2.39	—	—	17.80 ± 2.39
Protocatechuic	12.30 ± 1.53	0.16 ± 0.05	0.16 ± 0.04	12.62 ± 1.53
Salicylic	—	0.20 ± 0.09	—	0.20 ± 0.09
Sinapic	—	2.34 ± 0.44	—	2.34 ± 0.44
Vanillic	—	—	0.53 ± 0.14	0.53 ± 0.14
Veratric	—	0.42 ± 0.09	0.10 ± 0.03	0.52 ± 0.10
Total	107.40 ± 4.24	74.75 ± 4.01	2.95 ± 0.47	185.10 ± 5.85

[a]—, not detected.

coconut, sesame, and cottonseed flours (17), up to three times lower than those found in soybean and flax flours (17, 18), but up to 80 times lower than those reported for rapeseed and canola meals (19). Free phenolics were the principal phenolic acids present, as they constituted 58 and 69.3% of the total phenolic acids in evening primrose and borage meals, respectively (Tables 1,2). The total content of free phenolic acids in these meals was up to eight times lower than those found in canola flours (18). On the other hand, phenolic acids liberated from glycosides contributed only 1.6 and 9.1% of the total phenolic acids of evening primrose and borage, respectively. Furthermore, the total content of phenolic acids liberated from esters in borage was similar to those found in coconut and glanded cottonseed flours (17).

Protocatechuic acid was the predominant free phenolic acid in evening primrose. It constituted 58.5% of the total free phenolic acids present. The level of protocatechuic acid in evening primrose exceeded the reported taste threshold (20); therefore it may contribute to the flavor of this meal. Salicylic, *p*-hydroxybenzoic, 2-hydroxy-4-methoxybenzoic, vanillic, *m*- and *p*-coumaric, gallic, ferulic, and caffeic acids were found in smaller quantities (above 1 mg per kg) (Table 1). On the other hand, ferulic acid composed 50.2% of the total free phenolic acids of borage. Protocatechuic, *p*-hydroxybenzoic, and *p*-hydroxyphenyllactic acids constituted 40.5% of free phenolic acids. Furthermore, 2-hydroxy-4-methoxybenzoic, gallic, *p*-coumaric, and cinnamic acids (Table 2) were also found at a level above 1 mg per kg in the free phenolic acids fraction of borage.

Phenolic acids liberated from soluble esters constituted 21.6 and 40.4% of the total phenolic acids in evening primrose and borage, respectively. Hydroxycaffeic acid was the major phenolic acid of borage, whereas protocatechuic and gallic acids were the predominant phenolic acids of esterified phenolic acids in evening primrose (Tables 1,2). Small quantities (1 to 3 mg per kg) of salicylic, *p*-hydroxyphenylacetic, and *p*-coumaric acids in evening primrose, and 2-hydroxy-4-methoxybenzoic, caffeic, and sinapic acids in borage were also present. In addition, up to eight minor esterified phenolic acids (below 1 mg per kg) were detected in these meals.

Phenolic acids linked to sugars by glycosidic bonds were a minor fraction of phenolic acids present in both evening primrose and borage (Tables 1,2). Sugar moieties of glycosides were not identified in this study. Gallic and protocatechuic acids were the principal phenolic acids identified in evening primrose, and eight phenolic acids were present at concentrations below 1 mg per kg. The concentration of all 11 phenolic acids detected in borage were below 1 mg per kg. Of these, only the gallic and vanillic acid contents exceeded 0.5 mg per kg.

ACKNOWLEDGMENT

Marian Naczk thanks the Natural Sciences and Engineering Research Council of Canada for support in the form of a research grant.

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[Received August 13, 2001; accepted January 8, 2002]