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High-Performance Liquid Chromatography Analysis of Black Currant (*Ribes nigrum* L.) Fruit Phenolics Grown either Conventionally or Organically

MIKKO J. ANTTONEN*,† AND REIJO O. KARJALAINEN†,‡

Institute of Applied Biotechnology, University of Kuopio, Yliopistonranta 5, P.O. Box 1627, FI-70211 Kuopio, Finland, and MTT Agrifood Research Finland, FI-31600 Jokioinen, Finland

Black currants (*Ribes nigrum* L.) contain a diverse range of phenolics and possess a high antioxidant activity, which makes them an interesting target for the functional food industry. In this study, phenolic profiles of organically and conventionally grown black currant fruits, collected from commercial farms within a climatically similar area, were compared. Compounds were identified using UV/vis and mass spectroscopy techniques and quantified with high-performance liquid chromatography equipped with UV/vis detection. Several different conjugates of hydroxycinnamic acids, flavonols, and anthocyanins were quantified. Statistically significant differences between farms were found for almost all compounds. Differences between the highest and the lowest measured values of major phenolic compounds of different phenolic classes ranged from 24 to 77%. Principal component analysis quite effectively separated farms from each other but did not cluster them according to cultivation technique. Thus, it was concluded that the biochemical quality of organically grown black currant fruits does not differ from those grown conventionally.

KEYWORDS: Black currant; *Ribes nigrum* L; HPLC; mass spectrometry; principal component analysis; phenolic compounds; hydroxycinnamic acids; flavonols; anthocyanins; cultivation; organic cultivation

INTRODUCTION

Epidemiological data (*1*) suggest that a high intake of fruits and vegetables offers a number of health benefits against degenerative diseases and can promote longevity. It is widely accepted that one mechanism behind the protective effect is related to the bioactive compounds in fruits and vegetables that reduce the oxidative stress symptoms. As compared with fruits and vegetables, berries possess a high antioxidant activity (*2*). Black currant (*Ribes nigrum* L.) fruits have long been known to be a good source of vitamin C, but they also contain high levels of polyphenolics (**Figure 1**), particularly of hydroxycinnamic acids, flavonols, and anthocyanins (*3*), which also contribute to the high antioxidant activity of black currant (*4*). In a recent in vitro study, black currant phenolics were shown to protect neuroblastoma and promyelocyte cells against oxidative stress (*5*).

The area of organically grown black currants has rapidly increased in Europe during the last 10 years, which is due to demand and support provided to growers by the European Union. In addition, many consumers perceive that organic foods are healthier than the conventional ones (*6*). Interestingly, although there is no consistent scientific evidence supporting the better nutritional quality of organically produced food (*7*, *8*), in an extensive European study, it was found that the first criteria to make the purchase decision is based on the belief that it is beneficial for human health (*6*).

In Europe, organic production is defined by the European Union Council Regulation (EEC) No. 2092/91 (*9*). Fertilization and plant protection practices are especially highly regulated. In organic farming, the use of mineral fertilizers and synthetic plant protection agents is forbidden. In addition, according to the legislation, organic farms are inspected every year. In theory, differences between organic and conventional cultivation may lead to enhanced contents of phenolics in organically produced foods. Many studies have shown the importance of fertilization on the phenolic content (*10*, *11*). Thus, differences in the fertilization practices may affect the phenolic content. Phenolic compounds are also involved in plant defense against biotic stress factors (*12*) and increased disease pressure due to the lack of the use of pesticides, which could elevate the phenolic content of organically produced foods (*13*).

There is limited information available on whether fruits and vegetables coming from organically managed farms contain different amounts of phenolics than those coming from farms using conventional cultivation practices. However, it has been shown that the environment and differences in the agricultural regimen affect the contents of phenolics (*10*, *14*, *15*). In addition, it was previously found with limited material (*16*) that genotype

* To whom correspondence should be addressed. Tel: +358-17-16 3198. Fax: +358-17-16 3148. E-mail: mikko.anttonen@uku.fi.
† University of Kuopio.
‡ MTT Agrifood Research Finland.

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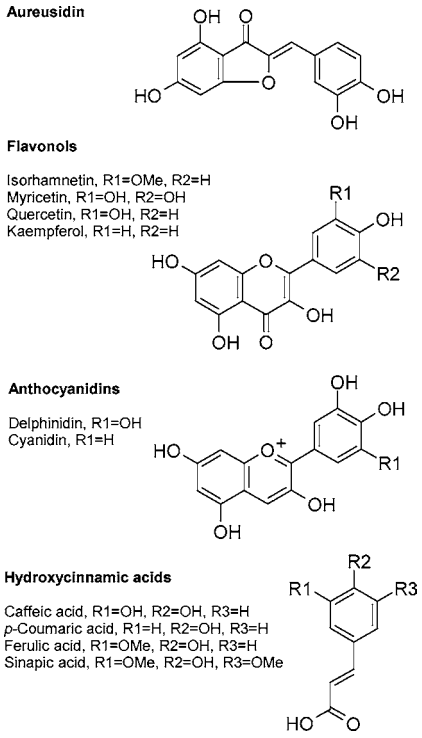

Figure 1. Aglycone structures of the black currant phenolics investigated.

is more important than the cultivation type for the determination of flavonol contents in black currants. In this study, the phenolic content of black currant fruits collected from organically and conventionally managed farms within a climatically similar area was evaluated. The aim of the study was to estimate whether the effect of cultivation technique on the phenolic profiles of black currant fruits could be distinguished from that caused by other factors.

MATERIALS AND METHODS

Plant Material. Black currant (*R. nigrum* L., Grossulariaceae, cv. Öjebyn) fruit samples were collected from commercial farms (**Table 1**) in eastern Finland within a climatically similar area in August 2004. The summer was very rainy, and thus, no additional irrigation was used on any of the farms. Bushes were grown on bare soil, whereas in the space between rows wild plants grew. Conventional cultivation techniques were used on five farms (CONV1−5), and organic cultivation techniques were used on three farms (ORG1−3). The longest distance between the farms was <100 km. The organic farms were managed according to the European Union Council Regulation (EEC) No. 2092/91 (*9*) and inspected by the responsible Finnish authority. Samples were collected from outer branches of the bush, south side of the row, and evenly matured according to surface color. One sample consisted of ∼500 g of fruits collected evenly from 10 different bushes. Five replicate samples were collected from systematically selected rows in the central area of the field. Samples were frozen immediately in dry ice and stored at −20 °C until analyzed.

Extraction. Before extraction, fruit samples were first homogenized as frozen at −20 °C using a food processor after which subsamples were taken. The subsamples were thawed in a microwave oven (650 W, <1 min) and further homogenized using a food processor. Three grams of the homogenate was weighed for the extraction of phenolic compounds. After the homogenate was thawed, the temperature of the berry homogenate was kept close to 0 °C until extraction solvent was added to limit deleterious enzyme activity and oxidation. The rest of the berry homogenate was used for separation of juice for the analysis of soluble solids and titratable acidity. The berry homogenate was warmed up to room temperature to aid juice separation, and juice was separated by centrifugation at ambient temperature (2000*g*, 30 min).

Phenolic compounds were extracted with 70% aqueous acetone containing 0.01 M hydrochloric acid. Twenty milliliters of solvent was added, and extraction was done by shaking vigorously for 20 min. Extraction was repeated twice with 20 mL of solvent for 10 min. Between extractions, solvent was separated by centrifugation at +4 °C (3000*g*, 8 min) and 15 mL of the extract was removed. Extracts were combined, and acetone was removed using a rotary evaporator. The final volume was adjusted to 25 mL with water in a volumetric flask, and the extract (the phenolic extract) was stored at −20 °C until analyzed.

Total Phenolics. The total phenolic content of the phenolic extract was measured using the Folin−Ciocalteu method (*17*) with minor modifications. Volumes of the sample, Folin−Ciocalteu phenol reagent (Merck KGaA, Darmstadt, Germany), and sodium carbonate were reduced to 1:10 as compared with the original method with a final volume of 20 mL. The modified method was found to give comparable results with the original method. Analyses were done in duplicate of each sample. Gallic acid (Sigma Chemical Co., St. Louis, MO) was used for the quantification, and results are expressed as g/kg.

The Folin−Ciocalteu method is based on the reducing (electron transfer) capacity of phenolic compounds in alkaline solution. Thus, the method also gives an estimate of the antioxidant capacity of the extract (*18*). For the determination of the antioxidant capacity of a sample, hydrogen atom transfer-based methods are considered to be

Table 1. Identification of Black Currant Cv. Öjebyn Fruit Samples Collected from Eight Farms within a Climatically Similar Area in Eastern Finland Using either Conventional (CONV) or Organic (ORG) Cultivation Practices during August 2004

	bush age in years	soil type	alignment of rows[a]	fertilization[b]	plant protection in 2004
CONV1	5	sand moraine	SW–NE	A2003, 235 kg/ha of PK S2004, 235 kg/ha of Y2	Karate[c]
CONV2	4	clay (rich organic)	SW–NE	S2004, row fertilization equaling 1200 kg/ha of Y2	Gusation,[d] Karate
CONV3	4	sand moraine (rich organic)	E–W	S2004, 130 kg/ha of Y2	Basta[e]
CONV4	5	silt clay	SW–NE	A2003, 300 kg/ha of PK S2004, 400 kg/ha of Y2	Karate, Dithane[f]
CONV5	5	sand moraine	SE–NW	S2004, 250 kg/ha of Y1	Roundup Gold,[g] Malasiini[h]
ORG1	5	sand moraine	SW–NE	2003, green fertilization and ash	none
ORG2	4	silt sand	SW–NE	biotite at establishment	none
ORG3	5	sand moraine	SW–NE	cow manure sludge, green fertilization and micronutrients at establishment.	none

[a] SW, southwest; NE, northeast; E, east; W, west; SE, southeast; and NW, northwest. [b] A, autumn; S, spring; PK, 0N:5P:20K; Y2, 6N:6Y:19K; and Y1, 9N:6P:17K. [c] Insecticide (Syngenta CropProtection A/S, Basel, Switzerland). [d] Insecticide (Makhteshim-Agan Industries Ltd., Tel-Aviv, Israel). [e] Herbicide (Bayer CropScience, Monheim, Germany). [f] Fungicide (Dow AgroScience LLC, Indianapolis, IN). [g] Herbicide (Monsanto Co., St. Louis, MO). [h] Insecticide (Kemira GrowHow Oyj, Helsinki, Finland).

Table 2. Identification of Black Currant Fruit Phenolics by HPLC Equipped with ESI-MS^n and Uv/vis Detection

		MS^n analysis [b]				
RT (min)	UV/vis[a]	mode	MS (*m*/*z*)	MS/MS (*m*/*z*)	MS^3 (*m*/*z*)	tentative identification
14.81	HCA	–	353	179 (44), 191 (100)	93 (65), 109 (30), 111 (29), 127 (100), 171 (31), 173 (76)	**1**, 3-caffeoylquinic acid
16.75	HCA	–	341	119 (11), 163 (100), 195 (94)	*p*-coumaric acid	**2**, *p*-coumaric acid derivate
18.07	HCA	–	341	161 (27), 179 (100)	caffeic acid	**3**, caffeoylglucose
19.62	HCA	–	371	163 (55), 325 (100)	163 (100)	**4**, coumaric acid glucoside (formate adduct)
20.36	HCA	–	337	163 (100), 191 (7)	*p*-coumaric acid	**5**, 3-*p*-coumaroylquinic acid
24.52	HCA	–	325	145 (99), 163 (100), 187 (54)	*p*-coumaric acid	**6**, *p*-coumaroylglucose
26.72	HCA	–	401	193 (16), 355 (100)	178 (3), 193 (100)	**7**, ferulic acid glucoside (formate adduct)
29.77	HCA	–	355	175 (32), 193 (100), 217 (57)	ferulic acid	**8**, feruoylglucose
31.57	HCA	–	431	223 (7), 385 (100)	223 (100)	**9**, sinapic acid glucose derivate (formate adduct)
58.27	HCA	±	NA	NA	NA	**10**, hyroxycinnamic acid derivate a
61.00	HCA	±	NA	NA	NA	**11**, hyroxycinnamic acid derivate b
47.65	FL	+	481	319 (100)	myricetin	**12**, myricetin glucoside
48.58	FL	+	627	319 (100), 481 (51)	myricetin	**13**, myricetin rutinoside
50.86	FL	+	567	319 (100)	myricetin	**14**, myricetin malonylglucoside
54.34	AUR	+	449	287 (100)	149 (14), 153 (100), 161 (12), 213 (11), 231 (42), 241 (57), 259 (47), 269 (74)	**15**, aureusidin glucoside
57.01	FL	+	465	303 (100)	quercetin	**16**, quercetin glucoside
58.16	FL	+	611	303 (100), 465 (38)	quercetin	**17**, quercetin rutinoside
60.86	FL	+	551	303 (100)	quercetin	**18**, quercetin malonylglucoside
62.73	FL	+	449	287 (100)	kaempferol	**19**, kaempferol glucoside
63.14	FL	+	595	287 (100), 449 (20)	kaempferol	**20**, kaempferol rutinoside
64.06	FL	+	625	317 (100), 479 (36)	165 (5), 243 (5), 271 (6), 274 (6), 285 (35), 299 (4), 302 (100)	**21**, isorhamnetin rutinoside

[a] According to standard compounds and literature data (*27*): HCA, hydroxycinnamic acid; FL, flavonol; AUR, aurone. [b] The ESI-MS^n was done using either positive (+) or negative (–) ionization mode. The table shows detected ions (*m*/*z*) with their relative intensities in parentheses. The molecular ions in the MS analysis were fragmented to produce a MS/MS spectrum of which the most intense ion was further fragmented to produce a MS^3 spectrum. Compound names in the MS^3 refer to correspondence of spectra with those of standard compounds; NA, not applicable.

biologically more relevant. However, the Folin−Ciocalteu method is widely used and considered a useful method for measuring antioxidant capacity.

Soluble Solids. Soluble solids of the juice were measured by a PR-32 Digital Refractometer (Atago, Tokyo, Japan). Four replicate measurements were done for each juice sample, and results are given as % of Brix.

Titrarable Acidity. Single measurements of titratable acidity of each juice sample were made according to AOAC official method 942.15 (*19*). Results are given as g/kg of juice in citric acid equivalents.

Anthocyanin Analysis. Anthocyanins of the phenolic extract were analyzed the day after the extraction using HP 1090 series high-performance liquid chromatography (HPLC) (Agilent Technologies, Palo Alto, CA) equipped with a diode array detector and HP Chemstation Rev. A. 10.02 controlling and data evaluation software. Compounds were separated on a 60 mm × 4.6 mm i.d., 3 μm Hypersil ODS column (Agilent Technologies), using gradient elution with 5% v/v formic acid (A) and acetonitrile (B). The column temperature was set to 40 °C. The flow rate was 1.0 mL/min, and the gradient program was as follows: 0−8 min, 5−14% B; 8−13 min, 14−95% B; 13−14 min, 95% B; and 14−19 min, 95−5% B followed by 5 min equilibrium time. The injection volume was 15 μL, and compounds were detected at a wavelength of 520 nm. Spectroscopic data were recorded between 190 and 600 nm with 2 nm steps. The extracts were thawed and filtered immediately before analysis. Quantification was based on peak areas, and cyanidin (Fluka Chemie GmbH, Buchs, Switzerland) was used as a standard. New standard dilutions of the stock solution stored at −20 °C were done and analyzed daily. Single analyses of each extract were done, and results are given as mg/kg.

Analysis of Other Phenolics. The same HPLC apparatus as in the anthocyanin analysis was used. Compounds in the phenolic extract were separated on a 250 mm × 4.6 mm i.d., 5 μm Vydac 218TP54 RP-18 column (Separations Group, Hesperia, CA) using gradient elution with 1% v/v formic acid (A) and 10% v/v acetonitrile in methanol (B). The column temperature was set to 40 °C. The flow rate was 0.9 mL min^{-1}, and the gradient program was as follows: 0−5 min, 2% B; 5−35 min, 2−15% B; 35−48 min, 15−26% B; 48−55 min, 26−30% B; 55−70 min, 30−50% B; 70−73 min, 50−100% B; 73−75 min, 100% B; and 75−80 min, 100−2% B followed by a 10 min equilibrium time using the initial gradient. The injection volume was 25 μL. Spectroscopic data were recorded between 245 and 600 nm with 2 nm steps. Phenolic acids were detected at a wavelength 320 nm and quantified according to peak areas whereas 360 nm was used for other compounds. Standards were used as follows (compound numbering according to **Table 2**): caffeic acid (Sigma Chemical Co.) for compounds **1**, **3**, and **9**−**11**; *p*-coumaric acid (Sigma Chemical Co.) for compounds **2** and **4**−**6**; ferulic acid (Sigma Chemical Co.) for compounds **7** and **8**; myricetin (Fluka Chemie GmbH) for compounds **12**−**14**; quercetin (Sigma Chemical Co.) for compounds **15**−**18** and **21**; and kaempferol (Fluka Chemie GmbH) for compounds **19** and **20**. Every day, new standard dilutions of stock solutions stored at −20 °C were made and analyzed.

Single analyses were made of each phenolic extract, and results are given as mg/kg.

Identification. Anthocyanins were identified using HPLC system equipped with UV/vis detection. For the identification of other compounds, electrospray ionization tandem masspectrometric (ESI-MS^n) detection was also used. The HPLC ESI-MS^n system consisted of a Finnigan Surveyor HPLC and Finnigan LTQ linear ion trap mass spectrometer (Thermo Electron Corporation, Waltham, MA). The HPLC conditions were as presented above. Simultaneous UV detection at 320 nm was done by splitting the solvent flow after the column. The ionization parameters in the negative mode were optimized by direct infusion of caffeic acid, chlorogenic acid (Sigma Chemical Co.), and *p*-hydroxybenzoic acid (Fluka Chemie GmbH). Similarly, in the positive ionization mode, chlorogenic acid, myricetin, and quercetin 3-*O*-rhamnosylglucose (Sigma Chemical Co.) were used. The optimized conditions in the positive and negative ionization mode were as follows: needle voltages, 4.5 and −4.5 kV; sheath gas flow, 40 and 70 units; heated capillary temperatures, 275 and 280 °C; heated capillary voltages, 44 and −10 V; tube lens voltages, 70 and −38 V; and collision energies for both modes, 35% in the MS/MS and MS^3 analyses. In the MS analysis, ions in the range of *m*/*z* 280 and 700 were measured. In MS/MS analysis, the most intense ion in the MS spectrum was chosen for collisionally induced dissociation (CID). Following MS/MS analysis, the most intense ion in the MS/MS spectrum was chosen for CID to produce the MS^3 spectrum, after which the sequence was repeated for the second most intense peak in the MS spectrum. The specific fragmentation of compounds was used for identification. The identity of aglycones was confirmed by comparing the fragmentation pattern to that of standard compounds when available.

Validation. The extraction procedure was developed according to maximum yield of phenolics measured by the Folin−Ciocalteu method. Methanol and acetone were tested in different combinations with water (50, 70, and 90%). Seventy percent acetone was most efficient in extracting phenolics. Hydrochloric acid was added to inhibit anthocyanin degradation (*20*). The stability of different compounds in the phenolic extract at −20 °C was tested by HPLC. No quantitative changes were detected during 9 days of storage. The repeatability of the extraction procedure (measured using the Folin−Ciocalteau method and pH differential method for anthocyanins) was 1.5 and 1.2% (relative standard deviation, RSD; $n = 5$). In the HPLC analysis of anthocyanins, the system suitability was 0.6−0.7% RSD ($n = 5$), repeatability was 1.7−2.6% RSD ($n = 5$), and linearity was consistently >0.999 (R^2). Specifity was very good in the anthocyanin analysis due to their unique absorption properties at higher wavelengths as compared with other phenolics. In the HPLC analysis of other phenolic compounds, the system suitability was 0.2−3.3% RSD ($n = 3$), repeatability was 0.7−5.7% RSD ($n = 5$), and linearity was consistently >0.999 (R^2). Specifity of the method was confirmed by comparing the spectra of compounds at three different points of the peak (up slope, apex, and down slope). Repeatability in the analysis of soluble solids was 0.8% RSD ($n = 3$) and 0.5% RSD ($n = 3$) in the analysis of titratable acidity.

Statistics. Statistical analyses were performed using the SPSS for Windows version 11.5.1 (SPSS Inc., Chicago, IL). The effect of growing location was evaluated using one-way analysis of variance (ANOVA). Multiple comparison was done using either Tukey's HSD or Dunnett's T3 test. Differences at $P < 0.05$ were considered to be significant. The phenolic content data were analyzed using factor analysis with principal component extraction and varimax rotation. Regression method was used to calculate factor scores. Factor scores were used to test the clustering of farms by one-way ANOVA in combination with either Tukey's HSD or Dunnett's T3 test.

RESULTS AND DISCUSSION

In this study, the phenolic profiles of organically and conventionally grown black currant fruits were compared. Samples were collected from professional farmers in eastern Finland within a climatically similar area. Phenolic compounds were identified using UV/vis and tandem mass spectrometry. HPLC equipped with UV/vis detection was used for quantification. Principal component analysis (PCA) was used to evaluate the similarity of the phenolic profiles between different farms and cultivation techniques.

Identification. *Anthocyanins.* Four major anthocyanins (delphinidin 3-*O*-glucoside, delphinidin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside, and 3-*O*-cyanidin rutinoside) contributing ∼97% of the total content of anthocyanins were identified in this study according to their retention order, UV spectra, and relative abundances (*21*, *22*).

Other phenolic compounds were identified using HPLC equipped with ESI-MS^n and UV/vis detection (**Table 2**). In the HPLC-ESI-MS^n analysis, negative ionization was found most suitable for phenolic acids and positive ionization was found most suitable for flavonoids.

Phenolic Acids. Hydroxycinnamic acids are the main group of phenolic acids in black currant fruits (*23*). The most abundant acids are caffeic acid, *m*-coumaric acid, *p*-coumaric acid, ferulic acid, and sinapic acid. Compounds are usually found as quinic acid or glucose derivates (*24*). In this study, the major compounds identified were derivates of caffeic acid, coumaric acid, and ferulic acid.

Using the hierarchical scheme for LC-MS^n identification of chlorogenic acids (*25*), compounds **1** and **5** with molecular ions $[M - H]^-$ at *m*/*z* 353 and 337 were identified as 3-caffeoylquinic acid (neo-chlorogenic acid) and as 3-*p*-coumaroylquinic acid.

The MS/MS analysis of compound **2** with $[M - H]^-$ at *m*/*z* 341 produced fragment ions at *m*/*z* 119, 163, and 195. The most intense ion at *m*/*z* 163 was indicative of coumaric acid, and the fragmentation of this ion in the MS^3 analysis corresponded to that of *p*-coumaric acid standard. However, other structural units could not be identified. Thus, the compound is suggested to be a *p*-coumaric acid derivate.

Fragmentation of compound **3** with $[M - H]^-$ at *m*/*z* 341 produced in the MS/MS analysis an ion at *m*/*z* 179 as base peak indicating loss of a glucose unit (162 amu). The ion at *m*/*z* 179 corresponds to caffeic acid. In addition, the fragmentation in the MS^3 analysis was comparable with that of the caffeic acid standard. In the UV/vis spectrum, the absorption maxima of compound **3** was at a higher wavelength than that of the caffeic acid standard (bathochromic shift), which is characteristic for an ester conjugate (*21*). Thus, the compound is suggested to be caffeoylglucose.

The MS/MS spectrum of the compound **4** with a molecular ion at *m*/*z* 371 showed ions at *m*/*z* 163 and 325. The MS^3 analysis of the most intense ion at *m*/*z* 325 produced an ion at *m*/*z* 163 corresponding to coumaric acid and a loss of glucose unit (162 amu). As compared with *p*-coumaric acid standard, the UV/vis absorption maxima are shifted to a shorter wavelength (hypsochromic shift), suggesting a *O*-glucoside derivate of *p*-coumaric acid (*21*), which has been identified in black currants. However, as compared with the expected *m*/*z* value for the molecular ion, a 46 amu higher value was detected. Phenolics have been shown to form formate adducts ($[M + 45]^-$) especially in the negative ion mode (*26*). In addition, the compound runs earlier than the corresponding glucose ester, as shown previously (*21*). Thus, the compound is suggested to be a formate adduct of *p*-coumaric acid glucoside.

The most intense ion in the MS/MS analysis of compound **6** with $[M - H]^-$ at *m*/*z* 325 was at *m*/*z* 163 indicating a loss of glucose unit (162 amu). The ion at *m*/*z* 163 corresponds to coumaric acid. In addition, the fragmentation in the MS^3 analysis was comparable with that of the *p*-coumaric acid standard. In the UV/vis spectrum, a bathocromic shift is observed when

Table 3. Contents of Soluble Solids (Brix, %), Titratable Acidity (g/kg), and Total Phenolics (g/kg) in Conventionally (CONV) and Organically (ORG) Grown Black Currant Fruits[a]

	CONV1	CONV2	CONV3	CONV4	CONV5	ORG1	ORG2	ORG3
Brix	14.1 ± 0.2 bc6	14.5 ± 0.4 ab2	13.5 ± 0.2 c8	14.9 ± 0.1 a1	14.2 ± 0.4 a–c4	14.4 ± 0.3 ab3	14.2 ± 0.6 bc5	14.0 ± 0.4 bc7
titratable acidity	31.8 ± 0.3 bc4	32.0 ± 0.4 bc3	30.7 ± 0.6 cd8	33.1 ± 0.4 a1	30.9 ± 0.2 d7	31.9 ± 0.8 ab2	31.4 ± 1.1 a–d5	30.9 ± 1.1 a–d6
total phenolics	7.65 ± 0.25 ab5	7.09 ± 0.28 cd7	6.67 ± 0.30 d8	7.91 ± 0.20 a3	7.97 ± 0.14 a2	8.00 ± 0.14 a1	7.27 ± 0.25 bc6	7.91 ± 0.11 a4

[a] Results are given as averages ± SD. Significantly different results ($P < 0.05$) in rows are marked with different letters, and the number following indicates ranking.

compared with *p*-coumaric acid standard. Thus, the compound is suggested to be *p*-coumaroylglucose.

The MS/MS spectrum of the compound **7** with a molecular ion at *m*/*z* 401 showed ions at *m*/*z* 193 and 355. The MS^3 analysis of the most intense ion at *m*/*z* 355 produced ions at *m*/*z* 178 and 193. The most intense ion at *m*/*z* 193 corresponds to ferulic acid and indicates a loss of glucose unit (162 amu). In addition, the ion at *m*/*z* 178 indicates a loss of methyl group, which is characteristic for ferulic acid. As with compound **4**, the loss of 46 amu in the MS/MS analysis suggests a formate adduct. As compared with the ferulic acid standard, a hypsochromic shift was detected indicating *O*-glucoside of ferulic acid. Thus, the compound is suggested to be a formate adduct of ferulic acid glucoside, which is further supported by the elution before corresponding glucose ester.

In the MS/MS spectrum of the compound **8** with $[M - H]^-$ at *m*/*z* 355, the ion at *m*/*z* 193 was the most intense ion indicating a loss of glucose unit (162 amu). This ion corresponds to ferulic acid and the fragmentation in the MS^3 analysis was comparable with that of the ferulic acid standard. In the UV/vis spectrum, a bathochromic shift was observed when compared with ferulic acid standard. Thus, the compound is suggested to be feruoylglucose.

The MS/MS analysis of compound **9** with a molecular ion at *m*/*z* 431 produced a major fragment ion at *m*/*z* 388, which further produced in the MS^3 analysis an ion at *m*/*z* 223. The fragmentation pattern indicated losses of 46 amu (formate adduct; see compound **4**) unit and glucose unit (162 amu). The ion at *m*/*z* 223 corresponds to sinapic acid. Identification as sinapic acid is also supported by the late elution of the compound. Thus, the compound is suggested to be a formate adduct of sinapic acid glucose derivate.

Compounds **10** and **11** have a very late elution time as compared with other phenolic acids. However, the UV/vis spectra of these compounds show that they are hydroxycinnamic acid derivates. The structures of these compounds could not be elucidated, because adequate MS data were not obtained.

Flavonols. Eight different conjugates of flavonols myricetin, quercetin, and kaempferol were found. Previous studies have identified in black currant fruits glucose, rutinoside (rhamnosylglucose), and malonylglucose conjugates of these flavonols (*21*, *24*). In this study, the fragmentation of compounds in the MS^n analysis revealed three types of conjugates. For compounds **12**, **16**, and **19** with $[M + H]^+$ at *m*/*z* 481, 465, and 449, MS/MS analysis revealed losses of a 162 amu unit, which is indicative of a glucoside conjugate. The major ions in the MS/MS corresponded to the flavonols myricetin, quercetin, and kaempferol. Identification was confirmed by comparing their fragmentation patterns in the MS^3 analysis to those of standard compounds.

The MS/MS spectrum of compounds **13**, **17**, and **20** with $[M + H]^+$ at *m*/*z* 627, 611, and 595 consisted of two fragment ions. The other ions matched the *m*/*z* values of flavonol aglycones myricetin, quercetin, and kaempferol and the others indicated losses of a 146 amu units of the parent ion. The difference between the aglycones and the other ions was 162 amu. This fragmentation pattern is indicative of a rutinoside (rhamnosylglucose) conjugate. Aglycones were identified by comparing their fragmentation patterns in the MS^3 analysis to those of standard compounds.

For compounds **14** and **18** with $[M + H]^+$ at *m*/*z* 567 and 551, losses of 248 amu were detected in the MS/MS analysis, which is indicative for malonylglucose unit. Aglycones were idenfied as myricetin and quercetin by comparing their fragmentation patterns in the MS^3 analysis to those of standard compounds.

Aureusidin. The MS/MS analysis of compound **15** showed an ion at *m*/*z* 287 indicating a loss of 162 amu from the molecular ion $[M + H]^+$ at *m*/*z* 449. The fragment ion *m*/*z* 287 suggested that the compound could be a kaempferol derivate. However, the MS^3 analysis of this ion did not confirm this. In addition, the UV/vis spectrum indicated that the compound could be an aurone derivate (band I maxima at 403 nm) (*27*). Määttä et al. (*21*) found a compound in black currant fruit extract with similar spectroscopic properties. This compound was suggested to be a tetrahydroxyaurone glucoside. In another study, the aurone aureusidin (MW 286) was identified in black currant seed residue by NMR spectroscopy (*28*). Thus, compound **15** is suggested to be aureusidin glucoside.

Isorhamnetin. The UV/vis spectrum of compound **21** was typical for a flavonol compound. The MS/MS analysis of the molecular ion $[M + H]^+$ at *m*/*z* 625 produced fragments at *m*/*z* 317 and 479 suggesting a rutinoside derivate of the compound at *m*/*z* 317. However, the fragment ion at *m*/*z* 317 does match the molecular weight of any previously known flavonols in black currants. The ion at *m*/*z* 317 corresponds to the molecular weight of isorhamnetin. Isorhamnetin has previously not been found in black currants; however, it was previously identified in gooseberries (*3*), which belong to the same genus as black currants. In a previous study, ions at *m*/*z* 302 and 285 were found to be the main fragments of isorhamnetin after CID (*29*). Similar fragmentation was also found in our study. In addition, the UV/vis spectrum of the compound corresponded well to that of quercetin 3-*O*-rhamnosylglucose standard, which has a similar aglycone hydroxylation pattern as isorhamnetin. Thus, the compound is suggested to be isorhamnetin rutinoside, which is also supported by the late elution of the compound.

Quantification. Sugar and acid contents of fruits contribute to their flavor. In addition, the ratio of sugars and acids indicates ripeness of fruits. In this study, Brix values were used as an indicator of sugar content and acid content was measured as titratable acidity (**Table 3**). Statistically significant differences were found between farms. The differences between the highest and the lowest measured values were 10 and 8% for Brix and titratable acidity, respectively. However, the difference in the ratio of sugars and acids was statistically nonsignificant, indicating even maturity of fruit samples (data not shown).

The total phenolic content of fruit extracts was measured using the Folin−Ciocalteu method (**Table 3**). Statistically significant differences were found between farms. However, the

Table 4. Content (mg/kg) of Hydroxycinnamic Acid Derivates in Conventionally (CONV) and Organically (ORG) Grown Black Currant Fruits[a]

	CONV1	CONV2	CONV3	CONV4	CONV5	ORG1	ORG2	ORG3
3-caffeoylquinic acid	3.37 ± 0.21 ab5	3.33 ± 0.22 ab6	2.73 ± 0.32 b8	3.50 ± 0.53 ab4	3.84 ± 0.09 a2	4.51 ± 0.97 ab1	2.89 ± 0.14 b7	3.54 ± 0.17 a3
p-coumaric acid derivate	2.31 ± 0.20 cd5	2.06 ± 0.16 d8	2.67 ± 0.25 bc4	2.22 ± 0.21 cd6	3.13 ± 0.17 ab2	2.94 ± 0.40 ab3	2.08 ± 0.15 d7	3.36 ± 0.36 a1
caffeoylglucose	37.55 ± 1.89 cd5	43.88 ± 1.30 a1	35.65 ± 0.71 d6	35.42 ± 1.79 d8	35.44 ± 1.56 d7	41.29 ± 1.57 ab2	38.60 ± 1.60 b–d4	39.37 ± 2.41 bc3
coumaric acid glucoside	5.14 ± 0.21 bc5	4.63 ± 0.21 cd7	4.77 ± 0.25 cd6	5.15 ± 0.37 a–d4	5.98 ± 0.13 a3	6.01 ± 0.62 a-d2	4.46 ± 0.23 d8	6.09 ± 0.45 ab1
3-*p*-coumaroyl-quinic acid	3.66 ± 0.16 b4	3.42 ± 0.21 b6	3.83 ± 0.24 ab2	3.54 ± 0.45 b5	4.35 ± 0.12 a1	3.67 ± 0.50 b3	3.28 ± 0.23 b8	3.36 ± 0.20 b7
p-coumaroylglucose	19.98 ± 1.16 cd5	20.44 ± 1.43 b-d4	17.93 ± 1.91 d7	21.59 ± 1.06 bc3	19.39 ± 0.74 cd6	22.86 ± 0.59 ab2	13.95 ± 0.89 e8	24.37 ± 1.38 a1
ferulic acid glucoside	4.09 ± 0.25 bc5	4.10 ± 0.39 bc4	3.80 ± 0.24 cd7	4.41 ± 0.31 bc3	4.58 ± 0.37 ab2	5.12 ± 0.53 a1	3.28 ± 0.34 d8	4.08 ± 0.20 bc6
feruoylglucose	4.72 ± 0.31 bc4	4.79 ± 0.20 bc3	3.73 ± 0.49 d8	5.65 ± 0.22 a1	4.47 ± 0.23 c5	5.22 ± 0.28 ab2	4.23 ± 0.44 cd7	4.40 ± 0.34 cd6
sinapic acid glucose derivate	6.19 ± 0.40 a7	6.56 ± 0.39 a5	6.24 ± 0.68 a6	7.27 ± 0.27 a1	6.71 ± 0.74 a2	6.65 ± 0.82 a3	6.10 ± 0.43 a8	6.59 ± 0.54 a4
HCA derivate a	16.10 ± 0.56 bc4	13.88 ± 0.61 d6	13.59 ± 0.86 d7	13.36 ± 0.85 d8	17.44 ± 1.25 ab2	16.49 ± 1.13 b3	14.52 ± 0.30 cd5	19.11 ± 1.38 a1
HCA derivate b	3.79 ± 0.10 a3	3.63 ± 0.23 ab4	3.27 ± 0.57 ab8	3.54 ± 0.35 ab6	3.57 ± 0.20 ab5	3.99 ± 0.16 a2	3.30 ± 0.11 b7	4.01 ± 0.31 ab1

[a] Results are given as averages ± SD. Significantly different results ($P < 0.05$) in rows are marked with different letters, and the number following indicates ranking.

Table 5. Content (mg/kg) of Flavonoids in Conventionally (CONV) and Organically (ORG) Grown Black Currant Fruits[a]

	CONV1	CONV2	CONV3	CONV4	CONV5	ORG1	ORG2	ORG3
myricetin glucoside	22.04 ± 0.58 b5	22.55 ± 1.15 ab2	21.70 ± 1.72 b6	20.67 ± 0.74 bc7	22.17 ± 0.99 ab4	18.46 ± 1.54 c8	24.59 ± 0.96 a1	22.44 ± 1.48 ab3
myricetin rutinoside	24.68 ± 1.52 bc3	23.56 ± 1.18 cd4	21.60 ± 1.29 de6	26.41 ± 1.47 ab2	22.64 ± 0.76 c–e5	20.62 ± 1.12 e7	27.66 ± 0.75 a1	20.20 ± 1.57 e8
myricetin malonylglucoside	7.26 ± 0.31 a–c4	7.21 ± 0.36 a–c5	6.89 ± 0.36 bc6	6.40 ± 0.42 c8	8.11 ± 0.47 a2	7.34 ± 1.38 a–c3	6.84 ± 0.33 bc7	8.23 ± 0.65 ab1
aureusidin glucoside	4.13 ± 0.42 a–c3	4.47 ± 0.27 ab2	3.89 ± 0.23 b–d5	3.46 ± 0.40 d8	3.71 ± 0.15 cd7	3.86 ± 0.14 b–d6	4.75 ± 0.37 a1	3.95 ± 0.27 b-d4
quercetin glucoside	17.48 ± 0.90 bc6	19.62 ± 0.87 a3	19.82 ± 0.46 a2	15.09 ± 0.87 d8	19.51 ± 0.84 a4	19.13 ± 1.37 ab5	16.38 ± 0.47 cd7	20.69 ± 1.38 a1
quercetin rutinoside	19.72 ± 1.38 ab5	19.21 ± 1.07 ab6	18.01 ± 0.95 b8	19.81 ± 2.10 ab4	21.65 ± 0.93 a2	23.47 ± 3.57 ab1	18.36 ± 0.46 ab7	19.91 ± 1.55 ab3
quercetin malonylglucoside	7.42 ± 1.07 ab3	6.04 ± 0.58 bc6	5.82 ± 0.50 bc7	7.29 ± 1.09 ab5	7.74 ± 0.59 a2	8.91 ± 1.21 a1	5.42 ± 0.42 c8	7.37 ± 0.74 ab4
kaempferol glucoside	4.65 ± 0.30 a5	3.77 ± 0.29 b6	4.67 ± 0.40 a4	3.60 ± 0.28 b7	5.13 ± 0.38 a1	4.77 ± 0.21 a3	3.14 ± 0.24 b8	5.00 ± 0.33 a2
kaempferol rutinoside	2.50 ± 0.24 a-c4	2.15 ± 0.15 c6	2.07 ± 0.24 c7	2.42 ± 0.41 bc5	2.88 ± 0.27 ab2	3.07 ± 0.49 a1	2.07 ± 0.10 c8	2.54 ± 0.20 a-c3
isorhamnetin rutinoside	2.31 ± 0.37 bc4	1.73 ± 0.25 bc7	1.65 ± 0.20 c8	2.72 ± 0.62 ab2	2.66 ± 0.30 b3	3.69 ± 1.13 a1	2.02 ± 0.21bc6	2.16 ± 0.28 bc5

[a] Results are given as averages ± SD. Significantly different results ($P < 0.05$) in rows are marked with different letters, and the number following indicates ranking.

cultivation technique did not seem to affect the ranking of the farms. The difference between the highest and the lowest measured phenolic content was 20%.

Several different phenolic acid derivates were quantified in fruit extracts (**Table 4**). Statistically significant differences were found between farms for all other compounds except for the sinapic acid glucose derivate. The differences between the highest and the lowest measured values ranged from 19 to 75%. Three major compounds (caffeoylglucose, *p*-coumaroylglucose, and hydroxycinnamic acid derivate a) contributed ~70% of the total content measured phenolic acid derivates. In many cases, high contents of compounds were found in fruits from organic farms 1 and 3, whereas the contents were low in fruits from organic farm 2. However, the content of the major compound (caffeoylglucose) was high in all organically grown fruits, although the highest content of this compound was measured in fruits from conventional farm 2. Thus, the cultivation technique did not seem to systematically affect the ranking of the farms.

The major flavonols found were derivates of myricetin and quercetin (**Table 5**). In addition, small amounts of kaempferol derivates and tentatively identified isorhamnetin rutinoside and aureusidin glucoside were quantified. Statistically significant differences were found between farms in all cases, whereas the sum of compounds was similar between farms. The major compounds were glucosides and rutinosides of myricetin and quercetin, which contributed ~75% of total content of these compounds. The differences between highest and lowest measured values of the major compounds ranged from 30 to 37%. The differences for other compounds with lower contents ranged from 29 to 124%. The cultivation technique did not seem affect to the ranking of the farms.

Anthocyanins were clearly the major group of phenolics in black currant fruits (**Table 6**). Anthocyanins contributed ~80% of total amount quantified compounds. Statistically significant differences were found between farms for all other compounds than for cyanidin 3-*O*-rutinoside. The differences between highest and lowest measured values ranged from 12 to 77%. As with other compounds, the cultivation technique did not seem to affect the ranking of the farms. Because of high amounts of anthocyanins, the effect of variation in their contents is more significant as compared with other compounds. The difference in the total amount of anthocyanins between the first and the last farm was >400 mg/kg, which is two times higher than the total amount of all other compounds.

PCA. The phenolic content data were analyzed with PCA to test clustering of farms. The first five PCs had eigenvalues >1 and explained 86.3% of the total variation. The clustering of farms according to factor scores of different PCs was tested using ANOVA (**Table 7**). The farms were quite efficiently

Table 6. Content (mg/kg) of Anthocyanins in Conventionally (CONV) and Organically (ORG) Grown Black Currant Fruits[a]

	delfinidin 3-*O*-glucose	delfinidin 3-*O*-rutinoside	cyanidin 3-*O*-glucose	cyanidin 3-*O*-rutinoside
CONV1	383.86 ± 13.66 a2	1057.53 ± 54.95 b2	185.95 ± 10.26 b6	925.24 ± 37.01 a4
CONV2	330.51 ± 23.97 bc7	840.18 ± 46.88 c6	200.00 ± 18.38 b4	877.74 ± 46.06 a7
CONV3	360.12 ± 29.09 ab6	862.94 ± 52.26 c4	236.28 ± 31.75 ab2	928.47 ± 69.51 a3
CONV4	370.86 ± 6.20 ab3	1174.39 ± 34.11 a1	151.31 ± 6.18 c8	947.24 ± 20.47 a1
CONV5	363.41 ± 22.13 ab5	857.80 ± 45.32 c5	218.44 ± 15.56 ab3	905.95 ± 54.97 a6
ORG1	303.86 ± 25.86 c8	808.69 ± 50.41 c8	192.87 ± 20.00 bc5	917.14 ± 67.61 a5
ORG2	370.59 ± 16.39 ab4	1043.19 ± 30.29 b3	156.12 ± 8.83 c7	844.69 ± 23.56 a8
ORG3	391.32 ± 23.15 a1	821.76 ± 60.76 c7	267.77 ± 21.19 a1	940.79 ± 46.26 a2

[a] Results are given as averages ± SD. Significantly different results ($P < 0.05$) in columns are marked with different letters, and the number following indicates ranking.

Table 7. Clustering of Farms According to Factor Scores (Regression) of Different Principal Components[a]

	PC1	PC2	PC3	PC4	PC5
CONV1	ab	c	a–d	a	bc
CONV2	b	c	a–c	ab	a
CONV3	b	ab	cd	ab	c
CONV4	ab	e	a	a	c
CONV5	a	b	b–d	ab	c
ORG1	ab	bc	ab	b	b
ORG2	b	d	d	a	ab
ORG3	ab	a	a	a	b

[a] Farms marked with the same letter in columns belong to the same cluster (ANOVA; $P < 0.05$).

separated especially by PC2. The PC2 explained 24.0% of the total variation and was most influenced (factor loading values >0.7 or <-0.7) by delphinidin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside, *p*-coumaric acid derivate, myricetin rutinoside, quercetin glucoside, and kaempferol glucoside. None of the PCs seem to separate cultivation techniques in different clusters. However, PC5 did not separate organic farms from each other, whereas they were to some extent separated from conventional farms, which on the other hand were not clustered together. PC5 explained only 9.7% of the total variation and was most influenced by caffeoylglucose and aureusidin glucoside.

Previous Studies. In other studies comparing differences between organic farming and other cultivation types, variable results has been obtained. Many comparisons have been made as controlled cultivation tests. This type of study eliminates the variation caused by environmental differences and gives a good estimate of the effects of cultivation techniques in similar conditions (*30*). In yellow plums, a higher total phenolic content was found in conventionally grown fruits than in the organically grown ones (*31*). However, it was also found that individual phenolic acids and flavonols were not systematically higher in either type of fruits. In a similar study, organically grown peaches and pears had slightly higher total phenolic contents as compared with the conventionally grown ones (*32*). In vegetables, no differences were found in the contents of total phenolics, individual flavonoids, or phenolic acids between organically and conventionally grown leaf lettuce (cultivars Kalura and Red Sails) or collards (*13*). However, a higher total phenolic content was measured in organically grown pac choi, which was associated with higher pest damages. Thus, it seems that different species are differently affected by the cultivation technique. This is supported by a study done on tomatoes (*33*). Slightly higher amounts of phenolic compounds and antioxidants were measured in organically grown fruits as compared with conventionally grown ones. In addition, a statistically significant interaction was found between cultivation technique and cultivar.

Increased disease pressure due to the lack of the use of pesticides is often suggested as a basis for higher polyphenol content in organically grown plants (*14*, *32*). The idea is based on the fact that phenylpropanoids act as defense compounds in plants (*12*). However, this theory cannot be applied to all cases. First, the lack of the use of pesticides does not directly mean higher disease pressure (*34*). Second, in some species, phenolics are constitutively expressed in high amounts and no changes in the contents are observed during pathogen attack (*35*). However, it is possible that due to pathogen pressure the contents of defense compounds are higher in organically produced plants. The use of organic soil amendments in organic cultivation may enhance plants defense responses (*36*). It has been shown that compost as soil amendment can result in the activation of systemically acquired resistance or induced systemic resistance. Systemic resistance primes plants to react more efficiently to stress (*37*), and because of this priming effect, phenolics can be produced more rapidly and in higher amounts (*38*).

Fertilization is another factor that may explain the differences in the contents of phenolic compounds between organically and conventionally grown plants. In organic farming, nutrients are supplied through crop rotation, compost, manure, and plant-derived byproducts. Organic nitrogen is transformed in the inorganic form by soil microflora. Thus, the nutrient availability to plants may be difficult to control. Many studies have shown the importance of fertilization on the contents of phenolics (*10*, *11*). The effect of nitrogen is especially well-studied. In a recent study, the nitrate availability was shown to directly affect the enzyme activity in the phenylpropanoid pathway (*39*). It was concluded that lower nitrogen availability leads to increased synthesis of phenylpropanoids. However, biotic and abiotic stress factors may change the metabolism of phenolic compounds in different situations. For example, it was shown that the decrease in the phenolic content in bilberry leaves induced by nitrogen fertilization was reversed by a fungal infection (*40*).

In the PCA, farms were most efficiently separated by PC2, which was most influenced by delphinidin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside, *p*-coumaric acid derivate, myricetin rutinoside, quercetin glucoside, and kaempferol glucoside. During the ripening of black currant fruits, the content of flavonols and anthocyanins changed significantly (*41*, *42*). Thus, small differences in the maturity of the fruits might explain a part of the variation between farms, although no differences were detected in the ratio of sugars and acids. In addition to maturation, phenolics are affected by many environmental factors, and variation in the phenolic content between growth locations has been shown in several studies (*14*, *15*). Variation in the availability nutrient and the relative amounts of different nutrients is probably one of the major factors affecting the

phenolic content (*10*, *11*). The phenolic content is also affected by light, temperature, and irrigation (*43*).

In our study, samples were collected from commercial farms within a climatically similar area. As compared with the above-mentioned cultivation tests, this type of study might not be optimal for the comparison of cultivation techniques because of the many differences in the environmental conditions and agricultural practices (*30*); however, it gives a good estimate of the practical significance of cultivation technique in the determination of phenolic contents of fruits and vegetables. Our data show that cultivation technique is not the major factor in the determination of the phenolic contents of black currant fruits. This observation is also supported by other studies (*34*, *44*). In a controlled cultivation test on oats (organic vs conventional cultivation), it was found that the hydroxycinnamic acid content of grains was influenced by cultivar and growing season but not by cultivation technique (*34*). In a similar study on potatoes, it was concluded that the contents of quality components including chlorogenic acid are equally or more affected by growth season, cultivar, and growth location than cultivation technique (*44*).

In conclusion, several different derivates of hydroxycinnamic acids, flavonols, and anthocynains were identified in black currant fruits. Most of the compounds have also been identified in previous studies (*21*, *22*, *24*, *28*). However, there are no previous reports of the tentatively identified isorhamnetin rutinoside in black currant fruits. The cultivation site was a major factor affecting the phenolic content in black currant fruits. The differences between the highest and the lowest measured values of major phenolic compounds of different phenolic classes ranged from 24 to 77%. Organically produced food is generally considered healthier than corresponding conventionally produced food. However, there is little scientific evidence to support this assumption. Our data show that the organically cultivated black currant fruits could not be distinguished from those conventionally grown.

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Supporting Information Available: Factor loadings for selected variables on the principal components. This material is available free of charge via the Internet at http://pubs.acs.org.

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