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Biochemical Profiling and Chemometric Analysis of Seventeen UK-Grown Black Currant Cultivars

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Black currant fruits are recognized as being an important dietary source of health-related compounds, such as anthocyanins and ascorbic acid. In the present study, the biochemical composition (viz., nonstructural carbohydrates, individual anthocyanins, total anthocyanins, total phenolics, and organic acids, including ascorbic acid) from 17 UK-grown black currant cultivars was analyzed. Berry composition was significantly affected by genotype. Nonstructural carbohydrates ranged from 85.09 to 179.92 mg g^{-1} on a fresh weight (FW) basis, while concentration for organic acids ranged from 36.56 to 73.35 mg g^{-1} FW. Relative concentrations of cyanidin 3-glucoside, cyanidin 3-rutinoside, delphinidin 3-glucoside and delphinidin 3-rutinoside were 3.1−7.9%, 35.4−47.0%, 7.6−12.5% and 36.9−50.9%, respectively. Differences in the biochemical profile among cultivars were emphasized by principal component analysis (PCA) and hierarchical cluster analysis (HCA). PCA was able to discriminate between cultivars, especially on the basis of health-related compounds. Initial exploration revealed that individual anthocyanins, total phenolics, and ascorbic acid could be used to characterize and classify different cultivars. HCA showed that the biochemical composition of the different cultivars was related to parentage information.

KEYWORDS: Nonstructural carbohydrates; anthocyanins; organic acids; total phenolics; principal component analysis; hierarchical cluster analysis

INTRODUCTION

Black currant (*Ribes nigrum* L.) is a species of Ribes berry native to central and northern Europe and northern Asia (*1*). Nowadays, most of the production of black currant berries is for use in processed products, including juices and cordials (*2*). Black currant fruits are one of the richest sources of antioxidants, notably anthocyanins, hydroxycinnamic acids (*3*), and ascorbic acid (*4*). A general agreement from health experts worldwide has stated that an increased dietary intake in antioxidant compounds from fruits and vegetables may protect against the oxidative damage caused by free radicals (*5, 6*). Moreover, recent studies suggest that consumption of products rich in certain phytochemicals (viz., phenylpropanoids) (*7–9*), such as berry fruits, helps toward reducing the incidence of certain cancers and chronic diseases (*5–7*).

Several methods are described in the literature for the extraction and quantification of specific quality-related target analytes in black currant fruit (*10–12*). Much of this information has been concentrated on quantification of anthocyanins and other phenylpropanoids from processed fruit or black currant-based beverages. Furthermore, the genotypic effects on the sensory properties of black currant juices have already been evaluated (*2, 12*). However, a recent study (*13*) demonstrated that processing black currant fruits dramatically reduced the content and bioavailability of certain phytochemicals when compared to fresh fruits. Given this situation, it is perhaps surprising that more research has not been conducted to characterize the biochemical profile of different black currant genotypes, especially for fresh fruits. The aim of the present study was to optimize different standard extraction and analytical methods for rapid quantification and comparison of a wide range of analytes, including nonstructural carbohydrates (NSCs), nonvolatile organic acids, individual anthocyanins, total phenolics (TP), and total anthocyanins (TA) in berries from a range of UK-grown black currant genotypes ($n = 17$). Cultivars (cvs) were classified and compared against one another using chemometric analysis, including principal component analysis (PCA) and hierarchical cluster analysis (HCA), and the intraspecific heterogeneity was assessed.

MATERIALS AND METHODS

Plant Materials. Seventeen different black currant cvs (**Table 1**) from 6-year-old bushes were hand-harvested at optimum ripeness (when fully black) in summer 2006 and used for this study. Bushes were grown under standard field conditions and were supplied by the Scottish Crop Research Institute (SCRI; Invergowrie, UK). Samples were transported at 4 °C by road to Cranfield University overnight. After reception, total soluble solids (TSS) was measured in the homogenate of each sample using a digital Palette refractometer PR-32α (Atago, Tokyo, Japan), after which the samples were immediately snap-frozen in liquid nitrogen and subsequently stored at −40 °C. From each cultivar, a subsample (150 g) was lyophilized using a Christ Alpha-RVC freeze drier with

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Table 1. Parentage Information of 17 UK-Grown Black Currant Cultivars

cultivar/genotype	parentage (♀ × ♂)
Ben Tirran	Ben Lomond × N29/17
Ben Alder	Ben Lomond × Ben More
Ben Dorain	Ben Alder × C2/1/62
Ben Starav	Ben Alder × C2/10/72
Ben Klibreck	S13/17/3 × (Ben More × 74020−16)
Ben Lomond	(Consort × Magnus) × (Brodtorp × Janslunda)
871-5	AB3/7 × C6/12/36
9199-4	S18-1-2 × B1834-120
9198-1	S18-15-1 open pollinated
9141-6	Ben Alder × S18-15-1
9137-2	Ben Avon × Ben Hope
9111-14	Ben Alder × B1610−68
S26-5-3	C2/4/51 × Ben Alder
9328-45	(Ben Alder × Ben Loyal) × S10-2-27/28
9148-9	Ben Dorain × S18-23-5
9311-82	S36−2−8 × B1834-120
91129-1	Ben Dorain × B1836-120

cooling-trap Alpha 1-4 (Christ, Lower Saxony, Germany) for 5 days at 0.015 kPa. Samples were subsequently ground in a pestle and mortar to a fine powder, weighed, and returned to the freezer (−40 °C) prior to analysis. All reagents were purchased from Sigma (Dorset, UK) unless otherwise stated.

Titrable Acidity (TTA) and pH. For the determination of these parameters, 150 g of fresh frozen berries were allowed to defrost at room temperature for 1 h (*10*) and were homogenized. The pH of the samples was determined using a Jenway 3020 pH meter (Jenway, Essex, UK). For TTA measurements, aliquots (1 mL) of homogenate were diluted into 80 mL of distilled water, and the titratable acidity was determined by titration with 0.1 M NaOH to an end point of pH = 8.1 (*14*). Results were converted to percentage of citric acid on a fresh weight (FW) basis.

Extraction and Quantification of Nonstructural Carbohydrates. Nonstructural carbohydrates were extracted according to ref *15*. Briefly, freeze-dried samples (150 mg) were mixed with 3 mL of 62.5% (v/v) aqueous methanol solvent and placed in a shaking bath at 55 °C for 15 min. Samples were vortexed every 5 min to prevent layering and then left to cool. Cooled samples were subsequently filtered through a 0.2 μm Millex-GV syringe driven filter unit (Millipore Corporation, Billerica, MA), and the clear extract was analyzed. Extractions (n = 17) were carried out in triplicate.

Fructose, glucose, and sucrose content in black currant extracts was determined using an HPLC system comprising a P580 pump, Dionex STH column thermostat, and GINA 50 autosampler (Dionex, Sunnyvale, CA) (*15*). The diluted black currant extract (1:10, v/v), was injected (20 μL) into a Rezex RCM monosaccharide Ca^{+} column of 300 mm × 7.8 mm diameter, 8 μm particle size (Phenomenex, Torrance, CA; part no. 00H-0130-K0) with a Carbo-Ca^{2+} guard column of 4 mm × 3 mm diameter (Phenomenex; part no. AJ0-4493). The mobile phase was degassed HPLC-grade water at a flow rate of 0.6 mL min^{-1}. Column temperature was set at 75 °C using a Dionex STH column thermostat. Eluted NSCs from all extracts were detected by evaporative light-scattering detection (ELSD 2420, Waters, MA) connected to the Dionex system using a UCI-50 universal chromatography interface. The presence and abundance of each sugar was calculated by comparing sample peak area with fructose, glucose, and sucrose standards using Chromeleon version 4.6 software (Dionex). Assays (n = 17) were carried out in triplicate (n = 51).

Extraction and Quantification of Nonvolatile Organic Acids. Extracts for organic acid determination were prepared according to ref *15* with slight modifications. Freeze-dried samples (150 mg) were dissolved into 3 mL of HPLC-grade water. Samples were kept at room temperature (20 °C) for 10 min, and the flocculate was filtered through a 0.2 μm syringe filter. Extractions (n = 17) were performed in triplicate. L-Ascorbic, citric, malic, oxalic, and tartaric acid concentrations in black currant extracts were determined using the same Dionex HPLC system as described for NSCs. Extracts (20 μL) were injected into an Alltech Prevail organic acid column 250 mm × 4.6 mm diameter, 5 μm particle size (Alltech, Los Alomitos, CA; part no. 88645) with appropriate guard column of 7.5 mm × 4.6 mm diameter (Alltech; part no. 96429). The mobile phase was degassed 0.2% (w/v) metaphosporic acid in water (*16*). The flow rate of the mobile phase was 1.0 mL min^{-1}. The column temperature was set at 35 °C. Eluted organic acids from extracts were detected at 210 nm by a UVD 170S/340S (Dionex). The presence and quantity of each acid was calculated by comparing the peak area obtained with standards using Chromeleon version 4.6 software. Assays (n = 17) were carried out in triplicate (n = 51).

Extraction and Identification of Individual Anthocyanins. Individual anthocyanins from fruits are commonly extracted using an acidified organic solvent, since this solvent destroys the cell membrane at the same time that it dissolves and stabilizes the anthocyanins (*17*). Different solvent combinations, mainly water-, ethanol-, or methanol-based solvents, were tested according to those previously reported (*17–19*) (**Figure 1**). Individual anthocyanins were extracted by mixing freeze-dried sample (150 mg) with 3 mL of 70% (v/v) methanol and 0.5% (v/v) HCl in HPLC-grade water. The slurry was held at 20 °C for 1.5 h, mixing the samples every 15 min. Finally, the flocculate obtained was filtered as previously described.

The anthocyanin profile of black currant extracts was determined using an Agilent 1200 series HPLC binary pump system (Agilent, Berks, UK), equipped with an Agilent 1200s DA G1315B/G1365B photodiode array with multiple wavelength detector. Black currant extracts (20 μL), were injected into an Alltech Allsphere ODS-1 column of 250 mm × 4.6 mm diameter, 5 μm particle size (Alltech; part no. 778357) with an Alltech Allsphere ODS-1 guard column of 7.5 mm × 4.6 mm diameter (part no. 96402). Different methods for the identification and quantification of individual anthocyanins were initially tested according to what was previously reported (*20, 21*), and finally, the method described by ref *20* was adapted with some modifications. The mobile phase consisted of degassed 2.5% (v/v) acetonitrile and 5% (v/v) formic acid in HPLC-grade water (A) and acetonitrile (B). The program followed a linear gradient from 0 to 13% B in 10 min and then from 13 to 23% B in 17.4 min. Flow rate was 1 mL min^{-1}, and the column temperature set at 40 °C using an Agilent G1316A thermostatted column compartment. The temperature of the autosampler was set at 4 °C using an Agilent cooled autosampler G1330B.

The eluted anthocyanins from the extracts were detected at 520 nm, and the presence and quantity of each anthocyanin was calculated by comparing the peak area with external standards (delphinidin 3-glucoside, cyanidin 3-glucoside, and cyanidin 3-rutinoside, (Extrasynthese, Lyon, France)) using Agilent ChemiStation Rev. B.02.01. Due to the lack of standard for delphinidin 3-rutinoside, the concentration of this compound was calculated from the calibration curve of delphinidin 3-glucoside. Assays (n = 17) were carried out in triplicate.

Determination of Total Phenolics and Total Anthocyanins. Freeze-dried powdered samples (150 mg) of each cultivar were dissolved into 3 mL of 80% aqueous ethanol (v/v) and were extracted in a Haake SWB 20 water bath at 70 °C (Haake, Berlin, Germany) for 2 h, mixing every 20 min as described elsewhere (*15*). The solution was filtered using a syringe filter of 0.2 μm, and the clear filtrate was analyzed. Total phenolics were measured according to the Folin−Ciocalteu method (FCM), based on the reduction of a phosphowolframate-phosphomolibdate complex by phenolics to blue reaction products (*22*). Briefly, 20 μL of filtrate or gallic acid and 3.2 mL of HPLC-grade water was mixed with 200 μL of Folin−Ciocalteu's phenol reagent followed by 600 μL of sodium carbonate (1.9 M). After 2 h of incubation at room temperature (20 °C) in the dark, absorbance was measured at 760 nm using a Camspec M501 UV/vis spectrophotometer (Camspec Ltd., Cambs., UK). TP content was estimated from a standard curve of gallic acid, and results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (DW). Nine-point calibration was linear to a concentration of 2.5 mg mL^{-1} ($r^2 > 0.99$), and reproducible results were obtained for all samples.

Total anthocyanins were extracted using the same extraction solvent as described for individual anthocyanins. Measurement of TA was adapted from that previously described by ref *23*. Briefly, freeze-dried black currant powder (150 mg) was dissolved into 3 mL of extraction solvent and incubated at 4 °C for 1 h. The flocculate was filtered as

Table 2. Concentration of Nonstructural Carbohydrates in 17 UK-Grown Black Currant Cultivars on a Fresh Weight (FW) and Dry Weight (DW) Basis[c]

	mg g^{-1} FW (DW)					
genotype	sucrose	glucose	fructose	total	sugar/acid[a]	(G + F)/S[b]
871-5	17.64 (48.23)	82.78 (226.35)	79.50 (217.51)	179.92 (492.10)	4.39	9.21
Ben Alder	3.38 (9.59)	78.02 (221.09)	85.37 (241.53)	166.78 (472.61)	2.61	48.58
Ben Lomond	16.38 (43.78)	74.85 (200.05)	68.52 (183.14)	159.75 (426.97)	2.96	8.76
Ben Dorain	15.38 (42.40)	60.36 (166.58)	73.48 (202.77)	149.22 (411.79)	2.49	8.71
9199-4	10.93 (29.49)	57.24 (154.40)	72.06 (194.35)	140.24 (378.24)	2.57	11.83
Ben Starav	36.88 (127.72)	45.53 (156.75)	57.35 (198.63)	139.76 (483.10)	3.20	2.78
9198-1	31.54 (90.48)	45.96 (131.84)	61.53 (176.50)	139.04 (398.82)	1.89	3.41
9141-6	19.26 (53.54)	49.92 (138.79)	60.10 (167.09)	129.28 (359.42)	2.47	5.72
9137-2	14.04 (47.71)	45.96 (156.19)	57.98 (197.04)	117.99 (400.94)	2.54	7.41
Ben Tirran	3.83 (11.99)	51.75 (162.02)	61.37 (192.11)	116.95 (366.12)	1.99	29.55
9111-14	17.86 (64.01)	39.52 (141.66)	54.03 (193.68)	111.40 (399.36)	2.22	5.24
S26-5-3	9.06 (30.92)	40.38 (137.88)	61.89 (211.30)	111.33 (380.11)	2.27	11.29
Ben Klibreck	17.72 (67.05)	43.19 (163.39)	50.10 (189.53)	111.01 (419.97)	2.85	5.24
9328-45	8.34 (28.58)	44.83 (153.67)	53.45 (183.22)	106.62 (365.47)	2.92	11.79
9148-9	11.86 (42.48)	36.86 (132.07)	55.76 (199.79)	104.48 (374.30)	1.83	7.82
9311-82	5.46 (17.03)	43.62 (135.99)	50.81 (158.39)	99.89 (311.41)	1.90	17.29
91129-1	3.57 (12.46)	38.09 (132.76)	43.43 (151.38)	85.09 (296.60)	2.18	22.82
mean	14.30 (45.15)	51.68 (159.50)	61.58 (191.67)	127.19 (396.32)	2.51	12.73
LSD ($P = 0.05$)	1.040 (3.328)	1.025 (3.103)	1.633(5.393)	2.842 (9.216)	0.082	1.956

[a] Total sugars/total organic acids. [b] (glucose + fructose)/sucrose. [c] Cultivars were arranged in descending order according to total sugar content on a FW basis.

previously described, and the absorbance of the resulting clear filtrate was measured at 520 nm, which corresponds to the maximum absorbance for anthocyanins. The anthocyanin content was based on a cyanidin 3-glucoside molar extinction coefficient of 26 900 and a molecular weight of 449.2 (*24*). Resultant values were expressed as milligrams of cyanidin 3-glucoside per gram of fresh weight (FW), as this is a common anthocyanin found in many small berries.

Data Analysis. All statistical analyses were carried out using Genstat for Windows, version 9.1.0.147 (VSN International Ltd., Hertz., UK). Least significant difference values (LSD; $P = 0.05$) were calculated for mean separation using critical values of t for two-tailed tests. Tests for correlations between mean values for analyte concentrations were made using Spearman's rank correlation. Correlations are presented with the Spearman's correlation coefficient (r) and a P value based on a two-tailed test.

Chemometric data analysis was performed using the same software described above; the triplicate per sample values were used as an input for chemometric analysis (PCA and HCA). Chemometric studies were carried out considering the different compounds measured as analytical data. Dimensions of the corresponding data matrix were 51 samples, corresponding to the triplicate values of each sample and different sets of variables. Data were autoscaled to provide similar weights for all the variables, as described elsewhere (*25*). Cluster analysis was therefore performed using Euclidean distance and average linkage algorithms (*25*). Results from cluster analysis are shown in terms of similarities between the genotypes examined and compared with the parentage information (**Table 1**).

RESULTS AND DISCUSSION

Effect of Genotype on Fruit Biochemistry. Biochemical composition of black currant berries depends mainly on the genotype and the agroclimatic conditions during their cultivation (*26*). Between the black currant genotypes studied (**Table 1**), significant differences were found in terms of their biochemical composition for most cvs and analytes studied (**Tables 2**, **3** and **4**). Generally, concentration of target analytes was comparable to that previously reported (*4, 27–29*).

Nonstructural Carbohydrates and Nonvolatile Organic Acids. For the extraction of NSCs and nonvolatile organic acids, the methods described herein were successfully adapted and slightly modified from previous reported methods applied to other fresh produce types (*15, 16, 30*). Although little information is available describing the sugar and acid composition of fresh black currant berries, concentrations were in accordance with those reported in the literature for black currant derivative products (*27*).

The content of NSCs in black currant fruits was significantly influenced by genotype (**Table 2**) on both a FW and DW basis. However, sugar concentrations were correlated with water content ($r^2 = 0.631$), and therefore, some of the sugar variation between cvs was most probably caused by a "dilution effect" (*15*). Sucrose, glucose, and fructose were identified in all cvs, and concentrations ranged between 3.38 and 36.89 mg g^{-1} FW, 36.86−82.78 mg g^{-1} FW, and 43.43−85.73 mg g^{-1} FW, respectively. The proportion of each sugar also varied with genotype. Glucose and fructose were the dominant sugars found in all cvs, making up ∼40% and 49%, respectively, of total sugars. Sucrose, on the other hand, made up ∼10% of the total sugar concentration but was more variable within cvs. Fructose/glucose ratios ranged from 0.91 (cv. Ben Lomond) to 1.53 (cv. S26-5-3) and therefore were in agreement with those reported by ref *27* for black currant concentrates. Concomitant to this, significant differences in the fructose/glucose ratio were observed by ref *27* when different origins and harvest years were studied. Some cvs, for example, 871-5, Ben Alder and Ben Lomond had a total sugar concentration that was 1.25-fold higher than the mean of the 17 cvs tested.

Nonvolatile organic acids on both a FW and DW basis were also influenced by genotype (**Table 3**). Five different organic acids were identified in all cvs, and significant genotypic differences were observed (**Table 3**). In all genotypes, citric acid was the predominant organic acid and accounted for ≥73% (FW basis) of the total organic acid content in black currant fruits. Comparable results, in terms of proportion of individual organic acids, were obtained by ref *27* when black currant concentrates were analyzed for citric, ascorbic, and malic acids. Concentration on a FW basis of oxalic, tartaric, malic, ascorbic, and citric acids varied from 0.23 to 0.47 mg g^{-1}, 0.46 to 1.12 mg g^{-1}, 0.70 to 6.90 mg g^{-1}, 1.92 to 5.41 mg g^{-1}, and 28.84 to 59.79 mg g^{-1}, respectively. Results from this study confirmed that black currant fruits are known to be a good source of ascorbic acid (AsA) (*4, 28*), and to the best of our knowledge, for the first time, minor organic acids, such as oxalic and tartaric acid, have been reported in fresh black currant berries. The concentration of AsA ranged from 1.922 to 5.415 mg g^{-1} FW and therefore was far higher as compared to other common berry

Table 3. Concentration of Main Organic Acids in 17 UK-Grown Black Currant Cultivars on a Fresh Weight (FW) and Dry Weight (DW) Basis[a]

	mg g^{-1} FW (DW)					
genotype	oxalic	tartaric	malic	ascorbic	citric	total
9198-1	0.42 (1.22)	0.82 (2.34)	6.90 (19.81)	5.41 (15.53)	59.79 (171.50)	73.35 (210.4)
Ben Alder	0.47 (1.33)	0.76 (2.16)	4.05 (11.47)	2.53 (7.18)	56.05 (158.83)	63.86 (180.96)
Ben Dorain	0.45 (1.25)	0.91 (2.50)	5.04 (13.91)	3.31 (9.15)	49.98 (137.93)	59.69 (164.74)
Ben Tirran	0.37 (1.17)	0.70 (2.19)	3.74 (11.70)	3.10 (9.72)	50.61 (158.45)	58.53 (183.23)
9148-9	0.30 (1.08)	0.53 (1.91)	2.59 (9.29)	2.75 (9.84)	50.74 (181.79)	56.92 (203.92)
9199-4	0.38 (1.02)	1.12 (3.03)	6.59 (17.77)	3.76 (10.14)	42.74 (115.28)	54.59 (147.23)
Ben Lomond	0.31 (0.82)	0.69 (1.84)	3.34 (8.94)	3.96 (10.60)	45.69 (122.13)	54.00 (144.34)
9311-82	0.30 (0.95)	0.95 (2.97)	6.37 (19.86)	3.51 (10.94)	41.37 (128.97)	52.51 (163.70)
9141-6	0.37 (1.04)	0.91 (2.53)	1.75 (4.86)	3.30 (9.19)	46.02 (127.95)	52.36 (145.55)
9111-14	0.35 (1.26)	0.46 (1.66)	1.33 (4.76)	2.38 (8.55)	45.78 (164.10)	50.30 (180.33)
S26-5-3	0.32 (1.11)	0.65 (2.23)	0.99 (3.38)	3.49 (11.90)	43.68 (149.13)	49.13 (167.74)
9137-2	0.36 (1.22)	0.73 (2.49)	3.35 (11.37)	4.00 (13.60)	38.05 (129.31)	46.49 (158.00)
Ben Starav	0.29 (1.02)	0.60 (2.80)	1.30 (4.52)	2.26 (7.82)	39.09 (135.38)	43.55 (150.83)
871-5	0.30 (0.82)	0.77 (2.09)	0.82 (2.23)	2.26 (6.19)	36.84 (100.75)	40.99 (112.09)
91129-1	0.29 (1.04)	1.12 (3.89)	5.03 (17.53)	3.79 (13.23)	28.84 (100.53)	39.08 (136.21)
Ben Klibreck	0.24 (0.90)	0.54 (2.06)	0.70 (2.65)	2.05 (7.74)	35.46 (134.16)	38.99 (147.52)
9328-45	0.23 (0.78)	0.61 (2.076)	4.35 (14.93)	1.92 (6.59)	29.45 (100.95)	36.56 (125.32)
mean	0.34 (1.06)	0.75 (2.36)	3.42 (10.53)	3.16 (9.88)	43.54 (136.60)	51.23 (160.12)
LSD ($P = 0.05$)	0.029 (0.096)	0.108 (0.335)	0.377 (1.275)	0.145 (0.483)	0.814 (2.628)	1.018 (3.346)

[a] Cultivars were arranged in descending order according to total organic acid content on a FW basis.

fruits in which the AsA content is commonly >1 mg g^{-1} FW (*15, 31*). Significant differences for AsA concentrations were also found between cvs on both a FW and DW basis (**Table 3**). Although AsA was not significantly correlated to total organic acid content, results suggest a strong relationship between both parameters for some cvs.

Sugar and acid contents are important indicators of fruit flavor (*32, 33*). Moreover, the sugar acid ratio is often used as an indicator of fruit ripeness (*33*). TSS and, to a lesser extent, TTA are commonly used during routine quality control in the black currant industry as a measure of sugar and acid content, respectively. In the present study, TSS and TTA were measured and compared to results obtained by HPLC. In agreement with that previously reported for other fresh produce types (*32, 34*), poor correlations were found between TSS and total sugar concentration ($r^2 = 0.53$) or between sugar/acid ratio measured as TSS/TTA with the ratio obtained from HPLC analysis ($r^2 = 0.54$). Sugar/acid ratios reported in the present work were slightly higher than those previously reported (*27*). However, these authors did not quantify sucrose content, and therefore, total sugar content may have been underestimated.

Individual Anthocyanins. Different solvent combinations appear in the literature describing the extraction of individual anthocyanins from black currant berries. Some authors have referred to the use of methanol as the most efficient solvent for the extraction of these compounds in black currant samples (*3, 9, 18, 16*); others, instead, have shown that aqueous acetone extracts were more efficient in extracting these compounds from black currant and other berries (*35*). In the method described herein, different methanol-, ethanol-, and water-based solvent combinations were tested (**Figure 1**). Results showed that, within the solvents tested, 70% (v/v) methanol and 0.5% HCl (v/v) in HPLC-grade water was the best solvent mixture for the extraction of these target analytes from freeze-dried black currant samples. Methanol solubilizes the anthocyanins mainly present in the skin of the berries (*3*) while the HCl fraction helps maintain these compounds in their stable flavylium cation form (*17, 20, 35*). The choice of extraction solvent was in agreement with that previously described (*19*), since methanol extracts from black currants had approximately 2 and 1.5-fold higher values of anthocyanins as compared to water- and ethanol-based solvents, respectively. For the identification and

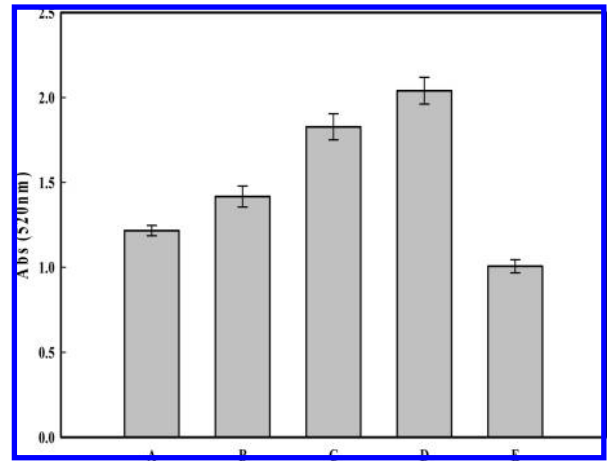

Figure 1. Effect of different solvent combinations (v/v) on the extraction of total anthocyanins at 20 °C for 1.5 h, from freeze-dried black currant (cv. Ben Dorain) samples. (A) EtOH/H_2O/HCl (50:49.5:0.5); (B) EtOH/H_2O/HCl (70:29.5:0.5); (C) MeOH/H_2O/HCl (50:49.5:0.5); (D) MeOH/H_2O/HCl (70:29.5:0.5); (E) H_2O/HCl (99.5:0.5). Error bars represent standard error for $n = 3$.

quantification of anthocyanins in black currant extracts, a method based on that previously published (*20*) was adapted and optimized. A final run of less than 28 min was required to elute all the anthocyanins present in the samples investigated (**Figure 2**). Four major anthocyanins (delphinidin 3-glucoside, delphinidin 3-rutinoside, cyanidin 3-glucoside, and cyanidin 3-rutinoside) were identified in this study according to their retention time, UV spectra, and comparison with standards. These four anthocyanins were also previously reported as the main anthocyanins in black currant berries and concentrates contributing to more than 80% of the total content of anthocyanins (*20, 26, 35, 36*). The content of individual anthocyanins among the 17 genotypes studied varied considerably (**Table 3**). The relative concentration of cyanidin 3-glucoside, cyanidin 3-rutinoside, delphinidin 3-glucoside, and delphinidin 3-rutinoside was 3.1−7.9%, 35.4−47.0%, 7.6−12.5%, and 36.9−50.9%, respectively, and was comparable to that previously reported (*28, 29, 35*). Consequently, the rutinoside forms of anthocyanins were predominant in all the cases. In contrast, ref *26* also reported similar relative concentrations of these pigments in black currant

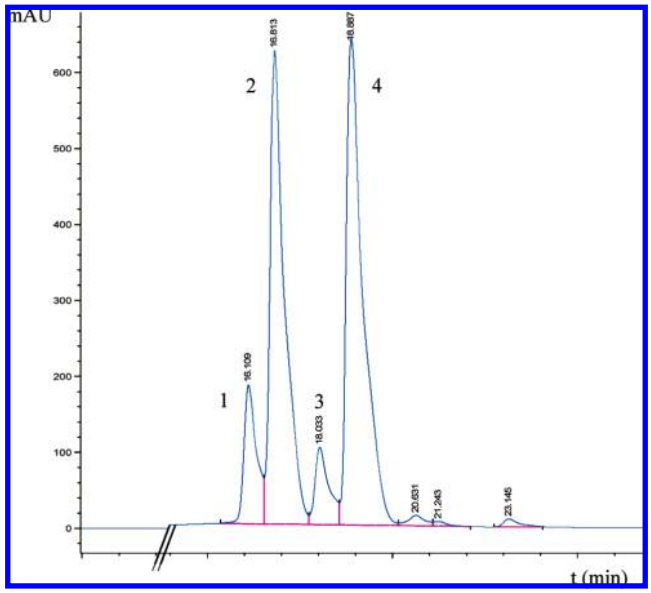

Figure 2. Example of typical chromatographic profile of main anthocyanins extracted from black currant berries. (1) delphinidin 3-glucoside (16.1 min); (2) delphinidin 3-rutinoside (16.8 min); (3) cyanidin 3-glucoside (18.0 min); (4) cyanidin 3-rutinoside (18.8 min).

fruit; however, the authors concluded that cyanidin was the major anthocyanin in all the UK-grown cvs examined. Conversely, the results presented herein show that although having similar concentrations, in most of the cvs, delphinidin was the main pigment identified. This dichotomy could be explained because during the past few years, black currant breeding programs have tended to select cvs with high delphinidin/cyanidin ratio due to the higher stability of delphinidin (*1*). The overall quantification of anthocyanins in black currant samples, expressed as the sum of individual anthocyanins (0.83−1.99 mg g^{-1} FW) (**Table 4**), was slightly lower than that previously reported (*35*). Although different harvest years, cvs, and locations are known to be a source of variation, most of the differences encountered are probably due to the extraction procedure because the authors (*35*) combined the extracts from several exhaustive extractions using 70% aqueous acetone containing 0.01 M HCl. Acetone was not assessed in the present study, and therefore, it is not possible to state whether the differences are due to the solvent used or the single step in the extraction procedure. Although anthocyanin content may be understimated, the method reported herein for the extraction and subsequent quantification of individual anthocyanins required less time than most of those cited in the literature (*20, 26, 38*) and therefore could be adopted as a simple method for fast and routine screening of major anthocyanins in black currant samples.

Total anthocyanins and total phenolics. The content of TP and TA from plant materials has been studied in great detail using several methods (*15, 24, 39, 40*). In the present study, previously described spectrophotometric techniques were adapted (*22, 23*) and results obtained were in the range of those previously reported (*40*). However, the exact assessment and comparison of results obtained is difficult due to the heterogeneity among experimental conditions applied and differences in chemical properties of oxidizable substrates within the methods found in the literature (*39–43*). Despite anthocyanins being one of the main groups of polyphenols present in black currant (*3, 38*), no correlation was found between TP and TA. In this context, the contents of phenolic compounds determined by the FCM may have been detrimentally affected by the presence of high concentrations of ascorbic acid and saccharides (mainly fructose), which are both relatively high in black currant berries (*39, 42, 43*). In addition to this, some of the differences encountered may be also influenced by the use of different solvents, temperatures, and time during the extraction of these target analytes in black currant freeze-dried samples.

As for other analytes studied, TA and TP content were significantly affected by the genotype (**Table 4**). Values for TA, expressed as milligrams of cyanidin 3-glucoside per gram FW, ranged from 3.49 to 9.14, while values for TP ranged from 6.38 to 13.33 mg GAE g^{-1} FW. It was found that values for TA, determined spectrophotometrically, were 3- to 4-fold higher when compared to those quantified by HPLC. Although the same extraction solvent was used, the comparison between both values is questionable because differences exist between both extraction methods and analysis. TA values were based on the molar extinction coefficient and molecular weight of cyanidin 3-glucoside. Even though this is not the main anthocyanin found in black currants (**Table 4**), other authors have already used it as a reference for total anthocyanin content in berries, including black currants (*24*).

Chemometric Data Analysis. Chemometric interpretation of analytical data is increasingly applied to food analysis (*25, 44–47*). For instance, PCA has been used for the differentiation and classification of food products according to geographical origin or for the chemotaxonomic approach to botanical classification. In this work, the chemometric interpretation of the biochemical profile of 17 UK-grown black currant cvs was assessed to test the possible heterogeneity among different genotypes.

Principal Component Analysis (PCA). Considering 14 variables as analytical data (complete biochemical profile excluding correlated variables), PCA was able to discriminate among cvs. Generally, all replicates from each sample were clustered together and separated from other genotypes, confirming the repeatability of the methods used in this study. Three principal components (PCs) were required to capture almost 73% of the variance. PC1 explained 32% of the total variation and was mainly related, according to factor loadings values, to TP. Glucose, fructose, and especially each delphinidin anthocyanin were also important variables to define PC1. PC2 explained 27% of the variation captured between cvs and was related to ascorbic acid and both cyanidin 3-glucoside and cyanidin 3-rutinoside. PC3 captured 15.1% of the total variation between cvs and was mainly related to citric and oxalic acid; fructose; and again, TP. The inclusion of additional PCs failed to improve clustering between cvs. Consequently, the interpretation of clustering between cvs was mainly characterized by the information provided by PC1 and PC2 (**Figure 3**A), in which the genotypic differences among cultivars were emphasized. The study of the distribution of cvs, bearing in mind the parentage information, did not shown relevant patterns. However, most of the related cvs were grouped within the two main central clusters, whereas nonrelated cvs were clustered individually in the surroundings of the space plot (**Figure 3**A). Initial exploration of the clustering revealed that the content of minor compounds, such as individual anthocyanins, TP, and ascorbic acid, were relevant variables to characterize and classify different cvs. Accordingly, several authors have referred to use of minor constituents as providing a versatile and valuable tool for the characterization and, therefore, quality assurance of certain food products (*44–47*). For instance, minor polyphenols (*46*) or biogenic amines (*45*) have been used to satisfactorily characterize different wine types, and the phenolic profile has suitably classified different quince jams (*44*).

Table 4. Concentration of major anthocyanins, total anthocyanins (TA) and total phenolics (TP) in 17 UK-grown black currant cultivars on a fresh weight (FW) and dry weight (DW) basis[h]

	μg g^{-1} FW (DW)				mg g^{-1} FW (DW)		
genotype	Cya-3-glu[a]	Cya-3-rut[b]	Dp-3-glu[c]	Dp-3-rut[d]	Σ anthocyanins[e]	TA[f]	TP[g]
871-5	123.07 (336.52)	937.12 (1762.23)	197.14 (539.13)	735.20 (2010.32)	1.99 (4.65)	8.39 (22.95)	10.78 (29.48)
Ben Alder	99.07 (280.74)	677.80 (1921.38)	226.36 (641.44)	814.30 (2307.27)	1.82 (5.35)	9.15 (25.29)	10.16 (28.80)
9148-9	110.21 (394.97)	781.90 (2801.69)	171.47 (614.22)	644.42 (2309.59)	1.71 (6.12)	6.28 (22.51)	9.14 (32.76)
Ben Starav	92.55 (320.59)	623.74 (2160.10)	152.00 (526.47)	675.93 (2340.84)	1.54 (5.35)	6.12 (21.20)	8.17 (28.31)
S26-5-3	87.96 (300.36)	654.90 (2236.84)	140.31 (478.94)	616.20 (2104.35)	1.50 (5.12)	6.01 (20.51)	9.84 (33.59)
9311-82	61.46 (191.61)	577.25 (1799.26)	123.60 (385.48)	650.55 (2028.50)	1.41 (4.40)	4.92 (15.33)	13.33 (41.56)
Ben Klibreck	58.12 (219.90)	620.88 (2349.04)	104.48 (395.19)	563.19 (2129.70)	1.35 (5.09)	5.25 (19.87)	9.12 (34.51)
Ben Dorain	59.02 (162.94)	540.11 (1490.30)	119.92 (331.00)	627.30 (1731.04)	1.35 (3.72)	6.22 (17.17)	10.42 (28.75)
91129-1	105.59 (368.12)	568.53 (1982.51)	132.62 (462.36)	528.34 (1842.28)	1.33 (4.66)	3.49 (12.18)	8.77 (30.57)
9328-45	62.90 (215.60)	489.81 (1679.17)	155.77 (533.75)	611.84 (2097.27)	1.32 (4.53)	3.85 (13.155)	9.26 (31.73)
9111-14	59.77 (214.23)	488.30 (1750.22)	99.49 (356.39)	620.13 (2223.03)	1.27 (4.54)	4.52 (16.19)	6.38 (22.87)
Ben Lomond	41.86 (111.95)	489.12 (1307.92)	95.61 (255.76)	629.60 (1682.95)	1.26 (3.36)	6.04 (16.14)	10.37 (27.72)
9199-4	74.85 (201.48)	492.40 (1328.63)	146.10 (394.10)	499.67 (1348.45)	1.21 (3.27)	5.26 (14.18)	10.77 (29.04)
Ben Tirran	43.69 (136.84)	396.55 (1241.13)	111.32 (348.52)	564.88 (1768.16)	1.12 (3.49)	4.80 (15.03)	9.01 (28.20)
9198-1	34.17 (98.02)	397.16 (1139.23)	96.03 (275.54)	579.32 (1661.69)	1.11 (3.17)	5.03 (14.42)	11.23 (32.23)
9141-6	54.42 (151.33)	423.14 (1176.04)	112.65 (313.15)	503.14 (1399.03)	1.09 (3.03)	5.26 (14.62)	8.78 (24.41)
9137-2	28.87 (98.10)	304.30 (1034.54)	76.22 (259.08)	425.21 (1445.22)	0.83 (2.84)	2.99 (10.15)	6.80 (23.11)
mean	68.81 (223.72)	546.21 (1762.42)	133.06 (418.20)	605.23 (1907.12)	1.35 (4.25)	5.50 (17.14)	9.55 (29.86)
LSD ($P = 0.05$)	12.305 (38.4)	110.340 (365.0)	26.223 (83.30)	90.594 (285.8)	0.219 (0.682)	0.675 (0.897)	0.626 (1.910)

[a] Cyanidin 3-glucoside. [b] Cyanidin 3-rutinoside. [c] Delphinidin 3-glucoside. [d] Delphinidin 3-rutinoside as Dp-3-gluc equivalents. [e] Sum of individual anthocyanins (mg g^{-1} FW (DW)). [f] Total anthocyanins (mg cyanidin 3-glucoside g^{-1}). [g] Total phenolics (mg gallic acid equivalents (GAE) g^{-1}). [h] Cultivars were arranged in descending order according to total content of individual anthocyanins on a FW basis.

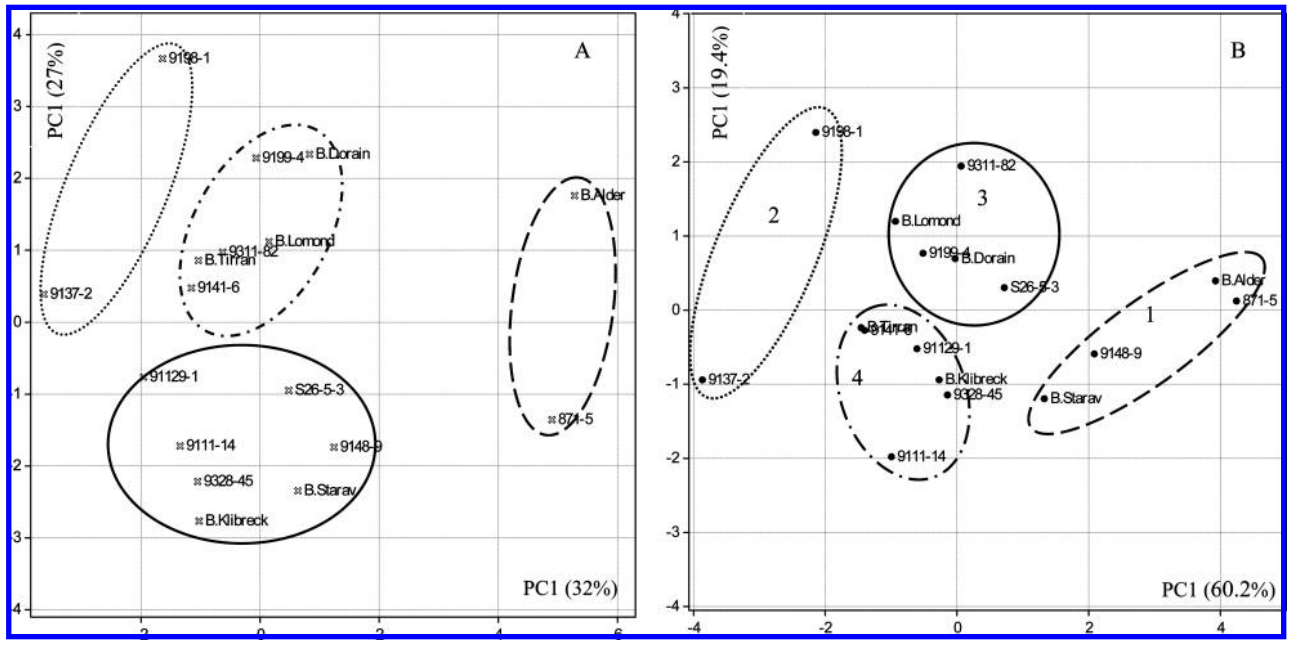

Figure 3. Principal component analysis characterization (PC1 vs PC2) of 17 UK-grown black currant cultivars using (A) the biochemical profile (14 variables) as the analytical data and (B) health-related compounds (7 variables) as the analytical data.

When considering health-related compounds/parameters only (7 variables; viz., individual anthocyanins, ascorbic acid, TP and TA) as analytical data for the chemometric analysis (**Figure 3**B), the clustering between cvs was slightly different from that obtained when all analytical data were considered (**Figure 3**A). However, the variation captured by these variables (health-related compounds) was significantly improved. Two main PCs were required to capture 80% of the variance between cvs. PC1 explained most of the variance observed (60.2%) and was closely related to individual anthocyanins. PC2 accounted for 19.4% of total variation and was related to TP and AsA. The inclusion of a third PC did not improve significantly the clustering between cvs (PC3 = 7.5% of the total variation). As described for general PCA, the interpretation of the results was mainly characterized by the information given by PC1 and PC2 (**Figure 3**B), in which the differences in AsA and anthocyanins among cvs were specially emphasized, since four different clusters were clearly identified. Bearing in mind all the analytical information, individuals from cluster 1 were characterized as having high anthocyanins and low ascorbic acid content. Generally significant differences in both sugar and acid content were found between the genotypes of this cluster. Cluster 2 grouped genotypes containing low anthocyanin and high AsA content. Although sugar concentration for individuals of group 2 was generally similar to the mean value between the different cvs studied, significant differences were found in their acid content. Genotypes from group 3 showed significant differences in both anthocyanins and sugar content. Similar concentration of AsA and generally mean acid content was found for all the genotypes grouped in cluster 3. Finally, genotypes from cluster

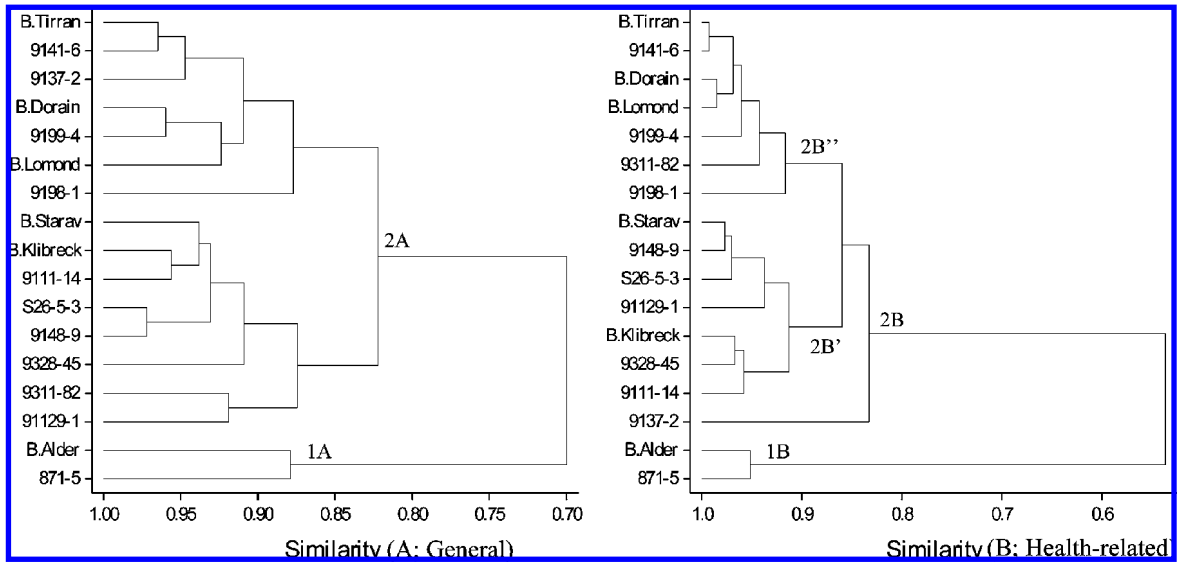

Figure 4. Hierarchical cluster analysis of 17 UK-grown black currant cultivars based on group average cluster analysis of (A) the biochemical profile (14 variables) as the analytical data and (B) health-related compounds (7 variables) as the analytical data.

4 showed low sugar and AsA content in comparison with other clusters. Similar concentrations of anthocyanins were found for the genotypes grouped in cluster 4.

Current black currant breeding programs are focused on selection of cvs with either high anthocyanin (*1*) or ascorbic acid content (*4*). Therefore, considering the results from this study, it is assumed that if both anthocyanins and AsA content are considered individually, black currant breeding programs may tend to increase the existent variability between cvs. For instance, the selection of new cvs with high AsA content may lead to a significant reduction in anthocyanin concentration.

Hierarchical Cluster Analysis (HCA). Like PCA, cluster analysis is an unsupervised data analysis method (*25*), meaning that prior knowledge of the sample is not required. Such methods allow the clustering of the samples according to intrinsic variance between them but without being biased by desired outcomes. As compared to PCA, HCA allows interpretation of the results in a fairly intuitive graphical way. Cluster analysis of the different black currant cvs, according to their biochemical profile (14 variables) and content in health-related compounds (7 variables), was used as an additional exploratory tool to assess the heterogeneity between different black currant genotypes and to relate the results obtained with parentage information (**Table 1**). Generally, HCA showed two clear clusters, with 70% of similarity, of 2 and 15 genotypes (**Figure 4**A), referred to as groups 1A and 2A, respectively. Inspection of the groups showed that individuals from group 1A, called Ben Alder and 871-5, although having different parentage, were reported as those containing higher concentrations of polyphenolic compounds, mainly cyanidin 3-glucoside, cyanidin 3-rutinoside, delphinidin 3-glucoside, and delphinidin 3-rutinoside, and also nonstructural carbohydrates. In terms of parentage, no clear relationships were observed between parentage information and biochemical profile when all the analytical data was considered. For instance, cvs descended from a cross with Ben Alder did not show substantial similarities (75%) in comparison with other genotypes. When considering only health-related compounds, results showed two clear clusters of 2 and 15 genotypes, respectively (**Figure 4**B). In this case, the similarity between the two main clusters (1B and 2B) was lower than 0.6, and clustering of cvs was slightly different from the HCA, which considered all analytical data (14 variables) (**Figure 4**A). Conversely to that described for cluster analysis of all analytical data, the use of health-related compounds was more suitable to classify cvs according to parentage. Within cluster 2B, two clear subclusters were identified (2B′ and 2B″). Most of the individuals from subcluster 2B″ (**Figure 4**B) were intimately related by genotype (Ben Lomond descendents) and showed the highest similarities (>0.95). Despite this, cvs 9199-4 and 9311-82, although being directly related by genotype, were not related to Ben Lomond, but grouped within the same cluster. On the other hand, cvs from subcluster 2B′ were intimately related to cv. Ben Alder (mainly first-generation progeny) and showed also high similarities (<0.9). This said, cv Ben Alder was clustered, using either all analytical data or health-related compounds, completely separate from its descendents. Although significant differences may exist among the parents and progeny in black currant breeding programs, it appears that a key set of characteristics is inherited that allows progeny to be closely grouped.

The work presented herein represents the first attempt to explore the genotypic variation in the major taste- and health-related compounds from a wide range of UK-grown fresh black currant berries. Wide variations in the biochemical profile of the target analytes examined were observed, and thus, it is clear that the differences in black currant cvs play an important role in determining fruit composition. The application of chemometric techniques is a suitable tool to compare and assess the variability among the biochemical profiles of different black currant genotypes. Chemometric data analysis revealed that the determination of health-related compounds alone was more suitable to discriminate among different genotypes than using other major components, such as citric acid and nonstructural carbohydrates. Thus, individual anthocyanins and ascorbic acid are key variables for capturing most of the genotypic variation among different black currant fruits.

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