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Phenolic Acids in Berries, Fruits, and Beverages

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The contents of soluble and total phenolic acids were analyzed in samples of 29 berries and berry products, 24 fruits and fruit peels, and 12 beverages. Variation of phenolic acids in berries was also studied. Soluble phenolic acids were extracted with methanolic acetic acid, and a tentative quantification was performed by high-performance liquid chromatography (HPLC). The total phenolic acid content was determined by HPLC after alkaline and acid hydrolyses. The content of total phenolic acids as aglycones in the above samples varied from 0 (pear cider) to 103 mg/100 g fresh weight (rowanberry). Besides rowanberry, the best phenolic acid sources among berries were chokeberry (96 mg/100 g), blueberry (85 mg/100 g), sweet rowanberry (75 mg/100 g), and saskatoon berry (59 mg/100 g). Among fruits, the highest contents (28 mg/100 g) were determined in dark plum, cherry, and one apple variety (Valkea Kuulas). Coffee (97 mg/100 g) as well as green and black teas (30−36 mg/100 g) were the best sources among beverages. Caffeic acid dominated in all of these samples except in tea brews. Variation in the phenolic acid contents of the berries was either small or moderate.

KEYWORDS: phenolic acids; berries; fruits; beverages

INTRODUCTION

Phenolic compounds constitute a large group of secondary plant products with an aromatic ring bearing one or more hydroxyl substituents. Phenolic acids are one major class of phenolic compounds, found widely in foods of plant origin (*1*−*3*). The four most common hydroxycinnamic acids are *p*-coumaric, caffeic, ferulic, and sinapic acids, while the corresponding hydroxybenzoic acids are *p*-hydroxybenzoic, protocatechuic, vanillic, and syringic acids. These derivatives differ in the patterns of the hydroxylations and methoxylations of their aromatic rings. Phenolic acids are present in plant foods mostly in bound form. Hydroxycinnamic acids occur frequently in foods as simple esters with quinic acid or glucose. Probably the most abundant soluble bound hydroxycinnamic acid is chlorogenic acid, which is combined from caffeic and quinic acids. Unlike hydroxycinnamates, hydroxybenzoic acid derivatives are mainly present in foods in the form of glucosides, whereas glucose esters have been found only occasionally (*1*, *3*−*5*).

Dietary polyphenols, such as phenolic acids, are considered to be powerful antioxidants. Their antioxidant activity is much higher in vitro than of well-known vitamin antioxidants (*6*). Antioxidation is, however, only one of the many mechanisms through which polyphenols can exert their actions. Polyphenols have been reported to demonstrate antibacterial, antiviral, antimutagenic, anticarcinogenic, anti-inflammatory, antiproliferative, and vasodilatory actions (*7*−*12*). The relationship between the intake of certain flavonoids and a reduced risk of many degenerative diseases (e.g., cardiovascular disease and cancer) has been shown in a number of epidemiological studies (*13*−*15*).

Despite the fact that phenolic compounds are purported to have health benefits, further studies are needed to fully understand their actions. It is essential to determine the nature and distribution of polyphenols in the human diet. Such knowledge will enable the evaluation of polyphenol intake and an epidemiological analysis to understand the relation between the intake of these substances and the risk to develop various diseases. Data compiled by Radtke et al. (*2*) and Clifford (*4*) indicate that many berries, fruits, and beverages are either moderate or good sources of phenolic acids. However, more recent data are limited, and the existing data on phenolic acid contents are inadequate and disconnected.

The aim of this study was to determine the content of phenolic acids in the most consumed fruits and beverages and in a wide range of berries. To our knowledge, no corresponding studies have been published in this extent. Our ultimate aim is to enter the obtained data into the Finnish Food Composition Database, Fineli, maintained by the National Public Health Institute of Finland.

MATERIALS AND METHODS

Standards. The standards of phenolic acids were obtained from various manufacturers. Chlorogenic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, *o*-coumaric acid, sinapic acid, caffeic acid, and ferulic acid were obtained from Sigma Chemical Co. (St. Louis, MO), while *m*-coumaric acid was from Fluka (Buchs, Switzerland) and *E*-cinnamic acid was

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from Aldrich (St. Louis, MO). All standards were prepared as stock solutions at 2 mg/ mL in MeOH. Stock solutions of the standards were stored in darkness at −18 °C. Standard solutions remained stable over 3 months.

Samples. For the study, we selected the most important fruits and beverages and almost all of the berries available in Finland (**Tables 1**−**3**). All of the samples except for fresh berries, cherry, rhubarb, and red wine were purchased from 10 retail stores in the Helsinki, Kuopio, and Forssa areas during 2003−2005. Each of the three major food chains in Finland was represented. Berries and rhubarb, all domestic, and cherries were purchased from retail stores, market stalls, and one wholesaler in the Kuopio area or, in the case of berries, picked from several areas in Finland during the summers of 2003−2005. Red wine samples were purchased from Alko Inc. in Forssa in fall 2004. (Alko has a monopoly in Finland over retail sales of beverages containing over 4.7% of alcohol by volume.) Generally, we obtained 10 subsamples weighing 0.5−2.0 kg of each food item. For berries, however, the number of subsamples was smaller (*2*−*10*).

Sample handling and pooling followed the protocols described earlier by Koivu et al. (*16*) and Mattila and Hellström (*17*). We prepared one pooled sample representing each item and analyzed these samples for consumption; that is, only their edible parts were included in the analyses. In the case of apples, the peels were also analyzed separately. Each subsample was diced when necessary, and identical amounts (usually 100 g) of each were added to the pool. The pooled samples were mixed and frozen, then freeze-dried (except for jams, bilberry soup, and beverages), and stored at −20 °C until analyzed. One tea bag from all 10 tea subsample packets was separately brewed at 95 °C for 3 min in 200 mL of water, after which 100 mL of each of these 10 brews was combined. The coffee sample was obtained by pooling 100 g of each of the 10 coffee packets. The sample of coffee beverage was prepared with a conventional coffee maker by brewing twice 35 g of pooled coffee powder and 700 mL of water and combining identical amounts from each brewing. The coffee and tea samples were analyzed immediately upon preparation.

To monitor variation of phenolic acid content in different types of berries, we collected samples of strawberry (cultivars Polka and Jonsok), raspberry, and bilberry in two or three different years. In addition, five chokeberry samples were obtained in summer 2005: two samples from St. Petersburg, Russia, two samples from eastern Finland, and one sample from western Finland. These samples were determined separately.

Moisture. To obtain their moisture contents, the pooled berry and fruit samples were weighed before and after drying at 97 °C overnight (16 h).

Analysis of Phenolic Acids. Soluble and total phenolic acids were analyzed according to the method by Mattila and Kumpulainen (*18*) as modified by Mattila and Hellström (*17*). First, 0.1−0.5 g of freeze-dried or 2 g of fresh homogenized sample was homogenized in 7 mL of a mixture of methanol, containing 2 g/L of butylated hydroxyanisole and 10% acetic acid (85:15). The mixture was sonicated for 30 min and made up to a volume of 10 mL with distilled water. After mixing, 1 mL was filtered for high-performance liquid chromatography (HPLC) analysis of soluble phenolic acids. The remaining 9 mL of the sample extract was used for a sequential hydrolysis experiment with base followed by acid. Next, 12 mL of distilled water containing 1% ascorbic acid, 0.415% ethylenediaminetetraacetic acid tetrasodium salt dihydrate (EDTA), and 5 mL of 10 M NaOH was added into the test tube, sealed, and stirred overnight (about 16 h) at 20 °C using a magnetic stirrer. The solution was adjusted to pH 2 with concentrated HCl, and the liberated phenolic acid aglycones were extracted with a mixture of cold diethyl ether and ethyl acetate (1:1; 3 × 15 mL). The organic layers were then combined. An acid hydrolysis was performed by adding 2.5 mL of concentrated HCl into the test tube and incubating in a water bath at 85 °C for 30 min. The sample was cooled, and further sample handling was performed in the same manner as after alkaline hydrolysis. The organic layers from the alkaline and acid hydrolyses were combined, evaporated to dryness, dissolved into 2 mL of methanol, filtered, and analyzed for total phenolic acids by HPLC.

An Agilent 1100 Series high-performance liquid chromatograph equipped with a diode array detector was used as the analytical HPLC system. The HPLC pumps, autosampler, column oven, and diode array system were monitored and controlled using the HP Chem Station computer program. Phenolic acids were quantified with the diode array detector using the following wavelengths: 254 nm for protocatechuic acid, *p*-hydroxybenzoic acid, and vanillic acid; 280 nm for syringic acid, *p*-coumaric acid, *m*-coumaric acid, *o*-coumaric acid, and *E*-cinnamic acid; and 329 nm for caffeic acid, ferulic acid, sinapic acid, and chlorogenic acid. Phenolic acid separation was done with an Inertsil ODS-3 (4.0 mm × 150 mm, 3 μm) column (GL Sciences, Inc., Japan) equipped with a C-18 guard column. The temperature of the column oven was set at 35 °C. A gradient elution was employed with a mobile phase consisting of 50 mM H_3PO_4 at pH 2.5 (solution A) and acetonitrile (solution B) as follows: isocratic elution 95% A, 0−5 min; linear gradient from 95 to 85% A, 5−17 min; linear gradient from 85 to 80% A, 17−40 min; linear gradient from 80 to 50% A, 40−60 min; isocratic elution 50% A, 60−65 min; linear gradient from 50 to 95% A, 65−67 min; post-time 6 min before the next injection. The flow rate of the mobile phase was 0.7 mL/min, and the injection volume was 10 μL. All phenolic acids were quantified using an external standard. Unknown soluble phenolic acids were tentatively quantified as the phenolic acid, which their UV spectrum resembled. All quantifications were based on peak area, and all samples were analyzed in triplicate. The determination limits for soluble and total phenolic acids were 0.1 mg/100 g and 0.05 mg/100 g, respectively.

The overall validation of the method has been reported previously by Mattila and Kumpulainen (*18*) and Mattila and Hellström (*17*). Recovery tests performed for fruits and berries ($n = 10$) showed an acceptable accuracy of the method; recoveries typically varied from 99 to 115% and from 79 to 101% for soluble and total phenolic acids, respectively, depending on the phenolic acid.

RESULTS AND DISCUSSION

To determine the total phenolic acid content (sum of bound soluble and insoluble forms plus free phenolic acids as aglycones) in berries, fruits, and beverages, the samples were sequentially hydrolyzed by base and acid. Nardini et al. (*19*) found that the recovery of phenolic acids can be improved by adding EDTA and vitamin C to the hydrolysis medium. This analysis step was also used in the present study. Initially, the soluble phenolic acids (free and bound soluble forms) were extracted with methanolic acetic acid and quantified as reported earlier (*17*, *18*). Because of a lack of reference standards for soluble bound phenolic acids, the results are to be considered tentative and are reported only as percentual shares of total phenolic acids in **Tables 1**, **3**, and **4**. However, this information may be of interest because the bioavailability of soluble phenolic acids may differ from insoluble ones.

Phenolic Acids in Berries. Berries showed variation in the content and distribution of phenolic acids (**Table 1**). The highest contents of these compounds were found in rowanberry (103 mg/100 g), chokeberry (96 mg/100 g), blueberry (85 mg/100 g), sweet rowanberry (75 mg/100 g), and saskatoon berry (59 mg/100 g). Caffeic acid, which is a hydrolysis product of soluble chlorogenic acid and its isomers (*18*, *20*), dominated in these berries, in agreement with the earlier literature (*1*, *21*−*25*). In fact, the percentual share of soluble phenolic acids in these berries was high (70−90%). The phenolic acid contents of sweet rowanberry and chokeberry accorded well with studies by Määttä-Riihinen et al. (*21*)., who analyzed only soluble phenolic acids. On the other hand, we found higher contents in blueberry in the present study. This is logical, however, because while blueberry also contained insoluble bound phenolic acids (particularly syringic acid), the phenolic acids found in sweet rowanberry and chokeberry were mainly soluble (**Table 1**).

Moderate phenolic acid contents were determined in bilberry (40−51 mg/100 g), cloudberry (40 mg/100 g), southern crowberry (33 mg/100 g), rose hip (29 mg/100 g), raspberry (26−

Table 1. Total Phenolic Acid Contents in Berries as Aglycones (Means ± SD of Triplicated Samples in Milligrams Per 100 Grams of Fresh Weight)[a]

sample	Latin name	DM (%)	CAF	FER	SIN	PROTO	VAN	P-COUM	P-OH-B	SYR	CIN	GAL	TOT	SOL (%)
strawberry Polka 2003	*Fragaria ananassa*	9.8	0.171 ± 0.0065	ND	ND	ND	ND	4.6 ± 0.20	4.4 ± 0.14	ND	1.07 ± 0.058	3.3 ± 0.10	14	30
strawberry Polka 2004	*F. ananassa*	9.4	0.139 ± 0.0071	0.20 ± 0.020	ND	ND	ND	2.9 ± 0.28	4.5 ± 0.21	ND	0.66 ± 0.035	2.1 ± 0.21	10	30
strawberry Polka 2005	*F. ananassa*	10.9	0.20 ± 0.014	ND	ND	ND	0.24 ± 0.029	4.9 ± 0.44	4.6 ± 0.25	ND	2.7 ± 0.17	5.3 ± 0.45	18	30
strawberry Jonsok 2003	*F. ananassa*	8.3	0.25 ± 0.010	ND	ND	ND	ND	3.7 ± 0.35	6.3 ± 0.78	ND	0.61 ± 0.049	3.3 ± 0.10	14	30
strawberry Jonsok 2004	*F. ananassa*	8.6	0.42 ± 0.043	0.25 ± 0.033	ND	ND	ND	2.9 ± 0.28	4.4 ± 0.21	ND	0.54 ± 0.006	4.1 ± 0.21	13	30
strawberry Honeoye	*F. ananassa*	10.0	0.34 ± 0.059	0.32 ± 0.022	ND	ND	ND	4.5 ± 0.29	5.1 ± 0.49	ND	1.30 ± 0	2.6 ± 0.23	14	30
strawberry jam		53.3	0.48 ± 0.047	0.25 ± 0.041	ND	4.3 ± 0.12	ND	1.7 ± 0.12	2.93 ± 0.078	1.2 ± 0.20	0.91 ± 0.080	NA	12	<10
bilberry 2003	*Vaccinium myrtillus*	11.3	9.5 ± 0.45	1.1 ± 0.12	0.30 ± 0.035	ND	6.0 ± 0.25	6.1 ± 0.40	ND	13.9 ± 0.60	ND	3.2 ± 0.35	40	40
bilberry 2005	*V. myrtillus*	12.4	10.6 ± 0.35	1.2 ± 0.21	0.50 ± 0.067	7.35 ± 0.071	6.90 ± 0	8.1 ± 0.64	ND	15.2 ± 0.92	ND	1.53 ± 0.023	51	30
bilberry soup		11.0	0.92 ± 0.065	0.060 ± 0	ND	1.4 ± 0.14	0.63 ± 0.021	1.10 ± 0	0.08 ± 0.013	0.94 ± 0.14	ND	ND	5	10
lingonberry	*Vaccinium vitis-idaea*	14.1	4.5 ± 0.35	1.5 ± 0.12	0.26 ± 0.022	4.42 ± 0.042	0.87 ± 0.075	3.5 ± 0.26	1.34 ± 0.085	ND	3.6 ± 0.36	4.4 ± 0.35	24	30
sea buckthorn	*Hippophae rhamnoides*	16.3	ND	0.67 ± 0.085	2.4 ± 0.33	ND	<0.1	3.7 ± 0.25	0.126 ± 0	ND	ND	ND	7	0
raspberry 2003	*Rubus idaeus*	13.7	0.691 ± 0.0092	0.76 ± 0.076	0.20 ± 0.010	ND	ND	0.98 ± 0.057	1.64 ± 0.085	ND	0.21 ± 0.014	22.0 ± 1.6	26	<10
raspberry 2005	*R. idaeus*	14.3	1.08 ± 0.014	0.94 ± 0.078	0.345 ± 0	ND	1.04 ± 0.042	1.80 ± 0.038	2.0 ± 0.16	ND	0.33 ± 0.046	21 ± 1.3	29	<10
raspberry jam		51.2	0.73 ± 0.010	0.35 ± 0.023	ND	4.28 ± 0.055	ND	0.59 ± 0.059	ND	ND	ND	NA	6	10
cranberry	*Vaccinium oxococcus*	12.9	2.3 ± 0.23	0.605 ± 0	0.520 ± 0	2.35 ± 0.010	3.1 ± 0.22	1.4 ± 0.16	ND	0.99 ± 0.028	0.49 ± 0.020	ND	12	20
red currant	*Ribes rubrum*	14.4	1.4 ± 0.92	ND	ND	1.55 ± 0,021	1.25 ± 0.031	2.6 ± 0.17	1.21 ± 0.053	0.29 ± 0.046	ND	ND	8	40
black currant	*Ribes nigrum*	16.7	3.5 ± 0.22	1.33 ± 0.074	1.17 ± 0.025	4.5 ± 0.41	0.70 ± 0.042	4.7 ± 0.29	1.4 ± 0.13	ND	ND	5.5 ± 0.43	23	30
gooseberry, red	*Ribes uva-crispa*	11.3	0.26 ± 0.093	0.62 ± 0.021	ND	1.35 ± 0.046	0.76 ± 0.078	4.6 ± 0.30	0.26 ± 0.012	ND	ND	ND	8	40
gooseberry, yellow	*R. uva-crispa*	12.1	4.0 ± 0.14	0.290 ± 0.0078	0.269 ± 0.0042	ND	0.256 ± 0.0051	0.11 ± 0.010	ND	ND	ND	ND	5	80
chokeberry	*Aronia Medik.*	18.5	75 ± 3.3	2.70 ± 0	ND	10.6 ± 0.92	ND	6.9 ± 0.14	ND	ND	0.72 ± 0.030	ND	96	80
sweet rowanberry	*Crataegosorbus mitschurinii*	20.4	63.0 ± 0.58	1.7 ± 0.11	ND	3.4 ± 0.25	ND	6.7 ± 0.35	ND	ND	ND	ND	75	80
rowanberry	*Sorbus aucuparia*	21.0	87 ± 2.8	2.8 ± 0.20	ND	ND	0.53 ± 0.027	11.4 ± 0.69	1.04 ± 0.072	ND	ND	ND	103	90
bog whortleberry	*Vacciniun uliginosum*	12.6	1.76 ± 0.076	1.36 ± 0.081	ND	ND	1.38 ± 0.014	0.173± 0.057	ND	15 ± 1.4	ND	2.1 ± 0.21	22	<10
cloudberry	*Rubus chamaemorus*	14.3	1.8 ± 0.15	2.0 ± 0.21	0.34 ± 0.016	ND	0.80 ± 0.056	5.6 ± 0.23	0.93 ± 0.037	ND	2.6 ± 0.29	26.0 ± 0.10	40	30
blueberry	*Vaccinium* spp.	14.5	59,1 ± 0.20	1.29 ± 0.014	0.70 ± 0.019	2.0 ± 0.23	2.0 ± 0.13	1.65 ± 0.064	ND	15.6 ± 0	0.41 ± 0.029	2.70 ± 0	85	70
rose hip	*Rosa rugosa*	21.2	0.387 ± 0.0042	0.430 ± 0.0082	ND	ND	0.45 ± 0.041	3.08 ± 0.051	0.53 ± 0.035	0.37 ± 0.030	ND	24 ± 1.2	29	<10
saskatoon berry	*Amelanchier alnifolia*	12.8	43.2 ± 0	2.3 ± 0.14	0.77 ± 0.037	8.40 ± 0.032	0.7 ± 0.12	2.9 ± 0.18	0.87 ± 0.073	ND	ND	ND	59	70
southern crowberry	*Empetrum nigrum*	13.0	2.8 ± 0.20	0.51 ± 0.024	ND	4.4 ± 0.22	3.3 ± 0.21	6.1 ± 0.26	ND	14.2 ± 0.49	ND	1.47 ± 0.064	33	<10

[a] DM, dry matter; CAF, caffeic acid; FER, ferulic acid; SIN, sinapic acid; PROTO, protocatechuic acid; VAN, vanillic acid; P-COUM, *p*-coumaric acid; P-OH-B, *p*-hydroxybenxoic acid; SYR, syringic acid; CIN, cinnamic acid; GAL, gallic acid; TOT, total amount of phenolic acids after hydrolyses; SOL/%, percentual share of detectable soluble phenolic acids from the total amount; ND, not detected; NA, not analyzed.

Table 2. Total Phenolic Acids Contents in Five Individual Chokeberry Samples as Aglycones (Means ± SD of Triplicate Samples in Milligrams Per 100 Grams of Fresh Weight)

sampling region	DM (%)	CAF	FER	PROTO	VAN	P-COUM	CIN	TOT
Eastern Finland 1	19.2	71 ± 1.8	2.5 ± 0.28	10.0 ± 0.13	0.91 ± 0.250	6.5 ± 0.64	0.53 ± 0.007	91
Eastern Finland 2	18.8	60 ± 5.9	1.9 ± 0.14	8.3 ± 1.5	0.7 ± 0.18	5.5 ± 0.42	0.70 ± 0.022	77
Western Finland	18.8	71 ± 2.5	2.6 ± 0.13	8.4 ± 0.35	1.31 ± 0.085	7.61 ± 0.032	ND	91
St Petersburg, Russia 1	20.9	75 ± 1.6	2.2 ± 0.12	13.0 ± 1.0	ND	5.9 ± 0.28	0.2 ± 0.27	96
St Petersburg, Russia 2	22.3	74 ± 3.5	2.8 ± 0.31	12.1 ± 1.3	ND	6.2 ± 0.73	0.9 ± 0.16	96

29 mg/100 g), lingonberry (24 mg/100 g), black currant (23 mg/100 g), and bog whortleberry (22 mg/100 g; **Table 1**). Phenolic acid contents in other berries were less than 15 mg/100 g. In raspberry, bog whortleberry, crowberry, and rose hip, the percentual share of detectable soluble phenolic acids was low (<10%), while the other above-mentioned berries contained higher levels of soluble forms (30−40%). Bound gallic acid dominated in cloudberry, raspberry, and rose hip. Gallic acid was probably liberated as a result of alkaline and acid hydrolyses from gallotannins and galloyl esters, whose presence has been established in the literature as typical of the family Rosaceae (*26*). In addition to blueberry, the occurrence of bound syringic acid was typical of bilberry and bog whortleberry—a finding, which, to our knowledge, has not been reported previously.

Strawberries contained total phenolic acids 10−18 mg/100 g, of which 30% were soluble, predominantly *p*-coumaroyl esters. Other dominant phenolic acid aglycones in strawberries, besides *p*-coumaric acid, were *p*-hydroxybenzoic and gallic acid. The magnitude of the contents of *p*-coumaric acid in strawberry was consistent with previous reports (*27*−*29*). However, according to data compiled by Radtke et al. (*2*), strawberries contain no *p*-coumaric acid at all. Earlier studies on berries have generally reported variable contents of phenolic acids. Reasons for the high variation may lie in differences in the analytical methodology used and in the natural variation of sample material. Besides, most of the earlier reports deal only with soluble phenolic acids.

To monitor variation in the phenolic acid contents of the pooled samples, sampling of selected berries was repeated twice (bilberry, raspberry, and strawberry Jonsok) or three times (strawberry Polka; **Table 1**). Small to moderate variation was observed; the phenolic acid contents of bilberry, raspberry, strawberry Jonsok, and strawberry Polka varied from 40 to 51, 26 to 29, 13 to 14, and 10 to 18 mg/100 g, respectively. Individual samples of chokeberry were analyzed to monitor the variation further (**Table 2**). The variation was small. The total phenolic acid contents of five chokeberry samples varied from 77 to 96 mg/100 g fw (mean = 90 ± 7.8 mg/100 g fw, CV 8.7%).

Phenolic Acids in Fruits. Among fruits, the highest contents of phenolic acids were found in dark plum, cherry, citrus fruits, red grape, and some apple varieties (**Table 3**). However, the contents in these fruits (<30 mg/100 g) were clearly lower than in the best berries. Caffeic acid dominated in plum, cherry, and apples; syringic acid dominated in red grape; and ferulic acid dominated in citrus fruits. **Table 3** shows that nearly all phenolic acids in plum and cherry were in soluble form (as chlorogenic acid isomers). In contrast, a large part of the phenolic acids in citrus fruits and red grape was detectable only after the performed hydrolyses. Our results for total phenolic acid content in plum and cherry agreed well with data compiled by Radtke et al. (*2*), although previous studies have found considerable cultivar- and maturation-born variations in the phenolic acid contents of these fruits (*1*, *30*, *31*). However, our results for citrus fruits were higher and for red grape lower than reported earlier (*2*).

The predominant phenolic acid aglycones in apples were caffeic, *p*-coumaric, and gallic acids. In agreement with previous studies (*1*, *32*−*34*), the contents of phenolic acids varied a lot between different apple cultivars. The lowest content of total phenolic acids was determined from the cultivar Granny Smith (7 mg/100 g) and the highest from Valkea Kuulas (28 mg/100 g; **Table 3**). Apart from a higher content of total phenolic acids, Valkea Kuulas differed from other varieties by its higher percentual share of soluble phenolic acids, mostly of chlorogenic and *p*-coumaric acid isomers. Contrary to peels of potatoes (*17*), for example, apple peels contained lower levels of phenolic acids than whole fruits. This agrees with results reported by Awad et al. (*33*) and Alonso-Salces et al. (*32*). In a study by Awad et al. (*33*)., chlorogenic acid was mainly present in the core area and the seeds, with an intermediate level in the flesh and a low level in the skin. On the other hand, Goristein et al. (*35*) found significantly higher phenolic acid contents in the peels of apples (cultivar Golden Delicious) than in the peeled fruits.

Peach, nectarine, pear, rhubarb, banana, green grape, kiwi fruit, and watermelon contained moderately low levels (<10 mg/100 g) of phenolic acids. Most of the phenolic acids in pear, peach, and nectarine were in soluble form as chlorogenic acid isomers. In green grape, kiwi fruit, and watermelon, the share of detectable soluble phenolic acids was lower (10−60%), and in rhubarb and banana, all phenolic acids became detectable only after the performed hydrolyses. There is scant earlier information on the phenolic acid content in kiwi fruit, watermelon, banana, and rhubarb, and the values for peach, pear, and grapes vary considerably in the literature. For example, Gorinstein et al. (*35*) reported higher results for pears and peaches than obtained in the present study. On the other hand, cultivar-born variation has been found to be considerable in the case of these fruits (*1*, *36*). Our results for these fruits, however, fit well with the ranges reported earlier.

Phenolic Acids in Beverages. The contents of phenolic acids in beverages varied widely, ranging from 0 (pear cider) to 97 mg/100 g (coffee; **Table 4**). In most beverages, the hydrolysis procedures liberated a large amount of phenolic acids from their bound forms as compared with results obtained after methanolic acetic acid extracts (results not shown). Coffee and black tea were exceptions, however, as their phenolic acid levels calculated as aglycones were comparable after both treatments.

Coffee was the richest source of phenolic acids (97 mg/100 g) among the studied beverages. Over 10 different chlorogenic acid isomers were detected in the coffee sample from which caffeic acid had been liberated after the hydrolyses. According to Clifford et al. (*37*), green coffee beans are remarkably rich in chlorogenic acid, containing over 30 chlorogenic acid isomers. Our results agree with former studies, which suggest that a 200 mL cup of roast and ground coffee might supply from 70 mg up to 350 mg of chlorogenic acid, respectively (*2*, *4*). Nardini

Table 3. Total Phenolic Acid Contents in Fruits (mg/100 g Fresh Weight) as Aglycones (Means ± SD of Triplicate Samples in Milligrams Per 100 Grams of Fresh Weight)

sample	Latin name	DM (%)	CAF	FER	SIN	PROTO	VAN	P-COUM	P-OH-B	SYR	GAL	TOT	SOL (%)
peach	*Prunus persica*	10.9	4.9 ± 0.47	0.11 ± 0.013	ND	ND	0.25 ± 0.050	0.52 ± 0.014	0.22 ± 0.026	0.26 ± 0.051	ND	6	90
nectarine	*P. persica* var. *nectarine*	11.9	4.9 ± 0.28	0.14 ± 0.010	ND	0.65 ± 0.024	0.80 ± 0.036	0.39 ± 0.027	0.46 ± 0.037	ND	ND	7	80
apple, Lobo, whole fruit	*Malus domestica*	12.2	4.3 ± 0.38	0.27 ± 0.024	0.080 ± 0.0042	0.45 ± 0.012	0.092 ± 0.0058	0.66 ± 0.086	ND	ND	7.2 ± 0.92	13	40
apple, Lobo, peel	*M. domestica*	14.9	3.48 ± 0.085	0.85 ± 0.023	ND	1.4 ± 0.014	0.131 ± 0.0057	1.7 ± 0.14	ND	ND	0.318 ± 0.021	8	30
apple, Valkea Kuulas, whole fruit	*M. domestica*	10.5	18.3 ± 0.28	0.36 ± 0.016	0.089 ± 0.0090	ND	ND	4.7 ± 0.20	ND	ND	4.2 ± 0.40	28	80
apple, Valkea Kuulas, peel	*M. domestica*	12.3	9.7 ± 0.52	0.80 ± 0.089	ND	ND	ND	4.9 ± 0.45	ND	ND	3.3 ± 0.21	19	60
apple, Granny Smith, whole fruit	*M. domestica*	15.1	1.3 ± 0.13	ND	ND	ND	ND	0.41 ± 0.057	ND	ND	5.4 ± 0.35	7	20
apple, Granny Smith, peel	*M. domestica*	18.2	0.85 ± 0.067	0.44 ± 0.026	ND	ND	ND	1.9 ± 0.16	ND	ND	3.45 ± 0.021	7	10
apple, Red Delicious, whole fruit	*M. domestica*	13.7	5.4 ± 0.22	0.27 ± 0.044	0.10 ± 0.015	ND	ND	1.9 ± 0.17	ND	ND	6.5 ± 0.38	14	50
apple, Red Delicious, peel	*M. domestica*	19.5	1.02 ± 0.023	0.38 ± 0.025	ND	ND	ND	1.88 ± 0.036	ND	ND	2.7 ± 0.32	6	20
rhubarb	*Rheum*	6.4	0.053 ± 0.0028	2.0 ± 0.14	0.177 ± 0.0092	ND	ND	1.8 ± 0.10	ND	ND	5.0 ± 0.17	9	0
banana	*Musa sapientum*	25.4	0.20 ± 0.015	5.4 ± 0.42	ND	ND	0.445 ± 0.0071	0.46 ± 0.050	0.12 ± 0.028	0.22 ± 0.035	ND	7	0
grape, red	*Vitis vinifera* L.	14.8	3.4 ± 0.12	0.43 ± 0.031	ND	ND	1.07 ± 0.058	3.8 ± 0.31	ND	6.8 ± 0.32	3.1 ± 0.21	19	20
grape, green	*V. vinifera* L.	15.2	3.4 ± 0.10	ND	ND	ND	ND	1.17 ± 0.058	ND	ND	2.85 ± 0.071	7	60
plum, dark	*Prunus domestica*	10.7	23.4 ± 0.17	1.47 ± 0.058	0.14 ± 0.0058	ND	1.27 ± 0.058	2.1 ± 0.11	ND	ND	ND	28	90
cherry	*Prunus avium*, *prunus cerasus*	17.4	17.1 ± 1.2	0.46 ± 0.046	ND	3.0 ± 0.40	1.17 ± 0.050	5.1 ± 0.51	0.88 ± 0.064	ND	ND	28	80
pear	*Pyrus communis*	17.4	6.5 ± 0.45	0.29 ± 0.023	0.104 ± 0.0081	ND	0.27 ± 0.052	0.70 ± 0.070	ND	ND	ND	8	90
orange	*Citrus sinensis*	16.4	3.3 ± 0.15	9.4 ± 0.80	2.2 ± 0.22	ND	0.44 ± 0.050	1.78 ± 0.058	0.54 ± 0.059	ND	ND	18	30
orange, red	*C. sinensis*	15.7	5.6 ± 0.24	9.9 ± 0.52	0.87 ± 0.018	ND	1.28 ± 0.049	1.81 ± 0.087	0.35 ± 0.029	ND	ND	20	50
Mandarin (clementine)	*Citrus retilculata*	16.8	6.6 ± 0.20	9.24 ± 0.086	1.51 ± 0.020	ND	0.64 ± 0.023	0.88 ± 0.032	ND	ND	ND	19	30
grapefruit	*Citrus paradisi*	14.4	5.5 ± 0.24	11.6 ± 0.44	0.99 ± 0.078	ND	1.66 ± 0.064	1.35 ± 0.024	ND	ND	ND	21	40
grapefruit, red	*C. paradisi*	15.1	3.24 ± 0.097	10.7 ± 0.32	1.97 ± 0.099	ND	0.61 ± 0.028	3.8 ± 0.12	0.44 ± 0.014	0.098 ± 0.0042	ND	21	30
kiwi fruit	*Actinidia chinensis*	17.6	1.5 ± 0.12	0.19 ± 0.018	ND	0.66 ± 0.010	0.19 ± 0.014	0.25 ± 0.010	ND	ND	ND	3	30
watermelon	*Citrullus lanatus*	8.1	0.12 ± 0.022	0.35 ± 0.053	ND	ND	0.23 ± 0.053	0.37 ± 0.014	ND	0.86 ± 0.11	ND	2	10

Table 4. Total Phenolic Acid Contents in Beverages (mg/100 g Fresh Weight) as Aglycones (Means ± SD of Triplicate Samples in Milligrams Per 100 Grams of Fresh Weight)

sample	CAF	FER	SIN	PROTO	VAN	P-COUM	P-OH-B	SYR	CIN	GAL	TOT
apple juice	3.6 ± 0.14	0.10 ± 0.013	ND	0.756 ± 0.017	ND	1.20 ± 0	0.056 ± 0.0082	ND	0.12 ± 0.0085	10 ± 1.4	16
orange juice	0.25 ± 0.015	4.7 ± 0.42	0.47 ± 0.042	ND	0.52 ± 0.020	1.0 ± 0.13	0.100 ± 0.0035	ND	ND	ND	7
pear cider	0.106 ± 0.0010	ND	ND	ND	ND	ND	ND	0.090 ± 0	ND	ND	0
apple cider	1.16 ± 0.035	0.076 ± 0.0067	ND	ND	ND	0.39 ± 0.019	ND	0.12 ± 0.012	ND	ND	2
red wine	3.2 ± 0.14	ND	ND	ND	0.9 ± 0.13	5.0 ± 0.28	0.18 ± 0.015	5.6 ± 0.21	ND	5.3 ± 0.15	20
bear	0.12 ± 0.010	0.95 ± 0.059	0.23 ± 0.013	ND	0.15 ± 0.013	0.110 ± 0.0015	ND	0.069 ± 0.0028	ND	ND	2
cocoa powder	ND	ND	ND	40 ± 1.6	3.7 ± 0.20	ND	ND	4.1 ± 0.21	ND	ND	48
coffee	87 ± 2.2	9.1 ± 0.36	ND	ND	ND	1.27 ± 0.053	ND	ND	ND	ND	97
black tea	1.42 ± 0.052	0.16	ND	ND	ND	2.0 ± 0.13	ND	ND	ND	26 ± 2.0	30
green tea	1.340 ± 0.0028	ND	ND	ND	ND	1.00 ± 0.028	ND	ND	ND	34 ± 2.7	36
mixed berry juice concentrate	1.00 ± 0.072	ND	ND	1.07 ± 0.058	ND	0.83 ± 0.014	ND	1.15 ± 0.071	ND	ND	4
black currant juice concentrate	2.6 ± 0.30	0.78 ± 0.043	0.78 ± 0.018	1.7 ± 0.10	ND	3.2 ± 0.21	ND	ND	ND	ND	9

et al. (*38*) also obtained a very similar result as achieved in the present study for total phenolic acid aglycones after alkaline hydrolysis (99 mg/100 g).

Apart from coffee, high or moderate contents of phenolic acids were determined in green and black tea (30−36 mg/100 g), red wine (20 mg/100 g), and apple juice (19 mg/100 g), whereas the phenolic acid contents in other beverages were below 10 mg/100 g. High contents of phenolic acids were also analyzed in cocoa powder (48 mg/100 g), but when the powder is prepared into a cocoa beverage, the phenolic acid content is low. There is but little information on phenolic acid content in different tea brews, although according to Clifford (*4*), black and green tea leaves contain large amounts of theogallin and small amounts of *p*-coumaroylquinic and caffeoylquinic acids. This is in agreement with the present study, as gallic acid aglycones dominated in the tea brews whereas the contents of caffeic and *p*-coumaric acids were lower. In wine and apple juice, the contents of phenolic acids vary widely (*4*, *39*, *40*), and the use of different methodologies makes comparison with previous data difficult. However, our results for red wine and apple juice were higher as compared with data introduced by Radtke et al. (*2*) and Amakura et al. (*41*). On the other hand, our total phenolic acid result for orange juice was at the same level as reported earlier (*42*). Commercial black currant and mixed berry juice concentrates contained moderately low levels of phenolic acids (9 mg/100 g and 2 mg/100 g, respectively), especially considering that they have to be diluted further before consumption. To our knowledge, these two commercial products have not been studied earlier. Clifford (*4*) observed high phenolic acid variation in commercial ciders (1−48 mg chlorogenic acid isomers/100 mL). Our study results for the Finnish pear and apple ciders fit at the lower end of this range (<2 mg/100 g). The result for beer (2 mg/100 g) was of the same magnitude as reported by Nardini and Ghiselli (*43*).

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LITERATURE CITED

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

(2) Radtke, J.; Linseisen, J.; Wolfram, G. Phenolsäurezufuhr erwachsener in einen bayerischen teilkollektiv der nationalen verzehrsstudie. *Z. Ernährungswiss.* **1998**, *37*, 190−197.

(3) Shahidi, F.; Naczk, M. *Food Phenolics*; Technomic Publishing Co., Inc.: Basel, Switzerland, 1995.

(4) Clifford, M. Chlorogenic acids and other cinnamates—Nature, occurrence and dietary burden. *J. Sci. Food Agric.* **1999**, *79*, 362−372.

(5) Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: Food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727−747.

(6) Tsao, R.; Deng, Z. Separation procedures of naturally occurring antioxidant phytochemicals. *J. Chromatogr.* **2004**, *B 812*, 85−99.

(7) De Bruyne, T.; Pieters, L.; Deelstra, H.; Vlietnick, A. Condensed vegetable tannins: Biodiversity in structure and biological activities. *Biochem. Syst. Ecol.* **1999**, *27*, 445−459.

(8) Haslam, E. Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. *J. Nat. Prod.* **1996**, *59*, 205, 215.

(9) Lairon, D.; Amiot, M. Flavonoids in food and natural antioxidants in wine. *Curr. Opin. Lipidol.* **1999**, *10*, 23−28.

(10) Morton, L.; Abu-Amsha Caccetta, R.; Puddey, I.; Croft, K. Chemistry and biological effects of dietary phenolic compounds: Relevance to cardiovascular disease. *Clin. Exp. Pharmacol. Physiol.* **2000**, *27*, 152−159.
(11) Parr, A.; Bolwell, G. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content and profile. *J. Sci. Food Agric.* **2000**, *80*, 985−1012.
(12) Lule, S.; Xia, W. Food phenolics, pros and cons: a review. *Food Rev. Int.* **2005**, *21*, 367−388.
(13) Arts, I.; Hollman, P. Polyphenols and disease risk in epidemiological studies. *Am. J. Clin. Nutr.* **2005**, *81*, 317S−325S.
(14) Erlund, I. Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. *Nutr. Res.* **2004**, *24*, 851−874.
(15) Knekt, P.; Kumpulainen, J.; Järvinen, R.; Rissanen, H.; Heliövaara, M.; Reunanen, A.; Hakulinen, T.; Aromaa, A. Flavonoid intake and risk of chronic diseases. *Am. J. Clin. Nutr.* **2002**, *76*, 560−568.
(16) Koivu, T.; Piironen, V.; Henttonen, S.; Mattila, P. Determination of phylloquinone in vegetables, fruits, and berries by high-performance liquid chromatography with electrochemical detection. *J. Agric. Food Chem.* **1997**, *45*, 4644−4649.
(17) Mattila, P.; Hellström, J. Phenolic acids in potatoes, vegetables, and some of their products. *J. Food Compos. Anal.* In press.
(18) Mattila, P.; Kumpulainen, J. Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. *J. Agric. Food Chem.* **2002**, *50*, 3660−3667.
(19) Nardini, M.; Cirillo, E.; Natella, F.; Mencarelli, D.; Comisso, A.; Scaccini, C. Detection of bound phenolic acids: Prevention by ascorbic acid and ethylenediaminetetraacetic acid of degradation of phenolic acids during alkaline hydrolysis. *Food Chem.* **2002**, *79*, 119−124.
(20) Antolovich, M.; Prenzler, P.; Robards, K.; Ryan, D. Sample preparation in the determination of phenolic compounds in fruits. *Analyst* **2000**, *125*, 989−1009.
(21) Määttä-Riihinen, K.; Kamal-Eldin, A.; Mattila, P.; González-Paramás, A.; Törrönen, R. Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. *J. Agric. Food Chem.* **2004**, *52*, 4477−4486.
(22) Taruscio, T.; Barney, D.; Exon, J. Content and profile of flavanoid and phenolic acid compounds in conjunction with the antioxidant capacity for a variety of northwest *Vaccinium* berries. *J. Agric. Food Chem.* **2004**, *52*, 3169−3176.
(23) Zheng, W.; Wang, Y. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *J. Agric. Food Chem.* **2003**, *51*, 502−509.
(24) Slimestad, R.; Torskangerpoll, K.; Nateland, H.; Johannessen, T.; Giske, N. Flavonoids from black chokeberries, *Aronia melanocarpa*. *J. Food Compos. Anal.* **2005**, *18*, 61−68.
(25) Hukkanen, A.; Pölönen, S.; Kärenlampi, S.; Kokko, H. Antioxidant capacity and phenolic content of sweet rowanberries. *J. Agric. Food Chem.* **2006**, *54*, 112−119.
(26) Haddock, E.; Gupta, R.; Al-Shafi, S.; Layden, K.; Haslam. E.; Magnolato, D. The metabolism of gallic acid and hexahydroxydiphenic acid in plants: Biogenetic and molecular taxonomic considerations. *Phytochemistry* **1982**, *21*, 1049−1062.
(27) Määttä-Riihinen, K.; Kamal-Eldin, A.; Törrönen, R. Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (family Rosaceae). *J. Agric. Food Chem.* **2004**, *52*, 6178−6187.
(28) Wang, S.; Zheng, W. Effect of plant growth temperature on antioxidant capacity in strawberry. *J. Agric. Food Chem.* **2001**, *49*, 4977−4982.
(29) Schuster, B.; Herrmann, K. Hydoxybenzoic and hydroxycinnamic acid derivatives in soft fruits. *Phytochemistry* **1985**, *24*, 2761−2764.
(30) Mozetič, B.; Trebše, P.; Hribar, J. Determination and quantitation of anthocyanins and hydroxycinnamic acids in different cultivars of sweet cherries (Prunus avium L.) from Nova Gorica region (Slovenia). *Food Technol. Biotechnol.* **2002**, *40*, 207−212.
(31) Mozetič, B.; Trebše, P.; Simčič, M.; Hribar, J. Changes of anthocyanins and hydroxycinnamic acids affecting the skin colour during maturation of sweet cherries (Prunus avium L.). *Lebensm.-Wiss. Technol.* **2004**, *37*, 123−128.
(32) Alonso-Salces, R.; Barrando, A.; Abad, B.; Berrueta, L.; Gallo, B.; Vicente, F. Polyphenolic profiles of Basque cider apple cultivars and their technological properties. *J. Agric. Food Chem.* **2004**, *52*, 2938−2952.
(33) Awad, M.; Jager, A.; Westing, L. Flavonoid and chlorogenic acid levels in apple fruit: characterisation of variation. *Sci. Hortic.* **2000**, *83*, 249−263.
(34) McRae, K.; Lidster, P.; DeMarco, A.; Dick, A. Comparison of the polyphenol profiles of apple fruit cultivars by correspondence analysis. *J. Sci. Food Agric.* **1990**, *50*, 329−342.
(35) Gorinstein, S.; Martin-Belloso, O.; Lojek, A.; Ĉiž, M.; Soliva-Fortuny, R.; Park, Y.-S.; Caspi, A.; Libman, I.; Trakhtenberg, S. Comparative content of some phytochemicals in Spanish apples, peaches and pears. *J. Sci. Food Agric.* **2002**, *82*, 1166−1170.
(36) Escarpa, A.; González, M. Evaluation of high-performance liquid chromatography for determination of phenolic compounds in pear horticultural cultivars. *Chromatographia* **2000**, *51*, 37−43.
(37) Clifford, M.; Knight, S.; Surucu, B.; Kuhnert, N. Characterization by LC-MS of four new classes of chlorogenic acids in green beans: Dimethoxycinnamoylquinic acids, diferuloylquinic acids, caffeoyl-dimethoxycinnamoylquinic acids, and feruloyl-dimethoxycinnamoylquinic acids. *J. Agric. Food Chem.* **2006**, *54*, 1957−1969.
(38) Nardini, M.; Cirillo, E.; Natella, F.; Scaccini, C. Absorption of phenolic acids in humans after coffee consumption. *J. Agric. Food Chem.* **2002**, *50*, 5735−5741.
(39) Nikfardjam, M.; Márk, L.; Avar, P.; Figler, M.; Ohmacht, R. Polyphenols, anthocyanins, and trans-resveratrol in red wines from the Hungarian Villány region. *Food Chem.* **2006**, *98*, 453−462.
(40) Lee, H.; Wrolstad, R. Apple juice composition; sugar, nonvolatile acid, and phenolic profiles. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 789−794.
(41) Amakura, Y.; Okada, M.; Tsuji, S.; Tonogai, Y. Determination of phenolic acids in fruit juices by isocratic column liquid chromatography. *J. Chromatogr. A* **2000**, *891*, 183−188.
(42) Peleg, H.; Naim, M.; Rouseff, R.; Zehavi, U. Distribution of bound and free phenolic acids in oranges (*Citrus sinensis*) and grapefruits (*Citrus paradise*). *J. Sci. Food Agric.* **1991**, *57*, 417−426.
(43) Nardini, M.; Ghiselli, A. Determination of free and bound phenolic acids in beer. *Food Chem.* **2004**, *84*, 137−143.

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