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ARTICLE

Characterization, Quantification, and Yearly Variation of the Naturally Occurring Polyphenols in a Common Red Variety of Curly Kale (*Brassica oleracea* L. convar. *acephala* var. *sabellica* cv. 'Redbor')

Helle Olsen,*[,†,‡] Kjersti Aaby,[†] and Grethe Iren A. Borge[†]

[†]Nofima Mat AS, Osloveien 1, N-1430 Aas, Norway, and [‡]Department of Chemistry, University of Oslo, Postbox 1033, N-0315 Blindern, Norway

This study focuses on the characterization and quantification of polyphenols in the edible leaves of red curly kale (*Brassica oleracea* L. convar. *acephala* (DC.) Alef. var. *sabellica* L.), variety 'Redbor F1 hybrid'. The kale was grown at an experimental field (59° 40′ N) in the years 2007−2009. The analysis of kale extract by HPLC-DAD-ESI-MS has allowed the determination of 47 different acylated and nonacylated flavonoid glycosides and complex hydroxycinnamic acids. Those compounds included mono- to tetraglycosides of quercetin, kaempferol, and cyanidin and derivatives of *p*-coumaric, ferulic, sinapic, and caffeic acid. Among the compounds characterized, four flavonols, three anthocyanins, and three phenolic acids were identified in the *Brassica* family for the first time. Aglycones and conjugated polyphenols were quantified by HPLC-DAD using commercially available standards. The main flavonol, anthocyanin, and phenolic acid were kaempferol-3-sinapoyl-diglucoside-7-diglucoside, cyanidin-3-sinapoyl-feruloyl-diglucoside-5-glucoside, and disinapoyl-diglucoside, respectively, each representing 9.8, 10.3, and 4.9% of the total amount of 872 mg polyphenol equivalents per 100 g of fresh kale. Variations between individual plants and growing seasons were of the same order of magnitude for total phenolics and total monomeric anthocyanins.

KEYWORDS: Kale; *Brassica oleracea* L. convar. *acephala* var. *sabellica*; polyphenols; flavonoids; flavonols; anthocyanins; hydroxycinnamic acids; liquid chromatography; mass spectrometry; metabolite profiling

INTRODUCTION

Vegetables from the Brassicaceae family are among the most commonly grown vegetables worldwide. The family includes cabbage, broccoli, cauliflower, kale, collards, and Brussels sprouts. *Brassica* vegetables are rich in bioactive compounds, including polyphenols, tocopherols, ascorbic acid, carotenoids, and glucosinolates (*1*−*3*). Epidemiological data have shown the ability of *Brassica* vegetables to lower the risk of cardiovascular diseases and several types of cancer (*4*), especially cancers of the gastrointestinal tract, and to reduce the mortality compared to persons with low consumption of fruits and vegetables (*5*). These beneficial properties of *Brassica* vegetables have been associated with the presence of bioactive compounds, which often have antioxidant and free radical scavenging properties, and are also reported to affect gene expression, cell signaling, and cell adhesion (*6*).

Flavonoids, a group of secondary plant metabolites, are the most abundant and diverse group of polyphenols in our diet. To date, more than 8000 different flavonoids have been identified (*7*). The most widespread subclass of flavonoids is the flavonols, with the most abundant aglycones quercetin and kaempferol, which often occur as complex conjugates, with glycosylation and acylation of the aglycone (*7*). Another flavonoid subclass is the anthocyanins, responsible for the red, blue, and purple colors in the plant kingdom. In plant tissues these compounds are, like the flavonols, invariably present as sugar conjugates and often acylated with hydroxycinnamic acids. The most abundant hydroxycinnamic acids are *p*-coumaric, caffeic, ferulic, and sinapic acids, often found in conjugation with sugar or other hydroxycinnamic acids. It is the biosynthesis in the plant, which is influenced by many factors, such as cultivar, climate, postharvest treatments, and agricultural and environmental factors, that determines the polyphenolic content in the plant (*1*, *8*−*11*).

Kale is a vegetable belonging to the Brassicaceae (mustards) family and *Brassica oleracea* L. (cabbage) species and is closest in form to the wild cabbage. The *B. oleracea* group includes, besides kale, broccoli, Brussels sprouts, cabbage, cauliflower, collards, and kohlrabi. Kale is among the hardiest of vegetable crops, growing in colder climates than other cabbages, and tolerates temperatures as low as −15 °C. Kale tastes sweeter and more flavorful after being exposed to frost due to sugars increase in the tissues when exposed to cooler weather (*9*). In addition to the green varieties, there also exist red/purple-colored kale varieties.

*Corresponding author (at Nofima Mat AS, phone + 47 64970108; fax + 47 64970333; e-mail helle.olsen@nofima.no).

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One of the most used red kale varieties is the 'Redbor F1 hybrid', a strongly colored purple/red kale with frilly leaves. The red color is a consequence of the presence of anthocyanins, which have been thoroughly studied in red cabbage (*12*−*14*) and in violet cauliflower (*15*).

Among *Brassica* vegetables, kale has been reported to exhibit the highest antioxidant capacity and a high variety and concentration of vitamins, minerals, dietary fiber, glucosinolates, carotenoids, flavonoids, and phenolic acids (*1*−*3*, *16*−*18*). Recent studies have demonstrated that the structure of the flavonoids and hydroxycinnamic acids in *Brassica* species, such as cauliflower, broccoli, cabbage, and kale, often is complex with up to five sugar residues present, and some with further substitution with hydroxycinnamic acid residues (*12*, *19*−*21*). As far as we know, detailed characterization of the native polyphenols in red curly kale has not been reported. To be able to understand the human gastrointestinal absorption, the metabolism, and in vivo functions of the *Brassica* polyphenols, it is important to reveal their exact chemical structures, their reactive groups, and their abundance in the edible part of the plant. Also, when effects of processing and culinary treatments on *Brassica* vegetables are evaluated, it would be valuable to report the native polyphenols, not only the aglycones. To assess any health-promoting function of the *Brassica* polyphenols in humans, the bioavailability of a compound is crucial and is dependent on several parameters, that is, concentration, chemical structure, and the food matrix.

The aim of the present study was to characterize and quantify the naturally occurring polyphenols in a common red variety of curly kale used for human consumption, in order to increase the knowledge of polyphenol contents and yearly variation in a 3 year time frame. The analyses of the native polyphenols and flavonol aglycones were performed using up-to-date analytical tools: liquid chromatography with diode array and electrospray ionization multistage mass spectrometry (HPLC-DAD-ESI-MS^n) detection.

MATERIALS AND METHODS

Chemicals. Gallic acid, quercetin, quercetin-3-rhamnosylglucoside (rutin), and Folin−Ciocalteu's phenol reagent were purchased from Sigma Chemical Co. (St. Louis, MO). Kaempferol and chlorogenic acid hemihydrate were purchased from Fluka Chemie GmbH (Buchs, Switzerland). Sodium carbonate, methanol, acetonitrile, ethyl acetate, and formic acid were obtained from Merck KGAa (Darmstadt, Germany). Cyanidin-3-glucoside was purchased from Polyphenols Laboratories AS (Sandnes, Norway). All solvents were of HPLC grade, and the water used was of Milli-Q quality (Millipore Corp., Bedford, MA).

Plant Material. The red variety of curly kale (*Brassica oleracea* L. convar. *acephala* (DC.) Alef. var. *sabellica* L. 'Redbor') was grown at the experimental fields of the Norwegian University of Life Sciences (59° 40′ N) in the years 2007, 2008, and 2009. Each year, curly kale plants from the same growing field were harvested approximately 140 days after transplantation into the field, in mid-October. The same day as harvested, five curly kale plants were randomly picked and rinsed for stalks, and leaves without damage from each of the five plants, approximately 0.6 kg, were immersed in portions into a mortar filled with liquid nitrogen and ground into small, homogeneous pieces. The leaf material was stored at −80 °C until extraction.

Extraction of Phenolic Compounds. Extraction was performed as earlier described (*21*) with some modifications. Frozen ground leaf material (5 g) was mixed with 15 mL of acidified methanol (0.1% v/v formic acid) and homogenized with a Polytron homogenizer, PT3100 homogenizer (Kinematica AG, Littau Switzerland). After 15 min on ice, the samples were centrifuged (31000*g*, 10 min, 4 °C), the supernatants were collected, and the pellets were re-extracted with 10 mL of acidified 80% (v/v) methanol in water. The supernatants were combined and stored at −80 °C until analysis. The extractions of each plant were made in duplicate.

Dry Weight. Dry matter content was determined by freeze-drying (Christ Gamma 1-16LSC, Martin Chris Gefriertrocknungsanlagen GmBH, Germany) 3 g of plant material to constant weight.

Total Phenolics (TP) Determination. TP content in the kale extracts was determined according to the Folin−Ciocalteu method as described by Waterhouse (*22*) with some modifications. The methanolic extracts of kale were diluted 1:20 with water, mixed with 1.0 mL of Folin−Ciocalteu's reagent (1:10 v/v, diluted with water), and incubated for 2 min before 0.8 mL of sodium carbonate (7.5% w/v) was added. The mixtures were incubated for 60 min at ambient temperature before absorption was measured at 765 nm in a UV−vis spectrophotometer (8453 UV−visible, Agilent Technologies, Waldbronn, Germany). All extracts were analyzed in duplicate. TP contents were determined with the use of an external standard curve and expressed as milligram gallic acid equivalents (GAE) per 100 g of fresh weight of kale (mg GAE/100 g of fw).

Total Monomeric Anthocyanins (TMA) Determination. TMA were determined according to the pH differential method (*23*). The methanolic extracts were diluted in two different buffers, 25 mM potassium chloride pH 1.0 and 0.4 M sodium acetate pH 4.5. After 20 min of incubation at ambient temperature, absorption was measured at 520 and 700 nm in a UV−vis spectrophotometer (Agilent Technologies). TMA were calculated as described in the method protocol (*23*) using a molecular weight of 449.2 g mol^{-1} for cyanidin-3-glucoside and expressed as milligram cyanidin-3-glucoside equivalents (CGE) per 100 g of fresh weight of kale (mg CGE/100 g of fw). All extracts were analyzed in duplicate.

Acid Hydrolysis. The methanolic extracts of red curly kale were subjected to total acid hydrolysis (*9*) to obtain the flavonol and anthocyanidin aglycones for characterization and quantification. The extracts (2 mL) were mixed with 2 M hydrochloric acid (2 mL) in a headspace crimp-top vial (Agilent Technologies). The vials were flushed with nitrogen gas for 15 s, sealed with crimp caps with silicone septa, and kept in a water bath at 94 °C for 60 min. After cooling of the extracts, they were filtered through a 0.45 μm Millex HV filter and immediately analyzed by HPLC-DAD-ESI-MS^n.

Characterization of Phenolic Compounds by HPLC-DAD-ESI-MS^n. Phenolic compounds in the methanolic kale extracts and in the hydrolysates were characterized using an Agilent 1100 series HPLC system (Agilent Technologies) with an ESI interface and MSD XCT ion trap mass spectrometer (MS) (Agilent Technologies). Chromatographic separation was performed on an analytical Betasil RP-C_{18} column (250 × 2.1 mm i.d., 5 μm particle size) equipped with a C_{18} guard column (4.0 mm × 2.1 mm i.d., 5 μm particle size), both from Thermo Hypersil-Keystone (Bellefonte, PA). The column temperature was set to 30 °C, and the injection volume was 3 μL. Solvent A consisted of acetic acid/water (2:98, v/v), and solvent B consisted of acetic acid/water/acetonitrile (2:48:50, v/v/v). The elution gradient used was 10−41% B in 60 min and 41−100% B in 10 min, followed by 7 min at 100% B, with a flow rate of 0.20 mL/min. The column was allowed to equilibrate for 10 min between the injections. The Agilent 1100 series HPLC system was equipped with a degasser, an autosampler cooled to 4 °C, and a photodiode array detector. Spectral data were accumulated in the range of 180−600 nm. The LC eluate was introduced directly into the ESI interface without splitting, at a flow rate of 0.20 mL min^{-1}, and the phenolic compounds were analyzed in both negative and positive ionization modes. Fragmentation (MS$^{2-5}$) was carried out in the automatic mode; that is, the two most abundant ions in MS$^{1-4}$ were fragmented. The nebulizer pressure was 40 psi; the flow rate of nitrogen dry gas, 10 L min^{-1}; the dry temperature, 350 °C; and the capillary voltage, 3.5 kV. Analysis was carried out using scan from m/z 100 to 1800, with a scan speed of 27000 amu/s. Nitrogen gas was used as the drying gas and helium gas was used as the collision gas in the fragmentation experiments.

Quantitative Analysis. Due to the lack of commercial standards of native polyphenols, quantification of the aglycones is commonly used. Analysis of the aglycones in the extracts after acid hydrolysis was carried out on the Agilent 1100 series HPLC-DAD-MS^n system as described above. The HPLC-MS conditions used were as described above. Aglycones were quantified on the basis of external standards of quercetin, kaempferol, and cyanidin, in the concentration range of 20−100 μg mL^{-1}. Chromatograms were recorded at 370 nm (quercetin and kaempferol) and 530 nm (cyanidin). Naturally occurring flavonols, anthocyanins, and phenolic acids in the methanolic extract of red curly kale were quantified

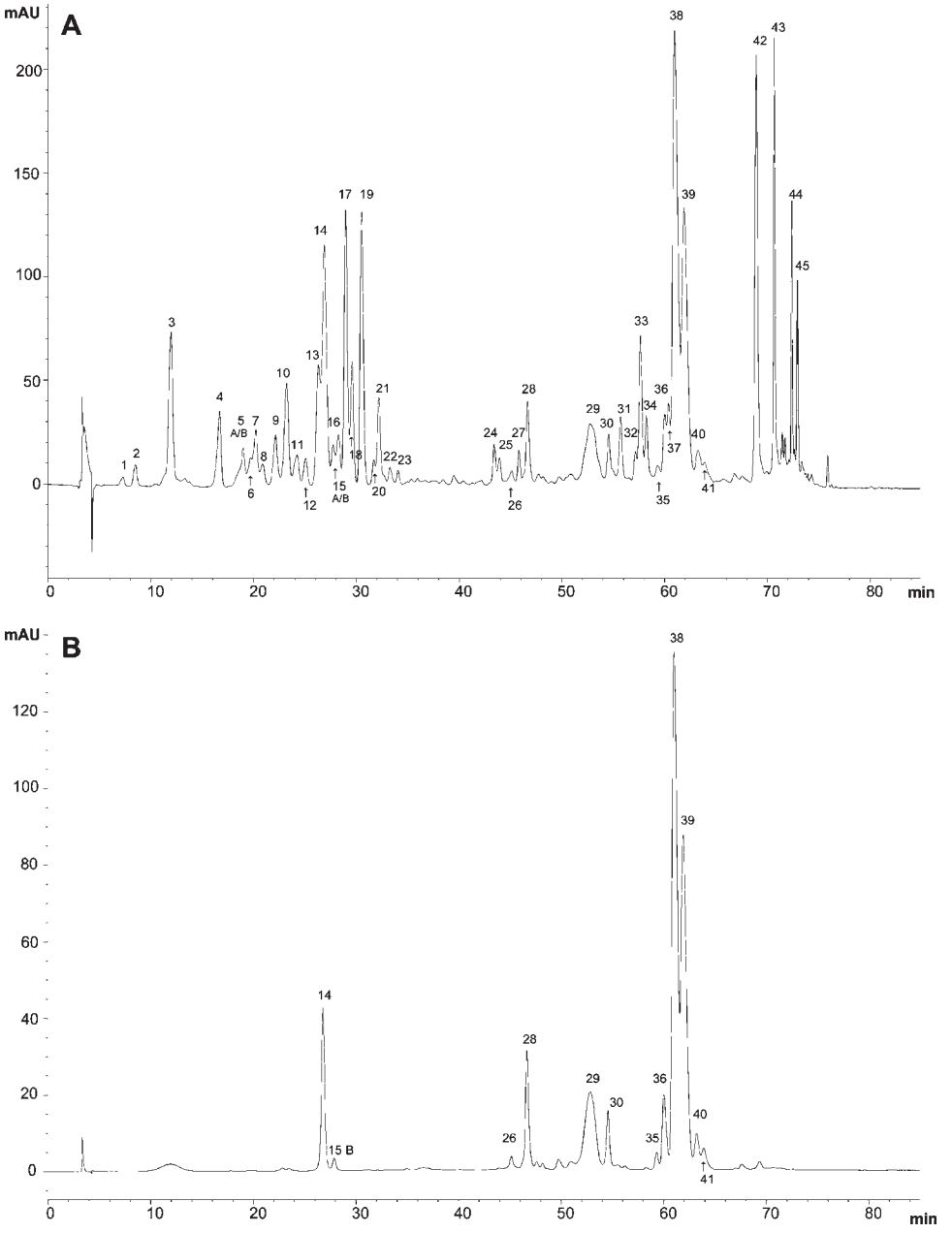

Figure 1. HPLC-DAD chromatogram of acidified methanolic extract of 'Redbor' curly kale recorded at (**A**) 330 nm and (**B**) 530 nm. Peak numbers refer to **Tables 1−3**.

using quercetin-3-rhamnosylglucoside (rutin) at 330 nm, cyanidin-3-glucoside at 530 nm, and chlorogenic acid at 330 nm, in the concentration range of 3−150 μg mL^{-1}. The results are expressed as rutin equivalents (RE), cyanidin equivalents (CGE), and chlorogenic equivalents (CAE), respectively.

Statistical Analysis. The differences in phenolic content between harvest years were explored using Levene's test for equality of variances, one-way analysis of variance (ANOVA), and Tukey and Games−Howell post hoc tests. All analyses were performed with a significance level of $\alpha = 0.05$ using the statistical software SPSS 18.0 (SPSS Inc., Chicago, IL). The statistical analyses were based on five plants ($n = 5$) each year.

RESULTS AND DISCUSSION

Characterization of Phenolic Compounds by HPLC-DAD-ESI-MS^n. Characterization of the phenolic compounds in extracts of red curly kale (*Brassica oleracea* L. convar. *acephala* (DC.) Alef. var. *sabellica* L. 'Redbor') was based on chromatographic behavior, mass spectra obtained under electron spray ionization (ESI) conditions, UV−vis spectra, and comparison with reference compounds and scientific publications (*12−15*, *19−21*, *24−28*). The chromatographic profile of the phenolic compounds is shown in **Figure 1**. Forty-seven complex structures were identified (**Tables 1−3**), including 10 novel phenolic compounds reported for the first time in *Brassica*. Within the flavonoid classes, both flavonols and anthocyanins were detected. In addition, a wide range of hydroxycinnamates and one hydroxybenzoate were detected. For most flavonoids, negative ionization mode provided the highest sensitivity and selectivity; therefore, mainly the mass spectra and fragmentation patterns of the flavonols obtained in negative mode are discussed. However, the anthocyanins have maximum sensitivity in positive mode MS, due to their inherent positive charge, and the information from both positive and negative modes has been used for characterization of the anthocyanins. The MS information of the phenolic acids was

Table 1. Chromatographic and Spectrometric Properties of Characterized Anthocyanins in Methanolic Extracts of Red Curly Kale Using HPLC with Diode Array and Electrospray Ionization MS^n Detection

					m/z of the main fragments					
peak[a]	tentative ID[b]	t_R (min)	MW	MS (*m/z*); ID	MS^2 ions[c]	MS^3 ions	MS^4 ions	λ_{max} (nm)	concentration[d] (mg CGE/100 g of fw)	SD
14	cyan-3-sin-diglu-5-glu	27.1	979	977 $[M-2H]^-$	815, 771, 609	609, 429, 339		236, 278, 340, 530	23.67	0.05
				979 $[M]^+$	**817**, 449, 287	287				
15B	cyan-3-fer-diglu-5-glu	27.8	949	949 $[M]^+$	**787**, 449, 287	287		256, 336, 530	3.54	0.01
26	cyan-3-triglu-5-glu	45.0	935	935 $[M]^+$	**773**, 449, 287	287		290, 536	3.50	0.01
28	cyan-3-sin-fer-diglu-5-diglu	46.7	1317	1315 $[M-2H]^-$	**1153**, 991	785, 495	285	238, 286, 326, 536	18.43	0.06
				1317 $[M]^+$	**1155**, 993, 449	**993**, 787, 447	**787**, 287			
29	cyan-3-caf-fer-diglu	52.9	949	947 $[M-2H]^-$	785	**609**, 285		236, 280, 330, 526	20.51	0.08
				949 $[M]^+$	**787**, 449, 287					
30	cyan-3-sin-caf-diglu-5-glu	54.6	1141	1139 $[M-2H]^-$	**977**, 771	285		240, 296, 330, 536	8.21	0.01
				1141 $[M]^+$	**979**, 449	287				
35	cyan-3-hydfer-fer-diglu-5-glu	59.4	1141	1139 $[M-2H]^-$	**977**, 785, 449	**785**, 285	**623**, 285	240, 284, 334, 536	4.14	0.01
				1141 $[M]^+$	**979**, 449	287				
36	cyan-3-sin-*p*-coum-diglu-5-glu	60.3	1125	1123 $[M-2H]^-$	961	**755**, 815, 285	609	236, 296, 322, 536	12.11	0.01
				1125 $[M]^+$	**963**, 449	287				
38	cyan-3-sin-fer-diglu-5-glu	61.1	1155	1153 $[M-2H]^-$	**991**, 785	**785**, 285	**609**, 285	238, 296, 330, 536	90.29	0.31
				1155 $[M]^+$	**993**, 449	**787**, 287	**625**, 445, 287			
39	cyan-3-disin-diglu-5-glu	62.0	1185	1183 $[M-2H]^-$	**1021**, 815	**815**, 285	609	238, 298, 332, 536	76.48	0.20
				1185 $[M]^+$	**1023**, 449	**287**, 817				
40	cyan-3-difer-diglu-5-glu	63.3	1125	1123 $[M-2H]^-$	**961**, 785	**785**, 285	**609**, 285	240, 296, 330, 536	7.73	0.02
				1125 $[M]^+$	**963**, 449	287				
41	cyan-3-fer-sin-diglu-5-glu	64.0	1155	1153 $[M-2H]^-$	**991**, 815	**785**, 285		240, 294, 330, 536	4.78	0.01
				1155 $[M]^+$	**993**, 449	287				

[a] Peak numbering refers to the peaks in **Figure 2**. [b] Abbreviations: cyan, cyanidin; glu, glucoside; diglu, diglucoside; triglu, triglucoside; hydfer, hydroxyferuloyl; fer, feruloyl; difer, diferuloyl; *p*-coum, *p*-coumaroyl; caf, caffeoyl; sin, sinapoyl; disin, disinapoyl. [c] The most abundant ions are shown in bold. [d] The concentration of the native anthocyanins is the average value of 3 years (2007−2009) and expressed as cyanidin-3-glucoside equivalents (CGE) per 100 g of fw. $n = 15$, five plants from three years (2007−2009).

rather weak, due to low sensitivity in both negative and positive ionization modes.

Polyphenols generally occur in nature as conjugates of sugars. The only sugar moiety found in *B. oleracea* is glucose, occurring as mono-, di-, tri-, tetra-, and pentaglucosides; all glycosidic bonds in flavonoids in *B. oleracea* are found to be *O*-glycosidic (*19*, *20*, *25*, *26*, *28*). In MS analysis, cleavage of the first glycosidic linkage is expected to take place at the *O*-glycosidic bond at the 7-position of the flavonols (*19*) and the 5-position of the anthocyanins (*27*), leading to the fragmentations $[(M-H)-162]^-$ for monohexosides and $[(M-H)-324]^-$ for dihexosides (*19*). The remaining glucose moieties of the flavonoid molecule are expected to be linked to the hydroxyl group at the 3-position of the aglycone. The disaccharide moieties of the flavonoids in *Brassica* species are mainly sophorosides (*21*,*26*,*29*). The interglucoside linkage can be determined by the MS fragmentation. Losses of 180, 162, and 120 amu indicate a sophoroside with a 1→2 interglucoside linkage (*29*), which has often been found in *Brassica* species. Fragmentation that only gives loss of 324 amu, and in some cases low abundance of 162 amu, corresponds to a diglucoside with a 1→6 linkage, that is, gentiobioside (*29*). A large number of the flavonoids detected had UV spectra suggesting that they were acylated derivatives, in which caffeic, ferulic, hydroxyferulic, *p*-coumaric, and sinapic acids were linked to the flavonoid glucoside molecules. Their characteristic spectra showed a flavonoid spectrum overlapped with a hydroxycinnamic acid spectrum with a broad maximum around 330 nm. In addition, complete acid hydrolysis releases the sugar moiety from the flavonoid aglycone by deglycosylation (*29*). Kaempferol, quercetin, and cyanidin were identified as the aglycones in red curly kale 'Redbor' on the basis of retention times, UV spectra, and MS information, using commercial reference compounds.

Anthocyanins. A total of 12 anthocyanins were detected in a red variety of curly kale (**Table 1**), and 3 of these (compounds **26**, **35**, and **40**) have not previously been reported in *Brassica* species. The anthocyanins were cyanidin glycoside acylated derivatives with different hydroxycinnamic acids showing λ_{max} at ∼330 and 526−536 nm. Only one nonacylated anthocyanin was identified.

In accordance with previously published data on anthocyanins in *Brassica* species, only cyanidin derivatives were found in red kale (*12*−*15*). The anthocyanins found in kale were mainly cyanidin-3-diglucoside-5-glucoside derivatives acylated with different hydroxycinnamic acids at the diglucosyl moiety in the 3-position. An HPLC-DAD chromatogram of kale extract recorded at 530 nm is shown in **Figure 1B**. Chromatographic conditions in the present study were not fully optimized for analysis of the anthocyanins, and therefore, some of the anthocyanin peaks in the chromatograms became broad. Characterization was based on chromatographic retention, UV−vis and mass spectra, and comparison with previous findings (*12*−*15*). Compound **14** was tentatively identified as cyanidin-3-sinapoyl-diglucoside-5-glucoside (**Table 1**), with a molecular weight of 979. Fragmentation in positive mode of *m/z* 979 caused ions at *m/z* 817 by loss of a glucosyl residue (162 amu) from the terminal 5-position. Additional fragmentation revealed a loss of 530 amu, corresponding to a sinapoyl-diglucosyl residue (206 + 324 amu), from the terminal 3-position. Compounds **15B** and **29** displayed similar fragmentation pattern in positive mode ($[M]^+$ *m/z* 949, MS^2 *m/z* 787 $[M-162]^+$, and MS^3 *m/z* 287 $[M-162-176-\text{diglucose}]^+$). However, the retention times of the two compounds on a reverse phase C_{18} column differed by 25.1 min, which indicates that compound **15B** must be an anthocyanin with much higher polarity. Considering the molecular weight, compound **15B** was tentatively identified as cyanidin-3-feruloyl-diglucoside-5-glucoside and compound **29** as cyanidin-3-caffeoyl-feruloyl-diglucoside. Compound **26** was the only anthocyanin without acylation with a hydroxycinnamic acid. On the basis of its UV spectra (λ_{max} 290 and 536 nm) and MS fragmentation pattern (*m/z* 935→773→287), compound **26** was tentatively identified as cyanidin-3-triglucoside-5-glucoside. To the best of our knowledge, the characterization of compound **26** has not previously been reported in any *Brassica* vegetables. Compound **28** gave

Table 2. Chromatographic and Spectrometric Properties of Characterized Flavonols in Methanolic Extracts of Red Curly Kale Using HPLC with Diode Array and Electrospray Ionization MS^n Detection

					m/z of the main fragments					
peak[a]	tentative ID[b]	t_R(min)	MW	MS (*m/z*); ID	MS^2 ions[c]	MS^3 ions	MS^4 ions	λ_{max} (nm)	concentration[d] (mg RE/100 g of fw)	SD
4	quer-3-diglu-7-glu	16.9	788	787 $[M-H]^-$	625	445, 301	301	256, 352	30.44	0.08
				789 $[M+H]^+$	627	463,303				
5A	quer-3-diglu-7-glu	19.0	788	787 $[M-H]^-$	**625**, 463	**463**, 301	301	266, 342	3.65	0.01
				789 $[M+H]^+$	**627**, 463, 301	**465**, 303	303			
5B	quer-3-diglu-7-diglu	19.4	950	949 $[M-H]^-$	625 649	445, **301**		254, 354	—[e]	
				973 $[M+Na]^+$		347	302			
6	quer-3-hydfer-diglu-7-glu	19.7	980	979 $[M-H]^-$	817, 787, **625**	445, 301		264, 342	12.54	0.04
7	kaemp-3-diglu-7-glu	20.4	772	771 $[M-H]^-$	609	429, 285		266, 346	20.82	0.04
				773 $[M+H]^+$	**611**, 449	**449**, 287	287			
8	quer-3-caf-diglu-7-glu	21.1	950	949 $[M-H]^-$	787	625	**445**, 301	248, 340	8.10	0.01
9	kaemp-3-diglu-7-diglu	22.3	934	933 $[M-H]^-$	609	429, 285		266, 344	18.00	0.03
10	kaemp-3-hydfer-diglu-7-glu	23.4	964	963 $[M-H]^-$	801	609	429, 285	236, 330	38.33	0.05
11	kaemp-3-hydfer-diglu-7-diglu	24.4	1126	1125 $[M-H]^-$	801	609	285	234, 268, 330	11.05	0.02
12	kaemp-3-caf-diglu-7-glu	25.2	934	933 $[M-H]^-$	771	609	429, 285	248, 268, 332	9.06	0.02
13	quer-3-sin-triglu-7-diglu	26.4	1318	1317 $[M-H]^-$	993	787	**445**, 301	246, 270, 342	19.36	0.09
14	quer-3-sin-diglu-7-diglu	27.1	1156	1155 $[M-H]^-$	**831**, 787, 625	625	445, **301**	242, 270		
15A	quer-3-fer-diglu-7-glu	27.8	964	963 $[M-H]^-$	**801**, 787, 625	625	445, **301**	262, 336	—[f]	
16	quer-3-fer-diglu-7-diglu	28.3	1126	1125 $[M-H]^-$	**801**, 625	625	445, **301**	254, 294, 340	14.26	0.03
18	kaemp-3-sin-triglu-7-diglu	29.6	1302	1301 $[M-H]^-$	977	771	**609**, 429, 285	240, 270, 332	34.86	0.06
19	kaemp-3-sin-diglu-7-diglu	30.6	1140	1139 $[M-H]^-$	815	609	429, **285**	240, 270, 334	85.92	0.15
20	kaemp-3-fer-triglu-7-diglu	31.7	1272	1271 $[M-H]^-$	947	771	**609**, 429, 285	240, 270, 334	5.41	0.01
21	kaemp-3-fer-diglu-7-diglu	32.2	1110	1109 $[M-H]^-$	**785**, 609	609	**429**, 285	244, 268, 332	23.75	0.05
22	kaemp-3-diglu-7-glu	33.2	772	771 $[M-H]^-$	609	447, **285**		232, 266, 348	3.50	0.02
23	kaemp-3-diglu-7-diglu	34.0	994	993 $[M-H]^-$	**609**, 285	285		232, 266, 346	2.45	0.01
24	kaemp-3-fer-diglu-7-glu	43.3	966	965 $[M-H]^-$	803	285 361		236, 298, 328	6.37	0.01
				989 $[M+Na]^+$	**523**, 827					
25	kaemp-3-sin-diglu-7-glu	43.8	978	979 $[M+H]^+$	**817**, 449, 287	287		240, 330	2.81	0.01
31	quer-3-disin-triglu-diglu	55.6	1524	761 $[M-2H]^{2-}$	599	993	787, 445, 301	242, 334	14.42	0.04
33	quer-3-disin-triglu-7-glu	57.5	1362	680 $[M-2H]^{2-}$	599	993	787, 445, 301	242, 334	40.64	0.10
34	kaemp-3-disin-triglu-7-diglu	58.3	1508	753 $[M-2H]^{2-}$	591	977	771, 609, 285	240, 320	14.27	0.03
37	kaemp-3-disin-triglu-7-glu	60.4	1346	673 $[M-2H]^{2-}$	591	977	771, 609	240, 330	20.02	0.16

[a] Peak numbering refers to the peaks in **Figure 2**. [b] Abbreviations: kaemp, kaempferol; quer, quercetin; glu, glucoside; diglu, diglucoside; triglu, triglucoside; hydfer, hydroxyferuloyl; caf, caffeoyl; fer, feruloyl; coum, coumaroyl; sin, sinapoyl; disin, disinapoyl. [c] The most abundant ions are shown in bold. [d] The concentration of the native flavonols is the average value of 3 years (2007−2009) and expressed as rutin equivalents (RE) per 100 g of fw. $n = 15$, five plants from 3 years (2007−2009). [e] Coelution of compounds **5A** and **5B**. [f] Coelution of compounds **15A** and **15B**.

Table 3. Chromatographic and Spectrometric Properties of Characterized Phenolic Acids in Methanolic Extracts of Red Curly Kale Using HPLC with Diode Array and Electrospray Ionization MS^n Detection

					m/z of the main fragments					
peak[a]	tentative ID[b]	t_R(min)	MW	MS (*m/z*); ID	MS^2 ions[c]	MS^3 ions	MS^4 ions	λ_{max} (nm)	concentration[d] (mg CAE/100 g of fw)	SD
1	fer-triglu	7.3	680	679 $[M-H]^-$	517	337	193	232, 326	2.11	0.01
				703 $[M+Na]^+$	541, 347	347	329			
2	diprotocatechuic acid-diglu	8.5	632	631 $[M-H]^-$	315	153	108	236, 316	2.01	0.01
				657 $[M+Na]^+$	495, **333**	307, 214	214			
3	caffeoylquinic acid	12.9	354	353 $[M-H]^-$	353	**191**, 179	127	238, 300sh[e], 324	26.63	0.07
17	benzoyl-sin-triglu	28.9	794	793 $[M-H]^-$	673	511	206	240,330	31.38	0.06
				795 $[M+H]^+$	409	185				
27	sin-fer-triglu	48.8	908	909 $[M+H]^+$	**685**, 523	**523**, 329	329	240, 326	6.10	0.02
32	sin-fer-triglu	57.2	870	869 $[M-H]^-$	707, **369**	206		240, 320	5.38	0.01
42	disin-diglu	68.9	754	753 $[M-H]^-$	529	204		236, 326	43.09	0.11
				777 $[M+Na]^+$	553	329				
43	sin-fer-diglu	70.7	724	723 $[M-H]^-$	499, 529	193		236, 326	24.00	0.06
44	trisin-diglu	72.4	960	959 $[M-H]^-$	735	529, 511	223	240, 330	12.36	0.03
45	disin-fer-diglu	72.9	930	929 $[M-H]^-$	705	529, 499	499, 223	240, 330	9.63	0.02

[a] Peak numbering refers to the peaks in **Figure 2**. [b] Abbreviations: glu, glucoside; diglu, diglucoside; triglu, triglucoside; fer, feruloyl; sin, sinapoyl; disin, disinapoyl; trisin, trisinapoyl. [c] The most abundant ions are shown in bold. [d] The concentration of the native phenolic acids is the average value of 3 years (2007−2009) and expressed as chlorogenic acid equivalents (CAE) per 100 g of fw. $n = 15$, five plants from 3 years (2007−2009). [e] sh, shoulder.

an $[M]^+$ ion at *m/z* 1317 with fragment ions at *m/z* 1155 and 993, caused by the loss of two successive glucosyl moieties, respectively. Additional fragmentation MS^4 (1317→1155→993) gave rise to the ions at *m/z* 787, which revealed the presence of a sinapoyl residue (loss of 206 amu), and to the cyanidin aglycone ion (*m/z* 287 $[993-(206+176+324)]^+$). Compound **28** was thus

tentatively identified as cyanidin-3-sinapoyl-feruloyl-diglucoside-5-diglucoside, previously identified in red cabbage (*12*−*14*). Compounds **30** and **35** showed similar $[M]^+$ ions at *m*/*z* 1141, but the different fragmentation patterns in negative mode indicated two different compounds, namely, cyanidin-3-sinapoyl-caffeoyl-diglucoside-5-glucoside and cyanidin-3-hydroxyferuloyl-feruloyl-diglucoside-5-glucoside, respectively. The fragmentation pattern of compound **30** in negative mode $[M - 2H]^-$ *m*/*z* 1139, MS^2 *m*/*z* 977 $[(M - 2H) - 162)]^-$ and *m*/*z* 771 $[(M - 2H) - 368]^-$, indicated a glucosyl moiety at the 5-position and a sinapoyl residue at the 3-position. The last fragmentation gave ions at *m*/*z* 285 (loss of 3 × 162 amu + 206 amu), corresponding to a residue consisting of three glucoses and a sinapoyl moiety at the 3-position. Cyanidin-3-sinapoyl-caffeoyl-diglucoside-5-glucoside (compound **30**) has previously been reported in red cabbage (*12*). MS fragmentation in negative mode of compound **35** revealed the presence of both a feruloyl moiety (loss of 176 amu) and a hydroxyferuloyl moiety (loss of 192 amu). Cyanidin-3-hydroxyferuloyl-feruloyl-digluoside-5-glucoside (compound **35**) has not previously been reported in *Brassica* species. From the UV spectra, MS fragmentation patterns, and previous publications (*12*, *14*, *15*), compound **36** was tentatively identified as cyanidin-3-sinapoyl-*p*-coumaroyl-diglucoside-5-glucoside.

Compound **38** was the most abundant anthocyanin in the red curly kale extract and was identified as cyanidin-3-sinapoyl-feruloyl-diglucoside-5-glucoside with a molecular weight of 1155. Negative mode fragmentation of *m*/*z* 1153 caused a loss of a glucosyl residue (loss of 162 amu) from the 5-position. Further fragmentation MS^3 (1155→991) gave rise to ions at *m*/*z* 785 and revealed the presence of a sinapoyl residue (loss of 206 amu). The event MS^4 (1155→991→785) produced ions at *m*/*z* 609 due to loss of 176 amu (feruloyl residue), as well as ions at *m*/*z* 285 (cyanidin). The fragmentation pattern of compound **38** indicated a substitution of sinapoyl-feruloyl-diglucoside in the 3-position. A similar fragmentation pattern was observed for compound **41**, an acylated anthocyanin glycoside, which was assigned cyanidin-3-feruloyl-sinapoyl-diglucoside-5-glucoside. The fragmentation pattern indicated that the feruloyl and sinapoyl residues were linked to the diglucoside in positions different from those in compound **38**; thus, the two compounds are isomers. The MS analysis in negative mode of compounds **39** and **40** showed that the deprotonated molecular ions at *m*/*z* 1183 and 1123 lost a monohexose residue from position 5, giving fragments at *m*/*z* 1021 and 961, respectively. Further fragmentation of their respective ions revealed losses of 206 and 176 amu, corresponding to sinapoyl and feruloyl groups, in the glucosyl residues at position 3 of compounds **39** and **40**, respectively. Additional fragmentation revealed the presence of another sinapoyl residue (loss of 206 amu) and a feruloyl residue (loss of 176 amu), respectively. MS^3 fragmentation gave a loss of a dihexose residue (324 amu) at the 3-position, resulting in the cyanidin aglycone ion (*m*/*z* 285). Both compounds had λ_{max} 330 nm. Thus, compounds **39** and **40** were assigned as cyanidin-3-disinapoyl-diglucoside-5-glucoside and cyanidin-3-diferuloyl-diglucoside-5-glucoside, respectively. Cyanidin-3-diferuloyl-diglucoside-5-glucoside (compound **40**) was tentatively identified for the first time in *Brassica* species.

Flavonols. The LC-DAD-MS analysis of the kale extract revealed a total of 26 flavonols, summarized in **Table 2**. Four flavonols, that is, quercetin-3-sinapoyl-triglucoside-7-diglucoside (compound **13**), kaempferol-3-feruloyl-triglucoside-7-diglucoside (compound **20**), kaempferol-3-feruloyl-diglucoside-7-glucoside (compound **24**), and kaempferol-3-sinapoyl-diglucoside-7-glucoside (compound **25**) were identified for the first time in kale. Several of the flavonols in the red curly kale extracts had UV spectra with a broad maximum around 330−340 nm, suggesting an acylation with hydroxycinnamic acids.

MS analysis of compound **13** showed that the deprotonated molecular ion at *m*/*z* 1317 lost a dihexose residue from position 7, giving the fragment at *m*/*z* 993. Further fragmentation revealed a loss of 206 amu, corresponding to sinapic acid, which was further confirmed by UV spectra (maximum ∼330 nm). The MS^3 fragmentation gave a loss of a trihexose residue at the 3-position (486 amu), leading to the quercetin aglycone (*m*/*z* 301). Thus, compound **13** was tentatively identified as quercetin-3-sinapoyl-triglucoside-7-diglucoside. During the MS fragmentation of compound **20** a loss of 324 amu, corresponding to a diglucosyl moiety at the terminal 7-position, was observed. Further fragmentation of the acylated ion, *m*/*z* 947, resulted in the loss of the hydroxycinnamic acid residue (feruloyl) and, finally, the loss of a triglucosyl residue, producing the kaempferol aglycone ion (*m*/*z* 285). Compound **20** was assigned as kaempferol-3-feruloyl-triglucoside-7-diglucoside. The MS analysis of compound **24** showed a deprotonated molecular ion at *m*/*z* 965. Fragmentation produced ions at *m*/*z* 803, corresponding to loss of a glucose moiety. MS^3 of the produced ion (*m*/*z* 803) led to a loss of ferulic acid (194 amu) and the glucosidic fraction (324 amu), and the aglycone ion (*m*/*z* 285) was observed. Compound **25** gave a $[M + H]^+$ ion at *m*/*z* 979, and the presence of a glucose moiety was clear due to the fragment ions at *m*/*z* 817 (loss of 162 amu), and other ions at *m*/*z* 817, 449, and 287 revealed the presence of sinapic acid derivative and two glucose moieties. From these data, compound **25** was tentatively identified as kaempferol-3-sinapoyl-diglucoside-7-glucoside. The remaining flavonols (compounds **4**−**12**, **14**, **16**, **18**, **19**, **21**−**23**, **31**, **33**, **34**, and **37**) were identified on the basis of identical MS spectra, MS fragmentation pattern, UV maxima, and retention times as flavonols, previously characterized in a green variety of curly kale (*21*).

Phenolic Acids. Eight hydroxycinnamic acid derivatives, one benzoic acid derivative, and one compound containing both groups were characterized in the red curly kale extract (**Table 3**). Two phenolic acids, feruloyl-triglucoside (compound **1**) and diprotocatechuic acid-diglucoside (compound **2**) were characterized for the fist time in *Brassica* vegetables. In accordance with the literature, the hydroxycinnamic acid derivatives and the benzoic acid derivative had UV spectra with absorption maxima at ∼250 and ∼320−330 nm (*30*). Compound **1** was tentatively identified as feruloyl-triglucoside, with fragmentation pattern *m*/*z* 679→517→337→193, confirming the presence of a glucoside (loss of 162 amu), a sophoroside (loss of 180 amu), and a ferulic acid moiety. Compound **2** was tentatively identified as diprotocatechuic acid-diglucoside, a benzoic acid derivative, with MS fragmentation pattern in negative mode *m*/*z* 631→315→153 and UV maxima at 236 and 316 nm. According to UV spectra and mass spectral data and retention order, compound **17** had structural features similar to the nonidentified hydroxycinnamic acid derivative found in the green variety of kale (*21*). Compound **17** was tentatively characterized as benzoyl-sinapoyl-triglucoside, with fragmentation pattern in positive mode *m*/*z* 795→409→185, corresponding to the sequential loss of a sinapoyl and a glucosyl moiety (224 + 162 amu), and finally the MS^3 fragmentation spectrum consisting of sodium adducts of hexose (*m*/*z* 185), previously reported for hydroxycinnamic acids (*31*). Compound **32** gave a $[M - H]^-$ ion at *m*/*z* 869 and fragments at *m*/*z* 707, 369, and 206 in the MS^{2-3} experiments caused by the loss of feruloyl, diglucosyl, and glucosyl moiety (176 + 324 and 163 amu) and was tentatively identified as sinapoyl-feruloyl-triglucoside. Compounds **3**, **27**, **42**−**45** have been characterized in our previous study on a green variety of curly kale, with identical MS spectra, MS fragmentation patterns, UV maxima, and retention times (*21*).

Table 4. Concentrations of Native Flavonols, Native Phenolic Acids, and Native Anthocyanins, Aglycones (Quercetin, Kaempferol, and Cyanidin), Total Phenolics, and Total Monomeric Anthocyanins in Methanolic Extracts of Red Curly Kale in the Years 2007−2009

	2007[a]	2008[a]	2009[a]	average[b]
native flavonols[c,d]	446 ± 31 a	424 ± 35 a	406 ± 19 a	425 ± 32
native phenolic acids[c,e]	153 ± 35 a	165 ± 23 a	171 ± 5 a	163 ± 24
native anthocyanins[c,f]	306 ± 59 ab	325 ± 19 a	220 ± 13 b	284 ± 58
kaempferol[g]	40 ± 4 a	42 ± 7 a	62 ± 5 b	48 ± 12
quercetin[g]	43 ± 6 a	49 ± 8 a	48 ± 4 a	47 ± 6
cyanidin[g]	19 ± 2 a	22 ± 3 a	38 ± 3 b	26 ± 9
total phenolics[h]	730 ± 34 a	697 ± 60 a	614 ± 21 b	680 ± 64
total monomeric anthocyanins[i]	96 ± 4 a	90 ± 3 a	69 ± 7 b	85 ± 13

[a] Average of the five plants each year ($n = 5$) ± standard deviation. For each row, different letters indicate significant differences ($p < 0.05$) [b] Average of the 3 years ± standard deviation. [c] Analyzed by HPLC and calculated as the sum of individual compounds. [d] Expressed as mg RE/100 g of fw. [e] Expressed as mg CAE/100 g of fw. [f] Expressed as mg CGE/100 g of fw. [g] Analyzed by HPLC after acid hydrolysis, expressed as mg/100 g of fw. [h] Analyzed by the Folin−Ciocalteu method, expressed as mg GAE/100 g of fw. [i] Analyzed by the pH-difference method, expressed as mg CGE/100 g of fw.

Quantitative Determination of the Polyphenols. Quantification results are based on five plants of the curly kale variety 'Redbor' grown in 2007−2009. It is well established that the flavonoid profile of vegetables is influenced by genetic and environmental factors (*1*, *9*−*11*). **Table 4** shows the total content of native flavonoids and phenolic acids, which were quantified as equivalents based on their subclasses, as commercial standards are not available for these naturally occurring phenolic compounds. The results are expressed as milligrams per 100 g of fw. The average dry matter content in the curly kale leaf samples was 20% (w/w), with a variation between 16 and 24%.

The native anthocyanins were quantified as cyanidin-3-glucosides equivalents (CGE), using HPLC-DAD analysis at 530 nm. The total contents of cyanidin derivatives in plants from 2007 to 2009 varied between 220 and 325 mg CGE/100 g of fw, with a mean value of 284 mg CGE/100 g of fw (**Table 4**). The total content of cyanidin derivatives varied significantly ($p < 0.01$) in plants grown in three different years, that is, 2007, 2008, and 2009. The concentrations of the individual anthocyanins ranged from 4 to 90 mg CGE/100 of g fw (**Table 1**), with cyanidin-3-sinapoyl-feruloyl-diglucoside-5-glucoside (compound **38**) as the most abundant anthocyanin in red kale, contributing 10.3% of the total amount of phenolic compounds, calculated as the sum of individual compounds as measured by HPLC (**Table 4**).

The naturally occurring flavonols were quantified as rutin equivalents (RE), with an average concentrations in plants from 2007 to 2009 of the individual flavonols ranging from 2 to 86 mg RE/100 g of fw (**Table 2**). The flavonol present at the highest level in the curly kale extract was kaempferol-3-sinapoyl-diglucoside-7-diglucoside (compound **19**), representing 9.8% of the total amount of phenolic compounds in the kale, which is in accordance with earlier findings in the green variety of curly kale (*21*). The total content of naturally occurring flavonol glycosides in the five plants from three years (2007−2009) varied marginally between 406 and 446 mg RE/100 g of fw, with no significant differences between the years (**Table 4**). The mean value of the total flavonols was 425 mg RE/100 g of fw. This is approximately 30% lower than in the green variety of curly kale (*21*).

The native phenolic acids were quantified as chlorogenic acids equivalents (CAE), with an average concentration in plants from 2007 to 2009 between 2 and 43 mg CAE/100 g of fw of the individual compounds (**Table 3**). Compound **42**, disinapoyl-diglucoside, was present at the highest concentration of the phenolic acids, representing 4.9% of the total amount of phenolic compounds in the kale. The total content of naturally occurring phenolic acids in five plants from each of the years, 2007−2009, varied between 153 and 171 mg CAE/100 g of fw, with no significant differences between the years. The mean value of the total phenolic acids was 163 mg CAE/100 g of fw (**Table 4**). In the red variety of curly kale grown in 2007−2009, the average concentration of total phenolics quantified by HPLC and calculated as the sum of the individual compounds was 872 mg equiv/100 g fw (= 284 mg CGE/100 g fw + 425 mg RE/100 g fw + 163 mg CAE/100 g fw), which corresponds well with the sum of phenolic compounds found in the green variety, that is, 850 mg RE/100 g of fw (*21*).

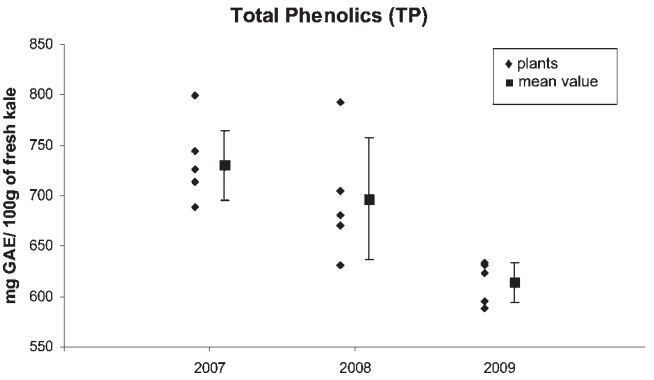

Figure 2. Concentrations of total phenolics (TP) in methanolic extracts of each of five plants of red curly kale over a 3 year period (2007−2009).

Naturally, free aglycones were not detected in the curly kale extracts. The concentrations of the three identified aglycones after acid hydrolysis of the extracts, that is, quercetin, kaempferol, and cyanidin, are shown in **Table 4**. The quercetin content varied between 43 and 49 mg/100 g of fw, with a mean value of 47 mg/100 g of fw, with no significant differences between the years 2007, 2008, and 2009. The kaempferol content ranged from 40 to 62 mg/100 g of fw, with a mean value of 48 mg/100 g of fw. There were significant ($p < 0.001$) differences in kaempferol content between the years 2007, 2008, and 2009, with significantly higher contents in plants from 2009. Previous studies on flavonol aglycones in green varieties of kale have reported a total content of quercetin between 7.7 and 82.2 mg/100 g of fw and kaempferol levels between 23.5 and 187.5 mg/100 g of fw (*11*, *21*, *32*−*34*). The cyanidin content of 'Redbor' varied between 19 and 38 mg/100 g of fw, with a mean value of 26 mg/100 g of fw. There were significant ($p < 0.001$) differences in cyanidin content between the years 2007, 2008, and 2009, with a significantly higher level in 2009 compared to the two previous years.

The average TP content in the red curly kale methanolic extract, determined with the Folin−Ciocalteu method, was 680 mg GAE/100 g of fw, with a variation from 614 to 730 mg GAE/100 g of fw in the years 2007−2009 (**Table 4** and **Figure 2**). There were significant ($p < 0.01$) differences in TP content between the years 2007, 2008, and 2009, with significantly lower TP content in 2009 than in 2007 and 2008 (**Table 4**). Zeitz et al. found a TP content of 996 mg GAE/100 g of fw in the genotype 'Redbor' (*10*). Reported TP content in different genotypes of kale ranged from 136 mg CAE/100 g to 996 mg GAE/100 g of fw (*9*−*11*, *35*, *36*).

The TMA in the red variety of curly kale were measured by means of their inherent pH-dependent color characteristics. The TMA in the red variety of curly kale extract was 85 mg CGE/100 g of fw, with a variation between 69 and 96 mg CGE/100 g fw in the years 2007−2009 (**Table 4** and **Figure 3**). There were significant

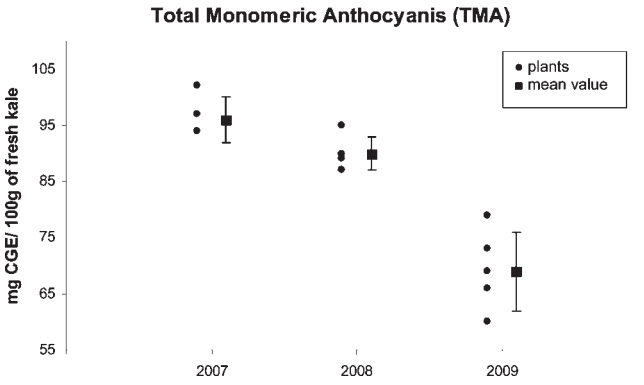

Figure 3. Concentrations of total monomeric anthocyanins (TMA) in methanolic extracts of each of five plants of red curly kale over a 3 year period (2007−2009).

($p < 0.001$) differences in TMA content between the years 2007, 2008, and 2009, with significantly lower TMA content in 2009 than in 2007 and 2008 (**Table 4**). The TMA level found in 'Redbor' curly kale is of the same magnitude as previously reported in other fresh red-colored *B. oleracea* species. In red cabbage, TMA contents from 41 to 322 mg CGE/100 g fw have been reported (*15*, *37*−*39*), and in two studies of purple cauliflower the range was 2−74 mg CGE/100 g fw (*15*, *40*).

In the present study, TP and TMA contents in curly kale plants from the same cultivar, grown at the same field in three different years, 2007−2009 (**Figure 2** and **3**), show that the variation between individual plants each year is of the same order of magnitude. However, many factors influence the composition and concentration of secondary plant metabolites, that is, genetic background, differences in climate (solar radiation, precipitation, and temperature), soil and its nutrient content, insect attacks, and maturity at harvest (*1*, *8*−*11*), and may make comparison of data from different studies and varieties difficult. This may explain the great variation in TP and TMA contents reported in the literature.

In summary, this is the first study characterizing and quantifying polyphenols in a red variety of curly kale. A total of 47 phenolic compounds were identified using complementary information from DAD and ESI-MS^n in negative and positive modes, revealing a large number of highly glycosylated and acylated quercetin, kaempferol, and cyanidin aglycones and complex hydroxycinnamic acids. Furthermore, the red variety of curly kale was a considerable source of polyphenols, with total contents of flavonols, 425 mg RE/100 g of fw; anthocyanins, 284 mg CGE/100 g of fw; and hydroxycinnamic acids, 163 mg CAE/100 g of fw. Information on the identity and content of the flavonoids and hydroxycinnamic acids present in a commonly used red variety of kale for human consumption *B. oleracea* L. convar. *acephala* var. *sabellica* cv. 'Redbor', grown at an experimental field and harvested and prepared for analysis under optimal conditions, should be useful in future databases.

ABBREVIATIONS USED

amu, atomic mass unit; ANOVA, analysis of variance; CAE, chlorogenic acid equivalents; CGE, cyanidin-3-glucoside equivalents; DAD, diode array absorbance detector; ESI, electrospray ionization; fw, fresh weight; GAE, gallic acid equivalents; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometer; MW, molecular weight; RE, rutin equivalents; TMA, total monomeric anthocyanins; TP, total phenolics; UV−vis, ultraviolet−visible light.

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