Effect of Different Organic Farming Methods on the Phenolic Composition of Sea Buckthorn Berries

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The effects of different organic cultivation methods on the berry phenolics of two Finnish sea buckthorn (*Hippophae rhamnoides* L. ssp. *rhamnoides*) cultivars, 'Terhi' and 'Tytti', were studied in an experimental field at a coastal area in Merikarvia, western Finland. Cultivation methods included different fertilizers (designed for organic cultivation), mulches (organic and plastic), and land contours (flat land versus ridged beds). Two experiments were conducted: The first, a fertilization experiment, allowed for the estimation of the effects of cultivar, fertilizer, land contour, and all of their interactions. The second experiment, a mulch experiment, allowed for the estimation of the effects of mulch, land contours, and their interactions for the cultivar 'Tytti'. Berry phenolics were analyzed by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. The results suggest that there are significant differences between the cultivars and cultivation methods. The concentrations of quercetin derivatives 1−3, isorhamnetin 3,7-diglucoside, quercetin-3-glucoside-7-rhamnoside, quercetin 3-glucoside, isorhamnetin 3-glucoside, and flavonoid derivative 3 were higher in 'Tytti' than in 'Terhi', while concentrations of isorhamnetin-glucoside 2 and 3 were higher in 'Terhi' than in 'Tytti'. Flat land increased the concentrations of isorhamnetin 3,7-diglucoside, isorhamnetin-glucoside 1, quercetin derivatives 2 and 4, and condensed tannins. Mulch did not have any significant effect on the concentrations of phenolic compounds. These results indicate that the phenolic accumulation in berries of studied sea buckthorn cultivars seems to be mainly dependent upon cultivar selection and soil structure.

KEYWORDS: *Hippophae rhamnoides*; organic farming; phenolic compound; berry; cultivar; fertilizer; mulch; land contour

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides* L. Elaeagnaceae) is a highly branched, deciduous, and usually spiny shrub or tree (*28*). Its natural habitat extends throughout the temperate zone of Asia and Europe and all over the subtropical zones, especially at high altitude (*28, 38*). All parts of sea buckthorn are considered to be a good source of bioactive substances. The berries have a high content of amino acids, fatty acids, organic acids, and vitamins (*2, 15, 26, 36, 39*). Furthermore, there are considerable amounts of phenolic substances, such as flavonoids in the berries (*30, 35*). The flavonoids of sea buckthorn are reported to have medicinal value, such as the prevention of cardiac disease, and antioxidant, immunomodulatory, anti-inflammatory, and antitumor effects (*1, 5, 7, 38*).

The importance of sea buckthorn as a crop has increased in many countries, such as Canada, Estonia, Germany, and Finland (*9, 25, 36*). There is particular interest in organically cultivated berries, because public concern for food quality and safety has increased in the markets for organically cultivated products. Sea buckthorn is a suitable plant species for organic farming because of its association with nitrogen-fixing bacteria, which enable it to grow in poor soils, while it improves soil fertility (*28, 32*).

Reproduction and fruiting are costly processes in plants, and the photosynthetic energy allocated to sexual reproductive efforts is not available for plant growth. Also, the production of phenolics, which are often regarded as defensive compounds, is expensive to a plant (*6*). This presents a dilemma: either to grow or to defend (*12*). Three well-known hypotheses predict allocation of resources to growth versus secondary chemistry in conditions with changing resource availability. All of these hypotheses, carbon/nutrient balance (CNB) (*3*), growth differentiation balance (GDB) (*12*), and protein competition model (PCM) (*16*), predict that fertilization with nitrogen should decrease the concentration of carbon-based secondary metabolites, such as phenolic compounds. These hypotheses provide a theoretical framework in which to study the effects of organic

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Sea Buckthorn Berry Phenolics

J. Agric. Food Chem., Vol. 57, No. 5, 2009 **1941**

Table 1. Statistical Tests for HPLC Compound Groups and Condensed Tannins in Fertilized 'Terhi' and 'Tytti' ($n = 4 =$ Number of Blocks)[a]

compound group	transformation	cultivar F	cultivar p	fertilization F	fertilization p	contour F	contour p	Cu × Co F	Cu × Co p	Fe × Co F	Fe × Co p
1 total HPLC phenolics	ln	0.601	0.467	1.025	0.392	1.845	0.182	3.840	0.057	3.980	**0.014**
3 quercetin derivative 1	ln	8.843	**0.025**	0.307	0.820	2.286	0.139	0.022	0.882	0.793	0.505
4 isorhamnetin 3,7-diglucoside	ln	146.245	**<0.001**	0.040	0.989	20.344	**<0.001**	0.012	0.913	0.635	0.597
7 isorhamnetin rhamnodiglucoside	none	4.553	0.077	0.906	0.447	0.486	0.490	0.819	0.371	1.993	0.131
8 quercetin 3-glucoside 7-rhamnoside	ln	65.999	**<0.001**	0.736	0.537	2.550	0.118	3.124	0.085	3.413	**0.027**
10 isorhamnetin-glucoside 1	ln	2.490	0.166	0.841	0.480	5.587	**0.023**	1.020	0.319	1.020	0.394
11 quercetin 3-glucoside	ln	46.423	**<0.001**	1.226	0.313	1.147	0.291	1.916	0.174	2.998	**0.042**
12 isorhamnetin 3-glucoside 7-rhamnoside	none	1.460	0.272	1.319	0.282	2.201	0.146	3.516	0.068	3.844	**0.017**
13 isorhamnetin-glucoside 2	none	9.418	**0.022**	0.692	0.562	0.002	0.967	1.912	0.175	1.385	0.262
14 quercetin derivative 2	none	683.678	**<0.001**	0.048	0.986	6.249	**0.017**	9.125	**0.004**	2.497	0.074
15 isorhamnetin 3-glucoside	sqrt	73.345	**<0.001**	0.925	0.438	1.851	0.181	3.937	0.054	4.422	**0.009**
16 isorhamnetin-glucoside 3	ln	95.745	**<0.001**	0.941	0.430	1.818	0.185	4.516	**0.040**	2.881	**0.048**
17 quercetin derivative 3	none	7.675	**0.032**	0.035	0.991	1.412	0.242	2.223	0.144	2.383	0.084
18 dicoumaroyl isoquercetin	none	0.532	0.493	0.632	0.599	0.035	0.852	4.054	0.051	1.457	0.241
21 quercetin derivative 4	ln	1.700	0.240	0.125	0.944	11.084	**0.002**	1.293	0.262	1.502	0.229
22 flavonoid derivative 3	ln	20.788	**0.004**	0.172	0.914	0.000	0.998	9.837	**0.003**	4.644	**0.007**
23 condensed tannins	none	5.095	0.065	0.423	0.738	4.610	**0.038**	0.022	0.884	0.152	0.928

[a] The transformations used for ANOVA are given. F values denote the ratio of variance because of a treatment and error variance. p values denote the probability of rejecting a true null hypothesis. The p values smaller than 0.05 are marked in bold. Cu, cultivar; Fe, fertilization; Co, contour.

farming methods on the concentrations of secondary compounds in sea buckthorn berries.

Although there has been lot of research of organic agriculture since the 1960s (*31*), little attention has been focused on the potential effects of organic farming methods on the accumulation of sea buckthorn berry phenolics, such as isorhamnetin, quercetin, and kaempherol derivatives (*14, 21, 29, 35*).

This paper aimed to test the effects of selected organic farming methods on the phenolic composition of berries of two sea buckthorn cultivars. Combinations of cultivation methods were tested, because they were expected to have a strong effect on resource allocation on berry phenolics. The methods used included different mulches, low nitrogen fertilizers, and land contours. The low nitrogen fertilizers were chosen to test their efficacy for this plant, which grows in symbiosis with nitrogen-fixing bacteria. Mulch and land contours studied are generally effective in organic cultivations.

MATERIALS AND METHODS

Experimental Design and Plant Material. The Finnish sea buckthorn cultivars 'Terhi', 'Tytti', and 'Tarmo' used in this study descended from the wild sea buckthorns originating in the Baltic Sea region in Finland (*18*). The saplings were grown at the study field in a coastal area in Merikarvia, western Finland (61° 52′ N, 21° 30′ E). Annual precipitation of the area was 734 mm in 2006.

The experiment was conducted in a flat square 90 × 90 m field. The top 30−40 cm of the soil was humus mixed with fine sand (1:2; v/v). Readily available P and K were 2.4 and 152 mg L^{-1}, respectively. Soil pH was 5.5. The field was tilled around 20 cm deep, before land contours (planting beds) were established. Ridged beds of the land contour treatment were performed using an excavator. The ridged beds were approximately 30 cm high and 100 cm wide. On the flat land, a 0.5 m^2 plot was tilled 20 cm deep for each sapling during planting. Cuttings that were 1.5 years old were used to start plants. In total, 560 saplings were planted in the field at the end of September 2003.

Five saplings were arranged in each row of five individuals, such that the first sapling in each row was a male ('Tarmo') and the four remaining ones were female ('Terhi' or 'Tytti'). Rows were oriented from south to north, because the prevailing wind direction was from the west. The study area consisted of nine blocks, so that each block received either of the two female cultivars 'Terhi' (4 blocks) or 'Tytti' (5 blocks) at random. In each block, four different fertilizer treatments (no fertilizer, apatite, bioapatite, or biolan) and two land contours (flat land or ridged bed) were arranged according to a fully crossed design in eight rows with four female plants in each block. In addition, each

row was covered with plastic mulch. The blocks with 'Tytti' also had eight rows each with four female plants, which were randomized to fully crossed combinations of the two land contours and five different mulches (control, straw, dry grass, conifer chips, and plastic mulch). These rows also received biolan. The plastic mulch used was an ultraviolet-stabilized woven black polypropene material that blocks sunlight but allows moisture, nutrient, and gas flow.

Two experiments were conducted. In the first, a fertilization experiment, the effects of cultivar, 'Terhi' and 'Tytti', fertilizer, land contour, and their interactions were studied. In the second, a mulch experiment, the effects of mulch, contour, and their interactions were studied using the cultivar 'Tytti'. To keep the experimental design balanced with both cultivars, only four randomly chosen 'Tytti' blocks were used in both studies.

Fertilizer was applied when the study field was established in 2003 and also at the beginning of the 2006 growing season. The chemical composition of commercial fertilizers and their added amounts were as follows: apatite (0:14:0 N/P/K at 1000 kg ha^{-1}), bioapatite (0:2:4 N/P/K at 5000 kg ha^{-1}), and biolan (3:3:15 N/P/K at 1670 kg ha^{-1}).

The mulch was applied when the study field was established in 2003 and also at the beginning of the 2005 growing season. Mulch was spread to form a thick layer covering an area of 1 m^2 around each plant. In the control treatment for mulching, grass growing in the area was cut and left to decompose. The chemical composition of mulch was measured at Viljavuuspalvelu Oy, Mikkeli, Finland. The observed nutrient contents were straw (N, 7.5 g kg^{-1}; P, 1.4 g kg^{-1}; K, 20 g kg^{-1}), dry grass (N, 16 g kg^{-1}; P, 2.2 g kg^{-1}; K, 19 g kg^{-1}), and conifer chips (N, 0.86 g kg^{-1}; P, 0.1 g kg^{-1}; K, 0.7 g kg^{-1}).

Berry samples for chemical analyses were collected from the cultivars at the end of the third growing season, in 2006. Five randomly selected mature berries were taken from one plant, and all berries from the plants grown in the same row were put together (20 berries) and analyzed as a single sample. The study area yielded a total of 96 samples of berries. Fresh berries were stored at −20 °C until analyses.

Sample Preparation. Seeds were separated from the berries by small forceps. Berries were then freeze-dried and crushed in a mortar. Samples mass for analyses of soluble phenolic compounds were homogenized with a sharpened glass rod for 5 min in 600 μL of acetone/water (3:1, v/v). Samples were then centrifuged at 11500g for 3 min. The supernatant was collected, while residues were re-extracted 3 more times (1× for 5 min and 2× for 5 s) in 600 μL of acetone/water. All supernatants were combined, and acetone was evaporated off under a nitrogen gas flow. The sea buckthorn oil was removed from the extract by washing the dry sample 3 times with 600 μL of petroleum ether. For analyses with high-performance liquid chromatography (HPLC), the samples were dissolved in 500 μL of methanol and water up to 1.4 mL.
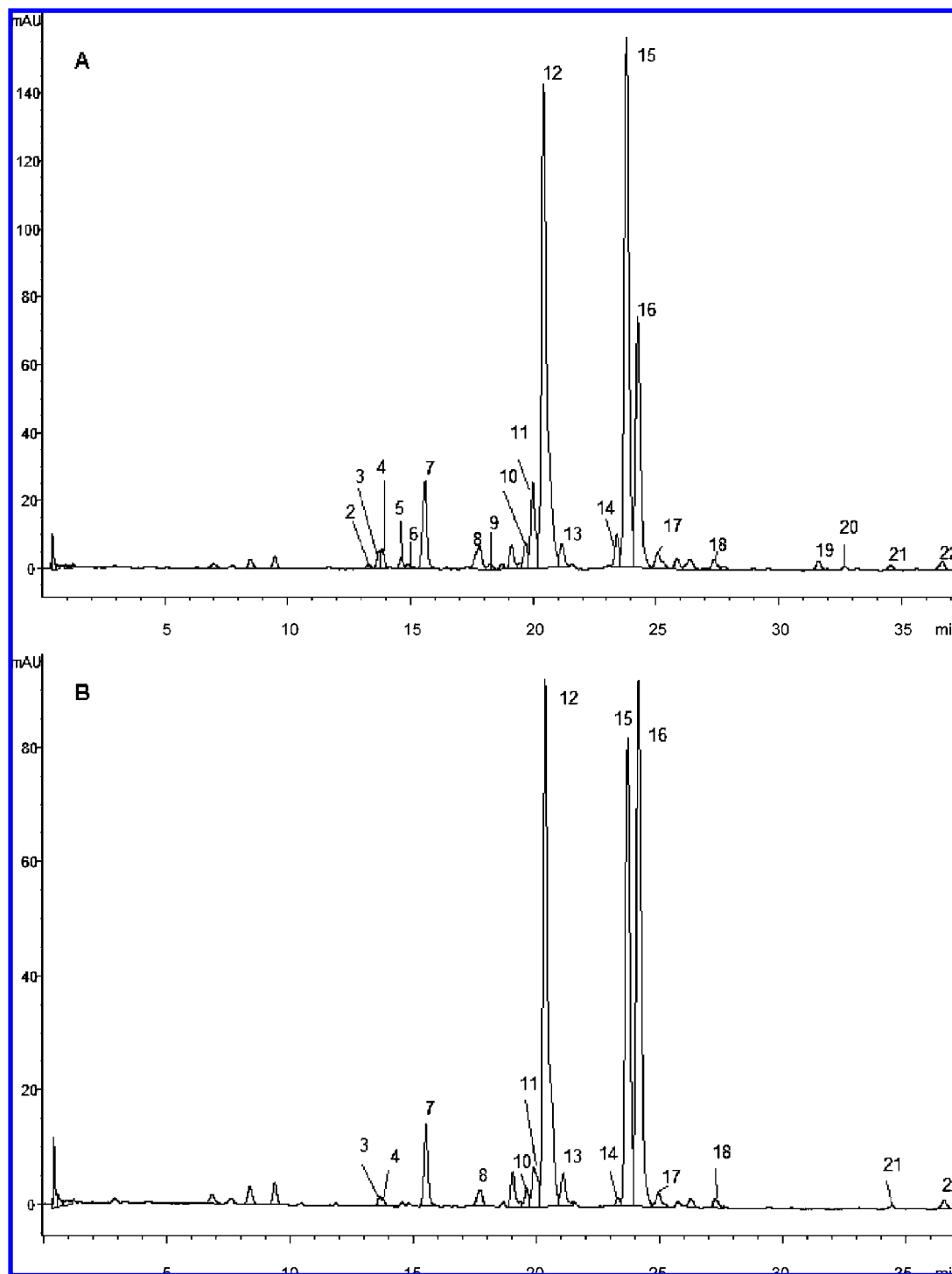
Figure 1. Comparison between HPLC chromatograms (320 nm) of sea buckthorn cultivars (A) 'Tytti' and (B) 'Terhi'.

HPLC Analyses. The phenolic compounds were analyzed by HPLC. The system used was a Hewlett-Packard (Avondale, PA) instrument with a quaternary pump (HP 1050), an autosampler (HP 1050), and a photo diode array detector (HP 1040A) controlled by HP Chem Station Software. A 3 μm HP Hypersil ODS column (60 × 4.6 mm inner diameter) was used. The gradient elution systems consisted of aqueous 1.5% tetrahydrofuran and 0.25% *o*-phosphoric acid (A) and 100% methanol (B). The samples were eluted as follows: 0−5 min, 100% A; 5−10 min, 85% A and 15% B; 10−20 min, 70% A and 30% B; 20−30 min, 65% A and 35% B; 30−50 min, 50% A and 50% B; 50−55 min, 100% B; 55−60 min, 100% A. The flow rate was 2 mL/min. The injection volume was 20 μL. The injector and column temperature were 22 and 30 °C, respectively. The phenolic compounds were identified using their retention times and the UV spectra and HPLC−MS. For HPLC−MS, eluent A contained 0.25% acetic acid instead of *o*-

phosphoric acid and a 3 μm HP Hypersil ODS column (100 × 2.1 mm inner diameter) was used. Other conditions were as reported by Julkunen-Tiitto and Sorsa (*17*).

Chemicals. Elution was monitored at 320 nm. Analyzed secondary metabolites were quantified against commercial standards: myricetin 3-rhamnoside (Roth, Karlsruhe, Germany) for myricetin derivatives and quercetin 3-galactoside (Roth, Karlsruhe, Germany) for isorhamnetin and quercetin derivatives.

HPLC−MS. The tentative identification by HPLC−MS gave the following ions: isorhamnetin 3,7-diglucoside 663 (M + 23), 317 (isorhamnetin M + 1); isorhamnetin-rhamnodiglucoside 809 (M + 23), 625 (M + 1, isorhamnetin, rhamnose, and glucose), 317 (isorhamnetin M + 1); isorhamnetin glycoside derivatives 793 (M + 23), 317 (isorhamnetin M + 1); isorhamnetin 3-glucoside-7-rhamnoside 625 (M + 1), 647 (M + 23), 317 (isorhamnetin M + 1); isorhamnetin

Sea Buckthorn Berry Phenolics
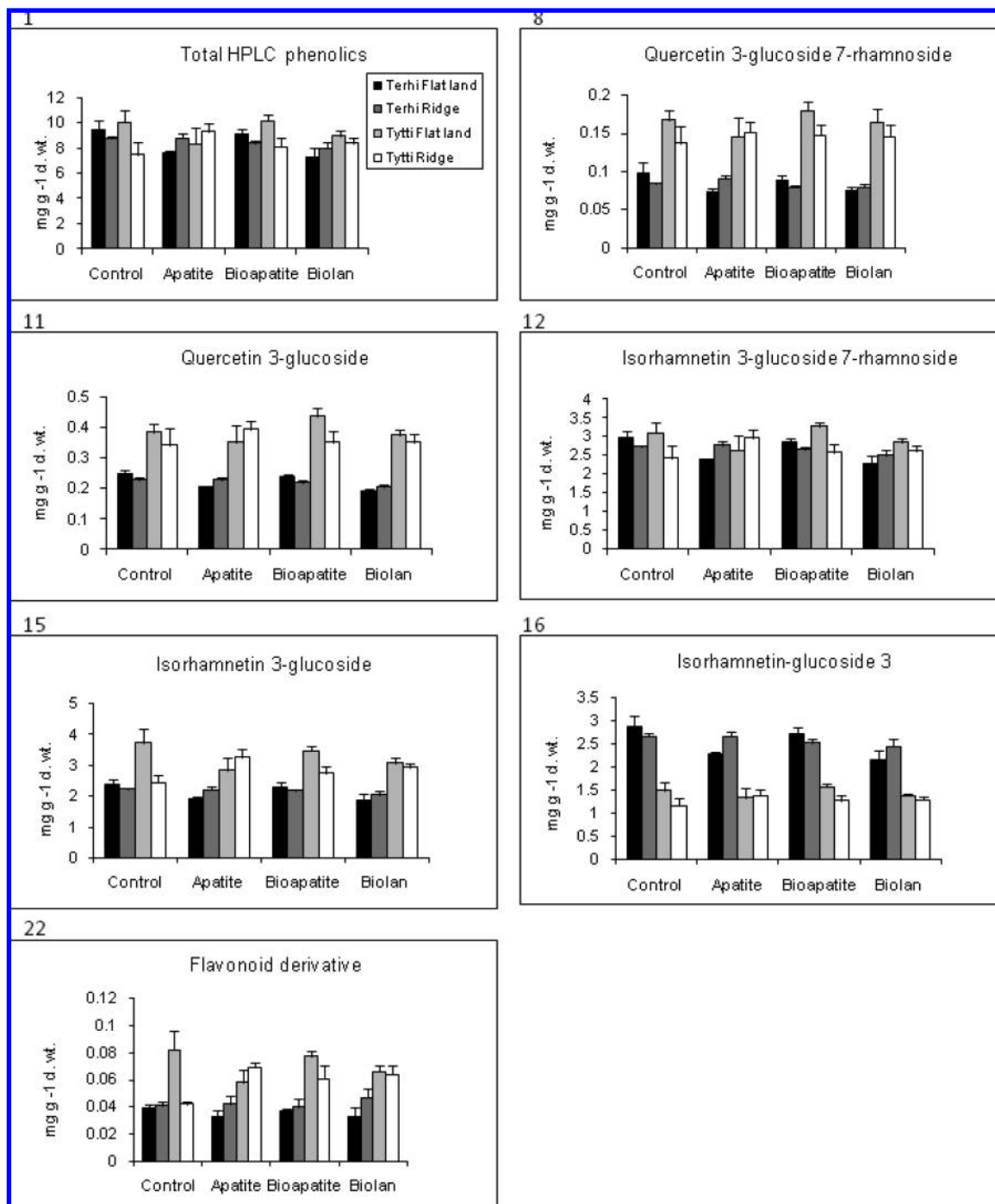
J. Agric. Food Chem., Vol. 57, No. 5, 2009   **1943**

Figure 2. Fertilization × land contour interactions in the fertilization experiment. Only compounds with significant effects are included. The bars denote means ± standard error (SE).

3-glucoside 479 (M + 1), 501 (M + 23), 317 (isorhamnetin M + 1); isorhamnetin glycoside derivative 647, 317 (isorhamnetin M + 1); quercetin 3-glucoside-7-rhamnoside 663 (M + 23), 303 (quercetin M + 1); quercetin 3-glucoside 465 (M + 1), 487 (M + 23), 303 (quercetin M + 1).

Condensed Tannins. The amount of condensed tannins was determined from both the methanol extract (HPLC sample) and the dried extracted plant residue using the butanol–HCl test according to Hagerman (*8*) and Porter et al. (*27*). Total tannin content was calculated on the basis of purified tannins extracted from sea buckthorn berries and is the sum of the extracted and residual tannins.

Statistical Tests. The experiment included (1) a split-plot design with two female cultivars (main plot factor) and combinations of two land contours and four fertilizers (split-plot factors) as well as (2) a

randomized block design for the cultivar 'Tytti' with combinations of two land contours and five mulches in each block. The data from these experiments were analyzed using appropriate models of analysis of variance (ANOVA). To meet the requirements of ANOVA, if the chemical data was not normally distributed, it was log- or square-root-transformed. The number of tests for each of the studied effects corresponded to the number of detected compounds and included ANOVA. The hypothesis concerning the effects of the experimental factors on the total concentration of phenolic compounds was considered separate from that concerning the individual compounds. The correlation tests were performed using Spearman's nonparametric two-tailed tests. The ANOVAs and correlation tests were analyzed with SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL).
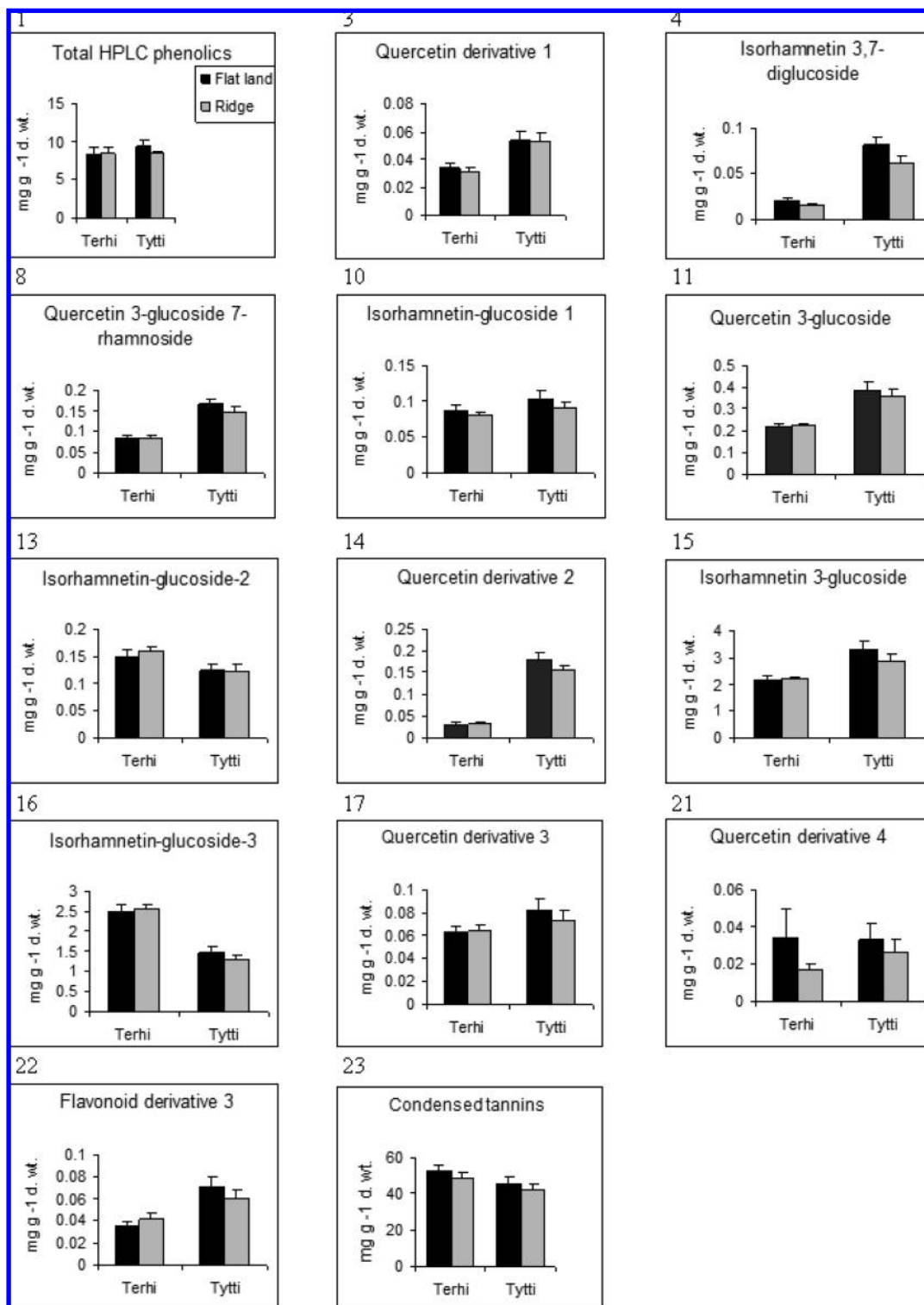
Figure 3. Effects of cultivar and land contour on the concentration of phenolic compounds in the fertilization experiment. Only compounds with significant effects are included. The bars denote means ± standard error (SE).

RESULTS AND DISCUSSION

The phenolic compounds of berries of two Finnish sea buckthorn cultivars grown under different organic cultivation conditions were analyzed. Sea buckthorn leaves have been found to contain more polyphenols (10−12%) compared to berries (0.13%) (*13*). The result in our study is comparable to this, showing 0.9% total polyphenolic content in berries and 6% in leaves, which we analyzed earlier (*10*). The compounds differ between leaves and berries: hydrolyzable tannins dominate in leaves but comprise only 0.02% phenolic compounds in

berries (*10, 30*). Flavonoids, such as isorhamnetin, kaempherol, and quercetin derivatives are found in low concentrations in sea buckthorn leaves, but they form the main flavonoid groups in berries (*10, 14, 21, 29, 35*). Two of these flavonoid groups, isorhamnetin and quercetin derivatives, comprised the main flavonoids found in our plants. We also tentatively identified three novel flavonoids: isorhamnetin-rhamnodiglucoside and two isomeric isorhamnetin glycosides. We did not detect kaempherol, which has been identified in several earlier studies of sea buckthorn berry phenolics (*13*).

Sea Buckthorn Berry Phenolics

J. Agric. Food Chem., Vol. 57, No. 5, 2009   **1945**

Table 2. Statistical Tests for HPLC Compound Groups and Condensed Tannins in Mulched 'Tytti' ($n = 4 =$ Number of Blocks)[a]

compound group	transformation	mulch		contour		mulch × contour	
		F	p	F	p	F	p
1 total HPLC phenolics	none	0.649	0.634	2.547	0.125	0.331	0.854
2 myricetin	none	0.919	0.471	1.664	0.210	0.540	0.708
3 quercetin derivative 1	sqrt	1.077	0.392	1.204	0.284	1.018	0.420
4 isorhamnetin 3,7-diglucoside	sqrt	0.369	0.828	0.255	0.691	0.839	0.515
5 isorhamnetin derivative 1	sqrt	0.491	0.743	0.013	0.912	0.303	0.873
6 flavonoid derivative 1	ln	1.327	0.291	3.809	0.064	1.743	0.177
7 isorhamnetin rhamnodiglucoside	none	0.826	0.523	1.541	0.228	1.013	0.422
8 quercetin 3-glucoside 7-rhamnoside	none	1.033	0.412	1.580	0.222	0.386	0.816
9 flavonoid derivative 2	ln	0.291	0.881	3.858	0.062	0.624	0.651
10 isorhamnetin-glucoside 1	sqrt	1.031	0.413	1.387	0.251	0.048	0.995
11 quercetin 3-glucoside	none	0.291	0.881	1.867	0.186	0.667	0.622
12 isorhamnetin 3-glucoside 7-rhamnoside	none	0.435	0.782	1.915	0.180	0.344	0.846
13 isorhamnetin-glucoside 2	none	0.777	0.552	0.806	0.379	1.262	0.315
14 quercetin derivative 2	ln	0.676	0.616	4.706	**0.041**	0.309	0.869
15 isorhamnetin 3-glucoside	none	0.644	0.637	1.917	0.180	0.138	0.966
16 isorhamnetin-glucoside 3	none	1.370	0.277	2.876	0.104	0.938	0.461
17 quercetin derivative 3	none	0.454	0.768	0.202	0.657	0.967	0.445
18 dicoumaroyl isoquercetin	none	1.900	0.146	0.304	0.587	0.082	0.987
19 rhamnetin derivative 1	sqrt	0.620	0.653	3.860	0.062	0.115	0.976
20 rhamnetin derivative 2	ln	1.215	0.333	2.951	0.100	0.543	0.706
21 quercetin derivative 4	ln	2.317	0.089	1.825	0.191	0.170	0.952
22 flavonoid derivative 3	none	1.746	0.176	3.243	0.085	0.483	0.748
23 condensed tannins	sqrt	0.698	0.601	0.914	0.349	0.489	0.744

[a] The transformations used for ANOVA are given. *F* values denote the ratio of variance because of a treatment and error variance. *p* values denote the probability of rejecting a true null hypothesis. The *p* values smaller than 0.05 are marked in bold.

In the fertilization experiment, the number of phenolic compounds in sea buckthorn berries varied between cultivars. In 'Terhi', 15 different phenolic compounds were found, and 21 compounds were identified in 'Tytti' (**Figure 1**). In addition, both cultivars had condensed tannins. Only those 16 compounds that were found in both cultivars were statistically compared in the fertilization experiment (**Table 1**). The concentration of total phenolics detected by HPLC was not affected by the tested single farming methods, such as cultivar, fertilization, or land contour. However, there was a significant fertilization × land contour interaction, which means that the effect of fertilization varied between land contours (**Figure 2**). The concentration of total berry phenolics was higher when cultivars were grown on the flat land compared to cultivars grown on a ridged bed either with no fertilizer or with bioapatite. Bioapatite contains phosphate and calcium, which are necessary for the function of symbionts in root nodules and, thus, growth and yield (*19*). Nevertheless, the results of our earlier experiments in this research field indicated that shoot length and berry production were decreased on the flat land compared to the ridged bed (*11*). Soil structure possibly decreased the nutrient use on the flat land, which according to the carbon/nutrient balance hypothesis induced the synthesis of phenolic compounds (*3*). The control treatment was growing in grass, which was assumed to decompose and fertilize the soil. Higher soil nitrogen content has been found to disturb the function of symbionts and thus will decrease nitrogen fixation (*19*). Nitrogen deficiency has been shown to increase the synthesis of several phenolic compounds (*22, 23*).

Fertilization did not have any significant effect on the concentrations of any single phenolic compound (**Table 1**). However, there were significant effects of cultivars and land contours. The concentrations of quercetin derivatives, such as 1−3, isorhamnetin 3,7-diglucoside, quercetin 3-glucoside 7-rhamnoside, quercetin 3-glucoside, isorhamnetin 3-glucoside, and flavonoid derivative 3 were higher in 'Tytti' compared to 'Terhi' (**Figure 3**). On the other hand, the concentrations of isorhamnetin-glucosides 2 and 3 were higher in 'Terhi' compared to 'Tytti'. It is well-known that the chemical composition of sea

buckthorn berries is genetically controlled (*20, 34*). Thus, genotype mainly explains the chemical differences between 'Terhi' and 'Tytti'. The previous results of our earlier experiments indicated that 'Tytti' bushes grew more slowly and produced smaller berries and fewer berries compared to 'Terhi' bushes (*11*). Retarded vegetative growth and berry production in 'Tytti' might have been in part due to the synthesis of phenolic compounds compared to that of 'Terhi'. The carbon/nutrient balance hypothesis supports this idea. According to the hypothesis, plants adapted to low-resource environments tend to show a tradeoff of assimilated carbon to carbon-based phenolics, instead of increased growth (*3*). However, berry yield ($n = 30$, $r = -0.037$, and $p = 0.845$) (in both land contours) did not show a significant correlation with phenolic production in 'Tytti'.

Growing on a flat land increased the concentrations of quercetin derivatives, such as 2, 4, isorhamnetin 3,7-diglucoside, and isorhamnetin-glucoside 1, and condensed tannins. In some cases, significant cultivar × land contour interactions indicate that the effect of land contour depended upon cultivar (**Table 1**). Cultivation on a flat land increased the concentration of quercetin derivative 2, isorhamnetin-glucoside 3, and flavonoid derivative 3 in 'Tytti' but not in 'Terhi'. In addition, there were six fertilization × land contour interactions. This indicates that the effect of fertilization on the concentrations of quercetin 3-glucoside 7-rhamnoside, quercetin 3-glucoside, isorhamnetin 3-glucoside 7-rhamnoside, isorhamnetin-3-glucoside, isorhamnetin-glucoside 3, and flavonoid derivative 3 depended upon land contour (**Figure 2**). Their concentrations were higher on the flat land compared to the ridged bed, when no fertilizer or bioapatite were used. There are some differences between flat land and ridged bed as a growth substratum, which can effect the accumulation of phenolic compounds. Sea buckthorn cannot tolerate flooding or standing water, and the 30 cm high ridged beds used in this experiment permitted up to about a 50 cm deep root system to develop in soil held above water-logged or compact zones (*24*). Sea buckthorn is a weak competitor and chokes easily with weeds (*28*). Ridged beds provide a growth

Heinäaho et al.

substratum above the weed level better compared to flat land, where plants on the ridge receive more sunlight (*33*). Ridged beds also have better drainage compared to the flat land, where evaporation speeds soil warm up time especially in the spring (*33*). The vegetative growth and berry yield were decreased on the flat land compared to the ridged bed (*11*). There was also a significant negative correlation between the berry yield and the content of phenolic compounds ($n = 30$, $r = -0.433$, and $p = 0.017$) (in both varieties). All of these differences indicate that flat land offered a low-resource environment compared to the ridged bed and thus increased the accumulation of phenolic compounds in berries as mentioned earlier with the carbon/nutrient balance by Bryant et al. (*3*). In addition, environmental stresses on the flat land, such as low temperature and limited photosynthesis, have been found to increase oxidative stress and therefore induce an accumulation of different phenolic compounds (*4*). Especially, some quercetin derivatives have been found to be highly active against radicals (e.g., ref *37*). On the other hand, apatite increased their concentrations on the ridged bed.

In the mulch experiment, a total of 22 phenolic compounds and condensed tannins were found in cultivar 'Tytti' (**Table 2**). Mulch did not have any significant effect on the concentrations of phenolic compounds. Instead of that, there was a significant land contour effect on the concentration of quercetin derivative 2. The concentration was higher when bushes were grown on the flat land compared to that on the ridged bed in every mulch.

In conclusion, there was a high variation in the number and concentrations of phenolic compounds in berries of sea buckthorn cultivars. 'Tytti' produced more phenolic compounds compared to 'Terhi'. Of the cultivation method tested, only the flat land increased accumulation of phenolic compounds. The results of this study indicate that organic farming methods cannot markedly increase the medicinal and nutritional quality of sea buckthorn berries.

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Sea Buckthorn Berry Phenolics

J. Agric. Food Chem., Vol. 57, No. 5, 2009 **1947**

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